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1st EUROPEAN PURINE MEETING
SANTIAGO DE COMPOSTELA
September 4-6, 2019

**Abstracts from First European
Purine Meeting
September 4-6, 2019**

Guest Editors/ Editores Invitados
María Teresa Miras-Portugal
Javier Gualix
Rosa Gómez-Villafuertes

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1st EUROPEAN PURINE MEETING
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Santiago University – Santiago de Compostela
(Spain)

Promoted by the Spanish Purine Club



Welcoming Address

Dear Colleagues:

For more than 1.000 years, since the 814, Santiago was the place where all the Europeans and their diverse cultural heritages met in peace. This is a powerful reason to organise here our foundational meeting, hoping to be inspired by the most stunning Romanesque art.

Over the last purinergic meetings, we were aware of the advances in the field and how many of the obstacles were overcome. Thanks to long road paved by the pioneers, like the pilgrims, we can now enjoy of a splendid sight. Notwithstanding, we still need to put emphasis in basic science, and the ways to translate its achievements into novel pharmacological and clinical applications.

The experience of senior scientists offers the necessary background to settle where we are in the purinergic field. We wait for their advice concerning proposals for Symposia and more relevant speakers. But, to go further, the brain of young scientists will make the difference, and hence we will pay special attention to them. We hope that for many of the young scientists assisting to this meeting, the forthcoming Meeting could be opportunity to display their first poster, or make their first presentation, and even get their first award.

Convinced that the **First European Purine Meeting** will remain in your memories forever, now we start the way to Santiago de Compostela. We hope that all of us will meet again at the end of the way from 4-6 September 2019.

With my best regards.

M^a Teresa Miras-Portugal

President of the Scientific and Local Committee

Honorary President of the Royal Academy of Pharmacy of Spain

Acknowledgments. The Guest Editors would like to thank the Royal Academy of Pharmacy of Spain and his president Prof. Antonio Doadrio, together with the Academy staff, very specially to Manuel Tirado and Carlos Fernández, for their support in the Abstracts Book edition. We would like also to thank the Santiago University, specially the Medicine Faculty and the Center for Research in Molecular Medicine and Chronic Diseases (CIMUS).

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In Memoriam

Jesus Pintor (Suso)

Professor and Chair of Biochemistry and Molecular Biology

Universidad Complutense de Madrid

Academic Secretary of the Royal Academy of Pharmacy of Spain

Our dear friend and colleague, Suso, passed away on April the 2nd of 2019.

He was born in Vigo (Galicia), December the 26th of 1964

All of us who have enjoyed the immense privilege of meeting him and working with him and his scientific friends from all the countries of the world today we feel his absence.

Conferences-Plenary Lectures

CPL-01

Lecture in Honour Prof Geoff. Burnstock

**PURINERGIC MODULATION OF MYELIN, A
NEW TARGET FOR NEUROREGENERATIVE
THERAPIES**

Mariapia Abbracchio. University of Milano, Italy



Oligodendrocytes (OLs), the myelin-forming cells, have emerged as new targets to implement CNS recovery not only in classical demyelinating diseases like multiple sclerosis, but also in Alzheimer's, trauma, stroke and Amyotrophic Lateral Sclerosis. Important, clinical studies have confirmed that implementation of myelin repair does correlate with neuronal recovery, thus validating myelin protection and reconstruction as a new neuroreparative approach. Quiescent Oligodendrocyte Precursor Cells (OPCs) are present in brain's parenchyma and spinal cord throughout life. Upon damage, OPCs proliferate and migrate to lesioned areas to start differentiating into fully mature myelin-producing cells. Unfortunately, this reaction is often insufficient to complete repair, likely due to the inflammatory local milieu that markedly blocks OPCs at immature stages. In recent years, we have shown that OPCs express various types of purinoceptors regulating their quiescence, reaction to injury and maturation. Among these receptors, the P2Y-like GPR17 receptor is needed to start OPC differentiation but, after the immature OL stage, has to be downregulated to allow cells' terminal maturation. Here, we utilized the first *in house* developed fluorescent GPR17 reporter mouse for fate mapping studies to follow the final destiny of GPR17-expressing OPCs (GPR17⁺ cells) in different models of disease. In models characterized by high acute inflammation levels, like stroke and Experimental Autoimmune encephalomyelitis (EAE), GPR17⁺ cells proliferate and are attracted to demyelinating lesions, but only a small number of these cells can enter the lesion area to generate new myelin producing OLs. Conversely, in the cuprizone demyelination model, which is characterized by milder inflammation, many of the recruited GPR17⁺ cells do express terminal myelination markers suggesting progression to more mature phenotypes. In line with these findings, the first synthetic GPR17 selective ligand significantly retarded EAE development *in vivo*, supporting the validity of the GPR17-based neuroreparative approach. Supported by ERANET Neuron EC project and Italian FISM.

CPL-02

PURINERGIC SIGNALING IN MICROGLIAL FUNCTIONS

Kazuhide Inoue. Kyushu University, Fukuoka, Japan



Microglia are thought to derive from primitive macrophages in the yolk sac. In normal, microglia are ubiquitously distributed throughout central nervous system (CNS) and have small cell body bearing branched and motile processes. In response to peripheral nerve injury (PNI), microglia are activated with hypertrophic morphology, an increase in cell number, and alteration in the expression of genes including neurotransmitter receptors such as purinergic P2 receptors in the spinal cord (Tsuda et al 2005). Extracellular ATP activate microglia to evoke various cellular responses such as the production and release of bioactive factors including cytokines and neurotrophic factors (Inoue 2006), which in turn leads neuropathic pain (Tsuda et al 2012) and several diseases in CNS. Neuropathic pain is a debilitating pain state, which is often caused by physical injury, diabetes, bone compression in cancer. One of the symptoms of neuropathic pain is tactile allodynia (strong pain by light touching) which is not healed by non-steroidal anti-inflammatory drugs and opioids. We found that activated microglia of spinal dorsal horn (SDH) over-expressed P2X4 receptors which cause to release brain-derived neurotrophic factor (BDNF) after the stimulation of ATP. BDNF causes downregulation of the chloride transporter KCC2 in a subpopulation of lamina I secondary sensory neurons in SDH, which then causes a depolarizing shift in the anion reversal potential. This shift inverts the polarity of currents activated by γ -amino butyric acid (GABA) and glycine, such that GABA and glycine cause depolarization, rather than hyperpolarization, in these secondary sensory neurons resulting in tactile allodynia (Nature, 2003, Nature, 2005). This finding is a breakthrough for this science area and many papers were reported after the breakthrough (Nat Commun. 2014, Nat Commun. 2016, Nature Review Neuroscience 2018). Now, we know that the purinergic signaling of microglia play main roles of CNS diseases.

CPL-03

**TOOLS AND DRUGS FOR PURINE RECEPTORS
AND ECTONUCLEOTIDASES**

Christa Müller. University of Bonn, Germany



The recently revived interest in drugs acting on purine receptors or otherwise interfering with purinergic signaling has led to the initiation and conduct of several clinical trials. Adenosine is one of the most potent immunosuppressive agents of the innate immune system, and the release of adenosine is a major contributor to the immune escape of cancer cells. This has caused a hype about purinergic drug development including P1 (adenosine) receptor antagonists for the subtypes A_{2A} and A_{2B} and ecto-nucleotidase inhibitors. Moreover, P2 receptor antagonists have shown great potential, e.g. for the treatment of inflammation and pain.

Since more than 25 years, our group has focused on the development of tool compounds and drug molecules for protein targets involved in purinergic signaling. Recent advances in the field of assay development, fluorescent- and radiolabeled tool compounds, and potent, selective as well as multi-target drugs for purinergic receptors and ectonucleotidases will be presented.

COMINGS AND GOINGS OF SIGNALLING CASCADES ACTIVATED BY NUCLEOTIDES

Raquel Perez-Sen. Complutense University of Madrid, Spain



Nucleotide signalling network expands beyond signalling kinases to the inactivation mechanisms via protein phosphatases. In particular, the regulation of MAP kinase phosphatases (MKPs) with substrate selectivity towards ERK, p38 and JNK, allow nucleotides to participate in the homeostatic control of the biological processes activated by MAPKs. The MKPs are dual specificity phosphatases (DUSPs) with the ability to dephosphorylate in serine/threonine and tyrosine residues. MKPs/DUSPs are self-regulated by MAPKs forming part of negative feedback loops that efficiently terminate MAPK signalling. Based on studies performed by DUSP genetic overexpression or deletion, the DUSPs reveal as critical regulators of the final biological outcome of MAPK activation during the cancer progression and the immune response. However, little is known about how the DUSPs are regulated by extracellular mediators in the nervous system. The neurotrophins NGF and BDNF represent well-known regulators of DUSPs function that limit MAPK proliferative signalling during differentiation and development. Besides, endogenous cannabinoids also behave as potent inducers of DUSP expression in spinal cord cells, in relation to the pain relief and the resolution of inflammation. The third players involved in DUSP regulation in neurons and glial cells are extracellular nucleotides. P2Y₁₃ and P2X7 receptors trigger different regulatory mechanisms, such as DUSP gene induction, protein turnover, and protein stabilization, and exert tight control of MAPK activity during neuroprotection and differentiation. Of relevance, the activation or inhibition of DUSP activity can be of therapeutic value, since it efficiently ameliorates the detrimental effects of MAPK dysregulation occurring in brain injury and neurodegeneration. By restoring proper levels of DUSP activity and expression, nucleotides contribute to arbitrate the full neuroprotective response in brain diseases. Overall, nucleotides behave as fine tuners of MAPK signalling through its participation in the spatio-temporal regulation of the DUSPs/MKPs that allows their dynamic adaptations to different conditions and cellular contexts.

References:

- Pérez-Sen R, Queipo MJ, Gil-Redondo JC et al., 2019. Dual-Specificity Phosphatase Regulation in Neurons and Glial Cells. *Int J Mol Biol.* Apr 23; 20(8).
- Queipo MJ, Gil-Redondo JC, et al. 2018. Nucleotide and EGF Receptors Exert Dual Modulation of the Dual-Specificity Phosphatase 6 (MKP-3) in Granule Neurons and Astrocytes, Contributing to Negative Feedback on ERK Signaling. *Front Mol Neurosci.* 2018, Jan 10;10:448.

CPL-05

Lecture in Honour Prof Bertil Fredholm

LIGHTING UP ADENOSINE RECEPTORS

Francisco Ciruela Alf rez. Universidad de Barcelona, Spain.



Adenosine, a ubiquitous extracellular signaling molecule, acts through cell surface G protein-coupled receptors. These receptors control many physiological functions, thus becoming promising therapeutic targets in a wide range of pathological conditions. Yet, the ubiquity of adenosine receptors and the eventual lack of selectivity of adenosine-based drugs often reduced the therapeutic expectations. Photopharmacology is a novel approach based on the use of photosensitive drugs allowing spatiotemporal control of receptor function in a light-dependent manner, thus circumventing some of the classical pharmacology limitations. Accordingly, we developed light-sensitive drugs to photocontrol adenosine receptor's function both *in vitro* and *in vivo*. To this end, two types of adenosine-based photosensitive drugs were developed: i) Photoswitchable; and ii) Photocaged.

MRS5543 is a photoisomerizable nucleoside derivative containing an aryl diazo linkage on the N(6) substituent. Interestingly, while in dark conditions (*i.e.* relaxed isomer) it behaves as a full adenosine A₃ receptor (A₃R) and partial adenosine A_{2A} receptor (A_{2A}R) agonist, but upon photoisomerization with blue light it turns into an A_{2A}R antagonist¹. Thus, MRS5543 is a photoswitchable purinergic drug that allow a light-dependent control of A_{2A}R intrinsic activity. Conversely, MRS7145 is a photocaged A_{2A}R antagonist which binds and blocks A_{2A}R in a light-dependent manner both in cells and *in vivo*. Thus, precise fibre optic brain irradiation allows MRS7145 uncaging and striatal A_{2A}R blockade, thus fine-tuning A_{2A}R-dependent spontaneous locomotor activity and reversing pharmacologically-induced Parkinsonian-like behaviour².

Overall, the design and synthesis of light-operated adenosine receptor ligands opens new opportunities to widen the phototherapeutic window of adenosine receptors.

References

- 1 Bahamonde MI, Taura J, Paoletta S, Gakh AA, Chakraborty S, Hernando J *et al.* Photomodulation of G protein-coupled adenosine receptors by a novel light-switchable ligand. *Bioconjugate Chemistry* 2014; **25**: 1847–1854.
- 2 Taura J, Nolen EG, Cabr  G, Hernando J, Squarcialupi L, L pez-Cano M *et al.* Remote control of movement disorders using a photoactive adenosine A_{2A} receptor antagonist. *Journal of Controlled Release* 2018; **283**: 135–142.

Symposia
(arranged by symposium number)

Symposium 1: NOVEL INSIGHTS INTO P2X3 AND A3 RECEPTOR CONTRIBUTION TO PAIN STATES

Chairs: Peter Illes (Leipzig, Germany) / Antonio R. Artalejo (Madrid, Spain)

Content: Purines signal sensory information involved in nociception. Although a variety of P1 and P2 receptors are known to mediate nociceptive transmission, recent findings on novel P2X3 receptor supramolecular complexes and locations as well as on the role adenosine A3 receptors in pain pathways are opening new avenues for the development of more effective and safer analgesics.

S1-01

OVEREXPRESSION OF P2X3 AND P2X7 RECEPTORS AND TRPV1 CHANNELS IN ADRENOMEDULLARY CHROMAFFIN CELLS IN A RAT MODEL OF NEUROPATHIC PAIN

A.R. Artalejo¹, M. Arribas-Blázquez¹, L. Alcides Olivios-Oré¹, M.V. Barahona¹, M. Sánchez de la Muela¹, V. Solar¹, E. Jiménez¹, J. Gualix¹, J.M. McIntosh², A. Ferrer-Montiel³, M.T. Miras-Portugal¹

¹. Universidad Complutense de Madrid. Madrid. Spain; ². University of Utah. Salt Lake City. Utah. USA; ³. Universitas Miguel Hernández. Elche. Spain.

We have tested the hypothesis that neuropathic pain acting as a stressor drives functional plasticity in the sympathoadrenal system. The relation between neuropathic pain and adrenal medulla function was studied with behavioral, immunohistochemical and electrophysiological techniques in rats subjected to chronic constriction injury of the sciatic nerve. In slices of the adrenal gland from neuropathic animals we have evidenced increased cholinergic innervation and spontaneous synaptic activity at the splanchnic nerve-chromaffin cell junction. Likewise, adrenomedullary chromaffin cells displayed enlarged acetylcholine-evoked currents with greater sensitivity to α -conotoxin RgIA, a selective blocker of $\alpha 9$ subunit-containing nicotinic acetylcholine receptors, as well as increased exocytosis triggered by voltage-activated Ca^{2+} entry. Altogether, these adaptations are expected to facilitate catecholamine output into bloodstream. Last, but most intriguing, functional and immunohistochemical data indicate that P2X3 and P2X7 purinergic receptors and TRPV1 channels are overexpressed in chromaffin cells from neuropathic animals. These latter observations are reminiscent of molecular changes characteristic of peripheral sensitization of nociceptors following the lesion of a peripheral nerve, and suggest that similar phenomena can occur in other tissues potentially contributing to behavioral manifestations of neuropathic pain.

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S1-02

THE ASIC3/P2X3 COGNATE RECEPTOR SENSES ACIDIC AND PURINERGIC PAIN IN A NOVEL MANNER

P. Rubini¹, G. Stephan¹, H. Lumei², Z. Ying², E. Fabbretti³, Y. Tang², P. Illes¹

¹University of Leipzig, Leipzig, Germany; ²TCM University of Chengdu, Chengdu, China; ³International School for Advanced Studies, Trieste, Italy.

Two subclasses of acid-sensing ion channels (ASIC3) and of ATP-sensitive P2X receptors (P2X3Rs) show a partially overlapping expression in sensory neurons. Although the amino-acid composition of the two receptor-channel types is different, their trimeric structure and ion conductive pathways are similar. Our aim was to clarify whether the ASIC3 and P2X3 subunits can compose a heteromeric channel or whether they build up a multiprotein complex responding to stimulation by protons and ATP in a novel manner. For this purpose we transfected COS cells with the respective plasmids to generate recombinant receptors and investigated dorsal root ganglion neurons in cultures. Patch-clamp measurements indicated that ASIC3 and P2X3Rs interact with each other in that the two receptor-channels have a lower ionic permeability than each channel alone. Further, ASIC3 stimulation strongly inhibited the P2X3R current partly by a Ca^{2+} -dependent mechanism. By contrast, the stimulation of P2X3Rs failed to modify the ASIC3 current. The proton-binding site was critical for this effect and the two receptor channels appeared to switch their ionic permeabilities during activation. Co-immunoprecipitation proved the close association of the two protein structures. The injection of either acidic medium or α,β -methylene ATP into the rat paw caused thermal hypersensitivity. Selective ASIC3 antagonists reversed both the acidic and purinergically-induced pain, while selective antagonists for P2X3Rs reversed only this latter pain condition. We conclude that the receptor subunits do not appear to form a heteromeric channel, but tightly associate with each other to form a protein complex, mediating unidirectional inhibition.

S1-03

MOLECULAR MECHANISMS FOR A₃ ADENOSINE RECEPTOR-MEDIATED PAIN CONTROL: AN *IN VITRO* STUDY ON DORSAL ROOT GANGLION NEURON EXCITABILITY

¹E. Coppi, ¹F. Cherchi, ¹I. Fusco, ¹P. Failli, ¹A. Vona, ¹I. Dettori, ¹L. Gaviano, ¹E. Lucarini, ²K.A. Jacobson, ²D.K. Tosh, ³D. Salvemini, ¹C. Ghelardini, ¹F. Pedata, ¹L. Di Cesare Mannelli and ¹A.M. Pugliese.

¹University of Florence, Florence, Italy. ²National Institutes of Health, Bethesda, Maryland 20892-0810, United States. ³Saint Louis University, St. Louis, Missouri 63104, United States.

Background. Interest has been focused in recent years on the analgesic effects exerted by adenosine and its receptors, A₁, A_{2A}, A_{2B} and A₃ subtypes, in different *in vivo* models of acute and chronic pain. In particular, recent studies have focused on the anti-hyperalgesic activity of the A₃ adenosine receptor (A₃AR) in several chronic pain models, but the cellular and molecular basis of this pain control is still unknown.

Material and methods. Here, we investigated the expression (by RT-PCR and immunocytochemical analysis) and functional effects (by patch-clamp technique) of A₃AR on ionic currents and cell excitability of small-medium sized, capsaicin-sensitive, dorsal root ganglion (DRG) neurons isolated from 3-4 week-old rats of both sexes.

Results. We demonstrated that A₃AR are expressed in rat DRG neurons and modulate different kinds of ionic currents. Two distinct A₃AR agonists were tested: CI-IB-MECA and the highly selective MRS5980. Both compounds concentration-dependently (0.1-100 nM) inhibited Ca²⁺ currents evoked by a voltage step depolarization protocol. This effect was sensitive to the A₃AR antagonist MRS1523 (100 nM) and to the N-type blocker PD173212 but not to the L-type blocker, lacidipine. The endogenous agonist adenosine also reduced N-type Ca²⁺ currents, and its effect was inhibited by 56% in the presence of A₃AR antagonist MRS1523, demonstrating that the majority of adenosine's effect is mediated by this receptor subtype. Current-clamp recordings demonstrated that neuronal firing of rat DRG neurons was significantly reduced by A₃AR activation in a MRS1523-sensitive but PD173212-insensitive manner.

Conclusion. We conclude that pain-relieving effects observed upon A₃AR activation could be mediated through N-type Ca²⁺ channel block and action potential inhibition in isolated rat DRG neurons. These findings indicate that A₃AR agonists utilized in the therapy of pain act at a first station reducing dorsal root ganglion neuron excitability.

S1-04

TARGETING ADENOSINE RECEPTORS TO TREAT NEUROPATHIC PAIN

L. Luongo¹, D. Salvemini²

¹ Department of Experimental Medicine, Division of Pharmacology, University of Campania “L. Vanvitelli”, 80138, Naples; ² Department of Pharmacology and Physiology, Saint Louis University School of Medicine, St. Louis, MO 63104

Chronic pain is a global burden that promotes disability and unnecessary suffering. The mechanisms underlying neuropathic pain are still poorly understood and efficacious treatment of has not been achieved. Thus, new therapeutic targets are needed. We demonstrate that increasing endogenous adenosine levels, through selective adenosine kinase inhibition, produces powerful analgesic effects in rodent models of experimental neuropathic pain through the A3 adenosine receptor (A3AR, now known as ADORA3) signalling pathway. Similar results were obtained by the administration of a novel and highly selective A3AR agonist. These effects were prevented by blockade of spinal and supraspinal A3AR, lost in A3AR knock-out mice, and independent of opioid and endocannabinoid mechanisms. A3AR activation also relieved non-evoked spontaneous pain behaviours without promoting analgesic tolerance or inherent reward. Further examination revealed that A3AR activation reduced spinal cord pain processing by decreasing the excitability of spinal wide dynamic range neurons and producing supraspinal inhibition of spinal nociception through activation of serotonergic and noradrenergic bulbospinal circuits. Critically, engaging the A3AR mechanism did not alter nociceptive thresholds in non-neuropathy animals and therefore produced selective alleviation of persistent neuropathic pain states. Preliminary data showed that both adenosine kinase and A3 receptors are overexpressed in astrocytes in peripheral neuropathic pain model as compared to the sham mice.

These studies reveal A3AR activation by adenosine as an endogenous anti-nociceptive pathway and support the development of A3AR agonists as novel therapeutics to treat chronic pain.

Symposium 2: NEW INSIGHTS INTO PURINERGIC SIGNALING

Chairs: Esmerilda G. Delicado (Madrid, Spain) / Jürgen Schrader (Düsseldorf, Germany)

Content: The symposium shows some examples of the implications of different components of purinergic signaling, ectoenzymes, nucleotide and adenosine receptors, in inflammation, neuroprotection and virus infection. It emphasizes the complexity of purinergic signaling and the multiple targets that could be considered for clinical interventions.

S2-01

Jürgen Schrader (IMC/HH University, Dusseldorf, Germany) CD73-derived adenosine in inflammation: mechanisms and relevance

S2-02

CROSSROAD BETWEEN BIOACTIVE LIPIDS AND PURINERGIC SIGNALLING IN MACROPHAGES

Lisardo Boscá, Marta Paz-García, Sergio Sánchez-García, Francisco J. de Castro and Rafael I. Jaén.

Instituto de Investigaciones Biomédicas Alberto Sols (CSIC-UAM). Madrid (Spain)

Receptors for purine and pyrimidine nucleotides (P2) are involved in many pathophysiological processes, including short-term purinergic signaling such as immune responses, neurotransmission, neuromodulation, neurosecretion, inflammation, platelet aggregation and vasodilatation; and long-term purinergic signaling of cell proliferation, differentiation, motility, death in development and regeneration. Under inflammatory conditions or in early stages of many diseases characterized by the occurrence of associated inflammatory processes, chemical mediators of lipidic nature accumulate at sites of tissue damage. One of these molecules is PGE₂ that activates specific G-protein coupled membrane receptors (EP receptors). Four different receptors, EP₁-EP₄, can be distinguished pharmacologically; however, in addition to EP-mediated effects PGs may exert other EP-independent actions. In addition to PGs, P₂ nucleotide agonists are released during the course of inflammatory responses and activate immune cells, such as monocytes, macrophages and lymphocytes. These extracellular nucleotides exert several effects on immune cells and are involved in cytoskeleton reorganization, cell migration, phagocytosis or exocytosis. Therefore, extracellular nucleotides acting on specific P₂ receptors are important modulators of inflammation. This regulation coexists with the temporal framework of pro-inflammatory and pro-resolution mediator lipids released by immune cells, including macrophages. Since inflammatory cells release large amounts of prostaglandins (PGs), such as PGE₂ due to COX-2 expression, we have investigated the impact of these bioactive lipids on the EP-dependent and independent receptors. Interestingly, in alternatively activated (anti-inflammatory) macrophages PGE₂ selectively impairs P₂Y but not P₂X₇ Ca²⁺-mobilization. This effect is absent in LPS-activated cells and is specific for PGE₂ as it cannot be obtained when cells are treated with other PGs, such as those with cyclopentenone structure that are chemically more reactive than the classic PGs. The inhibition of P₂Y responses by PGE₂ involves the activation of new protein kinase C isoforms (PKC ϵ) and/or PKD. Selective inhibitors of these PKCs or expression of dominant negative forms of PKD impairs the inhibitory effect of PGE₂ on P₂Y signaling. There are several examples of cross-regulation between the purinergic system and inflammatory molecules. In macrophages, it has been described that UTP potentiates PGE₂ release, which is involved in the enhancement of inducible nitric oxide synthase (NOS₂) induction by LPS. In platelets, a cross-desensitization between ADP and thromboxane receptor signaling has been reported, and there is mounting evidence supporting that these interactions play important roles in several inflammatory and degenerative disorders, such as multiple sclerosis, amyotrophic lateral sclerosis or Alzheimer's disease. In these pathologies, extracellular ATP exerts pro-inflammatory actions provoking cytokine release and PGs production. Therefore, the inhibition of P₂Y signaling by PGE₂ has an impact on the cell migration elicited by P₂Y-agonists in resting and alternatively activated macrophages, which provides new clues to understand the resolution phase of inflammation, when accumulation of PGE₂, anti-inflammatory and pro-resolving mediators occurs. In this presentation, we will provide data supporting a role for PGE₂ in resting and pro-resolution macrophages.

S2-03

ADENOSINE RECEPTORS AS A PRIMARY TARGET FOR RESVERATROL: FROM ANTITUMORAL TO NEUROPROTECTION ACTION

A. Sánchez-Melgar¹, J.L. Albasanz¹, V. Palomera-Ávalos², N. Saleh³, R. Guixà-González⁴, J. Selent⁵, M. Pallàs², M. Martín¹

¹ University of Castilla La-Mancha, Ciudad Real, Spain; ² University of Barcelona, Spain; ³ University of Copenhagen, Denmark; ⁴ Autonomous University of Barcelona, Spain; ⁵ Pompeu Fabra University, Barcelona, Spain.

Resveratrol (RSV) is a natural polyphenolic compound produced by plants under stressful conditions that has shown multiple beneficial properties for human health. However, the precise mode of action of this phytochemical remain still unclear. Therefore, we aimed to investigate whether RSV was able to induce modulation on adenosine-mediated signaling in rat C6 glioma cells. Our biochemical and computational analysis showed that RSV directly binds to adenosine receptors and acts as a non-selective agonist on these receptors. RSV-induced receptor activation can stimulate or inhibit adenylyl cyclase (AC) activity in a concentration dependent manner. RSV also modulates adenosine receptors, affecting gene expression, receptor levels, and the downstream AC pathway. Furthermore, RSV caused C6 cells growth inhibition in a time- and concentration-dependent manner. As adenosinergic system has been involved in cancer cell proliferation, we might hypothesize that RSV could induce inhibition of tumor cell growth through binding and modulation of adenosinergic system.

On the other hand, long-term diet supplementation with RSV induced changes on gene expression as well as transduction pathways mediated by adenosine receptors in brain from SAMP8 mice, an animal model of aging and Alzheimer's disease. RSV increased levels of A₁ receptor and potentiated its transduction-mediated pathway, whereas no changes on A_{2A} receptors were detected. Yet, A_{2A} receptor-mediated signaling was desensitized after RSV treatment -. In addition, the adenosine converting enzymes, 5'-Nucleotidase and Adenosine Deaminase, were found to be significantly reduced in RSV-treated mice, suggesting an alteration on adenosine metabolism.

In conclusion, beneficial effect of RSV could be mediated by direct activation and modulation of adenosine receptors. Therefore, new therapeutic strategies involving resveratrol and adenosine receptors should be aimed in the future for a variety of diseases such as cancer and neurodegeneration.

S2-04

CROSS-TALK BETWEEN P2 RECEPTORS AND TYROSINE KINASE RECEPTORS IN CEREBELLAR ASTROCYTES

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Cerebellar astrocytes have resulted to be a good model to study the multiple signaling pathways activated by nucleotide receptors, and their cross-talk with other extracellular relevant signals at the nervous system. At the beginning, studies focused on the identification of intracellular targets responsible for the activation of signaling pathways, such as MAP kinase cascades. We found that ERK activation was underlying the modulatory EGF receptor (EGFR) actions on P2Y signaling in these glial cells. EGFR plays a dual role in cerebellar astrocytes, depending on the cellular context and the mediators accumulating at the extracellular environment. EGFR potentiates ATP calcium responses allowing ineffective ATP concentrations *per se* to evoke calcium responses. The potentiation is maintained for long periods (up to 6 hours), assuring P2Y signaling at very low nucleotide concentrations. However, when nucleotides and PGE₂ accumulate at the extracellular space, EGFR contribute to the impairment of P2Y responses by the prostaglandin. Lately, we have centered on the characterization of the intracellular mechanisms responsible for dephosphorylating ERK proteins. Dual Specificity Phosphatases (DUSPs), which dephosphorylate both serine/threonine and tyrosine residues in the same substrate, are good candidates. In fact, DUSP6 appears to be one of the protein phosphatases inactivating ERK signaling at the cytosolic compartment. We have demonstrated that the metabotropic ATP nucleotide receptors, P2Y₂ and P2Y₄, the ionotropic P2X₇ receptors, and EGFR regulate the levels of the protein phosphatase with a biphasic pattern and in an ERK-dependent manner in rat cerebellar astrocytes. While short time stimulations of astrocytes with the agonists, UTP, BzATP or EGF, decrease the levels of protein phosphatase by proteosomal degradation, prolonged stimulations restore the basal levels of DUSP6 protein. We are currently investigating whether EGFR transactivation is involved in the modulation of DUSP6 levels elicited by nucleotide receptors.

S2-05

DRUG-INDUCED CARDIOVASCULAR TOXICITY MEDIATED BY P2X7 RECEPTORS: THE EXAMPLE OF ABACAVIR

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Abacavir (ABC), a guanosine analogue belonging to the nucleoside reverse transcriptase inhibitor family, is one of the most widely employed antiretroviral drugs in HIV therapy. However, a long-lasting controversy surrounding its association with an increased cardiovascular risk has been fueled by discrepancies in published clinical data and, in particular, by the lack of a credible mechanism of action that could explain such detrimental actions. Recent experimental evidence with human samples and in animal models relates ABC with vascular inflammation, a leading contributor to the atherosclerotic plaque and thrombosis. It has been demonstrated that the drug induces an increase in leukocyte-endothelial cell interactions and interactions of platelets with different vascular and blood cells involved in the early phases of thrombi formation, such as endothelium and leukocytes. Moreover, ABC has been shown to promote the formation of arterial thrombi and the complete obstruction of blood flow in resistance vessels. Finally, research has shown that the in vitro and in vivo actions of the drug are blocked by pharmacological antagonists of P2X7 ATP receptors, and that such actions are absent in P2X7-deficient mice. Likewise, the thrombus formation induced by ABC in vivo is prevented by selective pharmacological blockade of these receptors and is absent in P2X7-deficient mice. It has been hypothesized that the structural analogy between ABC and endogenous purinergic mediators such as ADP and ATP endows the drug with the ability to interfere with the purinergic system and to induce a vascular inflammatory response, an effect involving the activation of P2X7 ATP receptors. It remains to be determined if such an effect is unique to ABC or common to other drugs.

Symposium 3: PURINERGIC RECEPTORS AS FUTURE TARGETS IN ALZHEIMER'S DISEASE?

Chairs: David Blum (Lille, France) / Annett Halle (Bonn, Germany)

Content: Alzheimer's Disease is a major health concern with no treatment available to significantly slow down or halt cognitive symptoms. Facing approaches which target neuropathological lesions and that have not proven efficiency yet, it is mandatory to develop alternative strategies. Purine homeostasis is disrupted in Alzheimer's Disease involving immune and plasticity dysfunctions which favor the development of cognitive deficits. In the present symposium, speakers will give new insights into the role of purinergic signaling in the pathophysiological development of Alzheimer's Disease and discuss the therapeutic perspectives.

S3-01

INVOLVEMENT OF PURINERGIC P2X4 RECEPTORS IN ALZHEIMER DISEASE

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P2X4 receptors (P2X4R) are ATP-gated ion channels involved in several inflammatory processes and are expressed *de novo* in reactive microglia, the resident macrophages of the CNS, during neuroinflammation.

In light of this, we investigated the implication of microglial P2X4R in Alzheimer disease that is characterized by neurofibrillary tangles, amyloid deposits and chronic inflammation.

Using APP/PS1 and APP/PS1xP2X4^{-/-} mice, we demonstrate that mice lacking P2X4R present better mnemonic performances, associated with a lower level of soluble amyloid peptide. Immunostainings show that P2X4R are highly expressed in reactive microglia surrounding amyloid deposits in cortex and hippocampus and strongly co-localize with ApoE, a well-known risk factor for Alzheimer disease. Moreover, using proteomic studies, we found that P2X4 and ApoE interact and that the level of secreted ApoE is strongly increased in P2X4R^{-/-} microglia. Interestingly, we also show that cathepsin B, a lysosomal enzyme, is not active in P2X4^{-/-} microglia, leading to the increase of ApoE concentration. These results indicate that P2X4, by modulating enzymatic degradation of ApoE in reactive microglia, play crucial role in Alzheimer disease.

S3-02

ROLE OF ASTROGLIAL PURINERGIC TRANSMISSION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by a progressive loss of memory and cognitive functions. One of the pathological hallmarks of AD is the extracellular deposition of plaques containing amyloid- β (A β).

We have shown that reactive astrocytes around plaques become hyperactive, which is mediated by purinergic signaling through the P2Y1 receptor, but the consequences of long-term treatment with a P2Y1 receptor antagonist for network activity, amyloid metabolism and behavior have remained undetermined.

We administered the P2Y1 receptor antagonist MRS2179 intracerebroventricularly to APPPS1 mice for 6 weeks using osmotic minipumps. We recorded astroglial-neuronal network activity using in vivo two-photon microscopy of calcium indicators. Spatial learning and memory were tested using the Barnes Maze. Hippocampal long-term potentiation (LTP) was investigated in acute hippocampal slices. Plaque load, gliosis and soluble and insoluble amyloid levels were determined by immunohistochemical and biochemical analysis.

We have found that chronic treatment normalized astroglial hyperactivity. Deficits in spatial learning and memory were significantly ameliorated by MRS2179. The LTP suppression found in hippocampal slices of APPPS1 mice was prevented by MRS2179. These effects occurred without significant changes in plaque size or number. MRS2179 treatment also normalized network dysfunction, ameliorated cognitive abnormalities, and reduced synaptic dysfunction.

Our data indicate that aberrant astrocytic network activity contributes to synaptic and cognitive deficits in mouse models of AD, and that purinoreceptor signalling may represent a potential therapeutic target for future translational studies. Finally, we describe a novel RNAseq-based approach enabling the identification of novel astroglial calcium pathways for future studies.

S3-03

ATP-DERIVED ADENOSINE CONTROLS SYNAPTIC AND MEMORY DYSFUNCTION IN β -AMYLOID MODELS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is characterized by an initial synaptic dysfunction and damage causing progressive cognitive impairment. Amyloid- β peptides ($A\beta$) have been proposed as culprits of AD, since their production is bolstered in AD and they can trigger synaptic and memory dysfunction. Epidemiological and animal studies have converged to show that the intake of caffeine, an adenosine receptor antagonist, prevents memory deficits caused by $A\beta$, an effect mimicked by blockade of adenosine A_{2A} receptors ($A_{2A}R$), which are mainly synaptic. Since ecto-5'-nucleotidase (CD73), controlling the formation of extracellular adenosine from released ATP, provides the adenosine activating $A_{2A}R$ under physiological conditions, we now aimed to assess if tinkering with CD73 could rectify the synaptic and behavioural deficits triggered by $A\beta$. This was tackled using behavioural, neurochemical and slice electrophysiology approaches. We report that icv administration of $A\beta_{1-42}$ (2 nmol, 14 days) increased the synaptic release of ATP, upregulated the levels of CD73 and $A_{2A}R$ and blunted LTP amplitude in hippocampal synapses and impaired performance in memory tests. The genetic deletion of either CD73 (CD73-KO mice) or $A_{2A}R$ ($A_{2A}R$ -KO mice) counteracted this $A\beta_{1-42}$ -induced decrease of LTP and of memory. Similarly, direct exposure of naïf slices from wild type mice to oligomeric βA_{1-42} (50 nM for 40 min) also decreased LTP amplitude. This acute effect of $A\beta_{1-42}$ was reverted by the CD73 inhibitor α,β -methylene-ADP (100 μ M) or by the $A_{2A}R$ antagonist SCH58261 (50 nM) and was prevented in slices from CD73- and $A_{2A}R$ -KO mice. These results show that CD73 blockade prevents the impact of $A\beta$ on synaptic function and memory; this confirms that the formation of ATP-derived adenosine through CD73 is responsible for $A_{2A}R$ activation under pathological conditions, and prompts CD73 as a novel therapeutic target against the behavioural and synaptic deficits in early AD.

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S3-04

PURINERGIC SIGNALING SHAPES MICROGLIAL FUNCTIONS IN ALZHEIMER'S DISEASE

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Genome-wide association studies have highlighted the importance of microglia, the main immune cells of the CNS, in Alzheimer's disease (AD), implicating microglia as a promising target for AD treatment. Microglia in mouse models of AD show signs of functional impairment. Whether alterations in purinergic signaling, which under physiological conditions is critically involved in shaping microglial functions, contribute to this phenomenon and whether microglial dysfunction is reversible by targeting purinergic signaling has remained unclear.

Plaque-related microglia from APP/PS1 mice showed impaired phagocytic capacity and cell motility in intravital 2-photon microscopy and acute cerebral slice phagocytosis assays. Transcriptional analysis of microglial cells harvested by laser-microdissection revealed significantly altered expression of ectonucleotidases and purinoreceptors in plaque-associated cells. Importantly, short-term treatment of microglia with purinoreceptor agonists or pharmacological blockage of ectonucleotidases rapidly normalized phagocytic and dynamic function of plaque-associated microglia in acute cerebral slices from APP/PS1 mice, suggesting that microglial dysfunction indeed is reversible upon targeting purinergic signaling.

In summary, we have identified altered purinergic signaling as a potential cause for the impairment of microglial dynamics, morphology and phagocytic capacity of plaque-associated microglia in the APP/PS1 model and targeting microglial purinergic signaling may represent a promising novel approach for the treatment of AD.

Symposium 4: HOW PURINERGIC SIGNALING INFLUENCES THE ACTIVITY OF HUMAN MESENCHYMAL STROMAL/STEM CELLS

Chairs: Renata Ciccarelli (Chieti, Italy) / Lin-Hua Jiang (Leeds, UK)

Content: The aim of this symposium is to give an overview of recent advances on the influence of purines on the activity/function of mesenchymal stromal/stem cells (MSCs).

These cells, which can be isolated from different human adult tissues (i.e. bone marrow, adipose or dental tissues) and embryonic annexes (umbilical cord, placenta) or fluids (amniotic liquid) are today actively studied given the great differentiation potential of these cells, which represent a possible new therapeutic tool in regenerative medicine. Stem cells are also present in tumors as a restrict population generally provided with a great invasiveness and resistance to radio- and chemo-therapy. Thus, considering that purines are ancestral molecules present in and released from virtually all cell types, including normal or tumor MSCs, there is a growing interest to explore which purines are mostly present in the extracellular environment (due to the extensive metabolism of released nucleotides by ecto-enzymes) and how endogenous or exogenous purines can influence MSC potentialities by interacting with their own receptors as signaling molecules. Accordingly, this symposium will examine different aspects of the purine involvement in the modulation of physiological/pathological MSC functions and differentiation ability of these cells, including the control of intracellular calcium levels, which play a crucial role also in MSC homeostasis.

S4-01

INFLUENCE OF DIFFERENT PURINE RECEPTORS ON THE DIFFERENTIATION POTENTIAL OR MALIGNANCY OF NORMAL OR CANCEROUS MESENCHYMAL STEM CELLS, RESPECTIVELY

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Mesenchymal Stromal/Stem Cells (MSCs) are multi-potent cells located in differentiated organs to maintain tissue homeostasis. Stem-like cells are present also in tumors and contribute to their malignancy. Preclinical/clinical studies demonstrated that differentiation capabilities/ aggressiveness of normal/tumor MSCs, respectively, can be modulated by endogenous substances/ related drugs. Purines are ancestral molecules released from cells, including MSCs, whose extracellular concentration is increased during tissue injury or in tumor microenvironment. Hence, endogenous/exogenous purines may influence MSC properties by interacting with their own receptors. In particular, both adenosine and ATP regulate MSC osteogenic differentiation. Indeed, the A1 adenosine receptor stimulation enhances osteogenesis while decreasing adipogenesis in MSCs derived from human dental or adipose tissue, by activating the Wnt signal. Also the stimulation of P2X7 receptor (P2X7R) by endogenous ATP contributes to cell osteogenic differentiation, increasing the number/expression of these receptors. Conversely, P2X7R pharmacological stimulation by BzATP, mimicking the effects of high ATP levels occurring during tissue injury, decreases receptor expression/activity, thus impairing MSC commitment towards osteogenesis. Thereby, our findings confirm the fundamental role played by local extracellular purines in bone healing/remodeling that should be carefully monitored to favor/increase MSC usefulness in bone regenerative medicine. On the other hand, in human stem-like cells from glioblastoma (GBM) surgical specimens P2X7R activation by 500-1000µM BzATP exerted a cytotoxic effect while lower concentrations (100-200µM) increased cell migration/invasion and the expression of epithelial-to-mesenchymal transition-related genes and of P2X7R. Since in GBM microenvironment there are ATP levels able to activate P2X7R, our data support a role for these receptors in GBM recurrence/invasiveness.

S4-02

ENHANCED OSTEOGENESIS AND ANGIOGENESIS FOR PUTATIVE BONE GRAFTS BY ACTIVATING PURINERGIC RECEPTOR SIGNALING DURING MESENCHYMAL STEM CELL DIFFERENTIATION

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Critical size bone defects are an incapacitating clinical condition which remains a challenge in reconstructive orthopedic surgery. Bone restoration is of growing interest due to an increasing number of elderly, which might need reconstruction after tumor surgery. But the need for bone replacement also concerns the young after traffic or sports accident. Since the sources for autografts are limited and their access is associated with infection risks and donor site morbidity, much hope is placed in tissue engineering strategies using stem cells. Next to scaffolds which should provide substrate for cell growth and mechanical integrity, bioactive molecules are needed to induce or enhance differentiation of these cells. One approach which is following this strategy is using mesenchymal stem cells and artificial ligands for purinergic receptors. Human mesenchymal stem cells (MSCs) were isolated from liposuction material after plastic surgery. Their stem cell character was confirmed by specific stains after three lineage differentiations and the validation of a defined set of positive and negative markers. The purinergic (P) receptors pattern before and after differentiation of MSCs towards osteoblast, endothelial and smooth muscle cells was unraveled. Several P2 receptor subtypes were altered during the maturation of MSCs towards the respective cell types. These alterations are functional and artificial ligands of the individual receptors can influence the differentiation process. In detail, expression level regulation of P2Y4 and P2Y14 seems to influence the onset of osteogenesis at the branching point to adipogenesis. Administration of a selective P2X7 antagonist led to enhanced matrix mineralization, confirming the role of P2X7 during late osteogenesis. Some specific P2 receptors such as P2Y1 also play a significant role in angiogenesis. Since vessel formation is a prerequisite for a successful treatment of critical size bone defects, artificial P2 receptor ligands are promising bioactive molecules for functionalized scaffolds with MSCs in future bone tissue engineering.

S4-03

ATP-INDUCED CALCIUM SIGNALLING IN MESENCHYMAL STEM CELL FUNCTIONS

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Mesenchymal multipotent stem cells (MSCs) have many promising applications in tissue regeneration and cell-based therapies, and a good understanding of how extracellular chemical, physical and biological signals regulates MSC functions should provide useful information for better applications of MSC. ATP is one of the most commonly-used autocrine and paracrine signalling molecules and can interact with the ligand-gated P2X receptors and G-protein-coupled P2Y receptors to raise the level of cytosolic calcium. Increasing evidence shows that MSCs release ATP in vitro and in vivo. I will present our own study aimed to elucidate the molecular mechanisms that participate in ATP-induced calcium signalling in human dental pulp-derived MSC. I will also discuss the role of such calcium signalling mechanisms and downstream calcium-dependent signal transduction pathways in ATP-induced regulation of cell migration and differentiation.

References:

- [1] Peng, H.S., Hao, Y.J., Mousawi, F., Roger, S., Li, J., Sim, J. A., Ponnambalam, S., Yang, X.B., and Jiang, L.-H. (2016) Purinergic and store-operated Ca^{2+} signalling mechanisms in mesenchymal stem cells and their roles in ATP-induced stimulation of cell migration. *Stem Cells* 34: 2102-2114.
- [2] Jiang, L.-H., Hao, Y.J., Mousawi, F., Peng, H.S. and Yang, X.B. (2017) Expression of P2 purinergic receptors in mesenchymal stem cells and their roles in extracellular nucleotide regulation of cell functions. *Journal of Cell Physiology* 232: 287-297.

S4-04

EMERGING ROLE OF NUCLEOTIDE-METABOLIZING ENZYMES IN REGULATION OF MESENCHYMAL STEM CELLS FATE

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Extracellular purines, in particular adenosine triphosphate (ATP) and adenosine (Ado), are recognized as the most primitive and widespread chemical messengers. In view of the emerging data, it is evident and widely accepted that an extensive network of diverse nucleotide/nucleoside-metabolizing enzyme activities functions in the extracellular space as an integrated element of the purinergic signaling system (purinome). The concerted action of ecto-enzymes from different classes (hydrolases and transferases) allows for the effective maintenance of nucleotides/nucleosides equilibrium. The ATP-Ado balance is considered as a key regulator of mesenchymal stem cell (MSC) biology, triggering inter alia the differentiation or self-renewal of stem cells.

Intensive research efforts from our and other laboratories worldwide indicate that MSC and cells induced to differentiate exhibit different sensitivity to purinergic ligands as well as distinct activity and expression profiles of nucleotide-metabolizing enzymes than mature cells. Our results showed that undifferentiated mesenchymal stem cells present relatively low activity toward ATP and ADP which increases only in the course of differentiation. This is due to the fact that nucleotide signal in the form of ATP is sensed by MSCs as an incentive to their mobilization, increasing the proliferation and migration rates, effectiveness of differentiation and regeneration capacity of a local damage. On the other hand, the differentiated cells increase the expression and activity of ecto-NTPDases, while the ecto-5'-nucleotidase activity decreases. Thus, ATP and ADP hydrolysis becomes the dominant direction of the nucleotides metabolism and serves as a defense mechanism protecting mature cells from cytotoxic nucleotide influence.

In this talk, we will consolidate the most important and up-to-date information from the field of ATP- and Ado-mediated regulation of the MSC fate and the emerging role of ecto-enzymes metabolizing nucleotides. This specific hallmark of the MSC purinome should be linked with cell-specific biological potential and capacity for tissue regeneration.

Symposium 5: MEMBRANE TRANSPORTERS AND PURINERGIC SIGNALLING

Chairs: Marçal Pastor-Anglada (Barcelona, Spain) / Beatriz Lopez-Corcuera (Madrid, Spain)

Content: Membrane purine transporters contribute to the modulation of nucleotide and adenosine tones. Moreover, the biological function of selected membrane transporters, including adenosine and neurotransmitter transporters, is also regulated by P1 and P2 receptors. These concepts will be illustrated and discussed in this symposium.

S5-01

MODULATION OF NEURONAL GLYCINE TRANSPORT BY P2Y AND P2X RECEPTORS

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Glycinergic inhibitory neurons of the spinal dorsal horn exert critical control over the conduction of nociceptive signals to rostral brain areas. The high affinity sodium- and chloride-coupled glycine neurotransmitter transporters (GlyTs) control the availability of glycine at glycine-mediated synapses. GlyTs control intra and extracellular glycine concentrations in glycinergic (GlyT1 and 2) and glutamatergic (GlyT1) synapses and therefore, modulate glycine-mediated neurotransmission. GlyT1 and GlyT2 transport activity was differentially modulated by P2Y/13 receptors in rat brainstem and spinal cord primary cultures through a paracrine regulation mediated by NO. Here we monitored GlyT2 expression and transport upon pharmacological stimulation of P2X purinergic receptors in rat brainstem and spinal cord primary cultures combined with siRNA-mediated receptor knockdown and dorsal root ganglion cell enrichment to increase P2X expression. Spontaneous glycinergic currents, glycine release and GlyT2 uptake were concurrently measured in response to P2X receptor agonists. We found the stimulation of P2X purinergic receptors with $\beta\gamma$ -methylene adenosine 5'-triphosphate induces the up-regulation of GlyT2 transport activity by increasing plasma membrane expression and reducing transporter ubiquitination. We identified the receptor subtypes involved. Up-regulation of GlyT2 required the combined stimulation of homomeric P2X3 and P2X2 receptors or heteromeric P2X2/3 receptors. We proved the impact of P2X2/3 receptor activation on glycinergic neurotransmission involves the modulation of GlyT2 expression and activity. Moreover, we found GlyT2 expression is upregulated by many pro-nociceptive agents besides ATP. In conclusion GlyT2 is differentially modulated by P2Y receptors of suggested anti-nociceptive role and P2X2/3 receptors of recognized pro-nociceptive action promoting an opposite regulation of the transporter by ADP and ATP in nociceptive pathways. We propose this modulation has physiological consequences in nociceptive signal conduction.

S5-02

ROLE OF CONCENTRATIVE NUCLEOSIDE TRANSPORTERS (CNT) IN ADENOSINE TRANSPORT AND METABOLISM

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Extracellular adenosine concentrations are regulated by a panel of membrane transporters which, in most cases mediate its uptake into cells. Adenosine transporters belong to two gene families, *SLC28* and *SLC29*, encoding Concentrative and Equilibrative Nucleoside Transporter proteins (CNTs and ENTs, respectively). Both families share common features such as substrate selectivity and often tissue localization. This apparent biological redundancy may anticipate some different roles for hCNTs and hENTs in cell physiology. Thus, hENTs may have a major role in maintaining nucleoside homeostasis, whereas hCNTs could contribute to nucleoside sensing and signal transduction. Among all the nucleoside transporters hENT1 has often been identified as a major player in purinergic signalling. However, the role of CNTs cannot be disdained, specifically hCNT2 and hCNT3 that should be the best candidates for efficient removal of adenosine considering their apparent high affinity for this nucleoside and their concentrative capacity. Moreover, solid evidences exist demonstrating that both transporters are under purinergic regulation albeit to some extent they seem to exert their functions in different tissues. Nevertheless, the role of hCNTs in purinergic signalling seems not to be only restricted to their mere uptake ability. In this regard, our results point to a more complex network in which protein interactions would play a major role in the coordinate regulation of nucleotide metabolism. Using proteomic approaches, we have identified putative hCNT3 protein partners that suggest that hCNT3 would be part of a cellular sensor of purine bioavailability, likely to include nucleotide metabolic enzymes regulating nucleotide de novo and salvage pathways.

S5-03

LIVE IMAGING AND SINGLE CELL TRACKING REVEALS CELL DYNAMICS FROM EARLY CEREBELLAR NEURAL STEM CELLS. ROLE OF VNUT IN THEIR LINEAGE PROGRESSION.

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The cerebellum, among the structures within the central nervous system, is the one whose development has been more studied. Cerebellar morphogenesis starts as early as embryonic day 7.5 but holds the particular feature of finalizing at postnatal stage (day 15). Afterbirth, cerebellar neural progenitors reside in three specific proliferative niches; the external granular layer (EGL) that produces the granule cells of the internal granular layer, the Purkinje cell layer (PCL) and the prospective white matter (PWM), which constitutes the main source of cerebellar interneurons. However, despite active research on cerebellar development, major questions of cerebellar neural stem cell (NSC) biology remain unanswered. For example, are cerebellar NSCs dependent or independent of their local niche to modulate their lineage progression? In addition we don't know which are the cell dynamics exhibited by the NSCs located in these proliferative areas. We refer here to cell dynamics as the cell cycle length, mode of division (symmetric or asymmetric), multi/bi/unipotency of NSCs, self-renewal capacity, heterogeneity of NSCs populations etc. Most of the studies that tried to disentangle these questions were classically performed with cerebellar NSCs cultivated in the presence of high doses of growth factors that (i) do not ensure isolation from the niche and (ii) may modify the NSCs cell biology. In order to unravel these questions, we have adapted a previously developed preparation of the adult subependymal zone to the early (P0) postnatal cerebellum. In this preparation, cells are cultured in adherent conditions and in absence of growth factors or feeding layer, in isolation from their niche. Combining this preparation with continuous live imaging by timelapse videomicroscopy we were able to monitor the lineage progression of neural progenitors studying the cell-intrinsic processes that drive their transitions from neural progenitors to neurons (either glutamatergic or GABAergic). Moreover, the isolation from the niche provides a unique tool to study the effect of single factors on NSCs lineage progression. Among the different extracellular signaling pathways present in the cerebellar niche, purinergic signaling modulates crucial aspects of cerebellar granule neurons and astrocytes maturation and survival. Purinergic-mediated cell to cell communication begins with the loading of its fundamental molecule, the ATP, into secretory vesicles which is mediated by the recently cloned vesicular nucleotide transporter (VNUT). In this study we have assessed the role of VNUT in the modulation of cerebellar NSCs behavior.

Symposium 6: NEW GENETIC TOOLS FOR UNDERSTANDING CELL-SPECIFIC PURINOCEPTOR FUNCTION IN HEALTH AND DISEASES

Chairs: Annette Nicke (Munich, Germany)

Content: Alterations of purinergic signals are associated with numerous disorders of cardiac and respiratory function, inflammation, chronic pain syndromes and various CNS diseases including neurodegenerative diseases associated with neuroinflammation. Adenosine and purinergic receptors are expressed in various peripheral cell types as well as in various neuronal and glial cells within CNS, it is thus crucial to decipher the cell-specific function of each receptor in pathological contexts. We will cover in this symposium very recent and ongoing validation of new transgenic animal models and molecular tools useful to better understand P2X and adenosine receptor functions.

S6-01

INCREASED SURFACE P2X4 RECEPTOR EXPRESSION LEADS TO SYNAPTIC PLASTICITY AND LEARNING DEFICITS IN NON-INTERNALIZED P2X4MCHERRYIN KNOCKIN MICE

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ATP signaling and surface P2X4 ATP-gated receptor channels are upregulated in various neurological disorders including chronic pain, epilepsy and neurodegenerative diseases such as Alzheimer's disease (AD) or amyotrophic lateral sclerosis (ALS). P2X4 displays a widespread distribution in CNS neurons and glial cells as well as in multiple peripheral cell types throughout the body. A key question regarding the role of purinergic signaling in health and disease is the function of this upregulated surface P2X4 state observed in specific cell types.

To elucidate the cell-specific functions of P2X4 in a pathological context, we created a conditional transgenic knock-in P2X4 mouse line (floxed P2X4mCherryIN) allowing the Cre activity-dependent genetic swapping of the internalization motif of P2X4 by the fluorescent protein mCherry to prevent constitutive endocytosis of P2X4. We describe and characterize two distinct knock-in mouse lines expressing non-internalized P2X4mCherryIN either in excitatory forebrain neurons (CamK2) or in all cells natively expressing P2X4 (CMV). The genetic substitution of wild-type P2X4 by non-internalized P2X4mCherryIN in both knock-In mouse models does not alter the sparse distribution and subcellular localization of P2X4 but leads to a cell-specific increased surface P2X4 expression mimicking the pathological upregulated P2X4 state. Floxed, CamK2- and CMV-P2X4mCherryIN mice were viable, normal in size, reproduced normally and displayed no obvious physical or behavioral abnormalities. We provide evidence using a battery of behavioral tests that the increase in P2X4 at the surface of excitatory neurons decreases anxiety and impairs memory processing. In addition, we demonstrate that increased surface P2X4 expression blocks long-term potentiation (LTP) and alters LTD at hippocampal CA1 synapses. These effects are more pronounced when surface P2X4 expression is specifically increased in forebrain excitatory neurons. The key finding of this study is that the increased surface expression of P2X4 in forebrain excitatory neurons is a major regulator of hippocampal synaptic plasticity, learning and memory and anxiety functions.

Overall, we provide an innovative knock-in P2X4 model to study the functional contributions of upregulated P2X4 in specific cells of the nervous system but also in peripheral tissues throughout the body.

This work was supported by CNRS, University of Bordeaux, the grant LabEx BRAIN ANR-10-LABX-43, a grant Inserm for the generation of the mouse line.

S6-02

A NEW CONDITIONAL MODEL TO STUDY ADENOSINE A_{2A}R DYSFUNCTION IN ALZHEIMER'S DISEASE

D. Blum

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Adenosine A_{2A} receptors (A_{2A}Rs) are abnormally upregulated in various neurological conditions, notably characterized by cognitive alterations. Beneficial impact of A_{2A}R blockade towards cognition and pathological impairments observed in such disorders support that the dysregulation of A_{2A} receptors is instrumental in their pathophysiological development. To address this question, we have developed new transgenic tools to conditionally overexpress A_{2A}Rs in mice for instance in neurons or astrocytes. In our laboratory, by combining to transgenic models reproducing amyloid and Tau lesions, these new models have been used to uncover the role of A_{2A}Rs in the development of Alzheimer's Disease (AD). In the present contribution, we will describe our new genetic models and provide results linking A_{2A} receptor dysfunctions and synaptic impairments in animal models of AD.

Fundings: Fondation pour la Recherche Médicale, Fondation Alzheimer, ANR, Inserm, Région Hauts de France, Université de Lille, Labex DISTALZ

S6-03

IN-SITU CROSS-LINKING OF P2X7 COMPLEXES TO IDENTIFY PROTEIN-PROTEIN INTERACTIONS

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P2X receptors are trimeric non-selective cation channels, which are gated by extracellular ATP. The P2X7 subtype differs from all other P2X family members by a particularly long intracellular domain that constitutes about 40% of the protein and is supposed to be crucial for interactions with other proteins. Activation of P2X7 leads to the assembly of the NLRP3 inflammasome, resulting in caspase 1 activation and subsequent release of the mature proinflammatory cytokines IL-1 β and IL-18. Accordingly, P2X7 signaling has been associated with a variety of inflammatory diseases and the P2X7 receptor has gained increasing attention as a promising drug target.

Although more than 50 proteins were shown to interact with the P2X7 receptor, only few of these interactions have been verified and P2X7 induced signaling pathways remain largely unclear. This could partly be explained by methodical limitations: Endogenous P2X7 protein expression rate is rather low in native cells, available antibodies lack selectivity and/or sensitivity, and P2X7 interactions seem to have a weak or transient character and might get lost during the purification processes. In this study, a BAC transgenic P2X7-EGFP mouse model in combination with *in situ* cross-linking of P2X7 complexes was used to analyze covalently cross-linked P2X7 complexes from native mouse tissue. Initial analysis by mass spectrometry identified a number of significantly enriched proteins that are involved in leukocyte migration. Experiments are ongoing to link a previously described, P2X7-mediated leukocyte infiltration to a physical interaction of P2X7 with proteins involved in cell adhesion.

S6-04

IMMUNE CELLS FROM P2X4KO MICE EXHIBIT ALTERED P2X7 EXPRESSION AND FUNCTION

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P2rx4 and *P2rx7* are neighboring genes encoding for the ATP-gated ion channels P2X4 and P2X7. The first P2X4ko mice were generated more than 10 years ago by targeting *P2rx4* in 129 embryonic stem cells. However, this might potentially lead to the introduction of *P2rx7* passenger mutations upon backcrossing to the C57BL/6 (B6) background. By analyzing the naturally occurring P451L polymorphism in P2X7 we could demonstrate that B6-P2X4ko mice (*P2rx4*^{tm1Rass}) express a P2X7 variant (P2X7-451P) which differs from the B6-WT P2X7 variant (P2X7-451L). We investigated the impact of this passenger mutation on B6-P2X4ko immune cells and observed an increased P2X7 expression on T cells when compared to B6-WT. This affected T cell sensitivity towards adenosine triphosphate (ATP). Further, B6-P2X4ko T cells were more susceptible towards nicotinamide adenine dinucleotide induced cell death (NICD) occurring after cell preparation, which influences the results of functional assays. When backcrossing B6-P2X4ko (P2X7-451P) to the Balb/c background (P2X7-451P), the passenger mutation and T cell related differences in P2X7 expression and function were neutralized. On innate immune cells we found that P2X4-deficiency in B6 mice leads to a lower P2X7 protein and mRNA expression. This was most prominent in peritoneal mast cells and macrophages whereas brain microglia exhibited almost unaltered P2X7 level. Interestingly, backcrossing of B6-P2X4ko to the Balb/c background did not normalize these differences. P2X4-deficiency in B6 and Balb/c mice influences P2X7-mediated calcium flux and pore formation in macrophages and P2X7-dependent mast cell degranulation. In conclusion, P2X7 expression and function can be mouse strain and cell type-specifically altered in P2X4ko mice. Our findings need to be considered when using these P2X4ko mice for complex immunological *in vivo* studies.

Symposium 7: PURINES AND PURINERGIC RECEPTORS IN VASCULAR INJURY, CANCER, AND INFLAMMATION

Chairs: Evgenia Gerasimovskaya (Denver, USA) / Alexander Verin (Augusta, USA)

Content: Extracellular purines are central, although still not fully recognized players in the vascular system. They control vascular cell growth, differentiation, permeability, inflammatory and immune responses. Inflammation plays a critical role in the vascular response to injury. Acute inflammation is characterized by increased vascular permeability and rapid accumulation of immune cells at the site of tissue injury and also associates with tumor progression. In addition, impaired vascular barrier function is frequently associated with dysregulated angiogenesis of immature and leaky microvessels, such as vasa vasorum, that serve as a conduit for the inflammatory cells into the vascular wall, thereby amplifying vascular inflammation process. In this symposium, we will highlight several research topics that demonstrate a role of extracellular adenosine, ATP and purine converting enzymes in a context of devastating vascular inflammatory disorders such as acute respiratory distress syndrome, pulmonary hypertension, diabetes and atherosclerosis. Moreover, novel data on detection, regulation and functional role of guanine, guanosine and guanosine phosphates in invasion and metastasis will be presented to underlie the importance of intracellular and extracellular purine signaling for cancer progression.

S7-01

ADENOSINE AT THE CROSS-ROADS OF LUNG FIBROSIS AND PULMONARY HYPERTENSION

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Group III pulmonary hypertension (PH) is a deadly lung disorder with limited treatment options. Group III PH affects patients with ongoing chronic lung injury, such as idiopathic pulmonary fibrosis (IPF). PH affects over 40% of patients with IPF and contributes significantly to mortality, yet the molecular mechanisms leading to PH in patients with IPF are not fully understood. Our hypothesis was that the hypoxic-adenosinergic system is enhanced in patients IPF + PH compared with patients with IPF with no PH. Explanted lung tissue was analyzed for markers of the hypoxic-adenosine axis, including expression levels of hypoxia-inducible factor (HIF)-1A, adenosine A2B receptor (ADORA2B), CD73, and equilibrate nucleotide transporter-1. Increased expression of HIF-1A was observed in tissues from patients with IPF + PH. These changes were consistent with increased evidence of adenosine accumulation in IPF + PH. Our data demonstrate that the hypoxic-adenosine axis is up-regulated in IPF + PH. Next, to demonstrate whether the hypoxic-adenosinergic axis is a therapeutic target for IPF + PH, we utilized the bleomycin model of lung fibrosis and PH. Using this model we demonstrate that pharmacological deletion of ADORA2B protects and prevents mice from the development of PH in the setting of lung fibrosis. These results were correlated with experiments using conditional knockout mice lacking ADORA2B in myeloid and vascular smooth muscle cells. Taken together, these results highlight the hypoxic-adenosinergic axis as a causative mechanism in IPF + PH that can be targeted therapeutically.

(Funding: R01-HL-138510 to HKQ and P01-HL114457 to MRB, YX, HKE)

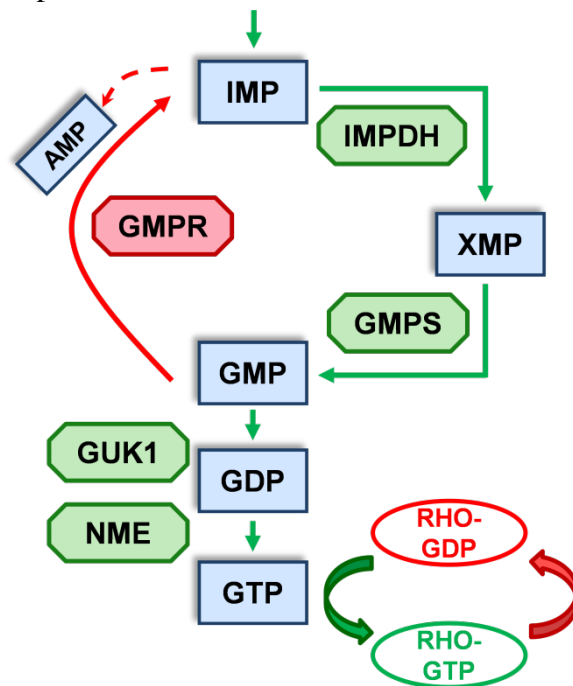
S7-02

GUANOSINE/GUANYLATE-DEPENDENT REGULATION OF SIGNAL TRANSDUCTION IN CANCER CELLS

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Recently, we have reported a connection between activity of RHO-GTPase and GTP metabolism enzymes (GMEs) including GMPR, GMPS, and IMPDH-1 and 2 (Fig. 1). In particular, we demonstrated that in cancer cells overexpression of **GMPR** or partial



inhibition of **IMPDH** led to a moderate ~25% depletion of intracellular GTP pools and a dramatic drop in the amounts of GTP-bound i.e. active RHO-GTPases. As a result, cancer cell invasion was significantly downregulated. Restoration of GTP levels or expression of RAC1^{G12V} mutant insensitive to changes in GTP, reverted these effects. Additionally, GMPR overexpression or inhibition of **GMPS**, a functional antagonist of GMPR, suppressed melanoma cell tumorigenicity.

Historically, changes in GTP levels have not been considered as a regulatory step in activation of RAC1 (or other G-proteins) in live cells. This is because average GTP concentration in the cell measured by HPLC (~500μM) is much

higher than the GTP dissociation constant of RAC1, which even in presence of GEF, is ~15μM GTP. However, HPLC cannot account for free vs bound GTP or for sub-cellular variations in GTP levels. Up until now no methods existed to detect such variations. We recently reported genetically encoded intracellular sensors of free GTP (Bianchi-Smiraglia *et al*, *Nature Methods*, 2017). These sensors for the first time made possible visualization of GTP changes in live cells and identified regions with low (~30μM) and high local GTP concentration. We will present data demonstrating that distribution of intracellular GTP levels is previously unrecognized major mechanism controlling activity of RHO-GTPases and possibly other G-proteins, and that physiological modulation of GME activity regulates GTP loading on RHO-GTP and therefore can be exploited therapeutically.

S7-03**DISSECTING THE MECHANISMS OF VASCULAR BARRIER STRENGTHENING IN ACUTE LUNG INJURY: NOVEL MECHANISMS FOR EXTRACELLULAR PURINES**

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Endothelial cells (EC) form a semi-permeable barrier between the interior space of blood vessels and the underlying tissues. In acute lung injury the EC barrier is weakened leading to increased permeability. The mechanisms involved in the preservation of EC barrier integrity are largely unknown. We have demonstrated that extracellular purines, ATP (stable analog, ATP γ S) and adenosine, strengthen EC barrier *in vitro* and *in vivo*. However, the mechanisms underlying the barrier protective effects of these purinergic agonists are different. Adenosine-induced EC barrier enhancement involves activation of P1 A2 receptors coupling to Gs trimeric G protein, followed by cAMP-dependent protein kinase A (PKA) activation, activation of myosin light chain (MLC) phosphatase (MLCP), and decrease in MLC phosphorylation. In parallel, adenosine strengthens EC barrier through Gs-mediated activation of small GTPase, Rac1. Unlike adenosine, ATP γ S activates EC-barrier-protecting signaling via P2Y receptors coupled to Gi and involved unconventional cAMP-independent PKA, MLCP and Rac1 activations. ATP γ S, Gi-mediated PKA and MLCP activations may be coordinated through the actions of adapter protein GAB1, Shp2 (a non-receptor Tyr phosphatase), and AKAP2 (PKA anchoring protein 2)-mediated signaling. AKAP2 was found in immune complexes with PKA and Gi and directly interacts with MLCP suggesting that the AKAP2/MLCP axis is a novel regulator of P2Y/Gi-mediated EC barrier enhancement. Further, our data show the involvement of a regulatory molecule ELMO1 (Engulfment and cell motility protein 1) in P2Y/Gi-mediated EC barrier strengthening, which together with the adapter protein Dock180 formed a bipartite GEF for Rac1. Collectively, our data suggested that while adenosine-induced EC barrier enhancement involves activation of Gs/cAMP-mediated signaling, ATP γ S-induced P2Y-mediated EC barrier strengthening requires Gi-mediated, coordinated activation of GAB1/Shp2 and Dock180/ELMO1 leading to activation of PKA and Rac1 pathways, respectively.

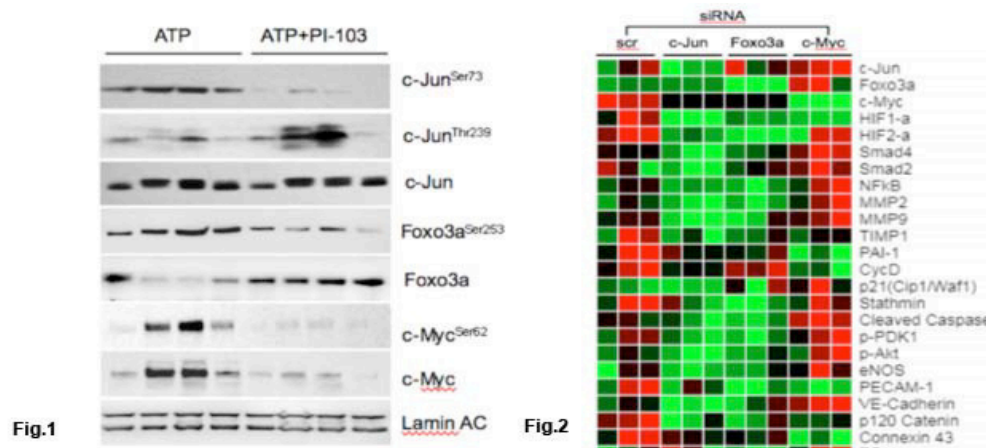
S7-04

EXTRACELLULAR ATP REGULATES ANGIOGENIC RESPONSES IN VASA VASORUM ENDOTHELIAL CELLS VIA THE ACTIVATION OF PI3K-AKT-mTOR SIGNALING AXIS AND C-JUN, FOXO3A, AND C-MYC TRANSCRIPTION FACTORS

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Angiogenic vasa vasorum (VV) expansion plays an essential role in the pathogenesis of hypoxia-induced pulmonary hypertension (PH). Previously we showed that extracellular ATP, released in hypoxia, plays an autocrine/paracrine role in angiogenic activation of VV endothelial cells (VVEC), acting via P2Y purinergic receptors (P2YR) and Phosphoinositide 3-kinase (PI3K)- Akt-Mammalian Target of Rapamycin) mTOR signaling axis. To further elucidate the mechanisms of ATP-induced angiogenesis, using TranSignal protein/DNA array we identified ATP-inducible transcription factors (TFs) in VVEC. C-Jun, Foxo3a, and c-Myc were found to be upregulated by extracellular ATP in most tested cell populations and form central nodes connecting



several signaling networks. Stimulation with ATP increased phospho-c-Jun^{Ser73}, phospho-Foxo3a^{Ser253}, and phospho-c-Myc^{Ser62} levels in the nuclear fraction, and this effect was reduced by a dual PI3K/mTOR inhibitor, PI-103 (100 nM, 1 h). In addition, PI-103 robustly increased inhibitory phosphorylation of c-Jun at Thr239, that causes a repression of DNA binding activity c-Jun (Fig.1). SiRNA-mediated knockdown of c-Jun, Foxo3a, and c-Myc revealed critical role for these TFs in VVEC angiogenic responses and the regulation of downstream proteins involved in tissue remodeling (MMP-2,9, TIMP-1, PAI-1), cell cycle control (Cyclin D, p21^{Cip1/Waf1}, stathmin), expression of endothelial markers (eNOS, PECAM-1), cell adhesion and junction proteins (VE-cadherin, p120 catenin, Connexin 43, ZO-1) (Fig.2). Our findings suggest that pharmacological targeting the components of P2YR-PI3K-Akt-mTOR signaling axis and specific TFs may attenuate VV angiogenesis and pathologic pulmonary vascular remodeling in PH. (Finding: R01-HL-086783 to E.V. Gerasimovskaya)

Symposium 8: NEW INSIGHTS IN P2X RECEPTOR STRUCTURE-FUNCTION

Chairs: Eric Boué-Grabot (Bordeaux, France) / Beata Sperlagh (Budapest, Hungary)

Content: ATP-gated P2X receptors are trimeric ion channels selective to cations. In this symposium, we will cover recent advances in the understanding of the structure-function of several members of P2X receptor family in terms of ion permeation, antagonist sites and allosteric modulation.

S8-01**PERMEATION MECHANISMS OF LARGE CATIONS IN ATP-GATED P2X RECEPTORS**

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The permeability of large cations through the P2X pore has remained arguably the most controversial and complicated topic in P2X-related research, with the emergence of conflicting studies on the existence, mechanism and physiological relevance of a so-called “dilated” state. Due to the important role of several “dilating” P2X subtypes in numerous diseases, a clear and detailed understanding of this phenomenon represents a research priority. Recent advances, however, have challenged the existence of a progressive, ATP-induced pore dilation, by demonstrating that this phenomenon is an artefact of the method employed. In this talk, I will present how modern chemical optogenetics combined to single-channel recordings have provided new insights into the permeation of large cations. The physiological relevance of such a large cation-permeable pore will also be discussed.

S8-02

MODULATION OF P2X7 RECEPTOR ACTIVITY BY EXTRACELLULAR Zn^{2+} : IMPLICATIONS FOR MOOD DISORDERS

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P2X receptors are ligand gated cation channels functioning in homo- or hetero-trimeric assemblies. The channel gating of different P2X receptor subtypes are allosterically modulated by divalent cations and their action is dependent on the individual subtype and the concentration of the cation. Zn^{2+} is an essential micronutrient that is concentrated in glutamatergic synaptic vesicles in the hippocampus and has antidepressant-like effect in animal experiments, whilst Zn^{2+} deprivation has opposite effect. We and others showed that P2X7R inhibition has also antidepressant effect, therefore we asked how extracellular Zn^{2+} modulates P2X7 receptor activity in the hippocampus, and whether the antidepressant effect of Zn^{2+} is mediated by inhibition of P2X7Rs.

The expression and activity of P2X7Rs was measured in primary mouse hippocampal culture and in acute mouse hippocampal slices, in vivo behavior tests were carried out in wild type and P2X7R deficient (P2rx7^{-/-}) mice.

We found that P2X7R immunofluorescence is expressed in both neurons and glia in primary mouse hippocampal culture. P2X7R/pannexin mediated pore formation measured by propidium iodide uptake was dose-dependently inhibited by Zn^{2+} . ATP or 3'-O-(4-Benzoyl) benzoyl ATP induced calcium influx in glial cells was also reduced by free zinc ions. Consistently, Zn^{2+} inhibited P2X7R mediated glutamate release from hippocampal slices. $ZnCl_2$ (1 mg/kg i.p.) increased immobility in the forced swim test (FST) and the tail suspension test (TST). P2X7R deficiency also exhibited antidepressant-like effect; however, the effect of $ZnCl_2$ was only slightly attenuated in P2rx7^{-/-} mice.

In conclusion, Zn^{2+} has an inhibitory effect on P2X7R function in the hippocampus, whereas its antidepressant-like effect in the TST and FST is largely independent from the overall activity of P2X7Rs.

S8-03

IDENTIFICATION OF A NON-COMPETITIVE ANTAGONIST SITE IN P2X RECEPTOR CHANNELS

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P2X receptor channels mediate fast ionotropic purinergic signaling in neurons and non-excitabile cell types. The highly calcium-permeable P2X4 subtype has been shown to play a significant role in cardiovascular physiology, inflammatory responses and neuroimmune communication. We previously reported the discovery of a P2X4-selective antagonist, the small organic compound BX430, with submicromolar potency for human P2X4 receptors and marked species-dependence (Ase et al., 2015). We investigated the molecular basis of P2X4 inhibition by the non-competitive blocker BX430 using a structural and functional approach relying on mutagenesis and electrophysiology. We provide evidence for the critical contribution of a single hydrophobic residue located in the ectodomain of P2X4 channel subunits, Ile312 in human P2X4, which determines blockade by BX430. We also show that the nature of this extracellular residue in various vertebrate P2X4 orthologs underlies their sensitivity or resistance to the inhibitory effects of BX430. Taking advantage of high-resolution structural data available on zebrafish P2X4, we used molecular dynamics simulation to model the docking of BX430 on an allosteric binding site around Ile315 (zebrafish numbering) in the ectodomain. We also observed that the only substitution I312D (human numbering) that renders P2X4 silent by itself has also a profound silencing effect on all other P2X subtypes. The generic impact of this mutation on P2X function indicates that the pre-TM2 subregion involved is conserved functionally and defines a novel allosteric inhibitory site present in all P2X receptor channels. This conserved structure-channel activity relationship might be exploited for the rational design of potent P2X subtype-selective antagonists of therapeutic value.

Funded by CIHR and NSERC.

S8-04

STRUCTURE-FUNCTION RELATIONSHIP OF P2X7 RECEPTORS

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The human P2X7 receptor (hP2X7R) is an ATP⁴⁺-gated nonspecific cation channel that is expressed in cells of the immune system. By activating this receptor, ATP, released during cell damage or hypoxia, serves as danger-associated molecular pattern to induce inflammatory reactions. In comparison to other P2X receptors, P2X7R-dependent ionic currents differ in their activation kinetics by an absence of desensitization and induction of large membrane pores upon long lasting agonist applications. Despite significant advances, the gating and permeation mechanisms of the P2X7R is not fully understood. We have found in a cysteine scanning mutagenesis study that Gly338 within the second transmembrane helix (TM2) is part of the narrowest channel region, which extends from Tyr336 to Gly345. The gate and selectivity filter of hP2X7R are colocalized and primarily determined by Ser342. Here we report that substitution of Gly338 to cysteine generates a constitutively open channel that closes in response to extracellular application of ATP or the typical P2X7R agonist benzoyl ATP (BzATP). Substitution of extracellular Na⁺ by the larger organic Tris⁺ significantly decreased the ion conductance and shifted the reversal potential of the ATP-induced current to more negative potentials indicating that the leak current is carried by cations. Homology modeling of hP2X7R^{G338C} during ATP application with the truncated zebrafish P2X4R X ray structure as template suggests that G338 is located near the narrowest part of the closed ion channel pore and substitution by cysteine pushes the TM2 of the three subunits apart, thus preventing a complete channel closure in the apo state. ATP application induces a conformational switch of hP2X7R^{G338C}, allowing Cys338 to interact with Tyr40 and Tyr343 in TM1 and TM2, respectively. This decreases the pore diameter of the trihelical TM2 bundle forming the ion channel of the hP2X7R^{G338C}, thus reducing the ion current. These findings point to a critical role of Gly338 in gating the P2X7R.

Acknowledgements

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Symposium 9: ADENOSINE-RECEPTOR CONTAINING COMPLEXES. FROM MOLECULAR DYNAMICS AND PHARMACOLOGY TO METABOLISM

Chairs: Kjell Fuxe (Stockholm, Sweden) / Rafael Franco (Barcelona, Spain)

Content: Purinergic G-protein-coupled receptors (GPCRs) were among the first to be discovered forming complexes with other cell surface receptors. More recently, they have been instrumental to advance in the understanding of GPCR pharmacology and physiological role. This symposium covers dynamics of dimer formation, and the role of GPCR-GPCR and GPCR-ionotropic (NMDA) receptor heteromers in metabolism/obesity and in the CNS/neurodegeneration.

S9-01

UNRAVELING THE GPCR DIMERIZATION BY SECOND TIMESCALE FREE-ENERGY CALCULATIONS

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Membrane proteins diffuse in the phospholipid bilayer forming functional dimers and oligomers which can play specific roles during cell cycle and in pathological condition. Unfortunately, the elucidation of membrane protein/protein binding interaction is difficult using standard experimental and computational techniques because of the size and complexity of the systems, and the slow diffusion of proteins in membrane. In the present talk, I introduce an innovative multiscale approach that combines Coarse-Grained molecular dynamics and MetaDynamics (CG-MetaD) [1], allowing to overcome both the size and the timescale limit of the state-of-the-art techniques. Specifically, in CG-MetaD the representation of the system as beads instead of atoms reduces the dimensionality of the system under investigation, while metadynamics enhances the phase space exploration, allowing practical investigation of long timescale and large-scale motions of proteins in membrane. As a result, CG-MetaD super-accelerates the sampling allowing to go over the second timescale, well beyond the timescale accessible by the state-of-the-art simulations. CG-MetaD has been used to disclose the free energy landscape underlying the dimerization mechanism of the transmembrane helices of the epidermal growth factor receptor. The characterization of the free energy minima allows identifying the active and inactive conformations of the receptor, shedding light on possible activation pathways [1]. Very recently, we have performed millisecond CG-MetaD calculations to describe the dimerization process of the chemokine GPCRs, CCR5 and CXCR4, and the adenosine GPCRs, A2A and A2B. The free diffusion of the proteins in membrane and the reproduction of several protein/protein binding events during the simulation, lead to a well-characterized free energy surface and the identification of the receptors' dimer states [2,3]. The dimerization interface in all the energy minima is characterized with atomistic resolution, elucidating the role of lipids and cholesterol molecules. Our findings pave the way to further investigations, especially structure-based drug design studies in diseases in which the dimer forms of these receptors are involved (i.e., neurodegenerative disorders, cancer and HIV).

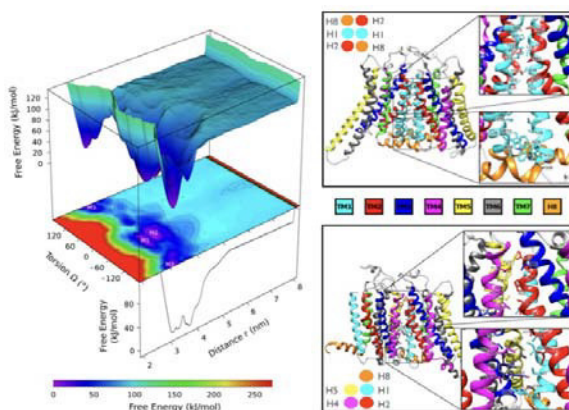


Figure. The free energy surface of the A2A GPCR dimerization (left) with the lowest energy dimer states (right).

Rererences

Lelimousin M, **Limongelli V**, Sansom MSP. *J. Am. Chem. Soc.* 138, 10611-10622 (2016). Di Marino D, Motta S, **Limongelli V**. *Biophys. J.* 116, 344a (2019). Di Marino D, Motta S, **Limongelli V**. (manuscript under preparation).

S9-02

ROLE OF ADENOSINE IN METABOLISM

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Background: Obesity has reached pandemic dimensions and is considered as one of today's most pressing public health problems. Obesity and overweight are associated with non-communicable diseases like type 2 diabetes, cardiovascular disease (stroke and myocardial infarction) and certain types of cancer.

Our laboratory is focusing on the role of adenosine signaling in adipose tissue and in obesity. Two types of fat can be distinguished: white adipose tissue (WAT) is the largest store of energy in our body. In contrast, brown adipose tissue (BAT) is specialized in dissipating energy by uncoupling ATP synthesis through its unique *uncoupling protein 1* (UCP1). BAT abundance is correlated with leanness in humans. We recently showed that adenosine activates human and murine brown adipocytes. This was not expected since previous studies in hamster showed an inhibitory effect of adenosine on BAT energy expenditure. Interestingly, adenosine receptor expression is different in hamster versus murine and human BAT, with high A₁ expression levels in hamster and low A₁ expression in murine and human BAT. Interestingly, mice are protected from diet-induced obesity when treated with an adenosine receptor A_{2A} agonist. [1] Besides A_{2A}, the adenosine A_{2B} receptor is abundantly expressed in murine BAT. Here, we analyzed the function of the A_{2B} receptor in murine and human BAT.

Material and methods: We analysed the role of adenosine receptor A_{2B} in BAT in mice using either pharmacological stimulation (Bay 60-6583) or mice deficient of A_{2B}. Moreover, we investigated expression of A_{2B} and its correlation with functional activity in human BAT.

Results: Adipose tissue-specific deletion of A_{2B} decreased BAT-dependent energy expenditure. Pharmacological stimulation of A_{2B} increased energy expenditure as well as glucose uptake and counteracted diet-induced obesity along with improved glucose tolerance. Moreover, A_{2B} stimulation induced browning of white fat. In humans, A_{2B} expression correlated with BAT activity. Moreover, we found that A_{2B} and A_{2A} receptor can interact and that this interaction regulates adenosine signalling in brown adipocytes.

Conclusion: Activation of A_{2B} has profound effects on whole-body metabolism by increasing BAT-dependent energy expenditure and browning of white fat. Moreover, A_{2B} expression might be crucial for human BAT activity. Thus, chronically increasing energy expenditure via A_{2B} stimulation might counteract obesity.

1. Gnad T, Scheibler S, von Kugelgen I, Scheele C, Kilic A, et al. (2014) Adenosine activates brown adipose tissue and recruits beige adipocytes via A_{2A} receptors. *Nature* 516: 395-399.

S9-03

ADENOSINE HETERORECEPTOR COMPLEXES IN THE BASAL GANGLIA ARE IMPLICATED IN PARKINSON DISEASE AND ITS TREATMENT

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There exists substantial evidence for the existence of G protein-coupled receptor (GPCR) homo and heteroreceptor complexes with allosteric receptor-receptor interactions in the Central Nervous System. The adenosine homo, isoreceptor and heteroreceptor complexes in the basal ganglia play a highly significant role in modulating the indirect and direct pathways and the striosomal projections to the nigro-striatal dopamine system. The major adenosine receptor complexes in the striato-pallidal GABA neurons can be the A2AR-D2R and A2AR-D2R-mGluR5 receptor complexes, in which A2AR protomers and mGluR5 protomers can allosterically interact to inhibit D2R protomer signaling. Through a reorganization of these heteroreceptor complexes upon chronic dopaminergic treatment a pathological and prolonged inhibition of D2R receptor protomer signaling can develop with motor inhibition and wearing off of the therapeutic effects of levodopa and dopamine receptor agonists. The direct pathway is enriched in D1R in and around glutamate synapses enhancing the ability of these GABA neurons to be activated and increase motor initiation. The brake on these GABA neurons is in this case exerted by A1R forming A1R-D1R heteroreceptor complexes in which they allosterically inhibit D1R signaling and thereby reduce motor initiation. Upon chronic levodopa treatment a reorganization of the D1R heteroreceptor complexes develops with the formation of putative A1R-D1R-D3 in addition to D1R-D3R complexes in which D3R enhance D1R protomer signaling and may make the A1R protomer brake less effective. Alpha-synuclein monomers-dimers are postulated to form complexes with A2AR homo and heteroprotomers in the plasma membrane enhancing alpha-synuclein aggregation and toxicity. The alpha-synuclein fibrils formed in the A2AR enriched dendritic spines of the striato-pallidal GABA neurons may reach the surrounding DA terminals via extracellular vesicle mediated volume transmission involving internalization of the vesicles and their cargo (alpha-synuclein fibrils) into the vulnerable DA terminals, enhancing their degeneration followed by retrograde flow of these fibrils in the DA axons to the vulnerable nigral DA nerve cells.

S9-04

ADENOSINE A_{2A} RECEPTOR ACTIVATION ATTENUATE NMDA RECEPTOR FUNCTION IN ALZHEIMER DISEASE

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Alzheimer disease is a neurodegenerative disorder of the central nervous system that manifests with memory, thinking and behavior deterioration. Symptoms usually develop slowly and get worse day after day, until patients are so severe that they interfere with daily tasks. Unfortunately, to date there is no curative or palliative treatment for this disease, only symptomatic interventions. Therefore, new strategies and treatments are required to treat this progressive neurodegenerative pathology.

Adenosine receptors play an important role in cognitive function. Adenosine receptors are divided in high affinity receptors: containing A₁R and A_{2A}R and low affinity receptors: containing A_{2B} and A₃. Interestingly, it has been observed that caffeine improve cognitive function in AD patients. And that antagonists of adenosine A_{2A} receptors mimic the beneficial effects of caffeine on cognitive function. Also, it has been described that A_{2A}R antagonist prevent amiloid- β neurotoxic effects.

On the other hand, NMDA receptors are implicated in the control of memory and learning, being one of the most analyzed targets for Alzheimer disease. However, is still deficient the demonstration of the mechanism underlying NMDA receptor role in AD.

Since nineties, it has been demonstrated that GPCR can interact forming oligomeric complexes that acquire new properties. We here provide data that demonstrate in transfected HEK-293T cells that NMDA receptors interact with adenosine A_{2A} receptors. Moreover, A_{2A}R stimulation block NMDA signaling in MAPK phosphorylation and calcium release. Moreover, A_{2A}R couple to G_s protein, thus analyzing cAMP accumulation it has been observed that NMDA stimulation also block A_{2A}R activity.

Finally, the A_{2A}-NMDA receptor complex has been identified in hippocampus neuronal primary cultures, showing an important increase in APP_{Sw,Ind} animal model in comparison with controls. It has been observed that in neuronal cells, A_{2A}R agonists induce an important decrease in NMDA signaling. Due to the increased expression of A_{2A}R in neuroinflation conditions, NMDA signaling in APP_{Sw,Ind} animal model is completely blocked by A_{2A}R stimulation. All together, we offer a new mechanism demonstrating a functional and structural regulation of adenosine A_{2A} receptor over NMDAR in Alzheimer disease.

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Symposium 10: PURINERGIC RECEPTORS AND CANCER

Chairs: Rosa Gomez-Villafuertes (Madrid, Spain) / Elena Adinolfi (Ferrara, Italy)

Content: Cancer is a major burden of disease worldwide. Each year, tens of millions of people are diagnosed with cancer around the world, and new disease markers and therapeutic targets are needed. P2 receptors are expressed by all tumours, in some cases to a very high level. Activation or inhibition of selected P2 receptor subtypes brings about cancer cell death or growth inhibition. This following symposium will try to show recent advances in the field of purinergic receptors in cancer development and progression.

S10-01

THE ROLE OF THE P2X4 RECEPTOR IN BREAST CANCER

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Purinergic signalling is associated with cancer cell invasiveness and metastasis formation with P2X receptors involved on the side of the host immune cells as well as the tumour. The unusually high levels of extracellular ATP within the tumour microenvironment activate the low affinity P2X7 receptor to promote both the growth and metastatic potential of breast cancers^{1,2}. P2X4 is commonly co-expressed with P2X7 and their synergistic activity is often questioned. However, unlike P2X7, P2X4 is targeted to endolysosomes³. In this study we assessed the role of P2X4 in mammary tumour growth and metastases, and its functional association with P2X7.

At the transcript level, P2X4 is over-expressed in human breast cancers compared to normal breast tissue. We showed by immunohistochemistry, elevated P2X4 expression in >50% of human breast cancer samples compared to normal breast tissue. A combination of *in vitro* and *in vivo* approaches was carried out, utilizing highly invasive human MDA-MB-435s and murine 4T1 mammary cancer cell lines, both endogenously expressing P2X4 and P2X7. Western blot and immunocytochemistry analyses showed expression of P2X4 and its targeting to lysosomes. Knocking down the expression of P2X4 using the CRISPR/Cas9 system reduced basal invasive capacities of cells through Matrigel-coated inserts by 50%. It also inhibited the 2-fold enhancement of invasion produced by BzATP and the secretion of cathepsin D, suggesting a role for P2X4 in lysosome exocytosis triggered by P2X7 stimulation. A comparison was made of tumour growth and metastases in BALB/c mice following implantation of either P2X4-CRISPR or CTL-CRISPR cells into mammary fat pads. For two clones of the P2X4-CRISPR cells, tumour growth was inhibited by 85.5% and 91.5% compared to control cells, and metastases development was prevented. In conclusion, our results show a prominent role of P2X4 in cancer cell invasiveness, tumour growth and metastases, which could be potentiated by P2X7 stimulation.

- 1) Jelassi J *et al.* P2X(7) receptor activation enhances SK3 channels- and cystein cathepsin-dependent cancer cells invasiveness. *Oncogene*, 30, 2108–2122 (2011).
- 2) Adinolfi E *et al.* Expression of P2X7 receptor increases *in vivo* tumor growth. *Cancer Res.* 72(12):2957-69 (2012).
- 3) Qureshi O *et al.* Regulation of P2X4 receptors by lysosomal targeting, glycan protection and exocytosis. *Journal of Cell Science.* 120, 3838-3849 (2007)

S10-02

P2X7 RECEPTOR IS A KEY REGULATOR OF ATP CONTENT AND IMMUNE INFILTRATION IN THE TUMOR MICROENVIRONMENT

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P2X7 is an ATP gated ion channel that attracted increasing attention as potential oncological target. P2X7 blockade reduces cancer growth in various preclinical models. However, and rather surprisingly, tumor growth and dissemination are increased in P2X7 null mice. This apparent paradox is due to reduced immune cell infiltration in tumors growing in the P2X7 null host compared to their wild type counterpart, suggesting that lack of P2X7 hampers tumor-immune cell infiltration. Here we investigated the mechanism by which P2X7 genetic deletion or pharmacological blockade modulate tumor immune contexture. In the P2X7 null host, an immunosuppressive infiltrate, characterized by fewer CD8⁺ and an increased number of Tregs, predominates. Furthermore, Tregs express high levels of fitness markers OX40, PD-1 and CD73. On the contrary, P2X7 pharmacological blockade in the P2X7 WT host, supports a tumor-aggressive infiltrate characterized by a high number of CD4⁺ effector T lymphocytes, reduced expression of OX40 on Tregs and of CD39 and CD73 on Teffs and dendritic cells. Genetic deletion versus pharmacological blockade of the P2X7 receptor also had a differential effect on the ATP content of the tumor microenvironment, as while in the former case we observed a large reduction in ATP concentration, no changes were observed in the latter. Our findings show that the P2X7 receptor is a crucial determinant of tumor-host interaction since its expression and function affect both immune cell infiltration and ATP content in the tumor milieu. P2X7 pharmacological blockade does not replicate the immunosuppressive effects due to genetic ablation, rather it enhances tumor infiltration by CD4⁺ T effector cells and diminishes CD39 and CD73 expression, thus reducing immunosuppression. Our observations support the hypothesis that administration of P2X7 antagonists may be a viable therapy for cancer, combining the direct inhibitory effect on tumor growth with the promotion of a tumor-aggressive immune infiltrate.

S10-03

ROLE OF PURINERGIC SIGNALIN IN PANCREATIC CANCER

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Pancreatic ductal adenocarcinoma (PDAC) is rising in incidence and has very poor prognosis. New disease markers and therapeutic targets are urgently needed. Our knowledge of the genetics and epidemiology of the disease is on par with many other cancers, but the molecular mechanisms that give rise to PDAC are unresolved. Therefore, we seek better understanding of cellular/molecular mechanisms involved in cross-talk between fibrogenic pancreatic stellate cells (PSC) and cancer cells. Chemical factors within the tumor microenvironment that may play significant roles are extracellular acid, nucleotides/sides, cytokines and hypoxia. Our studies aim to elucidate whether purinergic signaling is an important determinant of tumor development/progression.

Our study on mouse cancer model indicated that the P2X7 receptor (P2X7R) contributes to fibrosis and cancer progression (1). Therefore, we aimed to clarify mechanisms by which P2 receptors, in particular P2X7R, contribute to PSC-cancer cell cross-talk. We found that human and murine PSCs express P2X7R, which stimulates collagen secretion and cell proliferation, and these can be targeted by e.g. AZ10606120. Moreover, P2X7R also regulates secretion of IL-6, which promotes PDAC cell migration and cancer development. In PDAC cells, P2X7R also stimulates migration, while in PSCs it is the P2Y2 receptor that regulates cell migration. Extracellular ATP sources in the tumor are from metabolically active PDAC cells, as well as mechanosensitive PSC. Further stimuli of ATP release are bile acids and extracellular acid load, which are important factors in inflammation and pancreatitis, conditions associated with promoting pancreatic cancer development.

Our studies show that therapeutic targeting of purinergic signaling between pancreatic stellate cells and pancreatic cancer cells may be a promising avenue for co-/therapy of pancreatic cancer.

1. Giannuzzo et al. J Cancer 139, 2540, 2016.

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S10-04

REGULATION OF P2X7 RECEPTOR GENE EXPRESSION IN NEUROBLASTOMA CELLS

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In the nervous system, P2X7 receptors (P2X7R) are involved not only in physiological functions such as cell growth, differentiation and apoptosis, but also in brain pathologies including neurodegenerative diseases and cancer. P2X7R are highly expressed by nearly all human cancers so far investigated, including neuroblastoma cells. Noticeable, trophic deprivation induces a significant increase in *P2rx7* gene expression, facilitating proliferation of neuroblastoma cells in the absence of trophic support. Blockade of P2X7R results in increased neuritogenesis in neuroblastoma cells, whereas P2X7R overexpression significantly reduces the formation of neurites. In resting conditions, P2X7 transcript levels seems to be mainly regulated by the specificity protein 1 (Sp1), which is a multifunctional transcription factor constitutively expressed that directly binds to GC-rich DNA motifs to modify the expression of a wide variety of genes. We have demonstrated that the increase in P2X7R expression following serum withdrawal requires the activation of PI3K/Akt pathway and depends on nuclear Sp1 levels, since blockade of Sp1-dependent transcription with mithramycin A prevents upregulation of *P2rx7* gene. Now we have found a new regulatory mechanism of *P2rx7* gene expression independent of Sp1, which is mediated by dual specificity phosphatases (DUSPs), enzymes that regulate the activity of mitogen activated kinases (MAPKs). Inhibition of DUSP1/DUSP6 by (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI) strongly increases the expression of P2X7R, effect that is significantly reduced by p38/ERK inhibition. BCI treatment strongly enhances p38 and JNK phosphorylation, but decreases ERK1/2 phosphorylation, suggesting that BCI mainly inhibits DUSP1 activity in neuroblastoma cells. The observed decrease in ERK phosphorylation is mediated by activation of protein phosphatase 2 following p38 phosphorylation. Interestingly, the effect of BCI on P2X7R expression is not only independent of Sp1, but also independent of other transcription factors such as AP-1, CREB and Myc.

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References: Gómez-Villafuertes et al., 2015, Sci Rep 5:18417. García-Huerta et al., 2012, J Biol Chem 287(53):44628-44. Gutiérrez-Martín et al., 2011, J Biol Chem 286(13):11370-81. Gómez-Villafuertes et al., 2009, FEBS J 276(18):5307-25.

Symposium 11: STRUCTURAL INSIGHTS INTO LIGAND BINDING AND DYNAMICS OF P2X RECEPTORS

Chairs: Ralf Hausmann (Aachen, Germany) / Diego Dal Ben (Camerino, Italy)

Content: The publication of the hP2X3R structures in 2016 has created a solid basis for structural dynamics studies to provide further insights into the functioning of P2XRs. Molecular modelling, docking computations and molecular dynamics simulations allow us to gain a deeper understanding of how these ion channels function at the molecular level. These methods will facilitate molecular and atomistic insights into the mechanisms of ligand binding, gating, ion permeation and desensitization of P2XRs. Two talks will report on molecular dynamics simulations to provide insights into the closed-to-open and open-to-desensitized transition as well as ion permeation in P2XRs. The other two talks will focus on molecular modelling and docking computations to investigate molecular and structural determinants of small-molecule ligand binding and antagonist activity of novel ATP-derivatives. Thus, all talks will present computational studies including molecular modelling, ligand docking or molecular dynamics simulations to give structural insights into ligand binding and structural dynamics of P2XRs.

S11-01

CONFORMATIONAL CHANGE IN P2XR – INSIGHTS FROM MOLECULAR DYNAMICS SIMULATIONS

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With X-ray structures of different P2X receptor homologs (and homology models derived from them) in closed, open and desensitized states available (for review, see Pasqualetto et al., *Front. Pharmacol.*, 9, 58, (2018)) we can start to decipher the transitions between such states at an atomistic level. In my lab we use molecular dynamics simulations to monitor the response of receptors to “disturbances” such as mutations or (un)binding events over time, and to study the dynamics of structural variation. Recent progress in molecular dynamics algorithms and the advent of GPU hardware allows us to routinely extend atomistic simulations of P2XRs into the low micro second range. While this time resolution is by no means close to the millisecond to second time scales on which transitions between states typically occur, it enables us to investigate initial responses of a receptor to, for instance, binding or unbinding events. In my talk I will focus on two aspects our recent work on P2XRs. In the first part I will present data on binding and unbinding of agonists and antagonists to P2XRs, and how this affects receptor structure and dynamics. The second part covers effects of mutations on the stability and dynamics of the intracellular cap, and I will discuss consequences on transitioning between different receptor states.

S11-02

THE MOLECULAR DYNAMICS OF ION CONDUCTION IN P2X RECEPTORS

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P2X receptors are trimeric, ligand-gated cation channels that conduct Na⁺, K⁺, and Ca²⁺ ions across the cell membrane upon binding of extracellular ATP. Biochemical and electrophysiological studies have significantly advanced our understanding of P2X receptor function and regulation. In particular, high-resolution crystal structures of the human P2X3 receptor provided unprecedented insights into the architecture of the closed and open ion pore and suggested possible gating mechanisms. Furthermore, these structures visualized that opening and closing of the channel is not only determined by ATP-induced conformational changes in the extracellular domain, but also by the dynamic folding/unfolding of the so-called cytoplasmic cap, which stabilizes the open state. However, ion permeation and channel gating are inherently dynamic processes and thus challenging to study with static structures only. Here we investigate the mechanisms of ion conduction and channel gating using molecular dynamics simulations at atomic resolution. Simulations of P2X3 under physiological transmembrane voltages are used to directly observe ion permeation. Thereby we define the permeation pathways and illuminate the principles of the high cation selectivity, the ion current rectification, as well as the involvement of lipid headgroups in ion conduction. Finally, we demonstrate how the structural stability of the cytoplasmic cap determines the kinetics of receptor desensitization.

S11-03

THE MOLECULAR DETERMINANTS OF SMALL-MOLECULE BINDING AT P2X RECEPTORS

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P2X receptors are ATP-gated trimeric cation channels which are implicated in several diseases including neuropathic pain, cancer and arthritis, making them attractive drug targets. The availability of a series of P2X receptor crystal structures in resting, open and antagonist-bound states has transformed our understanding of the way small molecules bind to P2X receptors, and enabled us to perform molecular modelling studies to reconcile data from previously published structure-function experiments, describing potential binding conformations for the semi-selective synthetic P2X7 agonist 2'-(3')-O-(4-benzoyl)benzoyl ATP (BzATP), and the P2X4-selective positive allosteric modulator ivermectin. We find that the distal benzoyl group of BzATP lies in close proximity to Lys-127, a residue previously implicated in BzATP binding to P2X7, which may explain the increased potency of BzATP at rat P2X7 receptors. Ivermectin has been shown to bind between transmembrane domains in the crystal structure of the glutamate-gated chloride channel GluCl α , and a wealth of previous mutagenesis studies on P2X4 receptors suggest that it may bind in a similar location in P2X4 receptors. Our docking of ivermectin into a molecular model of rat P2X4 between the first and second transmembrane domains agrees very well with the previous data, and would likely have the effect of stabilizing the open channel structure, consistent with the mode of action of this positive allosteric modulator. From our docking simulations and analysis of sequence homology we propose a series of mutations likely to confer ivermectin sensitivity to human P2X1.

While several subtype-selective P2X receptor antagonists are known, no subtype-selective agonists, and few partially selective agonists have been described to date. In order to discover novel P2X receptor agonists, we have developed a screening platform using transgenic *Drosophila melanogaster* expressing P2X2 receptors in their taste neurons. Wild-type rat P2X2 expressed in *Drosophila* was fully functional (ATP EC₅₀ 8.7 μ M), and we were able to rapidly screen a library of very small volumes (2 μ l) of 80 adenosine nucleotide analogues, enabling us to determine the agonist potency and specificity profile for rat P2X2. We found that triphosphate-bearing analogues displayed broad activity, tolerating a number of substitutions, and that diphosphate and monophosphate analogues displayed very little activity. While several ATP analogues gave responses of similar magnitude to ATP, including the previously identified agonists, ATP γ S and ATP α S, we also identified a novel agonist, 2-fluoro-ATP, and confirmed its agonist activity on rat P2X2 receptors expressed in human cells. Docking of molecules displaying a range of agonist activity into a molecular model of rat P2X2 gave insights into where substituents are more likely to be tolerated, and will inform the future structure-based design of subtype-selective P2X receptor agonists.

S11-04

STRUCTURAL INSIGHTS INTO MODIFICATIONS TO THE ATP MOLECULE TUNING ITS AGONIST-TO-ANTAGONIST ACTIVITY AT THE P2X RECEPTORS

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P2XRs are potential therapeutic targets for a wide set of diseases related to inflammation, pain and cancer, including neurological and endocrinological diseases. Hence, the depictions of the mechanism of action of P2XR modulators and structure-activity relationships represent key steps for the rational design of novel molecules able to modulate the receptor function and to be developed as therapeutic agents.

Due to its chemical instability in the biological environment and to its lack of selectivity for specific subtypes, the P2XR endogenous ligand ATP was modified by changing the triphosphate chain and the ribose moiety or by inserting substituents in the purine moiety. These modifications led to the development of molecules endowed in some cases with subtype selectivity and increased potency respect to ATP. The modifications on the triphosphate chain were aimed mainly to develop chemically stable compounds without a remarkable change on the compound activity with respect to the corresponding unmodified analogues. Modifications on the sugar moiety by insertion of further groups or by simplification of the ribose ring led in many cases to compounds with a pharmacological profile shifted from full to partial agonist or antagonist. The insertion of substituents on the purine scaffold modulated the potency of the compounds and sometimes their intrinsic activity.

With the aid of available crystal structures of P2XRs in complex with the endogenous ligand ATP or analogues (i.e. its derivative TNP-ATP, endowed with an antagonist profile), an interpretation of the mechanism of action of these molecules and the depiction of structure-activity relationships for nucleotide ligands at the P2XRs will be described.

Symposium 12: GUANINE-BASED PURINES IN HEALTH AND DISEASE

Chairs: Francisco Ciruela (Barcelona, Spain) / Francesco Caciagli (Chieti, Italy)

Content: Guanine-based purines (GBPs) exert a multitude of beneficial (i.e., neuroprotective, neurotrophic, antidepressant, anxiolytic) effects throughout the CNS, being key players in cell metabolism and in several models of neurodegenerative disorders, such as Parkinson's Disease (PD) and Alzheimer's diseases (AD). Moreover, these compounds seem to be involved in tumor progression, and their converting enzymes can both activate some anti-cancer pro-drugs and play new pathophysiological roles independent of their enzymatic activities. Indeed, methyl-thioadenosine phosphorylase (MTAP) knockdown increases invasion and migration of several tumor cell lines; ii) an over-expression of the cytosolic 5'-nucleotidase (cN-II) has been observed in some cancer cells; iii) not frequent polymorphisms of gene encoding purine nucleoside phosphorylase (PNP) associated with a reduced enzymatic activity have been found in dysplastic or neoplastic alterations of marrow morphology or in prostate cancer. As no plasma-membrane receptor for GBPs have been described so far, these compounds are still considered orphan modulators of multiple functions, and many efforts should be made to elucidate the mechanism of action underlying their effects. In this context, the evaluation of the expression/activity of purine converting enzymes as well as the identification of specific receptors for GBPs could represent valuable diagnostic tools and innovative targets for the development of novel drugs and for new therapeutic approaches in neurological diseases and cancer. The talks of this symposium aim at giving a contribution to the knowledge of GBP roles in human diseases.

S12-01

GUANOSINE-INDUCED NEUROPROTECTION: A ROLE FOR ADENOSINE RECEPTOR OLIGOMERS?

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Guanosine is a guanine-based purine nucleoside with important trophic functions and promising neuroprotective properties. In line with this, we recently proposed a potential therapeutic role for guanosine in Parkinson's disease treatment¹. In animal models of parkinsonism (catalepsy, tremor, hemiparkinsonism), guanosine was effective not only for reversing parkinsonian motor impairments but also for reducing L-DOPA-induced dyskinesia¹. Despite these promising effects, the exact mechanism of action and molecular targets for guanosine are still unknown. Here, we aimed to elucidate a role for adenosine receptors (AR), which could be involved in mediating guanosine effects. To this end, we first investigated the neuroprotective role of guanosine in hippocampal slices from wild-type and A_{2A}R-deficient mice subjected to oxygen/glucose deprivation. Our results pointed to a crucial role of A_{2A}R expression in guanosine-mediated neuroprotective effects. Accordingly, we next aimed to assess possible guanosine binding and functional activation of A_{2A}R. We synthesized a new fluorescent selective A_{2A}R antagonist (MRS7396)², which could engage a Bioluminescence Resonance Energy Transfer process with NanoLuc-tagged A_{2A}R, and evaluated guanosine ability to block A_{2A}R ligand binding. Interestingly, we assessed guanosine effects in cells transfected with A_{2A}R alone or together with the A₁R, which may form functional oligomers. Guanosine partially displaced MRS7396 binding only in cells co-expressing A₁R/A_{2A}R. Similarly, we also observed that guanosine exerted inhibitory effects on functional activation of A_{2A}R (cAMP and label-free recording) in A₁R/A_{2A}R-expressing cells. Overall, our results suggest that A_{2A}R expression is crucial for guanosine neuroprotective effects and that the A₁R/A_{2A}R oligomer may be relevant for such guanosine-AR interaction.

References

- 1 Massari CM *et al.* Antiparkinsonian efficacy of guanosine in rodent models of movement disorder. *Frontiers in Pharmacology* 2017; **8**. doi:10.3389/fphar.2017.00700.
- 2 Duroux R *et al.* Bitopic fluorescent antagonists of the A_{2A} adenosine receptor based on pyrazolo[4,3- E] [1,2,4]triazolo[1,5- c] pyrimidin-5-amine functionalized congeners. *MedChemComm* 2017; **8**. doi:10.1039/c7md00247e.

S12-02

EXTRA- AND INTRACELLULAR METABOLISM OF ADENOSINE IN CANCER AND INFLAMMATION

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Current models of cellular purine turnover depend of functional interactions between distinct processes, including (i) the release of endogenous ATP, (ii) triggering of signaling events via nucleotide- and nucleoside-selective receptors, (iii) ectoenzymatic interconversion of extracellular nucleotides, (iv) cellular uptake of nucleotide-derived adenosine and other nucleosides via equilibrative nucleoside transporters and finally, (v) intracellular interconversion of the transported nucleosides into ATP through complex phosphotransfer reactions. Despite the significant progress in our understanding of the purinergic machinery as a multistep cascade, current knowledge on the whole sequence of “release-signaling-inactivation” remains rather limited. The adenosine pathway is currently viewed as a significant barrier to the effectiveness of immune therapies and becomes an important therapeutic target in cancer. Prior research has focused almost exclusively on adenosine producing ectoenzymes, nucleoside triphosphate diphosphohydrolase-1 (NTPDase1/CD39) and ecto-5'-nucleotidase/CD73, and on the role of different types of adenosine receptors (A₁, A_{2A}, A_{2B}, and A₃) with mixed findings of anti-tumor and pro-tumor effects of the different receptors. Major gaps in knowledge that impede the development of effective adenosine-based therapeutics include: (1) lack of consideration of redundant pathways controlling adenosine levels, and (2) lack of distinction between receptor-mediated and epigenetic receptor-independent effects of adenosine. In particular, along with “classical” CD39-CD73 axis, other membrane-bound and secreted enzymes contribute to the tuned control of extracellular adenosine levels, including the enzymes of the nucleotide pyrophosphatase/phosphodiesterase, alkaline phosphatase, adenylate kinase and nucleoside diphosphate kinase (NDPK/NME/NM23) families, CD38, adenosine deaminase and purine nucleoside phosphorylase. Here, we summarize recent advances in this rapidly evolving field, with particular emphasis on adenosine-producing and –inactivating pathways at such (patho)physiological states as inflammation, hypoxia and tumorigenesis.

S12-03

IMPACT OF IMP-GMP SPECIFIC 5'-NUCLEOTIDASE ON METABOLIC REGULATION AND DRUG RESISTANCE IN TUMORS

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Cytosolic 5'-nucleotidase II (cN-II) is an ubiquitous enzyme, highly expressed in replicative tissues and tumours. It catalyzes the dephosphorylation of purine nucleoside monophosphates into their corresponding nucleosides and inorganic phosphate. cN-II has a preference for IMP and GMP, followed by AMP, and it is highly regulated through the interaction of its two effector sites with adenosine derivatives or several phosphorylated compounds(1,2) It has been shown to be involved in the regulation of intracellular purine nucleotides, and the secretion of nucleosides is increased in cN-II-overexpressing cells (3). The role of cN-II in cancer and sensitivity to cancer treatments has been repeatedly demonstrated with a worse outcome of nucleoside-analogue-treated AML patients with high cN-II expression in their cancer cells as compared to those with low cN-II expression. Nucleoside analogues are dependent on intracellular activation by phosphorylation, and it was first hypothesized that cN-II could decrease the accumulation of active phosphorylated metabolites by dephosphorylating monophosphorylated cytarabine. However, as this metabolite is not a substrate for cN-II, the mechanism of cN-II-mediated resistance to nucleoside analogues remains unknown. The more recent observation of resistance to thiopurines in relapsed ALL patients with hyperactive mutants of cN-II suggests that a disturbance of nucleotide pools can be responsible of the mechanism of drug resistance (4). The direct role of cN-II in the sensitivity of cancer cells to nucleoside analogues was recently demonstrated by the sensitization of hematological malignant cell lines with shRNA-mediated decreased cN-II expression. A growing amount of results also indicate a direct or indirect role of cN-II in cell growth. First, yeasts expressing human cN-II grow faster than control strains, second, human neuroblastoma cells with decreased cN-II expression grow slower than control cells, whereas the same cells overexpressing wild-type cN-II or the hyperactive mutant R367Q grow faster than control cells (5). This is, however, not consistently observed as malignant hematological cell lines with decreased cN-II grow similarly to control cells. This might be explained by complex differences between cancer cells and normal cells or by a threshold effect. Based on the above mentioned evidences, cN-II has emerged as a potential target for cancer treatment. Furthermore, cN-II can interact with a number of other proteins, such as NRLC4 (IPAF) a protein involved in inflammasome formation, caspase.-1 activation and interleukines production when stimulated by intracellular or extracellular damage signals. Nevertheless the involvement of cN-II in inflammation is still to be unravelled. We finally, recently, demonstrated that a partial cN-II silencing resulted in a change in both metabolic and proliferative capacity in tumoral cancer cells. In fact, the decrease of cN-II activity caused an imbalance in purine and pyrimidine intracellular nucleotides, inhibition of AKT phosphorylation, a decrease of proliferation and cell motility and a more oxidative metabolism, associated to an increase of p53 phosphorylation (6).

References: Tzoneva G, Dieck CL, et al (2018) *Nature*. 553, 511-514. doi: 10.1038/nature25186. Cividini F, Cros-Perrial E, et al (2015) *Int J Biochem Cell Biol* 65:222–229. Pesi R., Petrotto E. et al (2018) *Int J Mol Sci*. 2018 Jul 20;19(7). pii: E2115. doi: 10.3390/ijms19072115.

S12-04

CONFLICTING EFFECTS OF GUANOSINE, GUANINE AND THEIR METABOLIZING ENZYMES ON NEUROPROTECTION AND TUMOR CELL PROLIFERATION

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Guanine-based purines and their converting enzymes stand out as key player in cell metabolism and in signaling pathways involved in neurodegenerative disorders and cancer. Guanosine (GUO) and guanine (GUA) are extracellular signaling molecules derived from the breakdown of GTP. GUA is generated from GUO in a reaction catalyzed by the purine nucleoside phosphorylase (PNP). Since several polymorphisms in the PNP gene as well as epigenetic mechanisms involving microRNAs expression have been reported in tumors, we investigated whether GUO-PNP-GUA system affected the expression of several markers involved in glioma cell proliferation and metastasis. To this aim, human glioma cell lines U87 were transfected with miR-200b or miR-133a mimics, and evaluated for EGFR, ZEB1, SNAIL, α -SMA, Fibronectin and PNP mRNA expression levels.

GUA, but not GUO, dose-dependently inhibited glioma cell proliferation and triggered a moderate up-regulation of Fibronectin, α -SMA, PNP and EGFR. A different trend was observed for ZEB1 and SNAIL expression. The hyper-phosphorylation of EGFR was restrained by a GUA-induced and AMPK1-mediated ubiquitination leading to receptor degradation.

The administration of miR-200b or miR-133a in association with GUA reduced the effects caused by GUA alone on marker expression. Moreover, cell pre-treatment with a selective PKG inhibitor, KT5823, reverted GUA-mediated effects, suggesting the involvement of this kinase in GUA-evoked signaling. Collectively, our data demonstrated that GUO activity is aimed at producing neuroprotective effects, whereas the nucleobase GUA inhibits the proliferation of glioma cells. Importantly, as glioma progression is driven by aberrant signaling of growth factor receptors such as EGFR, GUA, alone or in association with EGFR targeted therapies, might be a promising antitumor therapeutic tool.

Symposium 13: ROLE FOR PURINES IN BI-DIRECTIONAL GLIA-NEURON COMMUNICATIONS

Chairs: Yuriy Pankratov (Warwik, UK) / Alexei Verkhratsky (Manchester, UK)

Content: Communication between neuronal and glial cells is fundamental for brain function. There is a growing evidence that ATP acts as a ubiquitous neurotransmitter in glial-neuronal, glial-glial and neuronal-glial communications both in physiological and pathophysiological contexts. Importantly, ATP (as well as other purines) can mediate bi-directional communication between neurones and glia: first, by activating Ca^{2+} -signaling in glial cells via various P2 purinoceptors, and, in reverse, upon a release of various signalling molecules from astrocytes to modulate neuronal plasticity. Symposium speakers are international experts in most fundamental aspects of purinergic signalling integrating neuronal glial networks and contributing to neuropathology.

S13-01

ATP AS A DANGER SIGNAL IN THE BRAIN

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Brain noxious stimuli trigger a sustained increase of extracellular ATP, which plays a key role

as a danger signal in the brain. Indeed, the exposure of rat hippocampal neurons to glutamate (100 μ M) for 30 min, to mimic glutamate-induced neurotoxicity present in all neurodegenerative disorders, triggered a sustained increase of the extracellular levels of ATP that started prior to neuronal degeneration; glutamate exposure triggered neuronal death 24 h later, which was fully abrogated upon removal of extracellular ATP/ADP by apyrase (5 U/mL) or upon selective pharmacological or genetic blockade of P2Y1 receptors (P2Y1R). In vivo, the blockade of P2Y1R attenuated rat hippocampal neuron death upon systemic administration of kainic acid or upon intrahippocampal injection of quinolinic acid. This glutamate-induced excitotoxic process began with an early synaptotoxicity that was also prevented/attenuated by the antagonism of P2Y1R, both in vitro and in vivo.

Notably, the inhibition with α,β -methylene ADP of the extracellular catabolism of ATP into adenosine through the action of ecto-5'-nucleotidase or CD73, also abrogated the behavioral, electrophysiological and neurochemical modifications present either in a rat model of Parkinson's disease based on the intra-striatal administration of 6-hydroxydopamine, or in a mouse model of epilepsy triggered by intra-hippocampal injection of kainate or in a mouse model of Alzheimer's disease based on the intracerebral administration of β -amyloid peptides, these two later effects also mimicked by genetic elimination of CD73. This CD73-mediated neuroprotection was phenocopied by blocking adenosine A_{2A} receptors (A_{2A}R), in particular neuronal A_{2A}R, in the animal models of brain diseases.

Overall, these results re-enforce the role of ATP as a danger signal in noxious brain conditions and unravel a dual ability of ATP to promote synaptotoxicity that initiates neurodegeneration: directly through P2Y1R and indirectly through A_{2A}R upon CD73-mediated extracellular catabolism. However, the spatio-temporal interplay between these two parallel pathways remains to be determined.

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S13-02

ATP IN ASTROGLIAL CONTROL OF SYNAPTIC PLASTICITY IN AGEING AND AD

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Communication between neuronal and glial cells is thought to be very important for many brain functions. Acting via release of gliotransmitters, astrocytes can modulate synaptic strength. Still, the mechanisms of gliotransmission remain uncertain with SNARE-dependent exocytosis being the most intriguing and debated pathway.

Previously, we showed that SNARE-dependent exocytosis of ATP from neocortical and hippocampal astrocytes can be triggered by Ca^{2+} -elevation via direct UV-uncaging or via glia-specific receptors (PAR-1, CB1 or $\alpha 1$ -adrenoceptors). We also showed that activation of astrocytes initiated a burst of ATP receptor-mediated currents in adjacent pyramidal neurons. These purinergic currents can be inhibited by intracellular perfusion of astrocytes with Tetanus Toxin light chain (TeNTx), or by astroglia-specific expression of dn-SNARE protein, verifying their origin via astroglial exocytosis.

We found out that astrocyte-derived ATP can down-regulate phasic and tonic GABAergic currents, acting via Ca^{2+} -permeable P2X receptors on pyramidal neurons. Also, P2X receptors activated by astrocyte-derived ATP can facilitate trafficking of AMPA receptor into synapse. Hence, astroglia-derived ATP can strongly influence the balance between excitation and inhibition in neural networks and facilitate the long-term synaptic plasticity.

Our data show that synergetic action of astrocyte-derived ATP and D-serine is essential for synaptic plasticity. The LTP was impaired in the neocortex of dn-SNARE mice but could be rescued by application of exogenous or non-hydrolysable ATP analogs. We also have found out that weak sub-threshold theta-burst stimulation can induce LTP when astrocytes are additionally activated via endocannabinoid or noradrenaline receptors. This facilitation is dependent on the activation of neuronal ATP receptors and can be significantly reduced by perfusion of astrocytes with TeNTx. Moreover, we have found out the deficit in working memory of dn-SNARE mice; this is a first evidence of physiological relevance of glial exocytosis of ATP *in vivo*.

There is growing evidence that impairment of glial function can be related to the pathogenesis of many neurological disorders. We observed the considerable decrease in the astrocytic Ca^{2+} -signaling and release of ATP in the aged wild-type and Alzheimer's disease model mice. Impairment of glia-derived regulation altered the balance between excitation and inhibition leading to age- and AD-related deficit in the synaptic plasticity. Importantly, environmental enrichment (EE) or caloric restriction enhanced astroglial Ca^{2+} -signalling and Ca^{2+} -dependent release of ATP thereby ameliorating the age-related decline in purinergic glia-derived modulation of synaptic plasticity.

Combined, our data strongly support the importance of glia-derived ATP for astrocyte-neuron communication and show that age-related decline in gliotransmitter release can bring significant contribution to pathogenesis of neurodegenerative diseases.

S13-03

PURINERGIC SIGNALLING IN THE OLIGODENDROGLIAL LINEAGE

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Oligodendrocytes are the myelin-forming cells of the CNS and are derived from oligodendrocyte progenitor cells (OPCs). Myelinated axons are bundle together to form the white matter (WM) tracts of the CNS, providing for rapid communication throughout the CNS and integration. The main cells in myelinated tracts are oligodendrocytes and astrocytes, together with small populations of OPCs and microglia. The function of OPCs is to regenerate oligodendrocytes throughout life, to replace myelin lost through wear and tear and in pathology. A surprising aspect of WM physiology is the diversity of neurotransmitter signalling, with a key role for purinergic signalling. In physiology, astrocytes release ATP to activate purine receptors on OPCs and oligodendrocytes. In pathology, the release of ATP from damaged cells plays a role in both damage and repair by its direct effects on oligodendroglial integrity and survival, with prominent roles in ischemia, neuroinflammation, Multiple Sclerosis, and traumatic injury. The actions of ATP on oligodendroglia are mediated via a wide range of ionotropic P2X and P2Y G-protein coupled receptors (GPCR). In oligodendrocytes, predominant roles have been identified for P2X₇ and P2Y₁ receptor subtypes, which have a bipartite function, respectively mediating oligodendrocyte destruction and protection. In addition, glial ATP signalling may be altered with ageing and is implicated in impaired OPC regenerative capacity, which may be accelerated in Alzheimer's disease. In summary, oligodendroglial purine receptors have a 'Jeckyll and Hyde' nature in oligodendroglial cells and a comprehensive understanding of the roles of the different purine receptors is critical if they are to provide potential therapeutic targets in multiple neuropathologies.

Supported by a grant from the MRC

S13-04

Rashid Giniatullin (University of Eastern Finland, Kuopio, Finland) Role for glial purinergic signalling in migraine

Symposium 14: P2X7: A COMMON TARGET FOR BRAIN DISEASES?

Chairs: Miguel Diaz-Hernandez (Madrid, Spain) / Tobias Engel (Dublin, Ireland)

Content: Over the past recent years, the development of new transgenic animal models and highly specific pharmacological tools targeting the purinergic system has provided promising results in a great variety of different brain diseases. Purinergic signalling, in particular P2X7, has therefore gained an enormous interest, not only among brain researchers but also among pharma companies. Our symposium will include four experienced speakers at the forefront in their respective research field and who's main interest constitutes the study of purinergic signalling, in particular P2X7. Our symposium will also include four talks from early stage researchers who will contribute with talks focusing on purinergic signalling in different brain diseases including topics on translational and basic research.

S14-01

TARGETING OF ATP-GATED P2 RECEPTORS AS NOVEL TREATMENT FOR DRUG-REFRACTORY EPILEPSY

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Epilepsy, a heterogeneous group of neurological syndromes characterized by recurrent seizures, is one of the most common chronic neurological disease affecting ~70 million people worldwide. Despite the progress made in the development of new antiepileptic drugs (AEDs), the biggest challenges that epilepsy presents to drug development have remained unchanged for the last decades: reducing the percentage of patients resistant to all pharmacological interventions and finding a treatment with potential for modifying disease progression. Neuroinflammation is increasingly recognized as one of the key players in seizure generation and in the maintenance of the epileptic phenotype. Consequently, targeting signaling molecules involved in inflammatory processes may represent new avenues to improve treatment in epilepsy. Nucleotides such as adenosine-5'-triphosphate (ATP) are released in the brain into the extracellular space during pathological conditions such as increased neuronal firing or cell death driving neuroinflammatory processes and neuronal hyperexcitation. Mounting data has demonstrated a causal role for purinergic signaling during epilepsy, in particular for the ATP-gated P2X7 receptor with P2X7 antagonism protecting against acute seizures and potentially providing disease-modifying effects during chronic epilepsy (Jimenez Pacheco et al., J Neurosci. 2016). Emerging evidence is now also suggesting a prominent role for the metabotropic P2Y receptor family during epilepsy; here in particular for the P2Y1 receptor subtype (Alves et al., J Neurosci. 2019) with recent evidence demonstrating potent anticonvulsive and anti-epileptogenic properties of P2Y1 antagonists. In conclusion, P2 receptors may represent potential new targets for seizure control and disease modification.

S14-02

BAC TRANSGENIC MOUSE MODELS TO STUDY P2X7 EXPRESSION AND INTERACTIONS

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The P2X7 receptor is expressed in immune cells and plays an important role in cytokine release. However, the investigation of its function in various other cell types has been challenged by its complex pharmacology and a lack of specific antibodies. Thus, important questions such as its cell-specific localisation, function, and protein interactions remain unclear. To address this issue, we generated a BAC transgenic mouse model in which an EGFP-tagged P2X7 receptor is overexpressed under the control of its BAC-derived endogenous promoter. We will describe the evaluation of this mouse model and compare the P2X7-EGFP expression with the EGFP expression in another BAC transgenic P2X7 reporter mouse (Tg(P2rx7 EGFP)FY174Gsat) in which a soluble EGFP is expressed. In addition, the P2X7R-EGFP mouse model is used to investigate the interaction and mutual interrelation of the P2X7 and P2X4 subtypes in native mouse tissue. Our data indicate substantial differences in the cell type-specific expression of soluble EGFP and the P2X7-EGFP fusion construct in the two BAC transgenic mouse models and provide evidence that the proposed P2X4-P2X7 interaction is of minor physiological relevance in native tissues. Possible reasons for the observed differences will be discussed.

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S14-03

INVOLVEMENT OF P2X7 RECEPTOR ON THE ALZHEIMER DISEASE PROGRESSION, THERAPEUTIC IMPLICATIONS

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Alzheimer disease is a neurodegenerative disease characterized by the presence of senile plaques composed of amyloid- β (Ab) peptide, neurofibrillary tangles formed by hyperphosphorylated tau protein, neuronal loss, and neuroinflammation. Previous works from our group revealed that extracellular ATP, through its selective receptor P2X7 (P2X7R), promotes to the amyloidogenic processing of amyloid precursor protein (APP) via glycogen synthase kinase-3 (GSK3), favoring on this way the neuroinflammation and neurotoxicity induced by Ab. Other groups have also reported that P2X7R is upregulated on microglial cells around the senile plaques. This upregulation progressively rises with age and is parallel with an accumulation of senile plaques and correlates with the synaptic toxicity detected both in animal models reproducing AD and human patients of AD. Furthermore, the late onset of the first AD-associated symptoms suggests that aging associated-changes may be relevant to the disease progression. Since, microglia motility and its capacity to respond to exogenous ATP stimulus decrease with aging, we decided to evaluate whether the P2X7R age related-changes on microglia cells may also be relevant to AD progression. Our results indicate that neuroinflammation induced by Ab peptide causes changes in the P2X7R distribution pattern, increasing its expression in microglial cells at advanced and late stages, when microgliosis occurs, but not in the early stages, in the absence of microgliosis. Besides, we found that P2X7R activation promotes microglial cells migration to senile plaques but decreases their phagocytic capacity. We also found a significant reduction of P2X7R transcription on neuronal cells at the early and advanced stages, but not at the late stages. Since previous studies have reported that either pharmacological inhibition or selective downregulation of P2X7R significantly improve behavioral alterations and reduce the incidence and size of senile plaques in the early and advanced stages of AD, the results presented here provide new evidences, indicating that this therapeutic approach could also be efficient in the late stages of the disease.

S14-04

VALIDATING P2X7R AS RISK FACTOR FOR MOOD DISORDERS

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Mood disorders are prime contributors to global disease burden. The persistent lack of progress with regards to pharmacotherapy stands in stark contrast to the pandemic magnitude of the disease. For example, major depression is currently affecting 300 million people world wide. Alterations of inflammatory pathways in depressed patients including altered concentration of circulating pro-inflammatory cytokine levels have been put forward as a potential pathophysiological mechanism. The purinergic P2X7 receptor (P2X7R) has attracted considerable interest as a potential target for various central nervous system pathologies including affective and neurodegenerative disorders. The P2X7R plays an important role regulating the release of interleukin-1 β and other cytokines. Comprehensive investigation of the P2X7R Gln460Arg missense mutation (rs2230912), which has been associated with major depression and bipolar disorder, has substantially contributed to validate P2X7R as a potential genetic risk factor. Using genetic mouse models by introducing human P2X7R variants into the mouse as mammalian model organism we could demonstrate that the mood disorder associated variant P2X7R-Gln460Arg represents a genetic risk factor, which in conjunction with stress is able to convey susceptibility to mood disorders. Here, we will present latest results from cell type-specific inactivation of P2X7R in the central nervous system as well novel insights from different P2X7R reporter mouse lines.

Symposium 15: KEY ROLES OF ADENOSINE A_{2A} RECEPTORS THROUGHOUT BRAIN DEVELOPMENT

Chairs: Rodrigo A. Cunha (Coimbra, Portugal) / Sabine Levi (Paris, France)

Content: Purinergic signaling mediated by ATP and adenosine plays an essential role in the nervous system where it mediates fast neurotransmission and neuromodulation. This symposium will highlight recent and important discoveries regarding the contribution of the adenosine signaling pathway in brain development. We have gathered four speakers who will present evidences that Adenosine A_{2A} receptors govern neurogenesis, neuronal migration, axon formation and outgrowth, maintenance of newly formed synapses, and microglia morphology and function. Alterations in adenosine signaling during the critical period of development may lead to epilepsy, cognitive deficits and adult mood disorders.

S15-01

ADENOSINE A_{2A} RECEPTORS AND BDNF: AN INTIMATE INTERACTION REGULATING POSTNATAL HIPPOCAMPAL NEUROGENESIS

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In the postnatal mammalian brain, neurogenesis constitutively occurs in two main areas: the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG). Adenosine A_{2A} receptor (A_{2A}R) activation has the capacity to modulate several processes from neuronal maturation to synaptic plasticity. Importantly, most of these actions occur through the modulation of the neurotrophin brain-derived neurotrophic factor (BDNF). In this work we studied the role of A_{2A}Rs in regulating postnatal neurogenesis in rat hippocampal dentate gyrus (DG). Here we show that A_{2A}R exogenous activation with CGS 21680 promoted neural stem cell self-renewal, protected committed neuronal cells from cell death and contributed to a higher density of immature and mature neuronal cells, particularly glutamatergic neurons. Moreover, A_{2A}R endogenous activation was found to be essential for BDNF-mediated increase in cell proliferation and neuronal differentiation. Our findings contribute to further understand the role of adenosinergic signalling in the brain and may have impact in the development of strategies for brain repair under pathological conditions

Keywords: Adenosine A_{2A} receptor; BDNF; DG postnatal neurogenesis; Neurite outgrowth; SVZ postnatal neurogenesis

Conflict(s) of interest: Nothing to declare.

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S15-02

CORTICAL NEURONAL MIGRATION ENTAILS A2A RECEPTOR-DRIVEN NEURONAL POLARIZATION AND AXON FORMATION

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Neuronal migration is fundamental for the formation of the brain cytoarchitecture. Any defect in this process may have long-term deleterious effects ranging from mild cognitive impairment, to severe neurological and psychiatric conditions. (Neuron 60:273). It was recently shown that adenosine A2AR controls interneurons migration (Sci. Trans. Med. 5:197ra104). We now aimed to evaluate if A2AR is also involved in the migration of cortical principal neurons. We observed that mice embryos lacking the A2AR (A2AR-KO) displayed a delayed migration at E17.5 of cortical principal neurons labelled with BrdU at E14.5, in comparison with wild-type littermates. Similarly, mice embryos exposed to the A2AR antagonist SCH58261 (daily 0.1 mg/kg i.p. injection in pregnant females E13.5-E16.5) also displayed delayed migration. This should be due to A2ARs expressed by migratory neurons since in utero electroporation of a plasmid encoding shRNA for A2AR (E14.5-E17.5) also delayed migration. This delay or accumulation of a subset of neurons occurs mostly at the lower intermediate zone (IZ), where it is required a multipolar-bipolar transition and the establishment of an axon-like process in their migration towards the cortical plate (CP) (Nat. Neurosci. 12:1693). Accordingly, mice primary cortical neurons cultured in the presence of the A2AR antagonist SCH58261 (50 nM) either were unable to form an axon or displayed a reduced axonal length. Similarly, the knockdown of A2ARs in the migratory neurons leads to an impairment both in neuronal polarization and axon formation at the IZ. Moreover, we observed in embryos lacking ecto-5'-nucleotidase (CD73-KO mice), the enzyme that catabolizes AMP into adenosine and fundamental for the formation of ATP-derived adenosine, an accumulation of neurons also in the lower IZ (E14.5-E17.5). Accordingly, we detected immunoreactivity for the vesicular nucleotide transporter in the developing mice cortex at E13-E17, both in neurons and glial cells, indicating that they are endowed with the machinery to release ATP.

Altogether, these results show that A2ARs activated by ATP-derived adenosine are required for cortical principal neuronal migration, in particular for the transition from the IZ into the CP by controlling the establishment of neuronal polarity and axon formation.

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S15-03

Catarina Gomes (ICBR, Coimbra, Portugal) Adenosine A_{2A} receptor modulation of microglia morphologic remodeling in early neurodevelopment - gender bias in physiology and psychiatric disorders.

S15-04

A_{2A}R SIGNALLING REGULATES SYNAPTOGENESIS IN THE DEVELOPING BRAIN

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In the adult brain, adenosine, a degradation product of ATP, controls neurotransmitter release and synaptic plasticity through G protein coupled A₁ and A_{2A} receptors. However, its role in development remains to be elucidated. Here, we addressed the role of A_{2A}R-mediated signalling during GABAergic synaptogenesis in the hippocampus.

We found that A_{2A}R expression peaked during the period of synaptogenesis in the hippocampus *in vitro* and *in vivo*. This developmental expression of A_{2A}Rs was correlated with a role of A_{2A}Rs in the stabilization of nascent GABA synapses during synaptogenesis. We demonstrated that the A_{2A}R-mediated synapse stabilization is a cell autonomous process that requires A_{2A}R activation in the postsynaptic cell. We then characterized the molecular mechanism downstream A_{2A}Rs. We identified the Adenylyl cyclase/cAMP/Protein Kinase A (PKA) signalling cascade as the main molecular pathway and the postsynaptic scaffolding molecule gephyrin as a main target of PKA activation. Finally, we will provide evidences that the deleterious consequences of *in utero* and post-natal brain exposure to caffeine (Sci Transl Med. 2013; 5(197), an antagonist of A₁ and A_{2A} receptors, are primarily due to A_{2A}R-dependent alterations in synaptogenesis.

Our findings allow to propose that A_{2A}Rs act as coincidence detectors to stabilize newly formed GABAergic synapses during synaptogenesis and provide a better understanding of the pathological mechanisms engaged upon early-life exposure to caffeine.

Symposium 16: ROLE OF PURINES IN GLIAL CELL, PROLIFERATION, MATURATION AND CELL-TO-CELL COMMUNICATION IN THE CENTRAL NERVOUS SYSTEM

Chairs: Felicita Pedata (Florence, Italy) / Ana Sebastião (Lisboa, Portugal)

Content: This Symposium will integrate rapidly emerging information about the role of purinergic receptors expressed on astrocytes, oligodendrocytes and microglia as well as evidence for ATP-induced extracellular vesicles from glial cells. We will highlight the latest knowledge about the involvement of ATP and adenosine receptors in neuron–glia signalling, their functional significance under physiological and pathological conditions.

S16-01

EFFECTS OF ATP-INDUCED MICROGLIAL EXTRACELLULAR VESICLES ON OLIGODENDROCYTE PROGENITORS IN CEREBRAL ISCHEMIA

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Microglia are very plastic cells that can acquire multiple activated phenotypes in response to brain insults, participating not only in mechanisms of injury, but also in tissue remodeling. A dualistic role for distinct polarized cell populations has been associated with several neuropathological conditions, including ischemic stroke. In particular, during the acute phase of brain ischemia, microglia assume a protective phenotype, but at late stages, they acquire a prominent detrimental role that hinders the post-stroke reparative response sustained by oligodendrocyte progenitors (OPCs), the glial cell type able to generate myelinating oligodendrocytes. However, the mode(s) of action of microglia in supporting or inhibiting myelin repair is still unclear. Our *in vitro* data showed that extracellular vesicles (EVs) released from activated microglia promote OPC myelination, highlighting EVs as key players in microglia-OPCs cross-talk. Whether EVs shed from activated microglia could improve the endogenous remyelination capability of OPCs after brain ischemia is still not known.

Here, we investigated *in vivo* the effects of ATP-induced EVs isolated from primary microglia polarized toward pro-inflammatory or pro-regenerative phenotypes in a murine model of permanent middle cerebral artery occlusion (pMCAo). Briefly, pMCAo was performed in GPR17-iCreERT2:CAG-eGFP mice in order to visualize the sub-population of GPR17-expressing OPCs thanks to GFP expression following tamoxifen administration. Then, EVs produced by microglia with diverse activation state were infused in the ipsilateral corpus callosum of ischemic mice two weeks after MCAo, when proinflammatory cells dominate the injured area, in order to analyze their effects on GFP⁺-OPC by immunohistochemistry. Results pointed out that only EVs derived from pro-regenerative microglia increase OPC maturation *in vivo*.

Globally our results unveil EVs as a tool to implement tissue repair in brain ischemia. Dissecting microglial EVs content will be indispensable to design engineered-EVs for therapeutic purposes.

Supported by Fondazione Cariplo grant 2015-0910 to MF

S16-02

**ASTROCYTE PURINERGIC SIGNALING ENHANCES
EXCITATORY SYNAPTIC TRANSMISSION DURING
EPILEPTOGENESIS**

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Epilepsy is characterized by unpredictable recurrent seizures resulting from abnormal neuronal excitability. Increasing evidence indicates that aberrant astrocyte signaling to neurons plays an important role in driving the network hyperexcitability, but the underlying mechanism that alters glial signaling in epilepsy remains unknown. Increase in glutamate release by astrocytes participates in the onset and progression of seizures. Epileptic seizures are also accompanied by increase of tumor necrosis factor alpha (TNF α), a cytokine involved in the regulation of astrocyte glutamate release. Here we tested whether TNF α controls abnormal astrocyte glutamate signaling in epilepsy and through which mechanism. Combining Ca²⁺ imaging, optogenetics and electrophysiology, we report that TNF α triggers a Ca²⁺-dependent glutamate release from astrocytes that boosts excitatory synaptic activity in the hippocampus through a mechanism involving autocrine activation of P2Y1 receptors by astrocyte-derived ATP/ADP. In a mouse model of temporal lobe epilepsy such TNF α -driven astrocytic purinergic signaling is permanently active, promotes glial glutamate release and drives abnormal synaptic activity in the hippocampus. Blocking this pathway by inhibiting P2Y1 receptors restores normal excitatory synaptic activity in the inflamed hippocampus. Our findings indicate that targeting the coupling of TNF α with astrocyte purinergic signaling may be a therapeutic strategy for reducing glial glutamate release and normalizing synaptic activity in epilepsy.

S16-03

ROLE OF ASTROCYTIC ADENOSINE ON THE MODULATION OF SYNAPTIC PLASTICITY BY BDNF

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Astrocytes are key cellular partners to neurons, playing an important role in multiple processes in the brain. The concept of the tripartite synapse suggests that astrocytes are not only supportive cells with homeostatic functions, but that they also play a role in information processing by responding to neuronal synaptic activity with Ca^{2+} elevations that induce the subsequent release of gliotransmitters which in turn modulate neuronal excitability and synaptic plasticity. Hippocampal long-term potentiation (LTP) is a sustained enhancement of excitatory synaptic strength believed to underlie learning and memory processes; it has recently been described that astrocytes regulate synaptic transmission and play a role in shaping LTP. Specifically, the release of gliotransmitters, such as glutamate, ATP, and D-serine likely alters the viability and functioning of newly formed connections. Other very important molecule for the modulation of LTP is brain-derived neurotrophic factor (BDNF), a neurotrophin involved in the development and protection of different neuronal population in the nervous system. Furthermore BDNF has a facilitatory action upon hippocampal LTP, being this action dependent on the adenosine $\text{A}_{2\text{A}}$ receptor ($\text{A}_{2\text{A}}\text{R}$) activation. Thus, we evaluated 1) the involvement of astrocytes upon the modulatory effect of BDNF upon LTP, 2) the involvement of adenosine (and $\text{A}_{2\text{A}}\text{R}$) on this process and 3) the role of adenosine receptors activation on calcium signalling mediated by astrocytes. Main findings allow to suggest that BDNF effect upon synaptic plasticity is under the control of astrocytes. These results further highlight the role of astrocytes in the CNS, but more importantly the role of astrocytes on the glial–neuron communication involving synaptic plasticity modulation by neurotrophins.

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S16-04

ACTIVATED MICROGLIA REGULATE THE RESPONSE OF OLIGODENDROCYTE PROGENITORS EXPRESSING THE P2Y-LIKE RECEPTOR GPR17 FOLLOWING CEREBRAL ISCHEMIA

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Cerebral ischemia is a neurological disease representing a main cause of death and disability worldwide. Oligodendrocytes, the myelin-forming cells of the brain, are strongly affected by ischemia, leading to axonal demyelination which contributes to stroke-associated deficits. On this basis, enhancing myelin repair has recently emerged as a new therapeutic strategy to improve post-stroke recovery. Recent data obtained performing permanent middle cerebral artery occlusion (pMCAo) in conditional GPR17-iCreER^{T2}xCAG-eGFP transgenic mice showed that the subpopulation of adult oligodendrocyte precursor cells (OPCs) expressing the P2Y-like receptor GPR17 (GFP⁺-OPCs) actively contributes to reparative mechanisms after ischemia by increasing both proliferation rate and migratory ability. However, at late stages of the disease only a small percentage of these cells reaches complete maturation, resulting in remyelination failure. This limited post-stroke repair is likely due to the local unfavorable inflammatory milieu mediated by resident microglia and blood-borne macrophages.

Here, we aimed at understanding the time-dependent role of microglia/macrophages following cerebral ischemia and how these cells contribute to OPC responses after stroke. In this respect, our data showed that microglia/macrophages are activated early after ischemia and they assume both pro-inflammatory and pro-regenerative phenotypes; instead, at late stages after stroke the number of pro-regenerative cells remains stable while pro-inflammatory cells continue to increase and become prevalent. Furthermore, we exploited a gadolinium-based approach to deplete microglia/macrophages at different time points of the disease. Interestingly, partial depletion of microglia/macrophages during the early phase after ischemia exacerbates brain injury and reduces the amount of GFP⁺-OPCs in the peri-infarct region. On the other hand, late partial depletion of microglia/macrophages promotes GFP⁺-OPC reaction, but it is ineffective in fostering their differentiation.

Taken together, our results suggest that microglia/macrophages exert different roles in the regulation of OPC-mediated regenerative response during the course of the disease.

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S16-05

THE SELECTIVE STIMULATION OF ADENOSINE A₃ RECEPTOR INHIBITS N-TYPE CA²⁺ CURRENTS IN RAT DORSAL ROOT GANGLION NEURONS

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Introduction: Interest has been focused in recent years on the analgesic effects exerted by adenosine and its receptors, A₁, A_{2A}, A_{2B} and A₃ (AR, adenosine receptor) subtypes, in different in vivo models of chronic pain. We recently demonstrated that selective A₃AR agonists block pro-nociceptive N-type Ca²⁺ calcium channels in dorsal root ganglion (DRG) neurons isolated from rats (Coppi et al. 2019, Pain, 160(5):1103-18). In the present study, we investigate the effect of the first reported irreversibly binding A₃AR agonist, ICBM, on N-type Ca²⁺ currents in rat DRG neurons.

Material and methods: Whole-cell patch-clamp recordings from primary DRG neurons isolated from Sprague Dawley rats were performed as described (Coppi et al., 2019).

Results: The A₃AR agonist ICBM, which contains a reactive isothiocyanate group, significantly inhibited N-type Ca²⁺ currents. The effect was concentration dependent (0.01 - 3 µM), with an EC₅₀ of 22.9 nM, and blocked by the selective A₃AR antagonist MRS1523 (100 nM).

In order to demonstrate whether ICBM acts as an irreversible ligand on A₃AR, in a subsequent experimental series we pre-incubated (10 minutes) DRG neurons with 100 nM ICBM or Cl-IB-MECA, the most used A₃AR agonist, and then we washed out the A₃AR agonist for 15 min, before applying 30 nM Cl-IB-MECA again to test its ability to inhibit Ca²⁺ currents. ICBM pre-incubated cells maintained a prolonged suppression of Ca²⁺ currents, and no further effect of Cl-IB-MECA on these currents was detected. In contrast, after Cl-IB-MECA pre-incubation, the ability to inhibit Ca²⁺ currents upon a subsequent Cl-IB-MECA application was restored.

Conclusions: Present data demonstrate that ICBM, an A₃AR agonist designed for covalent binding to the receptor, concentration-dependently inhibits N-type Ca²⁺ currents in rat DRG neurons. This effect is irreversible and might represent an innovative, favourable strategy to achieve efficacious pain control upon selective A₃AR activation.

Symposium 17: ACUPUNCTURE AND PURINERGIC MECHANISM

Chair: Yong Tang (Chengdu, China)

Content: Acupuncture is a Traditional Chinese Medicinal Therapy commonly used to combat pathological conditions notably pain. In 2009, the purinergic hypothesis of acupunctural analgesia was proposed by Geoffrey Burnstock: sensory nerve activity initiated in the skin by acupuncture would exert an inhibitory modulating effect on the spinoparabrachial and spinothalamic tracts to the brain pain centers. The hypothesis is supported by the early pharmacological finding that adenosine receptor antagonists theophylline and caffeine blocked the acupuncture-induced elevation of the nociceptive threshold. In 2010, the study by Maiken Nedergaard and colleagues provides the direct evidence that the acupuncture-released ATP is degraded to adenosine to produce analgesia via activation of adenosine A1 receptors.

Since then, increasing evidence has demonstrated that purinergic signaling represent an important and crucial mechanism underlying the acupunctural analgesia. However, many central questions remain to be addressed experimentally to uncover the mode of the action of acupuncture. For example, are there dynamic changes in the concentrations of purines (ATP, ADP, AMP, adenosine) following acupuncture at the puncture site as well as pain-relevant areas of the brain? Is acupunctural analgesia mediated by purinergic signal at peripheral or central nerve systems? In addition to the A1 receptor, what are the contribution of other adenosine (such as A2A) and P2 receptors (such as P2X3, P2X4, and P2X7Rs) to acupunctural analgesia? Does purinergic signal interact with other nociceptive neuromodulators/receptors to modify acupunctural analgesia? This proposed symposium seeks to address these critical issues by scientists from multi-disciplines to provide the new insights into the purinergic mechanisms of acupuncture analgesia.

S17-01

ATP RELEASE FROM MAST CELLS BY ACUPUNCTURE STIMULI

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To treat various diseases selected points at the body surface (acupuncture points, trigger points, head zones) can be stimulated. In traditional Chinese medicine, activation of cellular structures in acupoints has been demonstrated to have e.g. analgesic effects. In acupuncture, various physical stimuli are used: during conventional needling cells within the connective tissue receive mechanical stress, in moxibustion heat is applied, and electrical fields as well as red laser light have been used. Recent investigations support that release of ATP and its metabolic products play a key role in acupoint responses.

An initial step in analgesia seems to be activation of mast cells within acupuncture points. Inhibition of mast-cell degranulation attenuates analgesia. Also the other physical stimuli activate mast cells.

By electrophysiological methods we demonstrate that physical stimuli activate ion channels associated with mast-cell degranulation. Again, inhibition of the ion-channel activity counteracts mast-cell degranulation and acupuncture-induced analgesia.

Physical stimulation of mast cells leads to extracellular Ca^{2+} entry. Fluorescence measurements illustrate that intracellular Ca^{2+} changes are associated with the ATP release, suggesting that the downstream purinergic signalling may be one of the underlying mechanisms.

Our focus is, therefore, on the release of ATP from mast cells embedded within the connective tissue of acupuncture points.

In conclusion, we suggest that initiation of analgesia by acupuncture as well as by moxibustion involves mast cell activation, and that the associated peripheral release of ATP forms a key step in this process.

S17-02

THE ROLE OF PURINE RECEPTOR IN THE TREATMENT OF DEPRESSION BY taVNS

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Background: Depression is a common affective disorder. An increasing number of studies have focused on neuroimmunology, and evidence suggests that many molecules and intracellular signal transduction pathways that are closely associated with inflammation and immune responses have been implicated in the biological origin of this illness. As a new method of combining acupuncture and neuromodulation technology, transcutaneous vagus nerve stimulation (taVNS) is currently used in the treatment of many diseases. We've previously confirmed through clinical trials that taVNS can be effective in treating depression, and the data from animal experiments suggest that the antidepressant mechanism is associated with inflammation and purinergic receptors in the brain.

Methods: This study was designed to assess the effectiveness of taVNS on depressive-like behavior caused by chronic stress in rats, and explore its potential mechanism of interaction between inflammation and purine receptors.

Results: The data showed that taVNS's antidepressant effect was accompanied by markedly decreased expression of NLRP3 and IL-1 β , and taVNS can significantly reverse stress-induced increases in P2X7R, IL-1 β and IL-18 expression in the hippocampus and prefrontal cortex.

Conclusion: taVNS may play an antidepressant role by reducing inflammation in the hippocampus and prefrontal cortex, and this anti-inflammatory effect is negatively correlated with the expression of P2X7R.

Keywords: taVNS, depression, inflammation, purine receptor, rats

S17-03

PURINERGIC SIGNALING IN INFLAMMATORY BOWEL DISEASE AND IT'S ROLE IN THE EFFECT OF ACUPUNCTURE AND MOXIBUSTION

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- **Background or Introduction**

Inflammatory bowel disease (IBD) is a chronic, relapsing, inflammatory disease of the gut. The exact cause of IBD is still unknown. A growing body of evidence indicates that purinergic signaling and its receptor system play important roles in IBD.

- **Material and methods**

Dextran Sulfate Sodium (DSS) induced colitis mice were set up and electro-acupuncture and moxibustion were used to treated the mice. The expression of CD39, CD73 and A2a proteins in the colon were detected by westernblot and the levels of CD4+CD39+ T cells, CD4+CD39+Foxp3+ T cells, CD4+CD73+ T cells and CD4+CD73+Foxp3+ T cells in peripheral blood, spleen, and local draining lymph node were detected by flowcytometry.

- **Results**

The protein expression of CD39, CD73, and A2a , the number of positive cells in the colons were decreased in the colitis model group. After acupuncture and moxibustion treatment, the above detections were significantly improved. The number of CD4+CD39+T cells, CD4+CD73+ T cells in the peripheral blood, lymph nodes and spleen of mice were all decreased in the model mice. However, after eletro-acupucture and moxibustion treatment, both of the number of CD4+CD39+T cells and CD4+CD73+ T cells significantly improved. Additionally, the ratio of CD4+Foxp3+CD39+ T cells to CD4+CD39+T cells,and the ratio of CD4+ Foxp3+CD73+ T cells to CD4+CD73+T cells in the peripheral blood, lymph nodes and spleen of mice were all increased after treatment (all P values were less than 0.01).

- **Conclusions**

Both moxibustion and electro-acupuncture have good therapeutic effects on DSS-induced colitis mice. Regulation of Treg cells may be one of the underline mechanisms of acupuncture and moxibustion.

Key words: Colitis, Electro-acupuncture,Moxibustion , Adenosine metabolic pathway, CD39 ,CD73 ,A2a

S17-04

PURINERGIC SIGNALLING AND ACUPUNCTURE-INDUCED ANALGESIA

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Acupuncture has been used in China from ancient times since more than 2,000 years ago. A variety of disorders can be treated effectively by inserting long, fine needles into specific acupuncture points (acupoints) on the skin of the patient's body. Since acupuncture was proposed by National Institutes of Health (NIH) consensus in 1997 as a therapeutic intervention of complementary medicine, acupuncture efficacy has become more and more accepted in the Western world. Among acupuncture therapies, the acupuncture-induced analgesic effect has been used widely to alleviate diverse types of pain, particularly chronic pain. To date, acupuncture analgesia has drawn the attention of many investigators and become an important research subject of international interest around the world. Numerous studies have also demonstrated that acupuncture analgesia has physiological, anatomical and neurochemical basis despite that there is still an ongoing debate about the mechanism by which acupuncture alleviates pain.

Since Professor Geoffrey Burnstock proposed that purinergic signalling, rather than a mystical subepidermal energy, may explain how acupuncture works in an article in Medical Hypotheses in 2009, the role of purinergic signalling in acupuncture research has gained much attention. So far, more scientists have got started to study the role of purinergic signalling in acupuncture-induced analgesia. In this talk, the work have been done by our group and other scientists will be summarized and where we are going and how we are going to get there in this amazing field will be described.

Symposium 18: MEDICINAL CHEMISTRY OF PURINE TARGETS

Chairs: Christa E. Müller (Germany) / Bilha Fischer (Israel)

Content:

S18-01

A QUEST FOR A DISEASE MODIFYING DRUG FOR THE TREATMENT OF OSTEOARTHRITIS-RELATED CALCIUM PYROPHOSPHATE DEPOSITION DISEASE

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Ecto-nucleotide pyrophosphatase-1 (NPP1) hydrolyzes phosphodiester bonds of ATP resulting mainly in the formation of AMP and pyrophosphate (PP_i). NPP1's activity plays a deleterious function in osteoarthritis (OA)/calcium pyrophosphate deposition (CPPD) disease. Hence, inhibitors of NPP1 represent a medical need. For this purpose, we developed several generations of potent and selective NPP1 inhibitors based on scaffolds of natural nucleotides. The design of the candidates was based on molecular modeling of NPP1, namely, on the catalytic site which contains two zinc ions, and the catalytic mechanism. The novel nucleotides have been prepared via rather facile syntheses trying to avoid the formation of a new chiral center. Later, the analogs have been evaluated; first as substrates of ecto-nucleotidases, NPP1,-3 and NTPDase1,-2,-3 and -8, and then, for non-substrates, as inhibitors of these enzymes. In addition, we explored the selectivity of the NPP1 inhibitors at relevant purinergic receptors. Promising NPP1 inhibitors effectively inhibited NPPase vs. TNAP activity in whole human cartilage and in human osteoarthritic chondrocytes, and reduced extracellular PP_i accumulation. Finally, NPP1 inhibitors were tested for toxicity to human chondrocytes and for stability in culture medium and human plasma. Docking simulations have indicated the origin of the enhanced NPP1 inhibitory activity and selectivity of most promising analogs. Among all tested analogs MN8 was identified as a potent NPP1 inhibitor both in assays using purified enzyme (IC₅₀ 0.645 µM) and in osteoarthritic human chondrocytes (IC₅₀ 0.033 µM). Furthermore, it efficaciously (10-fold vs. control) inhibited ATP-induced CPPD deposition in human chondrocytes. Therefore, we suggest several potent and selective inhibitors of NPP1 for lowering extracellular PP_i levels in cartilage for delaying and treating OA/CPPD.

S18-02

INTEGRATED APPROACHES TO MODEL THE CONFORMATIONAL EQUILIBRIUM AND THE LIGAND OPTIMIZATION ON ADENOSINE RECEPTORS

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In this talk, I will introduce our GPCR modeling and simulation approaches to explore ligand binding affinities, within the scope of structure-affinity relationships (SAR), on one side, and to characterize the effect of single point mutations (commonly coming from site-directed mutagenesis, SDM). I will show how this approach can provide a mapping of the key receptor-ligand interactions that aided in the design of novel chemotyper for the family of adenosine receptor.

The new structural landscape of GPCRs provides an excellent starting point to model receptor ligand interactions, either with experimental structures of the receptor of interest, either obtaining high quality homology models. For this, in our lab we have developed the GPCR-ModSim web server, which allows the homology modeling and MD simulation of any GPCR of interest. Once the structure of the receptor-ligand complex is clear, we have developed protocols via advanced free energy perturbation (FEP) simulations to quantitatively and routinely assess the effects of point-mutations on ligand binding as well as the SAR of congeneric series of ligands. The characterization of These data can be mapped into the structure of the receptor using computational modeling, providing the most complete picture of the molecular interactions responsible of high affinity and selectivity. Our method is based on an efficient molecular dynamics (MD) sampling of the protein-ligand binding site using spherical boundary conditions, and is now automated as part of our open source MD software Q.

and FEP simulation protocols and present recent applications on adenosine receptors. I will focus on three recent achievements with important methodological and practical applications: 1) The conformational selectivity of agonists and antagonists for the active and inactive forms of the A_{2A} and A_{2B} receptors; 2) The optimized design of A_{2B} antagonists derived from ISAM140 (currently commercialized as a reference antagonist). 3) and the recent series of A_{2A} antagonists initially reported by the company Sosei-Heptares, where our FEP-guided design allowed the synthesized, pharmacologically characterization and co-crystallization with the A_{2A} receptor of two antagonists, confirming the initial modeling hypothesis.

Our methodology is freely available under <http://open.gpcr-modsim.org/> and <https://github.com/qusers/Q6>

- [1] a) Keranen, H.; Aqvist, J.; Gutierrez-de-Teran, H. Chem Comm (2015), 51:3522; b) Boukharta, L.; Gutierrez-de-Teran, H.; Aqvist, J. PLOS Comp. Biol (2014) 10:e1003585
- [2] Jespers, W. et al. and Gutiérrez-de-Terán H. Trends Pharmacol. Sci. (2018) 39:75-89
- [3] Esguerra, M. et al. and Gutiérrez-de-Terán, H. Nucl. Ac. Res. (2016) 44: W455-62
- [5] El Maatougui, A., Azuaje, et al. and Gutiérrez-de-Terán, J Med Chem. (2016) 59:1967.

S18-03

EVOLUTIONARY APPROACHES FOR ADENOSINE RECEPTORS MODULATION

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The purine nucleoside adenosine is present in all body fluids. In addition to its well-known role as genetic code building block and energy transfer processes, adenosine participates in diverse biochemical events. It has a cytoprotective function, while its extracellular accumulation contributes to the regulation of inflammation, immunity and tissue repair. Adenosine exerts its action by interacting with P₁ receptors, a family of highly conserved receptor subtypes that belong to the family of seven transmembrane G-protein-coupled receptors.¹ The four adenosine receptors (A₁, A_{2A}, A_{2B} and A₃) have distinct localization, signal transduction pathways and different means of regulation upon exposure to agonists.¹

Recent advances on the physiology, pharmacology and the structural and molecular biology of adenosine and its receptors has provided solid evidences that supports the ability of adenosine to regulate diverse physiopathological events. These findings, along with the identification of potent and selective ligands, embrace the emergence of conceptually novel therapeutic strategies to address significant unmet medical needs.³

For several years we have been involved in a medicinal chemistry program aimed to develop novel A₁, A_{2A}, A_{2B} and A₃ ligands³ by employing advanced synthetic methodologies. In this talk, I will introduce our multicomponent-assisted platform for drug discovery using A_{2B} and A₃ receptors as case studies. The presentation will cover ligand discovery and optimization as well as preliminary evidences of the anticancer effect of some optimized ligands.

References:

- [1] Fredholm, B. B.; Ijzerman, A. P.; Jacobson, K. A.; Linden, J.; Müller, C. E. Nomenclature and classification of adenosine receptors – An update. *Pharmacol. Rev.* **2011**, *63*, 1–334.
- [2] Müller, C. E.; Jacobson, A. J. Recent developments in adenosine receptor ligands and their potential as novel drugs. *Biochim. Biophys. Acta*, **2011**, *1808*, 1290–1308.
- [3] Crespo, A. El Maatougui, A. Biagini, P. Azuaje, J. Coelho, A. Brea, J. Loza, M. I. Cadavid, M. I. García-Mera, X. Gutiérrez-de-Terán, H. Sotelo, E. *ACS Med. Chem. Lett.* **2013**, *4*, 1031–1036. El Maatougui, A., Azuaje, J., González-Gómez, M., Miguez, G., Crespo, A., Carbajales, C., Escalante, L., Gutiérrez de Terán, H., Sotelo, E., *J. Med. Chem.*, **2016**, *59*, 1967-1983. Carbajales, C., Azuaje, J., Oliveira, A., Loza, M. I., Brea, J., Cadavid, M.I., Masaguer, C.F., García-Mera, X., Gutiérrez de Terán, H., Sotelo, E., *J. Med. Chem.*, **2017**, *60*, 3372-3382. Jespers, W. Scheidel, A.C., Heitman, L.H, Cooke, R.M., Kleene, L., van Westen, G.J., Gloriam, D.E., Müller, C.E, Sotelo, E., Gutiérrez de Terán, H., *Trends Pharmacol. Sci.*, **2018**, *39*, 75–89.

S18-04

Anna Junker (Germany) P2 receptor ligands, PET tracers

Symposium 19: IMPACT OF PURINERGIC SIGNALLING IN MULTIPLE SCLEROSIS PATHOLOGY

Chairs: Carlos Matute (Leioa, Spain) / María Domercq (Leioa, Spain)

Content: Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by massive infiltration of immune cells, demyelination, and axonal loss. However, spontaneous myelin repair can occur during the course of the disease. A major component of this regenerative process is a robust innate immune response consisting of infiltrating macrophages and brain microgliosis. Therefore, specifically targeting myeloid cells could be an attractive therapeutic approach. Purinergic receptors control not only immune cell function together with the activation of microglia and astrocytes, but also neuronal and oligodendroglial survival in the pathology. Our symposium will include four different speakers talking about the role in MS of purinergic receptors, mainly P2X7 and P2X4. Claudia Verderio will focus on how microvesicles released by microglia in response to ATP influence oligodendrocyte progenitor cell function during remyelination. Susana Mato will talk about the role of P2X7 receptor in oligodendrocytes and microglia during primary demyelination. James Wiley will describe the scavenger activity of P2X7 receptors and its impact in MS disease. Finally, Maria Domercq's talk will be focused on the role of P2X4 modulating microglia/macrophage inflammatory responses and promoting the repair of myelin damage.

S19-01

DETRIMENTAL AND PROTECTIVE ACTION OF MICROGLIAL EXTRACELLULAR VESICLES ON MYELIN LESIONS: ASTROCYTE INVOLVEMENT IN REMYELINATION FAILURE

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Microglia are highly plastic immune cells which exist in a continuum of activation states. By shaping the function of oligodendrocyte precursor cells (OPCs), the brain cells which differentiate to *myelin-forming cells*, microglia participate in both myelin injury and remyelination during multiple sclerosis. However, the mode(s) of action of microglia in supporting or inhibiting myelin repair is still largely unclear. Here, we analysed the effects of extracellular vesicles (EVs) produced *in vitro* by either pro-inflammatory or pro-regenerative microglia on OPCs at demyelinated lesions caused by lysolecithin injection in the mouse corpus callosum. Immunolabelling for myelin proteins and electron microscopy showed that EVs released by pro-inflammatory microglia blocked remyelination, whereas EVs produced by microglia co-cultured with immunosuppressive mesenchymal stem cells promoted OPC recruitment and myelin repair. The molecular mechanisms responsible for the harmful and beneficial EV actions were dissected in primary OPC cultures. By exposing OPCs, cultured either alone or with astrocytes, to inflammatory EVs, we observed a blockade of OPC maturation only in the presence of astrocytes, implicating these cells in remyelination failure. Biochemical fractionation revealed that astrocytes are converted into harmful cells by the inflammatory EV cargo, as indicated by immunohistochemical and qPCR analyses, while surface lipid components of EVs promote OPC migration and/or differentiation, linking EV lipids to myelin repair. Despite the lipid species enhancing OPC maturation still remains to be fully defined, we provided the first demonstration that vesicular sphingosine 1 phosphate stimulates OPC migration, the first fundamental step in myelin repair. From this study, microglial EVs emerge as multimodal and multitarget signaling mediators able to influence both OPCs and astrocytes around myelin lesions, which may be exploited to develop novel approaches for myelin repair not only in multiple sclerosis but also in neurological and neuropsychiatric diseases characterized by demyelination.

S19-02

**ADDRESSING THE ROLE OF P2X7 RECEPTORS IN PRIMARY
DEMYELINATION AND REMYELINATION**

Susana Mato

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The contribution of P2x7 receptors to multiple sclerosis remains controversial as both detrimental and beneficial effects resulting from P2x7 receptor loss-of-function have been reported in autoimmune models of the disease. Here we investigated the relevance of P2x7 receptors to myelin damage and repair in the cuprizone model of T cell-independent demyelination. Toxic demyelination was associated to a robust upregulation of P2x7 receptors and NLPR3 inflammasome signaling molecules. Colocalization experiments indicated that upregulated P2x7 receptor expression in demyelinated white matter was mainly associated to microglial cells. Consistently, P2x7 knockout mice exhibited attenuated demyelination and reduced presence of microglia and reactive astrocytes and attenuated expression of inflammatory cytokines in response to cuprizone feeding. However, pharmacological blockade of P2x7 receptors during the remyelination phase did not improve the extent of myelin recovery nor attenuated glial reaction in damaged white matter in the time-window analyzed in this study. These results identify P2x7 receptors as mediators of primary demyelination and suggest potential applications, but also limitations, of the P2x7 receptor in supporting recovery from progressed pathological states.

S19-03

THE P2X7 RECEPTOR HAS A SCAVENGER ROLE IN THE CENTRAL NERVOUS SYSTEM WHICH IS LITTLE AFFECTED BY ANTAGONISTS OF P2X7 PORE FUNCTION

James S Wiley and Ben J Gu

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Most studies of P2X7 receptors have focused on the some aspect of the proinflammatory 'pore' function but the requirement for activation by high concentrations of ATP agonist suggest the receptor has an alternative non-inflammatory function in the CNS. Our group has proposed a major function of P2X7 is to phagocytose apoptotic neurones and debris in the absence of ATP and play a major role in early human neurogenesis which occurs in the non-inflammatory milieu of early life. Demonstration of the innate phagocytic ability of human monocytes or microglia requires the absence of serum proteins since as little as 1-5 % serum (either autologous or heterologous) abolishes all phagocytic function not only by P2X7 but also by other scavenger receptors. This inhibitory action of serum resides in a protein fraction containing copper and/or zinc containing glycoproteins such as ceruloplasmin and amyloid precursor protein. Genetic studies have identified a rare polymorphic haplotype spanning the P2X7-P2X4 gene loci which abolishes the phagocytic ability of the receptor not only in age-related macular degeneration (a disease of impaired innate phagocytosis) but also in a cohort with primary progressive multiple sclerosis (PPMS, n = 777) with a significant odds ratio of 1.82. A second unique P2X7-P2X4 haplotype has been shown to segregate with MS disease in six closely related members of a Canadian pedigree. This haplotype impairs surface expression of P2X7 receptors to values of only 5% of wild type and in turn nearly abolishes P2X7 mediated phagocytosis. This P2X7-mediated phagocytosis is not or little affected by three different selective P2X7 antagonists which abolish P2X7 pore function. These antagonists have few side effects and blocking neuroinflammation will have beneficial effects by stimulating innate phagocytosis in the brain.

S19-04

P2X4 RECEPTOR CONTROLS MICROGLIA ACTIVATION AND FAVOURS REMYELINATION IN AUTOIMMUNE ENCEPHALITIS

Alazne Zabala¹, Alejandro Montilla, Björn Rissiek², Jon Gejo¹, Alberto Perez-Samartín¹, Abraham Martín¹, Carlos Matute^{1*} and María Domercq^{1*}

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Microglia survey the brain microenvironment for signals of injury or infection, and are essential for the initiation and resolution of pathogen- or tissue damage-induced inflammation. Understanding the mechanism of microglia responses during pathology is hence vital to promote regenerative responses. We observed that purinergic P2X4 receptor (P2X4R) was overexpressed in activated microglia in EAE and in human MS optic nerve samples. Here we analyzed the role of purinergic receptor P2X4 (P2X4R) in microglia/macrophages during autoimmune inflammation. Blockade of P2X4R signaling exacerbated clinical signs in the experimental autoimmune encephalomyelitis (EAE) model as well as favoured microglia activation to a pro-inflammatory phenotype and inhibited myelin phagocytosis. Moreover, P2X4R blockade in microglia halted oligodendrocyte differentiation in vitro and remyelination after lysolecithin-induced demyelination. Conversely, potentiation of P2X4R signaling by the allosteric modulator ivermectin (IVM) potentiated myelin phagocytosis, promoted the remyelination response and ameliorated clinical signs of EAE. We are currently studying the molecular mechanisms linking P2X4 activation to myelin phagocytosis. Our results provide evidence that P2X4Rs modulate microglia/macrophage inflammatory responses and identify IVM as a potential candidate among currently used drugs to promote the repair of myelin damage.

Symposium 20: FIGHTING THE FIRE: NOVEL PURINERGIC APPROACHES TO MANAGE IMMUNE-MEDIATED DISORDERS

Chairs: Luca Antonioli (Pisa, Italy) / Carmen Montesinos (Valencia, Spain)

Content: Inflammation is a defense reaction aimed at eliminating the harmful agent from the organism and restoring tissue homeostasis. This process involves a series of immunological mechanisms able to modulate the activity of immune cells in order to preserve the integrity of our body. A number of studies revealed the critical role of the purine system (ATP and adenosine, mainly) in the modulation of inflammatory processes. Indeed, ischemia and hypoxic conditions at the inflammation site cause the release of high amounts of ATP, promoting the activation of the immune system through the interaction with purinergic receptors.

The present symposium will provide novel evidences about the role of purines in the pathophysiology of the inflammatory process, highlighting its potential as a possible target for the development of innovative strategies useful for the pharmacological treatment of immune-inflammatory disorders.

S20-01

P2X7 RECEPTOR EXPRESSION AND FUNCTION IN HUMAN SEPSIS

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Sepsis characterises by a systemic inflammatory response that is followed by an immunosuppression of the host. Metabolic defects and mitochondrial failure are common in immunocompromised septic patients. The NLRP3 inflammasome is important for establishing an inflammatory response after activation by the purinergic P2X7 receptor. Here, we study a cohort of individuals with intra-abdominal origin sepsis and show that patient monocytes have impaired NLRP3 activation by the P2X7 receptor. Furthermore, most sepsis-related deaths are among patients whose NLRP3 activation is profoundly altered. In monocytes from septic patients, the P2X7 receptor is associated with mitochondrial dysfunction; moreover, activation of the P2X7 receptor results in mitochondrial damage, which in turn inhibits NLRP3 activation by HIF-1 α . We also show that mortality increases in a mouse model of sepsis when the P2X7 receptor is activated in vivo. Our data reveal a molecular mechanism initiated by the P2X7 receptor that contributes to NLRP3 impairment during infection.

S20-02

Ryszard T. Smolenski (Department of Biochemistry Medical University of Gdansk, Gdansk, Poland) Therapies targeting ectoenzymes of nucleotide breakdown in chronic inflammation related vascular pathologies

S20-03

**ADENOSINE METABOLISM AND RECEPTORS IN
OSTEOARTHRITIS: TARGETING A2A RECEPTORS TO
REVERSE INFLAMMAGING**

B.N. Cronstein

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Aging and injury often lead to chronic low-grade inflammation, a condition known as Inflammaging, which contributes to the development of disease in the musculoskeletal system and is also thought to contribute to the development of diabetes and other diseases of aging. Previous studies demonstrate that chondrocytes from osteoarthritic cartilage have lower cellular ATP levels, lower biomass and diminished mitochondrial function and we have discovered that this leads to diminished ATP transport into the extracellular space resulting in lower extracellular adenosine levels. In other experiments we found that reduced extracellular adenosine levels or loss of A2A receptors (A2AR) lead to the development of premature osteoarthritis (OA). Intra-articular injection of liposomal adenosine or CGS21680 (A2AR agonist) both prevents development of OA after trauma in rats, an effect completely reversed by intra-articular injection of an A2AR antagonist (ZM241385), and reverses post-traumatic OA in rats and obesity-induced OA in mice. A2AR stimulation promotes chondrocyte homeostasis and reverses OA by multiple mechanisms which will be reviewed.

S20-04

TARGETING P2X7 IN AMYOTROPHIC LATERAL SCLEROSIS: WHERE AND WHEN?

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Amyotrophic lateral sclerosis (ALS) is a predominant motor neuron disease that irreversibly targets upper and lower motor neurons. Upper motor neurons carry motor information from the motor cortex through the brainstem and spinal cord to the lower motor neurons, which in turn directly signal to the muscles. The loss of somatic motor neurons and their innervation to voluntary skeletal muscles occurring in ALS, leads to paralysis that culminates into respiratory failure and death. In the majority of cases, ALS occurs as a sporadic form, while about 10-15% of patients suffer from a familial disease attributed to dominant, high-penetrance gene variants. More than twenty-five genes have been identified which are responsible for about 60% of familial forms and 10% of sporadic patients. Among the most common genetic mutations known to cause ALS, more than 180 different mutations are located on the SOD1 gene encoding the cytoplasmic enzyme superoxide dismutase 1, found mutated in about 20% of familial patients and 2% of sporadic cases. Although the SOD1-G93A (glycine 93 changed into alanine) is a relatively rare mutation, it is currently expressed in the animal model that best mimics some phenotypic and pathological feature of both familial and sporadic ALS, the SOD1-G93A mouse.

The concept that the purinergic P2X7 receptor could become a “key player” in ALS has recently emerged from in vitro and in vivo studies. What we have learned is that precocious genetic ablation of the receptor is certainly detrimental on disease progression, that pharmacological systemic inhibition at pre-symptomatic phase or after disease onset might still be too early or too late to elicit beneficial effects, finally that there is a precise time window of intervention (the late pre-onset) when pharmacological inhibition of the P2X7 provides successful outcomes on motor performance and survival. In other words, there is a precise time dependency concerning the role of P2X7 that likely acts as a dual modifier in ALS disease. In general, whether P2X7 activation is beneficial or detrimental depends on a lot of factors, including the nature and the duration of the toxic insult, and the specific cell population, both converging in a precise temporal window of the injury event. With our studies, we have provided clear evidence that a P2X7-targeted and site-specific modulation might be an additional strategy to interfere with the complex multifactorial and multisystem nature of ALS.

S20-05

Marco Idzko (Division of Pulmonology, Department of Medicine II, Medical University of Vienna, Vienna, Austria)

Symposium 21: TEACHING CAFFEINE NEW TRICKS FOR OLD PROBLEMS IN PARKINSON'S DISEASE

Chairs: Luisa Lopes (Lisboa, Portugal) / Jiang-Fan Chen (Boston, USA)

Content: Caffeine and adenosine receptor ligands have been on trial against motor symptoms in Parkinson's disease with very little efficacy, albeit their demonstrated benefits in epidemiological studies in patients. This hints at a predominant early, non-motor effect of the caffeine analogs that has been poorly discussed. We will cover very recent research and state-of-the-art tools that are providing new clues for these non-motor aspects, namely the effects on protein aggregation and spreading, receptor dimerization and cognition. We hope to contribute to a timely discussion while highlighting novel mechanisms that could be of general interest and applications beyond PD, to the wider neuropurine audience.

1. Jiang-Fan Chen (Boston School of Medicine, Boston, USA)
2. Poul Jensen (Arrhus University, Arrhus, Denmark)
3. Joana Coelho (iMM, Lisboa, Portugal)

**Oral Communications and Posters
(by areas of research)**

P1. STRUCTURE OF PURINE RECEPTORS AND ECTONUCLEOTIDASES

P.01

IDENTIFICATION OF P2X7 RECEPTOR EXTRACELLULAR VESTIBULE RESIDUES CONTRIBUTING TO RECEPTOR STRUCTURE AND CHANNEL GATING

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Extracellular vestibule, unexpectedly discovered for the first time in crystal structure of zfP2X4 receptor, exhibits multiple roles in P2X receptor function: it is important for ion entry to the channel pore, and vestibule widening upon ATP binding is important for channel gating. Extracellular vestibule is present also in the P2X7 receptor, but its role has not yet been studied. To address this task, we have substituted several conserved and/or unique residues in this region (residues K49, Y51, Q52, F322, G323, G326, K327, F328 and Q332) with alanine, and expressed wild type and mutated receptor in HEK293T cells. These positions have been selected based on previous studies where they have been identified as important for function and structure of P2X1 and P2X4 receptors. Western blotting and electrophysiology showed that four of nine residues (Y51, Q52, G323, and F328) are important for P2X7 trafficking and substitution of three residues (K49, G326 and K327), including receptor-specific residue Q332, did not alter or only slightly reduced receptor function. Alanine substitution of non-conserved F322, that is present also in the P2X4, dramatically increased P2X7 receptor sensitivity to agonist and enhanced the rate of receptor-mediated dye uptake. These results revealed that clusters of conserved residues above TM1 (Y51 and Q52) and TM2, (F328) play a role in receptor expression in the plasma membrane. Conserved residue G323 is important for P2X7 receptor trafficking and vestibule structure. Aromatic residue at position 322, in the top of extracellular vestibule just between extracellular and central vestibules, is important for pore opening. Overall, our data demonstrate that transition between extracellular and central vestibule represents a promising new target for the allosteric control of P2X7 receptor sensitivity and channel gating.

P.02

P2X7 RECEPTOR ACTIVATION INCREASES MESENCHYMAL TRANSDIFFERENTIATION AND INVASIVENESS OF GLIOBLASTOMA-DERIVED STEM-LIKE CELLS

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Stem-like cells (GSCs) with high self-renewal, resistance to radio/chemotherapy and metastatic potential contributes to glioblastoma (GBM) unfavorable prognosis. We previously showed high expression levels of P2X7 receptors (P2X7R) in GSCs isolated from human primary GBM. Here, using the same GSCs, we investigated the effects of P2X7R modulation on genes associated to epithelial-to-mesenchymal transition (EMT), a process likely contributing to GBM malignancy. The P2X7R agonist 2'(3')-O-(4-benzoylbenzoyl)-ATP (BzATP, 50-200 μ M) or the known EMT inducer, Transforming Growth Factor- β 1 (TGF β 1, 5-10 ng/ml) up-regulated mRNAs and increased the protein content of selected EMT markers during 24-72h following drug administration to GSCs. Both agents also enhanced GSC migration and invasiveness, evaluated by scratch and trans-well migration assays. BzATP effects were mediated by increased phosphorylation of SMAD2, a downstream TGF β signaling effector. A8301, an inhibitor of this pathway, abrogated them, suggesting TGF β involvement in P2X7R-mediated effects in GSCs. BzATP also decreased lactate dehydrogenase and caspase-3/7 activity in GSC medium. The P2X7R antagonist A438079 mostly counteracted BzATP effects. Finally, i) GSCs expressed two main human P2X7R splicing variants, the full length P2X7AR and the truncated P2X7BR, lacking the carboxylic tail, which have different functional properties; ii) BzATP up-regulated their expression, thus possibly favoring A/B subunit assembly into a heterotrimeric P2X7R with greater sensitivity towards agonists/support to cell energy. These results are in line with the increased expression of EMT markers, cell migration/invasion and GSC survival induced by P2X7R stimulation. Since in GBM microenvironment extracellular ATP levels may activate P2X7R, our data also suggest a role in GBM recurrence/invasiveness.

P.03

IDENTIFYING REGIONS OF P2X7 RECEPTOR INTRACELLULAR DOMAINS INVOLVED IN DOWNSTREAM SIGNALING

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The P2X7 receptor is an ATP-gated cation ion channel which displays a widespread expression in mammals, and has been implicated in several pathologies, including immune disorders and cancer. Unique among the P2X receptor family, P2X7 possesses a long (240 amino acid) intracellular C-terminal domain (CTD) which is thought to couple cation channel activation by extracellular ATP to signaling pathways including cell blebbing, pore formation and ERK phosphorylation. Truncation mutants of the CTD have been demonstrated to show impaired ATP-induced membrane blebbing, and impaired ATP-induced uptake of the cationic dye Yo-Pro1. Removal of the N-terminal domain (NTD) has been demonstrated to abolish ATP-induced ERK phosphorylation. P2X7-dependent cell blebbing has been proposed to arise from the ATP-induced dissociation of P2X7 and non-muscle myosin, and P2X7-dependent Yo-Pro1 uptake has been proposed to be cholesterol-dependent, with a cysteine-rich, palmitoylated portion of the CTD proximal to the second transmembrane domain (CRR) able to sequester cholesterol to prevent inhibition of dye uptake in the full-length receptor.

We set out to investigate which portions of the intracellular domains are involved in downstream signaling following P2X7 receptor activation, and whether or not the different signaling phenomena are coupled, by constructing a series of truncations, deletions and point mutants in the well-characterized rat P2X7 receptor fused to a C-terminal GFP-His tag. Constructs were transfected into HEK293 cells, and cell surface protein expression, ATP-induced cell blebbing, calcium uptake, Yo-Pro1 uptake, and ERK phosphorylation were measured. We found that any mutant which impaired blebbing also impaired Yo-Pro1 uptake, implying that these signaling phenomena are intrinsically coupled. Our data for ERK phosphorylation was more complex, but imply a role for both the NTD and CTD, and suggest that this pathway is distinct from the blebbing/dye uptake pathway. While both non-muscle myosin IIA and myosin V were expressed in HEK cells, we found no evidence for a physical interaction of either protein with P2X7, and so the molecular pathway for P2X7-dependent blebbing in these cells remains unclear. Cholesterol depletion by Methyl- β -cyclodextrin (MCD) was found to enhance ATP-induced Yo-Pro1 uptake in some CTD mutants, and further investigation of the CRR by deletion and multiple point mutagenesis revealed an essential role for this region for P2X7 dye uptake function.

P.04

STRUCTURAL MODELS FOR *DICTOSTELIUM DISCOIDEUM* P2X RECEPTORS

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Our understanding of P2X receptors at a structural level has been hugely advanced by series of X-ray structures of P2X receptors in closed, open, and desensitized states (for review, see Pasqualetto et al., Front. Pharmacol., 9, 58, (2018)). These structures can serve as templates for the generation of homology models for P2X receptors where structures have not been solved yet. Feasibility and accuracy of homology modelling is limited by the level of sequence similarity, typically sequence identity levels $\sim < 35\%$ are considered problematic. This threshold is not reached for P2X receptors from species very distant to mammals. The pairwise sequence identity to the probably best characterized protist P2X receptors from *Dictostelium discoideum* is $\sim 22\%$. Nevertheless the low, but significant sequence similarity implies common ancestry, and indeed it has been shown that *Dictostelium discoideum* P2X receptors show characteristic features (e.g. activation by ATP, formation of stable trimers) and the overall shape with tri-fold symmetry has been demonstrated via electron microscopy. To gain further structural insight, we have used an integrated bioinformatics approach. For improving the quality of pairwise sequence alignments for homology derived structural constraints we employed profile Hidden Markov Models. Despite this, some regions, for instance the P2X receptor head region, remain unalignable and we applied *ab initio* modelling for “filling the gaps”. A series of alternative models are discussed in the context of the available functional data from literature.

P.05

UNCOVERING ‘HIDDEN’ CONFORMATIONS OF ATP-GATED P2X RECEPTORS USING BIOCHEMICAL AND BIOINFORMATICS METHODS

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Static structures of P2X receptors are available, but the extent of additional transitional states is unclear. Previous work on P2X1 suggests that the upper vestibule of the receptor is accessible to MTS-compounds that is not predicted by the apo homology model (Roberts et al. Proc. Natl. Acad. Sci USA 109; 4663-67, 2012). This work has been extended with the larger MTS-TAMRA to fluorescently label residues in hP2X1, which indicate that the upper vestibule is even more accessible than previously thought. Cysteine accessibility studies using MTS-TPAE and MTS-TAMRA confirms that access to residues inside the upper vestibule is size-dependent.

These biochemical data are difficult to reconcile with structural information derived from homology models based on the closed and open states of zfP2X4. We have therefore generated further P2X1 homology models to include antagonist-bound and desensitized state models derived from more recent hP2X3 and paP2X7 and used HOLE to identify any internal cavities or tunnels which might be accessible to MTS molecules. The models allowed us to compare the differences in the relaxation of the subunits and perform measurements between selected residues that defined the cavities.

Molecular dynamics simulations were used to further sample these models for more accessible states and variability in cavities. From the combination of our biochemical data and molecular modelling a picture emerges where dynamically accessible conformations of the P2X1 receptor allow bigger MTS-compounds access to the upper vestibule. This might point to further, looser, conformations states of the P2X proteins that cannot be captured using crystallographic work.

P.06

ALFAXOLONE-INDUCED P2X4R ALLOSTERIC AGONISM CONFIRMED BY MOLECULAR DYNAMIC CALCULATIONS

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The P2X4R is modulated allosterically by multiple ligands including trace metals, steroids and ivermectin an antiparasitic agent. While trace metals such as Cu(II) or Zn(II) have sites in the extracellular part of the P2XR domain, steroids and ivermectin interact with P2XR sites in the transmembrane domain. Neurosteroids based on pregnanolone derivatives, are brain synthesized that influence animal behavior by interacting with membrane receptor sites. Alfaxolone (A) is a synthetic steroid derivative formerly used as an anesthetic agent. 1-10 μ M A applications to oocytes or HEK cells transfected with rP2X4R, caused ATP-gated currents potentiation; however, at 30 μ M, A evidenced *per se* gating currents sensitive to suramin blockade. This finding allowed to propose that A is a P2X4R allosteric agonist. We now examined the molecular mechanism of the allosteric agonism, hypothesizing that this steroid must somehow open the receptor pore in the absence of ATP. Bioinformatic tools such steroid docking analysis, rP2X4R modeling and molecular dynamic simulations, and hole analysis, were used to generate rP2X4R model in the *apo* and *holo* states in the absence and in the presence of A. Molecular dynamics in the different rP2X4R states revealed allosteric-induced stability. Pore and lateral fenestration measurements of the different rP2X4R states showed that A can induce a larger pore opening in the absence of ATP, as expected and consistent with an allosteric agonist. Altogether, the present findings are compatible with A binding to a membrane localized allosteric site eliciting rP2X4R conformational changes compatible with the notion of allosteric agonism. Future studies will allow the design of novel allosteric agonists or human P2X4R antagonists with proved beneficial clinicals as analgesic drugs. Funded by Newton Picarte DPI Conicyt 20140080 grant, FONDECYT 117-0842; additional funds were provided by CEDENNA, FB 0807 grant.

P.07

DEVELOPMENT OF NPP1 INHIBITORS AS POTENTIAL DRUGS FOR THE TREATMENT OF OSTEOARTHRITIS/CPPD DISEASE

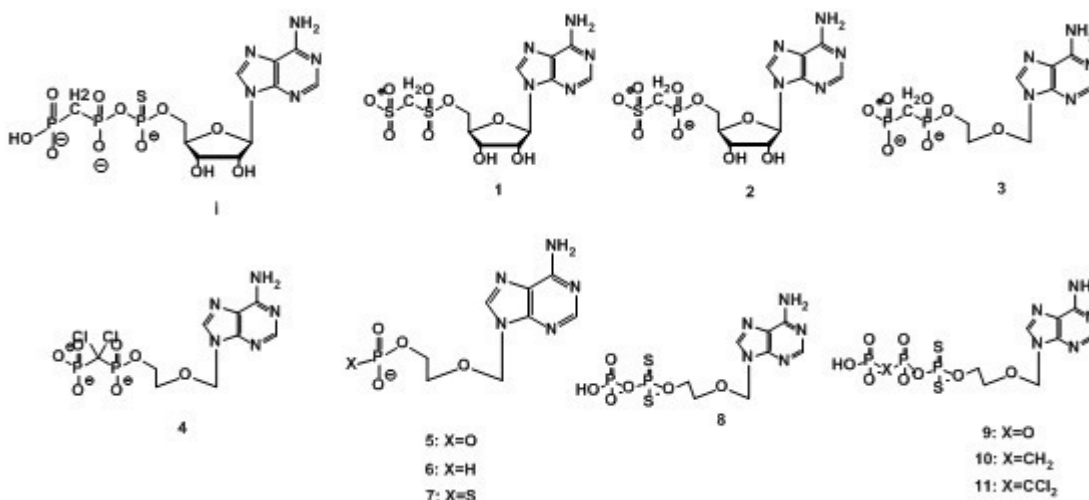
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Overproduction of extracellular pyrophosphate due to hydrolysis of ATP by NPP1 leads to deposition of pathological $\text{Ca}_2\text{P}_2\text{O}_7 \cdot \text{H}_2\text{O}$ (CPPD) in cartilage, resulting in a degenerative joint disease (CPPD disease) which has no cure. We explored the hypothesis that NPP1 inhibitors may be therapeutic agents for CPPD disease by inhibiting the hydrolysis of ATP. Specifically, we synthesized ADP derivatives where either $\text{P}_{\alpha,\beta}$ phosphate groups or only P_{β} phosphate, are replaced by sulfonate groups, analogs **1**, and **2**, respectively. In addition, we prepared acyclic ADP and AMP analogs in which the primary alcohol was substituted by bis-phosphonate, analogs **3-4** and **5-7**, respectively. Finally, we prepared acyclic ATP/ADP $\text{P}_{\alpha,\alpha}$ dithio-phosphate analogs, **8-11**, which are expected to exhibit high affinity to Zn(II) ions in the active-site of the enzyme, while avoiding a chiral center at P_{α} -phosphate, and the waste of half of the nucleotide. The novel analogs have been evaluated for their inhibitory effect on NPP1 and their selectivity to NPP1 vs. NPP3. Finally, we evaluated the effect of the inhibitors to reduce the formation of PPi and consequently to reduce CPPD formation, in human chondrocytes. Analogs **8** and **9** are the most promising inhibitors with K_i values of 300 nM, 330 nM, respectively vs. analog **i** previously reported as a selective NPP1 inhibitor, K_i 685 nM. 10 μM analogs **10** and **11** demonstrate 100% inhibition of NPPase activity in human chondrocytes. Therefore, we suggest potent and selective inhibitors of NPP1 for lowering extracellular PPi levels in cartilage for preventing and treating OA/CPPD.



P.08

POINT MUTATIONS IN P2X7 RECEPTORS

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P2X7 receptors are predominately expressed in the plasma membrane of cells of the hematopoietic lineages and of epithelial cells. They are nonselective Ca^{2+} -permeable cation channels activated by extracellular ATP. P2X7 receptors are involved in inflammation, pain sensation and bone homeostasis. Several point mutations of P2X7 have been associated with mental and infectious diseases, inflammatory and bone disorders and malignancies. After heterologous expression of different P2X7 point mutation constructs in *Xenopus* oocytes and HEK-293 cells we measured ATP-induced P2X7-mediated ion currents by two microelectrode voltage clamp in oocytes and by the tight seal whole cell voltage clamp method in HEK-293 cells. ATP-induced intracellular Ca^{2+} signals were measured in HEK-293 cells by single cell fluorimetry using the Ca^{2+} -indicator Fluo-4. Fluorimetry was also used to quantify the P2X7-dependent uptake of the large cationic fluorescent dye Yo-Pro by HEK-293 cells. The hR307Q, hT357S, rF218K and rF288S mutations significantly decreased ATP-induced ion currents, Ca^{2+} signals and Yo-Pro fluorescence compared to the P2X7 wt. The E496A mutation, known as loss of function variant, displayed no significant change in ATP-dependent ion currents and Ca^{2+} signals but a reduced Yo-Pro uptake. Cells expressing the construct H155Y, known as gain of function mutation, had unchanged ion currents and Ca^{2+} signals but an increased Yo-Pro uptake showing that different signaling pathways are involved in P2X7-mediated Ca^{2+} of Yo-Pro uptake, respectively. These results indicate that point mutations of P2X7 receptors may have different effects on P2X7-dependent cellular signaling and that the characterization of the mutation effect may depend on the experimental readout.

P2. PHARMACOLOGY AND BIOCHEMISTRY OF P1 RECEPTORS

P.09

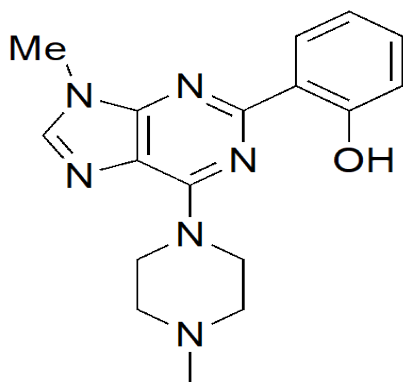
2-ARYLADENINE DERIVATIVES AS POTENT SCAFFOLDS FOR A₁, A₃ AND DUAL A₁/A₃ ADENOSINE RECEPTOR ANTAGONISTS: SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS

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In a previous work from our group, *in silico* target profiling of a chemical library of biologically-orphan molecules led to the identification of novel antagonists for all four members of the adenosine receptor family [1]. Subsequently *in silico* target profiling of the academic library of 1584 compounds led to the identification of compound **3a** containing a purine scaffold as a new hit for the adenosine A₁ receptor (Fig. 1). In order to expand this new chemical series targeting adenosine receptors and to study structure activity relationships, a new series of 24 purines (**3a-x**) were synthesized and their affinities at human adenosine A₁, A_{2A}, A_{2B} and A₃ receptors tested in radioligand binding assays.

Adenine derivatives were substituted at three different positions, N₉, C₆ and C₂, of the purine nucleus obtaining compounds with different affinity profiles towards the four adenosine receptors. From the synthesized compounds, five showed high affinity and selectivity for A₁ receptors and seven showed high affinity and selectivity for A₃ receptors. Two of the compounds showed high affinities for both A₁/A₃ receptors. SAR analysis indicate that, in order to generate high potent and selective ligands for A₁, A₃ and dual A₁/A₃ receptors, a hydrogen atom must be in N₉ and an aryl group must be in C₂, while the group in C₆ (piperidiny1 vs 4-methylpirazinyl) combined with a specific aryl group in C₆ seems to govern selectivity.



Functional studies indicated antagonist activity at A₁ and A₃ receptors for selected compounds of the series.

In conclusion, a number of purine-based compounds are described in this study, among them fourteen molecules with marked selectivity and antagonist activity at A₁, A₃ or dual A₁/A₃ adenosine receptors at submicromolar concentration. These purine scaffolds constitute an important source for novel biochemical tools and/or therapeutic drugs.

Figure 1. Novel purine hit (**3a**) for adenosine A₁ receptor identified by *in silico* target profiling.

References:[1] F. Areias, J. Brea, E. Gregori-Puigjane, M. Zaki, M.A. Carvalho, E. Dominguez, H. Gutierrez-de-Teran, M. Proença, M. Loza, J. Mestres, *Bioorg. Med. Chem.* 18 (2010) 3043-3052.

P.10

PHARMACOLOGICAL BLOCKADE OF ADENOSINE A_{2A} RECEPTORS RECOVERS MOTOR LEARNING AND GRIP STRENGTH DEFICITS IN AN ANGELMAN SYNDROME MOUSE MODEL

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Angelman syndrome (AS) is a rare neurodevelopmental disorder resulting from a loss of function of neuronal Ube3A protein. A_{2A} receptor (A_{2A}R) antagonists can counteract locomotor deficits in mouse models of different brain diseases. As AS therapeutics has been met with limited success, we here hypothesized that an adenosine-based strategy could also ameliorate the motor impairments in the AS animal model.

We used Ube3A m-/p+ modelling AS and wild-type (WT) littermates mice, at 8 weeks of age. In an accelerated rotarod test, AS mice had a lower performance as compared to their WT littermates (33.2±7.8 s in AS vs. 67.0±9.0 s in WT, when measuring latency to fall in the first day, with differences persisting across the days, n=13-16, p<0.01). Additionally, AS mice displayed decreased strength in a grasping protocol (2.9±0.1 kg-force/kg vs. 3.7±0.1 kg-force/kg, n=7-13, p<0.0001) and displayed lower locomotor activity in the open field (22.1±1.4 m vs. 26.4±1.3 m, n=19-25, p<0.05). Next, we checked if the by daily intraperitoneal injections of the A_{2A}R selective antagonist SCH58261 (0.1 mg/kg, for 21 days) could revert the impairments found in AS mice. SCH58261 attenuated the motor learning deficits of AS mice, since SCH58261-treated AS mice improved their rotarod performance (34.2±6.2 s in day 1 vs. 65.7±13.3 s in day 3, n=9, p<0.01) while the saline-treated AS mice were not able to learn in the rotarod (40.3±8.2 s in day 1 vs. 44.2±4.9 s in day 3, n=9). SCH58261 also rescued grip strength impairment (difference between grip strength values in the post and pre-treatment: 0.42±0.25 kg-force/kg in SCH58261-treated AS mice vs. -0.44±0.24 kg-force/kg in saline-treated AS mice, n=9-11, p<0.05).

Thus, we propose that the blockade of A_{2A}R can ameliorate the motor and coordination deficits found in this AS mice model.

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P.12

DEVELOPMENT OF A LIVE CELL NANOBRET BINDING ASSAY FOR ADENOSINE A_{2B} RECEPTORS

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Adenosine receptors (ARs, subtypes A₁, A_{2A}, A_{2B}, A₃) are G protein-coupled receptors which are widely distributed throughout the body and are involved in a number of physiological and pathological functions.¹ The adenosine A_{2B} receptor (A_{2B}AR) is currently in the focus of interest as a drug target in immuno-oncology, and in addition to immune cell activation, A_{2B}AR antagonists also display anti-proliferative and anti-angiogenic effects. Bioluminescence resonance energy transfer (BRET) assays have previously been applied to examine various aspects of receptor-ligand interactions. The novel bioluminescent donor NanoLuc luciferase, derived from *Oplophorus gracilirostris* luciferase, allows the use of BRET for the monitoring of ligand binding in living cells in real time.²

In the present study we developed a novel NanoBRET-based ligand binding assay. A_{2B}AR antagonists (xanthine scaffold)^{3,4} labeled with a fluorescent dye (a green BODIPY or a red cyanine dye) were synthesized and employed to observe BRET between the NanoLuc[®] tag attached to the extracellular N-terminus of the human A_{2B}AR and the receptor-bound fluorescent ligand. Saturation binding experiments showed specific binding of the ligands to the A_{2B}AR. Kinetic studies were performed, and the calculated kinetic K_D-values were in close agreement with the K_D-values obtained in saturation binding experiments. The BODIPY-labeled A_{2B}AR antagonists showed fast association at 37°C reaching equilibrium within less than 30 min, and equilibrium binding was stable for at least 180 min. Dissociation at 37°C was induced by the addition of 1,3-dipropyl-8-cyclopentylxanthine (DPCPX). Next, we performed competition binding experiments to determine the affinities of a structurally diverse set of A_{2B}AR ligands, agonists as well as antagonists. The obtained K_i-values were consonant with the K_i-values determined in radioligand binding studies. Our data showed that the novel A_{2B}AR NanoBRET assay will be highly useful for high-throughput screening and may replace traditional radioligand binding studies in the future.

¹Fredholm B. B. et al. International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors-an update. *Pharmacol Rev.* **2011**, *63*, 1-34.

²Dale C. N. et al. NanoBRET: The Bright Future of Proximity-Based Assays. *Front Bioeng Biotechnol.* **2019**, *7*, 1-13.

³Jiang J. et al. A_{2B} Adenosine Receptor Antagonists with Picomolar Potency. *J Med Chem.* **2019**, *62*, 4032-4055.

⁴Köse M. et al. Fluorescent-Labeled Selective Adenosine A_{2B} Receptor Antagonist Enables Competition Binding Assay by Flow Cytometry. *J Med Chem.* **2018**, *61*, 4301-4316.

P.13

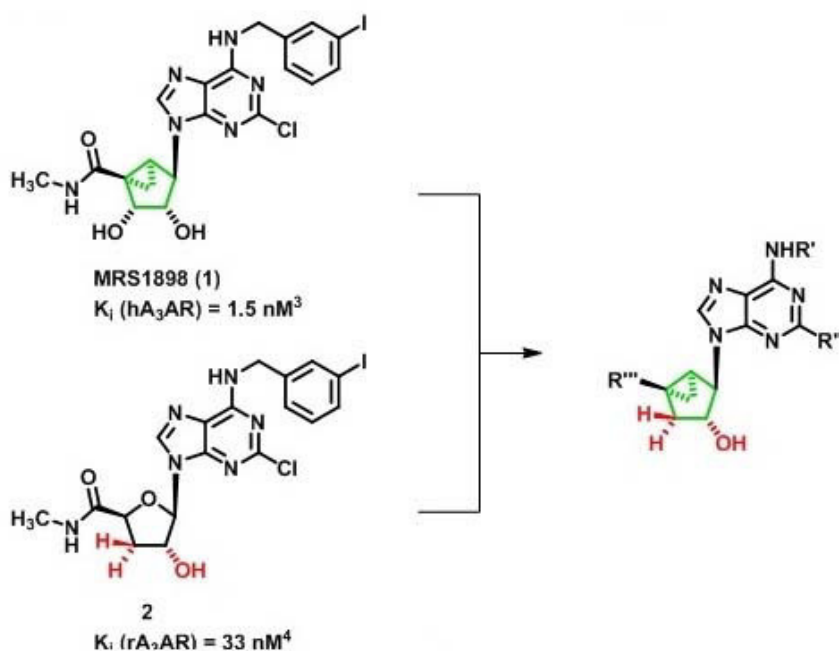
SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 3'-DEOXY-(N)-METHANOCARBA-BASED ADENOSINE RECEPTOR LIGANDS

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The bicyclo[3.1.0]hexane scaffold, often referred to as (N)-methanocarba, has been widely used as a ribose bioisostere in the development of nucleoside-derived A₃ adenosine receptor (A₃AR) ligands. It is known to improve selectivity towards the other adenosine receptor subtypes (A₁, A_{2A}, A_{2B}) while maintaining high affinity.¹

While the 2'-OH-group of the corresponding adenosine derivatives is known to be important for A₃AR affinity,² 3'-deoxy-(N)-methanocarba adenosine derivatives were never synthesized and therefore never studied.



The limited data for 3'-deoxyribose derivatives (e.g., compound **2**) indicate affinities in a similar range to ribose-based compounds (see MRS1898 (**1**)).^{3,4} However, the reduced polar surface area of the new compound class promises more favorable physicochemical properties and thus improved pharmacokinetic behavior. Therefore 3'-deoxy-(N)-methanocarba adenosine derivatives are highly desirable compounds for studying SAR of A₃AR ligands.

Herein we report the first synthesis and pharmacological evaluation of 3'-deoxy-(N)-methanocarba-based adenosine receptor ligands.

1. Müller, C. E.; Jacobson, K. A. *Biochem. Biophys. Acta* **2011**, 1808, 1290-1308.
2. Lee, K.; Ravi, G.; Ji, X.; Marquez, V. E.; Jacobson, K. A. *Bioorg. Med. Chem. Lett.* **2001**, 11, 1333-1337.
3. Tchilibon, S.; Joshi, B. V.; Kim, S.; Duong, H. T.; Gao, Z.; Jacobson, K. A. *J. Med. Chem.* **2005**, 48, 1745-1758.
4. Siddiqi, S. M.; Olah, M. E.; Ji, X.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O.; Jacobson, K. A. *J. Med. Chem.* **1995**, 38, 1720-1735.

P3. PHARMACOLOGY AND BIOCHEMISTRY OF P2 RECEPTORS

P.14

FLUORESCENT SENSORS FOR THE IMAGING OF ATP AT CELL SURFACES

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Signalling by extracellular adenine nucleotides is an important mechanism regulating activation and quiescence in the immune system. The prime event in this process is the release of ATP by stressed or damaged cells or by regulated secretion. Extracellular ATP either acts directly on P2 receptors (e.g. P2X7) to mediate activating effects or is converted to adenosine (ADO) by the sequential action of the ecto-nucleotidases CD39 and CD73 to mediate suppressive effects by acting on P1 ADO receptors (ARs). Since the relevant concentrations of eATP at the site of receptor signalling differ from bulk ATP levels measured in cell supernatants, it is important to develop methods to monitor ATP concentrations at distinct subcellular locations like the cell surface.

We engineered genetically encoded FRET-based sensors for expression in the cytoplasm or at the cell surface and monitored ATP levels in the cytoplasm by flow cytometry or live cell imaging. To avoid the dependence on genetic manipulation we also synthesized several ATP-responsive small-molecule fluorescent probes.

Transfection of the cytoplasmic FRET sensors into Yac-1 lymphoma cells revealed that gating of P2X7 resulted in a constant decrease in cytosolic ATP levels for the duration of exposure to eATP that was blocked if the cells were pre-incubated with the P2X7-inhibitory nanobody 13A7. This response could be monitored both by flow cytometry on a population level and by fluorescence microscopy imaging on the level of individual cells.

In a complementary approach we synthesized small-molecule fluorescent probes that change their emission intensity or spectra in response to ATP. Work is currently in progress to improve the sensitivity of these probes as well as their selectivity towards ATP and to target these probes to the cell surface.

* This work is part of SFB1328 “Adenine Nucleotides in Immunity and Inflammation” funded by the DFG

P.15

P2X7 RECEPTOR MODULATES THE OSTEOGENIC DIFFERENTIATION OF STROMAL STEM CELLS FROM HUMAN ADIPOSE TISSUE

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Since the role of P2X7 receptors (P2X7Rs) is still controversial in cell osteogenic differentiation, we investigated their expression and effects in mesenchymal/stromal stem cells derived from human subcutaneous adipose tissue (S-ASCs), which are of potential interest in bone regenerative medicine. Undifferentiated S-ASCs expressed higher mRNA levels for the P2X7AR full-length isoform, equipped with a ionic channel and a macropore, than for the truncated P2X7BR variant, lacking the pore. P2X7R stimulation by the agonist BzATP increased cell $[Ca^{2+}]_i$ and migration without impairing proliferation or causing pore opening. The antagonist A438079 reversed these effects. In contrast, BzATP, added to the osteogenic medium, decreased extracellular matrix mineralization and expression of osteogenic transcription factors (Runx2, alkaline phosphatase, osteocalcin) and of P2X7R A and B subunits. BzATP-induced effects were not due to a reduction in cell proliferation or to an increase in apoptotic/necrotic events, but rather to a decrease in P2X7R expression. Noteworthy, A438079, administered alone during cells differentiation, first restrained this process, enhancing it later. Accordingly, A438079 reversed Bz-ATP effects only in the second phase of S-ASCs osteogenic differentiation. Apyrase, an ATP diphosphohydrolase, had a similar behavior. These effects were confirmed by experiments of titanium scaffold colonization by S-ASCs. Our findings suggest that adenosine derived from purine metabolism contributes to a pro-osteogenic activity of endogenous ATP, in turn related to a prevalent P2X7RB expression. Conversely, P2X7R pharmacological stimulation by BzATP, like that induced by high ATP levels occurring during tissue injury, would compromise osteogenic differentiation of local/exogenous stem cells and thereby bone repair.

P.16

DESIGN, SYNTHESIS, AND PHARMACOLOGICAL EVALUATION OF NOVEL P2X7R ANTAGONISTS POTENTIALLY TARGETING THE CNS

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The purinergic P2X7 receptor (P2X7R) is a ligand-gated ion channel physiologically activated by high concentrations of ATP that occur after tissue or cell damage. Its activity is correlated with inflammatory signalling and the promotion of cell death, which are causes of several neurodegenerative, neurological, and psychiatric diseases. In this work, we plan to design and synthesise new antagonists capable of crossing the blood-brain barrier (BBB) to block the P2X7R activity in the central nervous system (CNS).

After a rational design supported by computational aids, twenty-six compounds have been synthesised so far and they are being pharmacologically screened in a cell line expressing the P2X7R. Fluorescence calcium dynamics and YO-PRO-1 dye uptake assays are used in HEK293-hP2X7R cells, while the P2X7R-induced IL-1 β release is measured in bone marrow-derived macrophages.

From a first screening in intraperitoneal macrophages, two compounds, *i.e.* ITH15004 and ITH15006, revealed a certain functional blockade of the receptor. However, further investigation is needed to confirm their mechanism of action. These first results allowed us to design novel optimised compounds based on the molecular structure of ITH15004 and ITH15006, so to define full structure-activity relationships.

The disclosure of a new P2X7R antagonist hit with appropriate characteristics for crossing the BBB will permit further *in vitro* and *in vivo* studies in models of the above mentioned CNS diseases, as well as precise structural optimisation to follow with the development of a potential final clinical candidate.

P.17

CHARACTERISATION OF P2Y₂ RECEPTORS IN HUMAN VASCULAR ENDOTHELIAL CELLS USING AR-C118925XX, A POTENT AND SELECTIVE P2Y₂ ANTAGONIST

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The physiological functions of many of the eight P2Y receptor subtypes are unclear due to the limited selectivity and low potency of most antagonists. A P2Y₂ antagonist, AR-C118925XX, is now available, so the aims were to quantify the action of AR-C118925XX at recombinant P2Y₂ receptors and then to determine the role of native P2Y₂ receptors in the actions of UTP in human vascular endothelial cells.

Human EAhy926 umbilical vein endothelial cells, and 1321N1 cells stably expressing recombinant human P2Y₁, P2Y₂, P2Y₄, P2Y₁₁ or rat P2Y₆ receptors, were grown on glass coverslips. Following incubation with the Ca²⁺-sensitive dye, Cal-520AM, they were placed in a fluorimeter and intracellular Ca²⁺ measured. Cells were superfused continuously and agonists applied in the superfusate before and after 5 min superfusion with AR-C118925XX. Data were quantified using the Hill equation, the Gaddum-Schild equation or a Schild plot, as appropriate.

UTP evoked a concentration-dependent rise in intracellular Ca²⁺ in 1321N1-P2Y₂ cells (EC₅₀=54nM). AR-C118925XX (10nM-1μM) had no effect *per se* on intracellular Ca²⁺, but shifted the UTP concentration-response curve progressively rightwards, with no change in maximum. Schild analysis gave a pA₂=8.30 and slope=0.985. In contrast, AR-C118925XX (1μM), a concentration 200x greater than its K_B at P2Y₂ receptors, had no effect at recombinant P2Y₁, P2Y₄, P2Y₆ and P2Y₁₁ receptors. UTP also increased intracellular Ca²⁺ in EAhy926 cells in a concentration-dependent manner (EC₅₀=680nM). AR-C118925XX (30nM), shifted the UTP curve rightwards (EC₅₀=7.6μM), with no decrease in maximum. Gaddum-Schild analysis gave a K_B=3.0nM.

These data show that AR-C118925XX is a potent and selective P2Y₂ antagonist, which enabled us to identify P2Y₂ receptors as the P2Y subtype that mediates UTP-evoked increases in intracellular Ca²⁺ in human endothelial cells. Currently, AR-C118925XX is the only selective P2Y₂ antagonist available and so will be invaluable in identifying the physiological functions of other native P2Y₂ receptors.

P4. ECTONUCLEOTIDASES

P.18

T CELL-DERIVED EXOSOMAL CD73 MEDIATES IMMUNE SUPPRESSION

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The ecto-nucleotidases CD39 and CD73 sequentially hydrolyze extracellular ATP to generate adenosine, which in turn engages to P1 receptors on immune cells dampening the inflammatory response. In humans, co-expression of CD39 and CD73 on T cells is a rare event, both *ex vivo* and after activation. Under steady-state conditions, CD39 is expressed on the membrane of a subset of regulatory CD4 T cells, while CD73 is mostly expressed by naïve CD8 T cells. *In vitro* activation of T cells induced an increase of CD39 on the cell membrane, while CD73 expression is dramatically reduced. This CD39⁺CD73⁻ phenotype is observed on T cells at sites of inflammation. We found CD73-specific AMPase activity in cell culture supernatants of activated CD8 T cells as well as in synovial fluid of patients with juvenile idiopathic arthritis, indicating that CD73 is present in a non-cell-bound form. CD73 is a GPI-anchored protein, however, inhibitors of phospholipases and metalloproteases did not prevent activation-induced shedding from the cell surface. We then considered the possibility that the activity is contained in exosomes/extracellular vesicles (EVs). Indeed, differential centrifugation of T cell culture supernatants revealed most AMPase activity in the EVs found in the pellet after ultracentrifugation. The use of specific inhibitors confirmed that the activity is CD73-specific. Furthermore, we could show that these EVs are able to suppress T cell proliferation *in vitro*. We conclude that CD73-containing EVs released upon T cell activation provide AMPase activity *in trans*. The occurrence of CD73 as non-cell-bound molecule widens the range of action of this enzyme at sites of inflammation and contributes to the control of inflammatory responses.

P.19

EXTRACELLULAR NUCLEOTIDE METABOLISM IN THE CONTEXT OF YEAST-HOST INTERACTION

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Ectophosphatase and ectonucleotidase activities are recognized to influence the infectious potential of several microbes, including *Candida albicans* and other non-*albicans Candida* species, which are important human agents of infection.¹ Several studies have revealed that the conversion of extracellular ATP into adenosine determines the efficiency of microorganisms infection.^{2,3} In fact, in recent years and due to its anti-inflammatory and immunosuppressive effects, adenosine and its sensing devices, namely adenosine A_{2A} receptors, have emerged as important targets in the context of infection.

The present work aims to characterize the ectonucleotidase activities in *Candida* spp. and to explore its relevance in the evasion of the immune system by yeasts. We found that *C. albicans* does not have a classical ecto-5'-nucleotidase enzyme⁵ since, together with *C. glabrata* and *Saccharomyces cerevisiae*, it can also use 3'AMP as a substrate, under acidic pH conditions. In resemblance with other previously described mechanisms⁴, this 3'-nucleotidase/nuclease activity seems to be important for yeasts to escape Neutrophils Extracellular Traps.

In conclusion, although the exact nature and specificity of yeasts ectonucleotidases are not completely established, we highlighted the importance of those enzymes in the context of infection, helping yeasts to overcome host defenses, through its involvement in yeast-to-hypha transition, survival and persistence either in the epithelial surfaces and/or once internalized by phagocytic cells.

1) Mayer et al. Virulence.2013;4:119–128.

2) Németh et al. J Immunol.2006;176:5616-5626.

3) Thammavongsa et al. J Exp Med.2009;206:2417-2427.

4) Guimarães-Costa et al. Infect Immun.2014;82:1732-40.

5) Rodrigues et al. FEMS Microbiol Lett.2016;363(1):fnnv212.

P.20

P.A METABOLIC IMMUNE CHECKPOINT: ADENOSINE IN BRAIN METASTASES TUMOR MICROENVIRONMENT

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Brain metastases are the most common intracranial neoplasm in adults and represent the major cause of death in tumor patients. Given the fact that purines i.e. ATP and adenosine play key roles in neurotransmission and act as danger signals, we seek to understand the involvement of glial crosstalk in brain metastasis from different primary entities via adenosine and purinergic signaling and investigate how standard of care modulates this pathway.

We observed cell-type specific expression of the key enzymes that convert purines to adenosine in a syngeneic breast-to-brain metastases model. While the cancer cells mainly expressed CD73, the myeloid compartment showed preferential expression of CD39. For functional analyses, we depleted tumor and/or stromal-cell derived CD73 or CD39 by CRISPR/Cas9 (for tumor cells) and knock-out mouse models (for stromal cells). In line with our data, we found no difference in brain metastases incidence and tumor growth in CD73 proficient and deficient mice if CD73 activity is unaffected in the tumor cells. Preliminary data indicate that genetic targeting of CD73 in the tumor and stromal compartment led to a delay in tumor onset, reduced growth rates and prolonged survival. However, in contrast to our genetic approaches, we found that CD73 pharmacological inhibition led to decreased median survival. Strikingly, the combination of CD73 inhibition with fractionated whole brain radiotherapy reverted this effect. Of clinical relevance is the observed expression of CD39, CD73, ADA, ADK, A2AR and A2BR in tumor cells and tumor-associated stromal cells in human brain metastases. Interestingly, A2BR expression inversely correlated with CD3, CD4 and CD8 infiltration, indicating that an adenosine driven environment might be implicated in establishing an immune suppressive environment.

The long-term goal of this project is to interrogate if blockade of adenosine generating enzymes and adenosine receptors might establish a purine-driven pro-inflammatory environment that is more sensitive to standard of care treatment or immune therapies.

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ANALYSIS OF CD73 IN SERUM OF CANCER PATIENTS AND HEALTHY DONORS

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CD73 is an ectoenzyme able to hydrolyze AMP into extracellular adenosine and inorganic phosphate (Pi). In pathological conditions, adenosine has been observed to have anti-inflammatory and immunosuppressive effects. CD73 is up-regulated in several human cancers, and its overexpression is often related to poor prognosis. A soluble form of CD73 has been individuated in biological fluids. The aim of this study was to characterize CD73 expression and activity in serum of cancer patients and healthy donors.

Serum samples were collected at National Cancer Institute “G. Pascale” (Naples, Italy). Specifically, this study involved 91 patients with malignant melanoma (MM), 40 patients with other solid tumors (including head and neck cancer, bladder, gastric and kidney cancer), and 115 healthy donors. All the individuals provided written informed consent for blood donation.

CD73 enzymatic activity was determined by Malachite Green assay, which allows the measurement of Pi released after adding AMP as substrate. The Pi neat value was obtained subtracting the basal Pi, determined in absence of AMP. CD73 activity was expressed as Pi released (pmol/min/mg protein) and the values were associated with clinical pathological characteristics of patients. The CD73 protein levels in serum was determined by ELISA assay.

The enzymatic activity of CD73 resulted increased in serum of cancer patients (n=131, median= 39.41 pmol/min/mg protein), compared to healthy donors (n=115, median= 0 pmol/min/mg protein). Furthermore, the highest CD73 activity levels among cancer patients were measured in serum of MM patients (n=91, median= 56.47 pmol/min/mg protein). Elevated enzymatic activity was associated with male gender but not with other variables. CD73 expression showed the same trend, resulting higher in serum of cancer patients, compared to healthy donors.

Overall, our results suggest that CD73 could be a potential therapeutic target for MM patients.

INVESTIGATION OF HEPARIN-INDUCED ECTONUCLEOTIDASE-INHIBITION TO COUNTERACT ADENOSINE-MEDIATED IMMUNOSUPPRESSION IN THE TUMOR MICROENVIRONMENT

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Background: The tumor microenvironment is of crucial importance for cancer cell survival and proliferation. A multitude of different host cells, such as granulocytes, macrophages and different lymphocyte subtypes are recruited to the growing tumor nodules or the sites of metastatic niche. To escape or mitigate the attack of the immune surveillance, certain tumor cells express ectonucleotidases that generate high levels of extracellular adenosine by degradation of released ATP in the tumor microenvironment. Since adenosine is known for its potent suppressive activities on immune cells, while ATP shows the opposite effect, the pharmacological inhibition of ectonucleotidase activity of cancer cells appears an intriguing immuno-oncological approach to foster an anti-tumor immune response.

Aim: Early reports described an inhibition of crude ecto-nucleotidase preparations by heparin. We have recently studied various heparin preparations on purified ecto-nucleotidase subtypes, confirming potent effects on certain ecto-nucleotidases (manuscript in preparation). To investigate whether these effects have an impact on ATP degradation and on adenosine levels in cancer cells, we established methods i) to analyze ATP degradation and adenosine formation; and ii) to study potential consequences on immune cell activities.

Methods: Various human carcinoma cell lines were investigated by qPCR with respect to their mRNA expression of various ectonucleotidases. The supernatant of the glioblastoma cell line U87 was treated with ATP, and the effect of heparin on ATP degradation and the formation of its hydrolysis products, including adenosine, was analyzed by capillary electrophoresis (CE). Moreover, activated CD4⁺-T-lymphocytes were treated with adenosine to monitor concentration-dependent effects on their stage of proliferation by flow cytometry.

Results: The glioblastoma cell line U87 was selected as a cell model due to its high expression of ectonucleotidases. A suitable CE method was established for detecting nucleotide degradation and adenosine formation, as well as the effect of heparin on extracellular nucleotide metabolism. Proliferation of CD4⁺-T-lymphocytes was slightly affected by adenosine in preliminary studies, but further experiments are required to confirm these effects.

Conclusions: Heparin is a well-accepted, and guideline-based component of the clinical treatment of cancer patients. Discussions on further heparin effects, going beyond the inhibition of the coagulation system in oncology are still ongoing. Our data provide evidence that heparin is able to reduce the degradation of ATP to adenosine and accordingly impact multiple cellular effects of adenosine. It is tempting to speculate that heparin fosters the immune response towards cancer cells in an immunosuppressive tumor microenvironment.

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DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING ASSAY FOR THE IDENTIFICATION AND CHARACTERIZATION OF NUCLEOTIDE PYROPHOSPHATASE / PHOSPHODIESTERASE 4 (NPP4) INHIBITORS

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Nucleotide pyrophosphatase/phosphodiesterase 4 (NPP4) is a membrane-bound enzyme that hydrolyzes extracellular diadenosine polyphosphates such as Ap₃A and Ap₄A. The NPP4, which is present on the surface of endothelial cells, was reported to promote platelet aggregation by hydrolyzing Ap₃A to AMP and ADP, since ADP activates pro-thrombotic G protein-coupled P2Y₁ and P2Y₁₂ receptors. Thus, inhibitors of NPP4 have potential as novel anticoagulant drugs. To discover NPP4 inhibitors, a high-throughput screening method is required. In the present study, we developed a luminescence-based assay using recombinant human soluble NPP4 expressed in Sf9 insect cells, and diadenosine tetraphosphate (Ap₄A) as a substrate. The reaction product ATP was quantified by luciferin-luciferase reaction in a 96-well plate format. The method is highly sensitive with a limit of detection (LOD) and a limit of quantification (LOQ) of 16.6 nM, and 44.3 nM, respectively. The determined Z'-factor was 0.68 indicating that the developed assay is suitable for high-throughput screening. Subsequently, we applied it to studying the enzyme kinetics of NPP4 and for inhibitor screening. This led to the discovery of the first NPP4 inhibitors, which are required for target validation studies.

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URIDINE- AND CYTIDINE-BASED *ECTO*-5'-NUCLEOTIDASE-INHIBITORS: A NOVEL CLASS OF HIGHLY ACTIVE AND SELECTIVE CD73-INHIBITORS

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In 2018 *J. P. Allison* and *T. Honjo* won the Nobel Prize in Medicine “for their discovery of cancer therapy by inhibition of negative immune regulation.”¹ *Ecto*-5'-nucleotidase (CD73), an enzyme which catalyzes the hydrolysis of extracellular AMP to adenosine, is a possible target for immunotherapy. Unfortunately, inhibition of CD73 often suffered either from low inhibitory activity² of the compounds or from degradation products, which can activate P1 receptors.³ To overcome these issues, we developed and synthesized a novel uridine- and cytidine-based class of CD73 inhibitors.⁴ Our inhibitors display inhibitory activities in the low nanomolar range and the most active compound **9h** (and its degradation products) additionally shows selectivity over the P1, P2Y₆ and P2Y₁₄ receptors.⁴

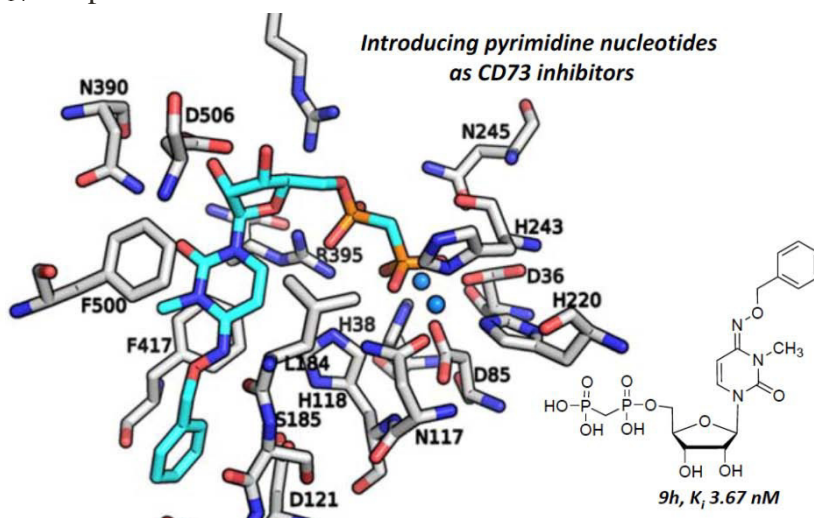


Fig. 1: Interactions of the CD73 inhibitor **9h** with the active site of CD73.⁴

1. Press release: The Nobel Prize in Physiology or Medicine 2018. NobelPrize.org. Nobel Media AB 2019.
2. Baqi, Y. *et al. J. Med. Chem.* **2010**, 53, 2076–2086. b) Ripphausen, P. *et al. J. Med. Chem.* **2012**, 55, 6576–6581.
3. Bhattarai, S. *et al. J. Med. Chem.* **2015**, 58, 6248–6263.
4. Junker, A.[∞]; Renn, C.[∞]; Dobelmann, C.[∞] *et al. J. Med. Chem.* **2019**, 62, 3677–3695.

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ECTO-NUCLEOTIDE

PYROPHOSPHATASE/PHOSPHODIESTERASE (E-NPP)

ACTIVITY IN NEURO-2A NEUROBLASTOMA CELLS

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Diadenosine polyphosphates (Ap_nAs) comprise a group of compounds formed by two adenosine moieties linked by a phosphate chain of variable length. Ap_nAs fulfill with the requirements to be considered as signaling molecules in the central nervous system: they are co-stored with ATP and other neurotransmitters in storage granules of neural and neuroendocrine cells. The exocytotic release of these compounds permits them to interact with P2 receptors, both metabotropic and ionotropic. Moreover, Ap_nAs can also activate specific receptors termed dinucleotide receptors. Extracellular actions of Ap_nAs are finished by ectonucleotidases that degrade these compounds yielding adenosine as the final product.

N2a cells display an ectoenzymatic hydrolytic activity able to degrade diadenosine polyphosphates. The Ap_nA-cleaving activity of these cells has been analyzed with the use of the fluorogenic compound BODIPY-FL-GTP γ S. Hydrolysis of this dinucleotide analog showed a hyperbolic kinetic with a K_m value of 4.9 ± 1.3 μ M. Ap₅A, Ap₄A, Ap₃A as well as the nucleoside monophosphate AMP behaved as inhibitors of BODIPY-FL-GTP γ S extracellular degradation. Ectoenzymatic activity shared the typical characteristics of the E-NPP family, as hydrolysis reached maximal activity at alkaline pH and was dependent on the presence of divalent cations, being strongly inhibited by EDTA and activated by Zn²⁺ ions. Both NPP1 and NPP3 isozymes are expressed in N2a cells, their expression levels substantially changing when cells differentiate into a neuronal-like phenotype. It is relevant to point the expression pattern of the NPP3 protein, whose levels were drastically reduced in the differentiated cells, being almost completely absent after 24 h of differentiation. Enzymatic activity assays carried out with differentiated N2a cells showed that NPP1 is the main isozyme involved in the extracellular degradation of dinucleotides in these cells, this enzyme reducing its activity and changing its subcellular location following neuronal differentiation.

MONO-ADP-RIBOSYLATION OF CD73 – A NEW LEVEL OF PURINERGIC CONTROL

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CD73-derived adenosine plays a major role in damage-induced tissue responses by modulating inflammation and tissue remodelling. Damage-associated stimuli, such as hypoxia and mechanical stress, induce the release of ATP and NAD and upregulate the expression of the nucleotide-degrading purinergic ecto-enzyme cascade, including CD73. Extracellular NAD can either be degraded to adenosine by this cascade or serve as substrate for mono-ADP-ribosylation. In humans, this post-translational protein modification of arginine residues at the cell surface is mediated by ADP-ribosyltransferase 1 (ART1) and has been shown to regulate protein function of membrane proteins as well as soluble factors. To explore, whether CD73 activity is regulated by mono-ADP-ribosylation, human recombinant CD73 was studied in the presence of ART1 using etheno-labelled NAD as substrate. Multi-colour Western Blot analysis showed an ART1-mediated transfer of ADP-ribose onto CD73. Mass spectrometry of *in vitro*-ribosylated CD73 identified six ribosylation sites of which two are likely to inhibit the enzyme in model analysis. UPLC analysis of the adenosine-generating activity of *in vitro*-ribosylated CD73 revealed that activity is inhibited by about 60% in comparison to non-ribosylated CD73. Flow cytometric analysis of human peripheral blood immune cells identified a CD73+/ART1+ double-positive B cell population, in which ART1-mediated mono-ADP-ribosylation of CD73 might be of functional relevance. Our study is the first to identify human CD73 as target for ART1-mediated mono-ADP-ribosylation, which importantly can modulate its adenosine-generating activity. Therefore, CD73 activity on ART1-expressing cell types might be subject to an NAD-dependent regulation, implicating that high CD73 expression, e.g. after tissue damage, is not necessarily associated with enhanced adenosine generation and signalling.

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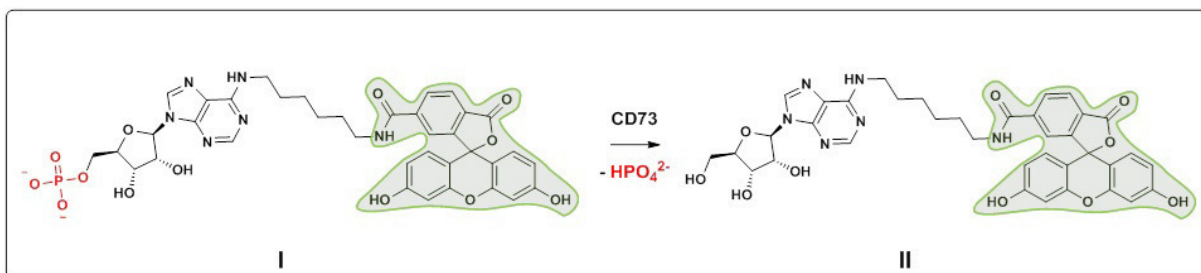
SYNTHESIS OF FLUORESCENCE-LABELED AMP AND ADENOSINE DERIVATIVES AS TOOLS FOR MONITORING CD73 ACTIVITY

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Ecto-5'-Nucleotidase (*eN*, CD73) is an ecto-enzyme which catalyzes the hydrolysis of extracellular nucleoside-5'-monophosphates, mainly AMP, leading to the formation of adenosine. Adenosine activates G protein-coupled adenosine receptors which results in anti-inflammatory, immunosuppressive and tumor-promoting effects. Blockade of CD73 has been proposed as a novel strategy in immuno-oncology.

The aim of the current work was to develop and establish a new fluorescence-based CD73 assay which would avoid the use of radioactive substrate and allow high sensitivity. To this end, we designed a fluorescence-labeled CD73 substrate (**I**), and developed a synthetic pathway to obtain **I** and its hydrolysis product **II**. Preliminary biological experiments showed that CD73 converts fluorescence-labeled AMP derivative **I** to the corresponding adenosine derivative **II**. In future studies, we will utilize **I** to monitor CD73 reactions to establish a new fluorescence-based CD73 assay.



P5. VESICULAR AND MEMBRANE TRANSPORTERS

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ANALYSIS OF THE EXPRESION AND FUNCTION OF VNUT IN POSTNATAL CEREBELLUM

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Adenosine triphosphate (ATP) is a relevant extracellular neurotransmitter which is involved un different processes such as cell differentiation, neuroprotection or axon guidance. ATP is store in secretore vesicles before being released through the Vesicular Nucleotide Transporter (VNUT). This transporter is expressed heterogenously in the mouse brain, being particulary abundant in the cerebellar cortex, where granular neurons represent the majority of neuronal population. These neurons are glutamatergic cells which express a variety of functional P2Y and P2X nucleotide receptors. Regardless the importante of the purinergic system in granular neurons neuroprotection, there is no information about the vesicular ATP storage and its relationship with VNUT expresion. In this present work, we analyzed the involment of VNUT in the vesicular release of ATP from primary cultures of cerebellar granule cells. Besides VNUT is expressed very early in the neuronal lineage of the cerebellar progenitors, indicating that it could contribute to the postnatal development of granular neurons. Therefore we also analyzed the role of VNUT in the lineage progression of neural progenitors isolated from early (P0) postnatal cerebellum.

P6. PURINERGIC SIGNALING IN NERVOUS SYSTEM

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ANATOMIC STUDY OF THE CENTRAL NERVOUS SYSTEM DEVELOPMENT EMPLOYING THE MOUSE STRAIN *P2rx7-EGFP*.

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The development of mammalian central nervous system (CNS) is a complex and dynamic process that requires an accurately orchestrated sequence of genetic, environmental and biochemical events. The generation of neural cells, i.e. neurons, astrocytes and oligodendrocytes, comprise a precise control of crucial processes as cell proliferation, cell fate determination, migration, maturation, synapse formation, network implementation and a final controlled apoptosis to define the correct neuronal number and location. The control of these processes accounts for multiple mechanisms including extracellular signaling molecules. Amongst these regulatory extracellular signaling, the purinergic system is one of the most recently discovered and least investigated. Purinergic signaling is driven by cell-surface purinoceptors (P2 receptors) classified in two different subfamilies, P2X and P2Y receptors. In the CNS, purinergic receptors regulates cell growth and migration during development, modulating, subsequently, glia-glia/neuronal-glia interactions, mechanosensory transduction and control of autonomic functions as the CNS matures. Among the P2 receptors, P2X7 receptor (P2X7R) constitutes one of the most promising target to regulate both physiology and pathology in the brain. However, the lack of specific and reliable technical and pharmacological approaches has classically been one of the major hurdles in the study of purinergic receptors and, in particular, the P2X7R. Transgenic mice expressing fluorescent proteins under the specific promoter of purinergic receptors are tools that facilitates the identification of the expression patterns of individual receptors during development. Specifically, the *P2rx7*-EGFP transgenic mice express EGFP immediately downstream of the *P2rx7* mouse promoter allowing for a detailed study of its distribution in the CNS. Here, we have employed this mouse strain to perform a comprehensive analysis of the pattern of expression of P2X7R during murine CNS development.

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NICOTINIC $\alpha 7$ RECEPTOR-INDUCED ADENOSINE RELEASE FROM PERISYNAPTIC SCHWANN CELLS CONTROLS ACETYLCHOLINE SPILLOVER FROM THE RAT MOTOR ENDPLATE

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Acetylcholine (ACh) spillover from the motor endplate region may occur after long nerve firing bursts. It may also appear in the presence of cholinesterase inhibitors that are commonly used to improve the neuromuscular transmission in patients with *Myasthenia gravis* or to reverse the residual neuromuscular blockade in the context of general anesthesia. Despite nicotinic $\alpha 7$ receptors ($\alpha 7$ nAChR) localized on perisynaptic Schwann cells (PSCs) can sense and control ACh spillover from the neuromuscular synapse, the mechanisms underlying communication between PSCs and the nerve terminal are not entirely understood. Here, we investigated whether adenosine could be the gliotransmitter mediating inhibition of transmitter release following $\alpha 7$ nAChR activation. Rat phrenic hemidiaphragms were used to measure nerve-evoked (i) myographic recordings, (ii) [³H]ACh release, and (ii) transmitter exocytosis using the FM4-64 fluorescent dye. The selective $\alpha 7$ nAChR agonist, PNU282987, decreased tetanic (50 Hz-bursts)-induced muscle contractions. This effect, which was mimicked by the cholinesterase inhibitor neostigmine, derives from inhibition of transmitter exocytosis detected as decreases in [³H]ACh release and FM4-64 dye unloading. The $\alpha 7$ nAChR antagonist, methyllycaconitine, and the gliotoxin, fluoroacetate, prevented the inhibitory effects of neostigmine and PNU282987. Removal of endogenous adenosine with adenosine deaminase (ADA, 2.5 U/ml), inhibition of adenosine release via ENT1 with S-(4-nitrobenzyl)-6-thioinosine (NBTI, 10 μ M), and blockade of A₁ receptors with 1,3-dipropyl-8-cyclopentylxanthine, all prevented inhibition of ACh exocytosis by PNU282987. Data suggest that $\alpha 7$ nAChR controls tetanic-induced ACh spillover from the neuromuscular synapse by favoring adenosine outflow from PSCs via NBTI-sensitive ENT1 transporters and activation of presynaptic A₁ inhibitory receptors.

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INHIBITION OF THE DOPAMINE TRANSPORTER AS AN ANIMAL MODEL OF MANIA MODULATES ADENOSINE RECEPTORS AND INFLAMMATORY RESPONSES IN THE BRAIN

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In this work, we characterized the adenosinergic receptors and enzymes as well as the inflammatory status in an animal model of mania induced by GBR12909 – a selective dopamine transporter inhibitor – as well as the effect of the mood stabilizer lithium carbonate.

Young adult C57Bl/6 mice (n = 20) received 12.5 mg/kg GBR 12909 (Sigma Aldrich) I.P. or vehicle (NaCl 0.9%; n = 30) I.P or lithium carbonate in water (Li₂CO₃ 1000 mg/L; n = 5). Prefrontal cortex, hippocampus, striatum and cerebellum were collected and immediately digested or frozen in dry ice and stored in -80°C until further experiments. Gene expression was analyzed by RT-qPCR; nucleotides levels were obtained by luciferase assay; cytokine profile was assessed by protein blotting and data was analyzed *in situ* with IPA software (Qiagen).

Our data show that lithium and GBR12909 effect vary according to the brain region. After GBR12909, adenosine receptors and ectonucleotidases showed significant alterations especially in striatum and cerebellum. Those regions also showed higher levels of pro-inflammatory cytokines and chemokines. ATP, ADP and AMP levels were also affected, but could be reversed by lithium treatment, especially in striatum. Hippocampus didn't show significant alterations, resembling resilient to both induction of disease and treatment. However, prefrontal cortex, showed high levels of anti-inflammatory cytokines, indicating a possible auto-immune response. Adenosine receptors A2a and A2b and the enzymes CD39 and CD73 seemed to be important targets for further investigation on the adenosinergic effect over neuroinflammatory responses.

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COMPARISON OF P2X7 PROTEIN EXPRESSION IN TWO BAC-TRANSGENIC REPORTER MOUSE MODELS

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High concentrations of extracellular ATP are sensed as a danger signal by the P2X7 receptor, a non-specific cation channel which is highly expressed in immune cells. Activation of this receptor in microglia or macrophages mediates the release of pro-inflammatory cytokine IL-1 β and its blockade or deletion has shown ameliorating effects in numerous inflammatory diseases. In addition, P2X7 plays a role in diverse brain diseases such as depression, Alzheimer's disease and epilepsy. Here, its pathophysiological role is less clear but P2X7-mediated effects on neurotransmitter release, neuronal excitability, and cell death have been shown. However, P2X7 expression and function in neurons and astrocytes have been challenging to demonstrate due to the poor specificity of the available antibodies and a complex pharmacology. In recent years, two BAC-transgenic reporter mouse models have been generated. In one line (Tg(P2rx7 EGFP)FY174Gsat), a sequence encoding a soluble EGFP has been inserted into Exon 1 of the P2rx7 gene while in the other line (Tg(RP24-114E20P2X7^{451P}-StrepHis-EGFP)Ani) the EGFP sequence has been fused into the last exon of the P2rx7 gene and a P2X7-EGFP fusion protein is expressed. Although both EGFP constructs are expected to be expressed under the control of the P2rx7 promoter, preliminary data indicate substantial differences in their specific cell-type expression. Thus, the P2X7-EGFP fusion protein is dominantly expressed in microglia and oligodendrocytes but was not detected in neurons. In contrast, the soluble EGFP shows an expression pattern that can be reconciled with neuronal expression. In this study, we perform immunofluorescence staining and co-labelling for cell type-specific marker proteins to provide a detailed comparative analysis of the EGFP expression in the CNS of both mouse models. In addition, characterization of P2X7 expression during mouse development is ongoing. Preliminary data will be presented.

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IDENTIFICATION OF P2X7-DEPENDENT TRANSCRIPTOME AFTER SEIZURES

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Current epilepsy treatments are targeting excitatory or inhibitory pathways without any disease-modifying effect, moreover these therapies are ineffective in over 30% of patients, suggesting a need to investigate new targets with different mechanisms of actions. The ATP-gated P2X7 receptor, implicated in seizure generation and driving inflammatory processes, has recently been shown to be a promising target for epilepsy, showing anticonvulsant and neuroprotective properties in animal models. However, there are important gaps in our knowledge about the mechanism of action of this receptor that must be filled. P2X7 down-stream targets must be identified to establish accessible biomarkers to predict a pathological P2X7 activation in the brain. Mounting evidences have showed that microRNA may be a key player in the pathogenesis of epilepsy, suggesting not only a therapeutic but also a diagnostic potential of microRNAs. Therefore, the aim of our study is to identify the P2X7 dependent transcriptome after status epilepticus focusing on microRNA changes. For this purpose we used intraamigdala injection of kainic acid model to induce status epilepticus in two groups of mice, P2X7 knock out and wild type. We collected the hippocampus 8 hours after the status epilepticus and extract the mRNA to perform miRNA open array. We found significant changes in knock out mice when compared to wild type in several miRNA, suggesting that P2X7 is involved in transcriptional changes during seizures.

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REGULATION OF THE UBIQUITIN-PROTEASOME SYSTEM BY P2X7 RECEPTOR, RELEVANCE ON NEUROLOGICAL PATHOLOGIES.

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The ubiquitin-proteasome-system (UPS) is the major intracellular pathway leading to the degradation of misfolded, unassembled, or damaged soluble proteins that could otherwise form potentially toxic aggregates. Dysregulation of UPS has been proposed as a common mechanism underlying several neurological pathologies, including Alzheimer's Disease (AD) and epilepsy. Taking into account that extracellular nucleotides through their selective purinergic P2Y2 receptor (P2Y2R) modulates the UPS activity, we wonder if the purinergic P2X7 receptor (P2X7R) is also involved in the UPS impairment associated with the neuroinflammation process. Using murine neuroblastoma cell line N2a, we found that the stimulation of native P2X7R by its selective P2X7 agonist 2'(3')-O-(4-benzoylbenzoyl) ATP (BzATP) induced a significant reduction in two of three peptidase proteasome activities, Chymotrypsin (CT) and Postglutamyl (PG) activities. Similar results were obtained using *in vivo* approaches, finding that intracerebroventricular administration of BzATP to wild type mice caused a significant reduction on both hippocampal proteasomal activities, but not in P2X7R Knock-Out mice (P2X7R KO). Accordantly, specific P2X7R antagonist (A438073) increased the chymotrypsin activity in the hippocampus of wild-type mice. Furthermore, additional analysis revealed that P2X7R KO mice present a decrease on polyubiquitinated conjugates, whereas P2X7R Overexpression mice (P2X7R OX) shown an increase in the accumulation of those proteins 24 hours after *status epilepticus* triggered by an intra-amygdala administration of kainic acid (KA). Altogether, our results suggest that P2X7R may be a novel selective target to regulate the proteasomal alterations related to neurological pathologies.

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THE CONTRIBUTION OF THE P2X7R TO DRUG REFRACTORY STATUS EPILEPTICUS

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1. Introduction

60 million people worldwide suffer from epilepsy and there are currently approximately 25 marketed anti-epileptic drugs (AED). With almost 30% of epilepsy patients becoming refractory to these drugs, it is paramount to focus on targeting specific therapeutic areas of the brain to fully impact on disease progression. P2X7R has been shown to play a pivotal role in inflammation but the question still remains as to its specific behavior and how it can affect sensitivity to anti-convulsant treatment. It has been shown previously that P2X7R antagonism reduces seizure duration, seizure-induced neuronal death and was also neuroprotective. Here, we are attempting to understand its influence on anti-convulsant drugs and whether P2X7R antagonism is a potential therapeutic strategy to treat drug-refractory epilepsy.

2. Methods

We are using a novel, transgenic P2X7R overexpressing mouse to determine the effects of the receptor on the efficacy of anti-convulsants and its cell-specific expression. Green fluorescent protein (GFP) is bound on the promoter region of the P2X7 protein so that it is observable using fluorescent microscopy where the receptor is expressed. Investigation of the effects of P2X7 overexpression is carried out using a combination of EEG analysis, immunohistochemistry, western blotting as well as qPCR to determine the differences/patterns of seizure activity, protein and mRNA expression, cell death and response in epileptic mice.

3. Statistical Analysis

GraphPad Prism is used to carry out statistical analysis, where group comparisons of will be analysed using unpaired t-tests and ANOVA analysis as appropriate.

4. Results and Conclusion

Firstly, we showed P2X7 activation occurs during inflammation, and observed increased P2X7 at 72hrs. We then carried out intra-amygdala kainic acid (KA) injections to induce epilepsy. Following KA administration, we found no effect of P2X7 overexpression on the severity of electrographic seizures. Despite no difference in seizure severity, however, overexpression of P2X7 led to a marked resistance to lorazepam, midazolam and carbamazepine with ongoing increased power in the EEG, compared to controls. We concluded that P2X7 may contribute to drug resistance during SE and epilepsy, thus, P2X7 antagonists may be a good as an adjunctive treatment for epilepsy.

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ADENOSINERGIC SYSTEM: NOVEL THERAPEUTIC TARGET FOR RETT SYNDROME

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Rett syndrome (RTT) is genetic disorder that originates severe intellectual disabilities besides other symptoms such as epilepsy, developmental stagnation, loss of hand skills, development of stereotypic hand movements and social withdrawal. RTT is mainly caused by mutations in the X-linked *MECP2* gene, which leads to impairment of signaling of the neurotrophin brain-derived neurotrophic factor (BDNF). The increase of BDNF signaling in RTT would be a significant breakthrough but it has been hampered by the difficulty to administer BDNF to the central nervous system. Adenosine, an endogenous neuromodulator, may accomplish that role since through A₁R it has antiepileptic actions and through A_{2A}R it potentiates BDNF synaptic actions in healthy animals. We thus characterized several hallmarks of the the adenosinergic and BDNF signaling in RTT and explored whether the activation of A_{2A}R could boost BDNF action in a RTT animal model, the *Mecp2* knockout (*Mecp2*^{-/-}) (B6.129P2 (C)-Mecp2tm1.1Bird/J) mouse. Whenever possible, parallel data was also obtained from post-mortem brain samples from a RTT patient.

The results show that BDNF facilitatory actions upon long-term potentiation (LTP) were absent in the RTT model. In the mice model there was also a significant reduction in TrkB full length (TrkB-FL) receptors levels and a tendency for compensatory alterations in TrkB-FL mRNA, detected both in mouse and human samples. Data obtained suggest a reduction on adenosine levels, and a consequent decrease of inhibitory adenosinergic tonus via A₁R and A_{2A}R, which was further aggravated by a significant decrease in the A_{2A}R receptor protein levels. Remarkably, activation of A_{2A}R with the selective agonist, CGS2168, rescued BDNF effect upon LTP. These findings set the stage for adenosine-based pharmacological therapeutic strategies for RTT, highlighting A_{2A}R as therapeutic targets in this devastating pathology.

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THE P2X7 RECEPTOR'S CONTRIBUTION TO NEONATAL SEIZURES AND THE DEVELOPMENT OF CHRONIC EPILEPSY AND NEURO-COGNITIVE DEFICITS.

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Neonatal seizures are a neurological emergency. Hypoxic-ischemic encephalopathy accounts for 60% of neonatal seizures and is associated with development of epilepsy and neuro-cognitive deficits in later life. Current treatments for neonatal seizures remain largely ineffective. The P2X7 receptor (P2X7R) is an ionotropic ATP-gated ion channel that is activated under pathological conditions, with a key role as a driver of inflammation. Antagonism of the P2X7R reduced the seizure burden in neonatal mice when exposed to global hypoxic conditions. The aim of this project is to further understand the mechanism of this action and the possible therapeutic use of P2X7R antagonism for neonatal seizures.

Seizures are induced in P7 mouse pups by exposure to global hypoxia (5% oxygen) for 15mins. This study uses multiple transgenic mouse models that either globally overexpress or knockout the P2X7R. Acute electroencephalography and behavioural seizures will be recorded to investigate the P2X7R's immediate role in neonatal seizures. At various times following seizure induction, markers of inflammation will be analysed by immunohistochemistry and ELISA methodology. Seizure related neuronal damage will be investigated with use of Silver Nissl staining. The overexpressing mouse model has P2X7R tagged with green fluorescent protein and will be used to locate where and what cell types P2X7R is upregulated following neonatal seizures. In a cohort of mice, a battery of behavioural assays and a seizure threshold test with kainic acid will be conducted 4 weeks post seizures after pharmacological antagonism of the P2X7R and in the transgenic models to investigate the P2X7R's role in the development of neurocognitive deficits and later life epilepsy respectively.

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CONTROL OF THE BLOOD-BRAIN BARRIER INTEGRITY DURING SEIZURES VIA THE ATP-GATED P2X7 RECEPTOR

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Epilepsy is a chronic neurological disease affecting more than 60 million people worldwide. Despite more than 25 anti-epileptic drugs available, 30% of patients are pharmacoresistant. The blood-brain barrier (BBB) is a specialized regulator that separates the bloodstream from the brain parenchyma. A functional BBB is crucial in maintaining brain homeostasis and preventing the entry of aberrant compounds and immune cells into the Central Nervous System (CNS). However, during epilepsy the permeability of the BBB may increase, resulting in blood-borne molecules and cells entering the CNS. Leakage of the BBB is one of the earliest characteristic disturbances following status epilepticus (SE) and may play an important role in the development of epilepsy. The purinergic P2X7 receptor (P2X7R) has been associated with numerous damaging mechanisms related to epileptogenesis, such as inflammation and inducing leakage of the BBB. However, we do not know whether seizure induced changes of the BBB are dependent on P2X7R signalling and whether this process can be targeted. P2X7R's impact on the BBBs integrity during seizures was studied using the intra-amygdala kainic acid model in a newly developed P2X7R overexpressing mouse model. Brains were removed at different time-points post-SE and were analysed by immunohistological techniques. Then, we evaluated changes in Podocalyxin (PODXL) that plays crucial roles in the proper functioning of the BBB as well as in disease progression. We identified an overall increase in the expression of P2X7R at 4 and 8 hours post-SE. Moreover, in these overexpressed P2X7R cells there was an enrichment in PODXL expression. Finally, our study demonstrates that following SE there is an increase of P2X7R in blood vessels suggesting P2X7R impacting on BBB permeability. This result indicates that P2X7R signalling may underlie pathological changes in BBB permeability and has an impact in the epileptogenesis process. Consequently, drugs targeting BBB function and P2X7R may represent novel treatment strategies in epilepsy.

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ADENOSINE-MEDIATED CONTROL OF SYNAPTIC AND BEHAVIOURAL ACTIONS OF CANNABINOIDS

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Cannabinoid use either for clinical or recreational purposes impacts in brain function and cognition. Therefore, it is of uttermost importance to understand in detail the synaptic and brain circuitry bases of the actions of cannabinoids as well as to discover strategies to mitigate the negative side-effects of cannabinoid-based therapies. The ubiquitous distribution of adenosine receptors in the brain, and the previous knowledge on the interaction between cannabinoid CB1 receptors and adenosine A_{2A} receptors (A_{2A}R) at the striatum (Fuxe et al., 2008; Ferré et al., 2009), inspired us to evaluate how adenosine receptors could affect synaptic and memory behavioural actions of cannabinoids. Prolonged intake of a cannabinoid receptor agonist affects brain metabolism and connectivity in brain areas relevant for memory processing (Mouro et al., 2018). A₁R overexpression in the forebrain caused by chronic caffeine intake exacerbates memory deficits induced by a single administration of tetrahydrocannabinol (THC) to rats or mice (Sousa et al., 2011). The synaptic mechanisms underlying this interaction remain to be fully understood but may involve GABAergic interneurons since A₁R activation attenuates the inhibitory action of the cannabinoid receptor agonist upon GABA release (Sousa et al., 2011) and selectively attenuates tonic inhibition of cannabinoid type 1 receptor positive (CB1R⁺) hippocampal interneurons (Rombo et al., 2016). The inhibitory action of CB1Rs on synaptic transmission (Serpa et al., 2009), the dual influence of endocannabinoids upon synaptic plasticity (Silva-Cruz et al., 2017) or the inhibitory action of CB1Rs upon cyclic AMP accumulation (Serpa et al., 2015), do not seem to be influenced by A₁R activity. Concerning A_{2A}R, we found out that they markedly influence cannabinoid actions in the forebrain. Thus, the inhibitory action of a CB1R agonist upon long-term potentiation (LTP), a synaptic correlate of memory processing, is attenuated by A_{2A}R blockade (Mouro et al., 2019). Accordingly, A_{2A}R antagonists are able to prevent memory consolidation deficits caused by both acute and chronic administration of a CB1R agonist (Mouro et al., 2017; 2019). The beneficial action of A_{2A}R antagonists to mitigate cannabinoid induced memory deficits are still evident upon prolonged agonist administration of the A_{2A}R antagonists (Mouro et al., 2019). Also, prolonged A_{2A}R antagonism does not cause significant alterations in A_{2A}R levels in the forebrain, as assessed by specific binding assays (Mouro et al., 2019). Taken together, our data highlights that selective A_{2A}R blockade, but not caffeine, is a promising strategy to mitigate cognitive side effects when therapeutic use of cannabinoids is desirable.

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Ferré et al. (2009) *Neuropharmacology*. 56 Suppl 1:226-34; Fuxe et al. (2008) *Brain Res Rev.*, 58:415-52; Mouro *et al* (2018) *J Neurochem.*, 47:71-83; Mouro *et al*. (2017) *Neuropharmacology*, 117:316-327; Mouro *et al*. (2019). *Neuropharmacology*, 155:10-21; Rombo *et al* (2016) *Cereb Cortex* 26:1081-1095; Serpa *et al* (2009) *Eur J Pharmacol.* 623:41-46; Serpa *et al*. (2015) *Biomed Res Int.*, ID 872684 eCollection; Silva-Cruz *et al* (2017) *Front Pharmacol.* 8:921.eCollection; Sousa *et al*. (2011) *Neuropsychopharmacology*, 36, 472-487.

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CONTEXT-SPECIFIC SWITCH FROM ANTI- TO PRO-EPILEPTOGENIC FUNCTION OF THE P2Y₁ RECEPTOR IN EXPERIMENTAL EPILEPSY

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Extracellular ATP activates inflammatory responses to tissue injury and is also implicated in establishing lasting network hyperexcitability in the brain by acting upon independent receptor systems. Here, we demonstrate that the metabotropic P2Y₁ receptor mediates both pro- and anti-convulsive responses, depending on context. Using different mouse models of status epilepticus and genetic and pharmacologic tools, we show that activation of the P2Y₁ receptor prior to seizure onset is anticonvulsant. Once seizure activity has been triggered, however, activation of the P2Y₁ receptor is pro-convulsive. Pharmacologic P2Y₁ blockade during status epilepticus reduced seizure severity and attendant brain damage, delayed the development of epilepsy and, when applied during epilepsy, suppressed the occurrence of spontaneous seizures, in mice. Our data demonstrates that the effect of targeting P2Y₁ is dependent on the switching behavior of this metabotropic receptor and provides evidence that drugs targeting P2Y₁ may be useful in the treatment of drug refractory status epilepticus and epilepsy.

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THE EFFECT OF P2X7 RECEPTOR DEFICIENCY ON C-FOS ACTIVATION IN AN ACUTE PCP INDUCED SCHIZOPHRENIA MODEL IN MICE

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P2X7 receptor (P2X7R) is an ATP-gated ionotropic channel expressed in the central nervous system by different cell populations. Genetic ablation or pharmacological antagonism of P2X7R shows protective effect in a wide range of models for psychiatric disorders.

Our group recently described (Koványi et al. 2016) that in a low-dose (2-5 mg/kg) phencyclidine (PCP) induced model of schizophrenia, the blockade or deletion of the p2rx7 gene attenuates PCP-induced hyperlocomotion, stereotype behavior and social withdrawal in mice. Moreover, it attenuates PCP-induced changes in the transcriptional regulation of proteins, affecting excitatory and inhibitory transmission and synaptic function in the prefrontal cortex.

A high dose of PCP delivered systemically, affects the activity and connectivity of different brain areas, mimicking the pattern of activation during schizophrenic episodes in humans (Parsonen et al. 2017). The medial prefrontal cortex (mPFC) in mice, corresponding to the dorsolateral cortex in humans, is of particular interest for what concerns schizophrenia-related cognitive deficits.

In this study, we have studied the effect of PCP in the mPFC using a sub-chronic (10 mg/kg ip per 7 days, + 3-10 days of washout) and an acute (10 mg/kg ip) model. In case of sub-chronic PCP model, we could not observe any notable signs of inflammatory reaction either in WT or P2X7R KO animals.

After a single injection of PCP (10 mg/kg ip), P2X7R KO mice displayed a lower number of c-fos immunoreactive neurons in the mPFC. The behavior of the treated P2X7 receptor KO animals was also different, displaying lower PCP-induced hyperlocomotion and stereotypic activity. Exploring how the genetic ablation of p2rx7 gene plays a role in counteracting the acute psychotomimetic effect of PCP could reveal novel function of the protein as well as help to disentangle the neurological substrate of the action of the arylcyclohexyl anesthetic drugs.

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INVESTIGATING THE ROLE OF GUANOSINE IN HUMAN NEUROBLASTOMA CELL DIFFERENTIATION.

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Neuroblastoma is the most common solid extracranial tumor of infancy. It arises from the neural crest cell precursors that fail to complete the process of differentiation; therefore, agents able to help tumor cells to differentiate into normal cells represent a valid therapeutic approach. To date, only few differentiating agents are available for therapy. Since guanosine, a natural purine nucleoside, increases the NGF-induced cell differentiation in rat hippocampal neurons and rat pheochromocytoma cells, the aim of this study was to evaluate whether extracellular guanosine was involved in the human neuroblastoma SH-SY5Y cell line differentiation and to investigate the molecular mechanisms involved. For this purpose, guanosine was added to cell medium and the percentage of differentiated cells (showing neurite *greater than one cell body length*) was evaluated. The analysis of signaling pathways involved was carried out using western blot and cGMP enzyme immunoassay while guanosine metabolism was evaluated by HPLC. We found that guanosine promoted neuronal differentiation in a concentration-dependent manner, an effect already evident after 24h and mainly induced by its extracellular action as demonstrated using transporter inhibitors. Guanosine increased phosphorylated ERK1/2 and Akt in a concentration-dependent manner and enhanced cGMP level via heme-oxygenase. The neuritogenic activity of Guanosine was significantly reduced by specific inhibitors of ERK1/2, heme-oxygenase or soluble guanylate cyclase but not by Akt inhibitors. Interestingly, in the medium, guanosine was largely metabolized into guanine by purine nucleoside phosphorylase (PNP) enzyme released from cells. Thus, our study suggests that guanosine, promoting neuroblastoma differentiation, may represent a potential therapeutic agent. However, due to its spontaneous extracellular metabolism, the role played by guanosine-PNP-guanine system needs to be further investigated.

INVOLVEMENT OF P2X7RS IN EXCITATORY NEUROTRANSMISSION IN THE MOUSE DENTATE GYRUS GRANULE CELLS

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P2X7 receptors (P2rx7) the ligand gated ion channels, regulate a diverse array of normal and pathological brain function, including learning and memory formation, mood and behavior. However, the cellular mechanisms underlying these functions are far from fully explored yet. Therefore, we studied here the involvement of P2X7Rs in excitatory neurotransmission in dentate gyrus granule cells. To address this question, we mainly utilized patch clamping technique with whole cell configuration to record miniature excitatory postsynaptic current (mEPSC) in both wild-type and P2rx7^{-/-} mice under the model of voltage clamp. During recording, Mg²⁺free/low Ca²⁺ ACSF solution was bath-applied together with TTX (Na⁺ blocker, 1 μM) and GABAzine (GABA_A antagonist, 10 μM). To isolate NMDA-mediated mEPSC, CNQX (AMPA/kainate receptor antagonist, 20 μM) was applied to block AMPA-related events. As for AMPA-mediated mEPSC recording, DL-AP5 (NMDA receptor antagonist, 50 μM) was used to block NMDA-related events. After each recording, CNQX or DL-AP5 was employed to confirm the events induced by NMDA or AMPA. Furthermore, selective antagonist of P2X7Rs (JNJ-47965567, 10μM) was perfused to replicate the effect of P2rx7 gene deficiency. Finally, the morphological features of studied cell filled with biocytin has been visualized based on confocal images. We found that the amplitude and frequency of NMDA-mediated mEPSC significantly decreased in P2rx7^{-/-} mice compared to WT counterparts whereas JNJ-47965567 decreased the frequency, but not the amplitude. AMPA-mediated mEPSCs were also decreased in in P2rx7^{-/-} mice and JNJ-47965567 replicated these results. To summarize, P2X7Rs participate in the modulation of excitatory neurotransmission partly through an action potential-independent mechanism in adult dentate gyrus granule cells.

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NLRP3 INFLAMMASOME PATHWAY MEDIATES THE EFFECT P2X7 RECEPTOR ACTIVATION IN THE MATERNAL IMMUNE ACTIVATION MODEL OF AUTISM

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Autism spectrum disorder (ASD) is a complex neurodevelopmental condition caused by interactions of environmental and genetic factors. We have recently shown that the activation of P2X7 receptors elicit ASD-like offspring phenotype in the maternal immune activation (MIA) model of ASD in mice. The NLRP3 inflammasome assembly is an important downstream signalling pathway mediating the effect of P2X7 receptor activation. Because MIA lead to the induction of pro-inflammatory cytokine response with the participation of P2X7 receptors and cytokines can compromise fetal brain development, the aim of this study was to clarify the role of the NLRP3 in this process.

MIA was induced by poly(I:C) (2 x 3mg/kg i.p) in wild-type and P2rx7 deficient pregnant mouse dams. Before poly(I:C) treatment, mice were injected with the selective NLRP3 inflammasome antagonist (MCC950, 1x50 mg/kg). We carried out autism relevant behavior tests on young adult male offspring and brain samples were collected for determination of the Purkinje cell density in the lobeVII. of the cerebellum and electronmicroscopic examination of synaptosome integrity.

Maternal poly(I:C) treatment elicited social deficit, increase in repetitive behaviors and impairment of sensorimotor coordination. In addition, elevated number of malformed synaptosomes and lowered Purkinje cell density was observed. Administration of MCC950 prevented the development of these autistic features. In P2rx7 deficient mice, poly(I:C) treatment did not induce autistic phenotype.

In conclusion, poly(I:C) treatment induced autism-like behavior and morphological changes in the offspring by the activation of P2X7 receptor and with the participation of NLRP3 inflammasome signaling pathway. These results may help to clarify the downstream signalling pathway of P2X7 receptor, thus finding potential pharmacotherapeutic targets of ASD in the future.

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ARE micro-RNAs THERAPEUTIC TARGETS FOR SPINAL CORD INJURY EXCITOTOXICITY? microRNA-135a-5p REDUCES P2X₇-DEPENDENT RISE IN INTRACELLULAR CALCIUM AND PROTECTS AGAINST EXCITOTOXICITY.

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Release of large amounts of neurotransmitters after traumatic spinal cord injury (SCI) causes excitotoxicity, contributing to extend neural damage and functional deficits. The P2X₇ receptor participates in the excitotoxic processes that follow SCI, leading to a massive influx of calcium and the activation of the apoptotic death. miRNAs are a family of small non-coding RNAs capable of post-transcriptionally silencing the expression of protein-coding genes to regulate major cell processes. We hypothesize that the downregulation of miRNAs targeting P2X₇ contributes to receptor overexpression after SCI and that restoring their levels to pre-injury conditions will reduce P2X₇-mediated excitotoxicity. We combined data from gene expression analyses, predictive algorithms, computational tools and luciferase reporter gene assays to identify microRNA-135a-5p (miR-135a) as a post-transcriptional modulator of P2X₇ expression. RT-qPCR confirmed that miR-135a expression decreases after SCI, in inverse correlation with P2X₇ overexpression. Transfection of Neuro2a neuroblastoma cell line with an inhibitory sequence (antagomiR-135a), simulating the miR-135a downregulation that occurs after SCI, led to an increase in mRNA and protein levels of P2X₇. Functionally, Fura-2AM and flow cytometry assays indicated that miR-135a inhibition results in an increase of P2X₇-dependent intracellular calcium concentration and excitotoxic cell death induced by ATP (300 μ M) and Bz-ATP (100 μ M). On the contrary, transfection with a miR-135a mimic reduced P2X₇ expression and calcium-dependent excitotoxic cell death. Therefore, we conclude that restoration of miR-135a expression after SCI may reduce the deleterious effects of ATP-dependent excitotoxicity. In this direction, we are developing a therapeutic strategy for the administration of miR-135a in the spinal cord of rat models of SCI using polymeric vehicles to reduce the extension of the secondary phase of neural cell death processes following SCI, that are induced, in part, by massive ATP release and P2X₇-dependent excitotoxicity.

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AGE-RELATED NUCLEAR TRANSLOCATION OF P2X6 SUBUNIT MODIFIES SPLICING ACTIVITY INTERACTING WITH SPLICING FACTOR 3A1

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P2X6 subunit expression and immunoreactivity is observed throughout the central nervous system, especially in hippocampus and cerebellum. The ability of this subunit to form homomeric receptors is very inefficient, but its presence associated with P2X2 and P2X4 subunits is well documented. The abundance of mRNA and protein expression of P2X6 in hippocampus is relatively high in neuronal bodies of CA1, CA2, CA3 regions and dentate gyrus. In this work we report, for the first time, the accumulation of the P2X6 subunit inside the nucleus of hippocampal neurons in an age-dependent way. Noticeable, a similar nuclear location of P2X6 subunits was observed in ependymal spinal cord-derived stem/progenitor cells from adult rats. This nuclear location is favored by its anchorage to endoplasmic reticulum through its N-terminal domain, and interestingly, we found that the extracellular domain of P2X6 subunit is responsible for its nuclear and perinuclear location. Nuclear P2X6 subunit presents a speckled distribution pattern and is retained by interaction with the nuclear envelope protein spectrin $\alpha 2$. *In vivo* results showed that, once inside the nucleus, P2X6 subunit interacts with the splicing factor 3A1, which ultimately results in a reduction of the mRNA splicing activity. Our data provide new insights into post-transcriptional regulation of mRNA splicing, describing a novel mechanism that could explain why this process is sensitive to changes that occur with age. Thus, the negative modulation that nuclear accumulation of the P2X6 subunit elicits on splicing activity, could explain, at least in part, the events associated with cellular reprogramming of neuronal consolidation.

References:

- Díaz-Hernández JI, Sebastián-Serrano Á, Gómez-Villafuertes R, Díaz-Hernández M, Miras-Portugal MT. (2015) Age-related nuclear translocation of P2X6 subunit modifies splicing activity interacting with splicing factor 3A1. PLoS One 10(4):e0123121.
- Purinergic Receptors in Spinal Cord-Derived Ependymal Stem/Progenitor Cells and Their Potential Role in Cell-Based Therapy for Spinal Cord Injury. (2015) Gómez-Villafuertes R, Rodríguez-Jiménez FJ, Alastrue-Agudo A, Stojkovic M, Miras-Portugal MT, Moreno-Manzano V. Cell Transplant 24(8):1493-509.

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PHYSICAL AND FUNCTIONAL INTERACTION BETWEEN GPR17 AND THE CHEMOKINE RECEPTOR 2: A PROMISING TOOL FOR MANAGING REMYELINATION

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The reconstitution of a functional myelin sheath in the central nervous system (CNS) is the cornerstone for the functional recovery in multiple sclerosis patients. Recent findings demonstrated that agonist compounds of the G-protein coupled receptor GPR17 can promote the activity of oligodendrocyte precursor cells (OPCs) towards CNS remyelination. Besides sugar nucleotides and leukotrienes, GPR17 can be promiscuously activated by pro-inflammatory oxysterols and chemokines released in demyelinating lesions, suggesting that a balanced interplay between GPR17 and the CXCR receptors may be central for OPC migration and successful remyelination.

Herein, the chemokine receptor CXCR2 was selected to establish the structural and functional interactions with GPR17. In particular, the relative propensity of GPR17 and CXCR2 to form heterodimers was assessed by immunoenzymatic assay, and the ability of CXCR2 to modify GPR17 functionality and *viceversa* was investigated by determining the receptor-mediated modulation of intracellular cAMP.

The CXCR2 and GPR17 receptors were found to physically interact in basal conditions. The receptor association was reduced in the presence of CXCR2 receptor ligands, and not affected by GPR17 receptor modulation. In contrast, when the GPR17 receptor agonist was combined with a CXCR2 antagonist, the significant decrease in GPR17-CXCR2 interaction persisted.

GPR17 functionality was modulated by the presence of the CXCR2 receptor only when CXCR2 is blocked by its antagonist. In contrast, CXCR2 functionality was modulated when GPR17 is stimulated by its agonist.

Overall, these results suggest that GPR17-CXCR2 interaction can be modulated differently by the activation or block of CXCR2 and confirm the importance of a functional interplay between the two receptors. Supported by FISM 2015 cod. 2015/R/11.

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P2X7 RECEPTOR ANTAGONISM WITH JNJ-47965567 REDUCED BODY WEIGHT LOSS AND DELAYED DISEASE ONSET IN FEMALE AMYOTROPHIC LATERAL SCLEROSIS SOD1^{G93A} MICE

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Neuroinflammation is one of the main physiopathological mechanisms of amyotrophic lateral sclerosis (ALS), a rapidly progressive and devastating neurodegenerative disease caused by the specific loss of motor neurons. The chronic activation of microglia is mainly responsible for this inflammatory process in the central nervous system (CNS), which is triggered by the activation of the ATP-gated P2X7 receptor (P2X7R). The present study aimed to evaluate the effect of the chronic treatment with the P2X7R antagonist JNJ-47965567 in the development and progression of ALS in the SOD1^{G93A} murine model.

SOD1^{G93A} mice, hemizygote for the mutated human SOD1 gene, were i.p. injected with either 30 mg/kg of JNJ-47965567 or vehicle four times a week, from pre-onset age (60-80 days of age) until study end-point. Body weight, motor coordination, phenotypic score, disease onset and survival were measured throughout the study and compared between vehicle- and drug-injected groups.

Treatment with the P2X7R antagonist JNJ-47965567 delayed disease onset in SOD1^{G93A} mice of combined sex (119±5 vs. 136±3 days), but had no effect on body weight loss, motor coordination, phenotypic score and survival. However, the treatment significantly reduced body weight loss and delayed disease onset in SOD1^{G93A} females (128±2 vs. 145±2 days), but not in males. In conclusion, our results suggest a partial, yet important effect of P2X7R in the development and progression of ALS. Moreover, they point out a possible gender effect of the treatment with P2X7R antagonists that could be of high relevance if translated into the clinic and raises new interesting questions about the physiopathology of ALS.

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CONTRIBUTION OF P2Y₁₂ RECEPTORS TO ANIMAL MODEL OF NTG-INDUCED MIGRAINE-ASSOCIATED PAIN

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Purinergic receptors are known to regulate different forms of pain as well as migraine headache. To explore the contribution of P2Y₁₂ receptors (P2Y₁₂Rs) to migraine pain, we used a nitroglycerin (NTG)-induced mouse model of migraine.

Migraine-like pain and behavioral alterations was induced by intraperitoneal NTG (15 mg/kg) in wild-type and P2ry12 gene-deficient (*P2ry12*^{-/-}) mice. In addition to test the effect of P2Y₁₂R inhibition the highly potent and direct-acting P2Y₁₂ receptor antagonist PSB-0739 was used.

NTG induced thermal hyperalgesia, increased head grooming time and caused photophobia in wild-type and *P2ry12*^{-/-} mice, followed by the induction of cytokines and c-fos in upper cervical spinal cord and trigeminal nucleus caudalis (TNC). PSB-0739 (0.3 mg/kg i.t.) reversed thermal hyperalgesia in wild-type mice but had no effect in *P2ry12*^{-/-} mice, and it was also effective when applied as a post-treatment. PSB-0739 attenuated the expression of c-Fos in TNC in wild-type mice after NTG treatment. NTG itself did not change ADP-induced platelet activation measured by CD62P upregulation in wild-type mice. Platelet depletion by an anti-mouse CD41 antibody attenuated NTG-induced thermal hypersensitivity and affected pro- and anti-inflammatory cytokine response in wild type mice.

In conclusion, P2Y₁₂ receptors regulate NTG-induced thermal hyperalgesia, the neurogenic inflammatory response, and expression of c-Fos in migraine related areas of the CNS. P2Y₁₂Rs might participate in the pathogenesis of migraine.

A POSSIBLE IMPLICATION OF THE ATP-GATED P2X7 RECEPTOR ON STATUS EPILEPTICUS-INDUCED ABERRANT NEUROGENESIS

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New neurons are generated continuously during the entire lifespan in the adult brain of mammals, including humans. Neurogenesis occurs in the subventricular zone, in the walls of the lateral ventricles, and the subgranular zone, in the dentate gyrus of the hippocampus, the two main adult neurogenic niches. This physiological process is upregulated following acute insult, such as status epilepticus, traumatic brain injury or stroke. Emerging data has demonstrated a prominent role of extracellular ATP in regulating neurogenesis. While P2X7 has been shown to alter the rate of neurogenesis, however, whether P2X7 also alters status epilepticus-induced neurogenesis has not been fully addressed.

Using an intra amygdala kainic acid mouse model of status epilepticus, in a transgenic mouse overexpressing P2X7 receptor, we used immunohistochemistry to visualize changes in the distribution of markers of neuronal development (e.g. Nestin, Doublecortin, NeuN) and trackers such as Iodo/Chloro-deoxyuridine (IdU/CldU), injected respectively 3 days and 17 days after the kainic acid injection.

The rate of neurogenesis in the dentate gyrus is increased in kainic acid-injected mice, with an evident presence of newly generated neurons, with a noticeable change in morphology, and located ectopically in the hilus. P2X7 overexpression increased not only the number of trackers positive cells in the subgranular zone and in the granular layer of the hippocampus, but also on status epilepticus-induced aberrant neurogenesis with several cells detected in the hilus.

In conclusion, using this animal model of epilepsy together with genetic manipulation permitted to investigate the involvement of P2X7 receptor in aberrant neurogenesis following status epilepticus.

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FACILITATION OF SYNAPTIC, BUT NOT EXTRASYNAPTIC, NMDA CURRENTS IN CA1 PYRAMIDAL NEURONS BY ADENOSINE A_{2A}R IN YOUNG ADULT RATS.

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NMDA receptors play a key role in both synaptic plasticity and neurodegeneration. Adenosine is an endogenous neuromodulator and through membrane receptors of the A_{2A} subtype can influence both synaptic plasticity and neuronal death. The present work was designed to evaluate the influence of adenosine A_{2A} receptors upon NMDA receptor activity in CA1 hippocampal neurons. We discriminated between modulation of synaptic versus extrasynaptic receptors, since extrasynaptic NMDA receptors are mostly associated with neurodegeneration while synaptic NMDA receptors are linked to plasticity phenomena. Whole-cell patch-clamp recordings were obtained to evaluate NMDA receptor actions on CA1 pyramidal neurons of young adult (5–10 weeks) male Wistar rat hippocampus. Activation of A_{2A} receptors with CGS 21680 (30 nM) consistently facilitated chemically-evoked NMDA receptor-currents (NMDA- PSCs) and afferent-evoked NMDA-currents (NMDA-EPSCs), an action prevented by an A_{2A} receptor antagonist (SCH58261, 100 nM) and a PKA inhibitor, H-89 (1 μM). These actions did not reflect facilitation in glutamate release since there was no change in NMDA-EPSCs paired pulse ratio. A_{2A} receptor actions were lost in the presence of an open-channel NMDA receptor blocker, MK-801 (10 μM), but persisted in the presence of memantine, at a concentration (10 μM) known to preferentially block extrasynaptic NMDA receptors. These results show that A_{2A} receptors exert a positive postsynaptic modulatory effect over synaptic, but not extrasynaptic, NMDA receptors in CA1 neurons and, therefore, under non-pathological conditions may contribute to shift the dual role of NMDA receptors towards enhancement of synaptic plasticity.

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SYNAPTIC DYSFUNCTIONS AT THE HIPPOCAMPUS OF AN AMYOTROPHIC LATERAL SCLEROSIS MOUSE MODEL (SOD1^{G93A}): REVERSAL BY ADENOSINE A_{2A}R BLOCKADE

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Amyotrophic Lateral Sclerosis (ALS) is mainly a motor disease, although extramotor neural and cognitive alterations have also been reported. Our aim was to access the changes in the activity and plasticity of synapses in SOD1^{G93A} mice through disease progression. We then assessed how those changes could be modified by an antagonist of a receptor frequently involved in excitotoxicity, the adenosine A_{2A} receptor, which is overexpressed and/or overactivated in SOD1^{G93A} mice and in ALS patients.

Field-excitatory post-synaptic potentials (fEPSP) were recorded in hippocampal slices from SOD1^{G93A} mice at pre-symptomatic (4-6 W) and symptomatic (14-18 W) stages, and from age-matched wild-type mice. θ -burst-induced LTP and paired-pulse facilitation (PPF) were recorded as previously. Statistical analysis was by the student's *t*-test while comparing two groups or one-way ANOVA for multiple comparisons.

There were no significant differences between the magnitude of LTP in pre-symptomatic and WT mice. In symptomatic mice, LTP magnitude was significantly decreased as compared with age-matched WT, indicating an impairment of synaptic plasticity. PPF was significantly decreased in pre-symptomatic mice when compared with age-matched WT, indicating an enhanced synaptic excitability in early disease stages. In symptomatic mice, PPF values were not significantly different from those obtained in WT mice.

In symptomatic SOD1^{G93A} mice chronically treated with the A_{2A}R antagonist, KW-6002, the decrease in LTP was no longer detected, as compared with WT mice similarly treated, suggesting that A_{2A}R blockade could prevent the impairment in synaptic plasticity in ALS. Treatment with the A_{2A}R antagonist did not affect basal synaptic transmission or PPF values in any group of animals.

The results suggest an enhanced transmitter release in pre-symptomatic stage followed by a decrease in synaptic plasticity in the symptomatic stage. This may result from A_{2A}R over activation in early disease stages.

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P2X7 RECEPTOR REGULATES THE INDUCIBLE NUCLEAR DUAL-SPECIFICITY PHOSPHATASE 1 IN RAT CEREBELLAR ASTROCYTES

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P2X7 is an ATP-gated ion channel belonging to the P2X receptor family. P2X7 receptors can activate several signaling pathways, including MAP kinase cascades. Mitogen Activated Protein Kinases comprise several serine-threonine kinases that mediate proliferation, differentiation and cell survival/death. Since these MAP kinases regulate important processes in the cell, it is necessary to know the signaling responsible for their dephosphorylation and deactivation. Several groups of phosphatases participate in this deactivation, being the MAP kinase phosphatases (MKPs) the main group. MKPs are Dual Specificity Phosphatases (DUSPs), which dephosphorylate both serine/threonine and tyrosine residues in the same substrate. There are different groups of MKPs, depending on the intracellular location of the phosphatase and the affinity for each MAP kinase. MKPs activity is regulated at different levels, translational and posttranslational modifications, by their MAPK substrates [1]. In addition, the MAPK itself is able to induce transcription of the genes encoding the phosphatases. In previous work, we showed that P2X7 receptors modulate the levels of the cytosolic ERK-selective protein phosphatase DUSP6 with a biphasic pattern and in an ERK-dependent manner in rat cerebellar astrocytes [2]. In the present work, we focus on the characterization of the intracellular mechanisms responsible for the regulation of the nuclear inducible phosphatase MKP-1/DUSP1 by P2X7 receptors. In contrast to that reported for DUSP6, the regulation of DUSP1 expression is dependent on ERK and p38 activation. DUSP1 appears to be involved in the dephosphorylation of p38 at the nuclear compartment.

[1] Caunt CJ, Keyse SM (2013) Dual-specificity MAP kinase phosphatases (MKPs): shaping the outcome of MAP kinase signalling. *FEBS J* 280:489-504.

[2] Queipo, MJ, Gil-Redondo, JC, et al. (2018) P2X7 Nucleotide and EGF receptors exert dual modulation of the Dual-specificity Phosphatase 6 (MKP-3) in granule neurons and astrocytes, contributing to negative feedback on ERK signaling. *Front Mol Neurosci* 10.

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ROLE OF ASTROCYTIC ADENOSINE A_{2A} RECEPTORS IN THE FACILITATION OF SYNAPTIC PLASTICITY IN CORTICO-STRIATAL SYNAPSES

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Adenosine A_{2A} receptors (A_{2A}R) control numerous striatal functions (locomotion, habits, addiction). A_{2A}R are mainly located in striatal medium spiny neurons (MSN), which are driven by cortical glutamatergic projection and modulated by dopaminergic inputs. We now tested if and how A_{2A}R control cortico-striatal plasticity, by comparing the amplitude of long-term potentiation (LTP) in cortico-striatal slices (% basal), in the absence and in the presence of the selective A_{2A}R antagonist, SCH58261 (50 nM) in 4 mouse lines: wild type (WT), global A_{2A}R knockout (g-KO), striatum-A_{2A}R-KO (st-KO with selective A_{2A}R elimination in MSNs) and forebrain-A_{2A}R-KO mice (fb-KO, with A_{2A}R elimination in glutamatergic and GABAergic neurons). In WT, LTP amplitude was 127±4% and SCH58261 inhibited LTP by -57±4% (n=4). To gauge the cellular site of action of A_{2A}R, we found that the effect of SCH58261 (n=4) on LTP was: 1-blunted in g-KO; 2-preserved in st-KO (-58±5%); 3-attenuated in fb-KO (-24±7%); 4-attenuated in WT mice injected in the pre-motor cortex with lentivirus expressing an sh-RNA to down-regulate A_{2A}R in glutamatergic terminals (-18±1%). Atropine (1 µM) did not affect the effect of SCH58261 on LTP (-6±4%, n=4), excluding the involvement of cholinergic modulation. Notably, the blockade of astrocytic glutamate uptake with DL-TBOA (50 µM) attenuated the effect of SCH58261 on LTP (-24±12%, n=4) in WT and blunted the effect of SCH58261 in fb-KO (n=4). This prompts the novel hypothesis that astrocytic A_{2A}R play a prominent role in the control of cortico-striatal plasticity.

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MICROGLIA VERSUS MACROPHAGE EFFECTS ON OLIGODENDROCYTE PRECURSOR CELLS: ROLE OF EXTRACELLULAR VESICLES

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Neuroinflammation plays a central role in multiple sclerosis (MS) by impairing remyelination and causing neuronal injury. Brain resident microglia (MG) and infiltrating macrophages (MP) are among the main effector cells of the inflammatory response associated to MS (Mallucci et al, 2015; Verderio et al, 2012). They contribute to MS onset and outcome, including secondary progressive phases, but also to the restorative phase of the disease (Rawji et al, 2013). However, it is still unclear whether all inflammatory myeloid cells are detrimental in the disease (Cao et al, 2013) and the possibility exists that the inflammatory activity of brain resident MG and peripheral MP may be distinct in MS, as reported in other brain diseases (Dibaj et al, 2011).

Recent data of the laboratory show that both inflammatory and proregenerative MG, through the secretion of extracellular vesicles (EVs) i) attract oligodendrocyte precursor cells (OPCs) in primary cultures, the glial cell type able to generate myelinating oligodendrocytes ii) favor OPC differentiation towards mature myelinating cells and ii) enhance myelin deposition in oligodendrocytes-DGR neuron co-cultures. To compare the action of MP and MG on oligodendrocytes I explored the action of EVs shed from pro-inflammatory MP or pro-regenerative MP on the migration and the differentiation of oligodendrocyte precursor cells (OPC).

OPC migration was assessed by a classical transwell-based chemokinesis assay. Quantification of migrating OPCs revealed that MP-derived EVs never attract OPCs, independent of the phenotype of donor MP. Specifically, EVs released by pro-inflammatory MP tend to limit OPC migration, albeit the difference didn't reach a significant difference. Sphingosine phosphate (S1P), a known chemoattractant agent, was used as positive control.

Immunocytochemistry analysis for the marker of mature oligodendrocytes MBP revealed that MP-EVs were not able to promote OPC differentiation as compared to MG-derived EVs. In particular, EVs released by pro-inflammatory MP significantly inhibited in vitro OPC maturation into myelin forming cells.

Collectively these results indicate that infiltrating macrophages, rather than resident microglia, may be responsible for block of OPC maturation and remyelination failure in the progressive phase of MS.

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PARACRINE SIGNALING VIA P2X2/3R IS REQUIRED FOR FUNCTIONAL DEVELOPMENT OF A CENTRAL AUDITORY SYNAPSE

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Understanding how early action potential discharges contribute to the maturation of neuronal networks is one of the major challenges in developmental neurophysiology. Following initial development including neuronal differentiation, migration, axon guidance and synaptogenesis, neuronal activity in maturing circuits is guiding the formation of precise sensory maps. In the auditory system, sensory map formation and sharpening depend on the spontaneous activity before hearing onset and extend to the period of early auditory experience. However, it remains elusive how neuronal activity affects different aspects of maturation in the auditory brainstem circuit that is encoding temporal features of sound to compute sound source location.

In the developing ventral cochlear nucleus, the first central station along the auditory pathway that receives inputs from the cochlea through the VIII nerve, paracrine ATP signaling enhances firing in a cell-specific and tonotopically-determined manner. Endogenously released ATP activates the P2X2/3R expressed only in bushy cells, and increases the synaptic efficacy of immature synaptic inputs, i.e. endbulbs of Held, by facilitating postsynaptic AP generation and prolonging APs. Developmental down-regulation of P2X2/3R currents occurs simultaneously with an increase in AMPAR currents from high-to-low frequency area.

Our in vivo and slice experiments in P2X2/P2X3Dbl^{-/-} mice demonstrate that the P2X2/3R is required for functional maturation of endbulb of Held synapses during the period of early auditory experience.

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ADENOSINE A_{2B} RECEPTORS AND SPHINGOSINE KINASE/SPHINGOSINE 1-PHOSPHATE SIGNALING AXIS CONTROL MATURATION OF OLIGODENDROCYTE PRECURSOR CELLS *IN VITRO*

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Introduction: Restoration of the myelin sheaths requires the differentiation of the oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs). Adenosine and sphingosine kinase/sphingosine 1-phosphate signaling axis (SphK/S1P) play important roles in remyelination processes. Remarkably, Fingolimod (FTY720), approved as orally active drug for relapsing Multiple Sclerosis, also modulates S1P receptors. An interaction between adenosine A_{2B} receptors (ADORA2B) and SphK activity has been demonstrated in mouse and human normal and sickle erythrocyte (Sun et al., 2015, Blood 125(10):1643-52). The voltage-dependent K⁺ current (I_K) block by tetraethylammonium (TEA) is also known to impair OPC maturation (Gallo et al., 1996, J Neurosci 16(8):2659-70).

Material and methods: The role of ADORA2B and SphK/S1P signaling on oligodendrogenesis in rat cultured OPCs was investigated by patch clamp, Real Time PCR and Western Blot techniques.

Results: The BAY60-6583 (0.1-30 μM), a selective ADORA2B agonist, reduced outward currents evoked by a voltage ramp protocol. This effect was blocked by MRS1706 (10 μM), a selective ADORA2B antagonist. The effect of BAY60-6583 on outward currents was prevented by TEA, indicating the involvement of I_K in BAY60-6583-mediated effect. The phosphorylated form of FTY720 (FTY720-P 1 μM) mimicked and partially occluded the effect of 10 μM BAY60-6583. SphK1 phosphorylation was enhanced after acute treatment with BAY60-6583, demonstrating an interaction between SphK/S1P pathway and ADORA2B activation. Prolonged ADORA2B stimulation reduced the expression of mature OL markers in cultured OPC. In contrast, SphK inhibitors produced an opposite effect. Finally, we found that downregulation of ADORA2B by siRNA significantly reduced the levels of NG2, S1P₅ receptors and SphK1 mRNAs and increased S1P lyase, suggesting that ADORA2B silencing stimulates OPC differentiation, modulates S1P-related target genes and reduces S1P levels.

Conclusions: Our data shows that ADORA2B stimulation inhibits K⁺ channels necessary to OPC differentiation and interferes with SphK/S1P pathway. Furthermore, either ADORA2B stimulation and S1P production inhibit OPC maturation.

ADENOSINE A_{2A} RECEPTOR ANTAGONISTS POTENTIATE CANNABINOID SIGNALING IN MICROGLIA

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Neuroprotective M₂-skewed microglia appear as promising to alter the course of neurodegenerative diseases and G-protein-coupled receptors are potential targets to achieve such microglial polarization. A common feature of adenosine A_{2A} and cannabinoid CB₂ receptors in microglia is that their expression is upregulated in Alzheimer's disease (AD). We here show a close interrelationship between those receptors in microglia that are able to physically interact and affect the signaling of each other due to allosteric interaction within an A_{2A}-CB₂ receptor heteromer (A_{2A}-CB₂Het). Particularly relevant is the increase in expression in samples from the APPS_{wt}, Ind AD transgenic model. The most relevant finding, confirmed in both heterologous cells and in primary cultures of microglia, was that blockade to A_{2A} receptors resulted in increased CB₂R-mediated signaling. This heteromer-specific feature suggests that A_{2A}R antagonists would potentiate, via microglia, the neuroprotective action of endocannabinoids with important implications for an AD therapy.

P2X7 AND BDNF RECEPTORS CONVERGE TO SHAPE ERK SIGNALLING IN NEURONS THROUGH THE MODULATION OF ERK-DIRECTED PROTEIN PHOSPHATASES

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Nucleotide receptors share signalling mechanisms with neurotrophins modulating several biological responses. In cerebellar neurons, P2X7 and BDNF receptors activate ERK signalling and promote neuronal differentiation and survival. The distinct pattern of ERK activation triggered by P2X7 and BDNF receptors reveals disparities in the extent they regulate the inactivation mechanisms, through the ERK-selective phosphatases. The family of dual specificity phosphatases (DUSPs) that dephosphorylate phosphothreonine and phosphotyrosine residues in MAP kinases are emerging as key regulators of the intensity and duration of MAPK signalling. The ERK-targeted phosphatases DUSP1 and DUSP6 were studied in primary cerebellar neurons, to analyse the changes in expression and activity of these proteins upon stimulation with P2X7 agonist and BDNF. Quantitative RT-PCR studies revealed that DUSP1 and DUSP6 transcripts were induced during neuronal differentiation and increased in response to BzATP and BDNF. Interestingly, P2X7 receptors correlated with DUSP6 protein along the culture, and dynamically regulated DUSP6 expression levels and activity. At short-term stimulations, DUSP6 protein decreased and was targeted to proteasome degradation, prolonging cytosolic ERK activity. This was followed by the recovery of protein levels through *dusp6* gene induction that terminated ERK signalling. Regarding the nuclear phosphatase DUSP1, whereas BzATP only elicited a transient response, BDNF produced a sustained increase in DUSP1 protein levels that involved several mechanisms, enhanced transcription of *dusp1* gene and protein stabilization, which was mediated by ERK-dependent Ser³⁵⁹ phosphorylation of DUSP1. The divergent behaviour in DUSP1 regulation by P2X7 and BDNF receptors could explain the different modifications on the dendritic and axonal branching observed with BzATP and BDNF. These effects were lost in cultured neurons in which *Dusp1* was down-regulated by shRNA. In conclusion, the spatio-temporal regulation of ERK signalling elicited by P2X7 and BDNF receptors through DUSP1 and DUSP6 phosphatases provides a mechanism for fine-tune modulation of the neuronal differentiation.

References:

Miras-Portugal MT, Queipo MJ et al. Brain Res Bull. 2018, Dec 26. doi: 10.1016/j.brainresbull.2018.12.012. "P2 receptor interaction and signalling cascades in neuroprotection".
Queipo MJ, Gil-Redondo JC, et al., Front Mol Neurosci. 2018, Jan 10;10:448. "P2X7 Nucleotide and EGF Receptors Exert Dual Modulation of the Dual-Specificity Phosphatase 6 (MKP-3) in Granule Neurons and Astrocytes, Contributing to Negative Feedback on ERK Signaling".

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MITOCHONDRIA ARE A KEY TARGET OF AMYLOID β -DEPENDENT DAMAGE IN MOUSE MICROGLIAL CELLS: ROLE OF THE P2X7 RECEPTOR AND PROTECTION BY NIMODIPINE.

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The pathogenic mechanism responsible for Amyloid β -dependent ($A\beta$) neurodegeneration is largely obscure despite several studies have evidenced a leading role of $A\beta$ -stimulated TNF α , IL-1 β or reactive species accumulation in Alzheimer's disease (AD) brains and the ensuing neuroinflammation. In a previous study we showed that the P2X7 receptor has a key role in $A\beta$ -dependent microglial activation and injury. We also showed that nimodipine (a L-type calcium blocker permeable across the blood-brain-barrier) is a powerful inhibitor of pro-IL-1 β intracellular accumulation and pro-IL-1 β cleavage and mature IL-1 β release. Moreover *in vivo* nimodipine significantly reduced IL-1 β accumulation triggered by intra-hippocampal $A\beta$ inoculation.

In the present study we investigate more in depth the pro-inflammatory effect of soluble $A\beta$ on mouse primary microglia cells (from *P2rx7-wt* and *P2rx7-deleted* mouse), on N13 mouse microglia cell line and on N13 microglial cells selected for the low expression of the P2X7 receptor referred as N13R (ATP Resistant).

Our data shows that $A\beta$ is a powerful stimulus for activation of NF κ B, Nalp3 inflammasome protein expression, Casp-1 activation and ROS production. These effects depend on P2X7 expression since they are strongly reduced in microglial cells derived from *P2rx7-deleted* mouse or N13R. We show that $A\beta$ exerts toxic effects on mitochondria such as induction of hyperpolarization, cytochrome *c* release and ATP content reduction. Moreover, in isolated mitochondria, $A\beta$ exerts an inhibitory effect on ATP-synthase activity. Nimodipine strongly reduced all $A\beta$ -dependent phlogosis as well as toxic effect on mitochondria but has not protective outcome on isolated mitochondria. Our data underline the key role of P2X7 receptor as permissive factor $A\beta$ toxicity in mouse microglia and demonstrate that nimodipine administration might be viable therapeutic strategy for AD.

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DENDRITIC OUTGROWTH ALTERATIONS DURING THE PATHOGENESIS OF SCHIZOPHRENIA IN GENETIC MOUSE MODELS OF P2X7R

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Neurons in some regions of the brain affected in schizophrenia (SCZ) show reduced dendritic length (Kalus et al. 2000) suggesting an abnormal dendritic outgrowth in schizophrenic patients. Correlation of abnormal dendritogenesis and human pathologies may offer some understanding to these currently untreatable diseases. P2x7 receptors (P2X7Rs) are potential therapeutic targets in SCZ. Genetic deficiency and pharmacological blockade of P2X7Rs shown to attenuate schizophrenia-like behaviour in rodents (Koványi et al., 2016). Our objective was to determine the regulation of neuronal outgrowth by P2X7Rs and the morphological correlates of P2X7R in primary cultures of murine hippocampal neurons derived from conventional wild-type (P2rx7+/+) and P2X7 receptor knockout (P2rx7-/-) mice in an animal model of SCZ.

Primary hippocampal neurons from P2X7R wildtype and knockdown mice obtained from E17.5–E18.5 embryos were dissected and processed. At day in vitro 10, transfection with GFP plasmid allows a clear visualization of the morphology of individual neurons in order to perform Neurolucida Software. Immunocytochemistry for the strengthening of the transfection was performed. One and Two-way ANOVA were used to determine statistically significant differences in the Sholl analyses, depending on the needs. All data are presented as mean \pm SEM. Deficits in dendritic outgrowth have been reported in P2X7R deficient mice, but also, in primary hippocampal neurons from wild-type animals co-cultured with P2X7R antagonist in a dose dependent manner. In conclusion, P2X7R depletion led to abnormal dendritic arborization in primary hippocampal neurons, demonstrating that P2X7R is needed for normal dendritic outgrowth during neuronal development, proliferation and maturation. The dendritic deficits in the disease model could constitute good correlates of the cognitive deficits in the schizophrenia. Additional genetic approaches should be tested to analyze and rescue these morphological deficits.

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BENEFICIAL EFFECTS OF P2X7R DEFICIENCY IN A MOUSE MODEL OF TAUOPATHY

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Alzheimer's disease (AD) is characterized by two neuropathological lesions: the accumulation of amyloid peptides into plaques and of hyperphosphorylated Tau proteins into neurofibrillary tangles. The latter combined with neuroinflammatory reaction underline synaptic and cognitive deficits. P2X7 purinergic receptor was found abnormally upregulated in the AD brain, notably by glial cells. We recently demonstrated (Martin et al., Molecular Psychiatry, 2019) that amyloid pathology is prone to activate P2X7R and that its deletion rescues cognitive deficits and mitigated lesions in the APP/PS1 model of AD amyloidogenesis. Protective effects of P2X7R deletion were ascribed to a reduction of CCL3 production and CD8 T-cells infiltration. In sharp contrast, the link between P2X7 and Tau pathology remains largely unknown. In the present study, we evaluated the role of P2X7R in a context of frontotemporal lobar degeneration (FTLD), a pure tauopathy using human tissue from FTLD patients with *MAPT* P301L mutation (FTLD-tau) and a mouse model based on FTLD-tau mutations (THY-Tau22), developing hippocampal Tau pathology along with plasticity and memory deficits. Our data demonstrate that P2X7R is upregulated in both the cortex of patients with FTLD-tau and the hippocampus of THY-Tau22 mice. Importantly, P2X7R deletion in THY-Tau22 mice improved long-term synaptic plasticity and hippocampal-dependent spatial memory. No major effect of P2X7R-deficiency on Tau phosphorylation were observed. However, lack of P2X7R improved the neuroinflammatory response by reducing microglia activation and related inflammatory markers. In conclusion, the present data are the first showing that P2X7R contributes to the pathological processes linked to the development of Tauopathy. Taken together, our findings support a detrimental role of P2X7R into the plasticity and memory deficits linked to the two AD lesions, paving the way towards P2X7R-based therapeutics in the context of AD patients.

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NEURONAL ADENOSINE A_{2A} RECEPTOR OVEREXPRESSION EXACERBATES MEMORY DEFICITS IN THE APP/PS1 MOUSE MODEL OF ALZHEIMER'S DISEASE.

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Epidemiological and experimental studies pointed-out that chronic caffeine consumption reduces Alzheimer's Disease (AD) risk and associated cognitive deficits. These protective effects are thought to be ascribed to the blockade of adenosine A_{2A} receptors (A_{2A}Rs). Interestingly, the latter are found abnormally upregulated in neurons of AD patient's brains in correlation with the development of cognitive deficits. These observations suggest a link between neuronal A_{2A}R dysregulation and memory impairments in AD. To get insights towards this relationship, we aimed at evaluating the pathophysiological impact of a neuronal A_{2A}R upsurge in a transgenic model of AD-like amyloidogenesis (APP/PS1dE9 mice). To this aim, we crossed APP/PS1 mice with our newly developed transgenic TRE-A_{2A} strain, carrying the mouse A_{2A}R under the control of a Tet-responsive-element promoter. This led rise to four genotypic groups: WT, APP, WT/TRE-A_{2A} and APP/TRE-A_{2A}. At 3m of age, all the animals were bilaterally injected in the hippocampus with an AVV2/5-CBA-ttA allowing the preferential overexpression of ttA transactivator in neurons, allowing neuronal A_{2A}R upsurge in TRE animals. At 6m of age, a time APP mice do not display major deficits, behavioral evaluations revealed that, neuronal A_{2A}R overexpression strongly worsens spatial memory impairments of APP animals without significantly altering neither amyloid burden nor neuroinflammation. Using Mass spectrometry-based high-throughput proteomics, we identified modifications in the molecular profile of APP/TRE-A_{2A} as compared to APP mice. Gene ontology analysis revealed that proteins the most highly differentially expressed were related to synaptic function. Further experiments are now ongoing to get further insights into these synaptic dysfunctions. These data support that pathological upregulation of A_{2A} receptors in neurons is instrumental towards the decline of cognitive functions in AD.

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THE ADP RECEPTOR P2Y₁₃ IS SELECTIVELY EXPRESSED IN BRAIN MICROGLIA AND CONSTITUTIVELY ATTENUATES HIPPOCAMPAL NEUROGENESIS

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Brain microglia represent a highly dynamic cell type involved in multiple functions in the CNS. They are involved in both physiological and pathological mechanisms and have been implicated in several neurodegenerative diseases. Purinergic mechanisms play an important role in the control of microglial function.^[1] Nucleotide receptors expressed by microglia in situ include the ATP-activated P2X7 and P2X4 receptors, which are strongly upregulated under diverse pathological conditions, the Gq-coupled and UDP-activated P2Y₆ receptor, which has been implicated in microglial phagocytosis as well as three closely related Gi-coupled receptors, the UDP-glucose/UDP-activated P2Y₁₄ receptor, the ADP-activated receptors P2Y₁₂ and - in spinal cord microglia - P2Y₁₃. These are upregulated in the spinal cord under conditions of neuropathic pain.

Using a combination of fluorescent in situ hybridization and immunostaining we allocate the P2Y₁₃ receptor specifically to brain microglia in situ. Since microglia is situated in close proximity to hippocampal neural progenitor cells and since we have previously demonstrated that purinergic mechanisms are involved in the control of adult neurogenesis^[2,3], we investigated the impact of the P2Y₁₃ receptor in adult hippocampal neurogenesis using *P2ry13* null mice^[4]. Disruption of *P2ry13* increased hippocampal progenitor cell proliferation and numbers, new neuron formation, and activity of mature granule cells. We have previously shown that the neurogenic niche has the potential to generate the P2Y₁₃ receptor agonist ADP from extracellular ATP by ectonucleotidases, which are associated with microglia itself, with type 1 cells and neural progenitor cells^[3,5,6]. This identifies a novel signaling pathway whereby microglia, via a P2Y₁₃ receptor-mediated mechanism contribute to the homeostatic control of adult hippocampal neurogenesis.

^[1]Calovi S. et al. (2018) Neuroscience (2018) doi: 10.1016/j.neuroscience.2018.12.021;

^[2]Zimmermann H. (2011) Semin Cell Dev Biol 22: 194-204; ^[3]Gampe K. et al. (2015) Stem Cells 33:253-264; ^[4]Stefani et al. (2018) Front Cell Neurosci 12:134. doi: 10.3389/fncel. 2018.00134; ^[5]Braun N. et al (1998) J Neurosci 18:4891-900; ^[6]Braun et al. (2000) Eur J Neurosci 12: 4357-4366

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PROTECTIVE EFFECT OF THE ADENOSINE A_{2B} RECEPTOR AGONIST, BAY60-6583, IN A RAT MODEL OF TRANSIENT CEREBRAL ISCHEMIA.

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Cerebral ischemia is today evaluated as the second leading cause of death in major industrialized countries and there is a strong demand for new therapeutic strategies.

After ischemic brain injury, extracellular adenosine concentration dramatically increases in the ischemic areas reaching μM concentrations that are able to stimulate all adenosine receptors including the A_{2B} receptor subtype to which adenosine deserves low affinity. The role of A_{2B}R in brain ischemia was less studied up to now because of paucity of A_{2B}R selective agonists and antagonists.

The aim of our study was to investigate the effect of the adenosine A_{2B} receptor agonist, BAY60-6583 (BAY), chronically administered (0.1 mg/kg, i.p., twice/day for 7 days), on ischemic damage parameters in the rat model of focal cerebral ischemia induced by transient (1hour) Middle Cerebral Artery occlusion (tMCAo) by the monofilament technique. Seven days after tMCAo rats were anesthetized and perfused transcardially. Brain infarct volume and cytoarchitecture of ischemic cortex and striatum were determined by cresyl violet (1%) and hematoxylin/eosin (H&E) staining.

BAY, 1, 5 and 7 days after tMCAo, significantly reduced the neurological deficit (score at 7 day: 2.8 ± 0.5 , $n=6$ versus 6.08 ± 0.9 , $n=4$ in vehicle group; $p<0.02$) evaluated by modified Neurological Severity Score (mNSS) test. Seven days after the ischemic injury, BAY has reduced the volume of the cortical damage ($12.60 \pm 2.30 \text{ mm}^3$, $n=4$ versus $21 \pm 1.70 \text{ mm}^3$, $n=3$ in vehicle group; $p<0.04$) and has reduced the number of heterochromatic nuclei both in cortex and striatum (respectively $p<0.005$; $p<0.02$).

Our preliminary results show that the A_{2B}R agonist BAY, systemically and chronically administered after ischemia, has reduced the ischemic brain damage and has improved the neurological deficit.

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NEURONAL ADENOSINE A_{2A} RECEPTOR OVEREXPRESSION EXACERBATES TAU PATHOLOGY-ASSOCIATED MEMORY DEFICITS.

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Tau pathology is defined by the accumulation of hyperphosphorylated and aggregated Tau protein in brain from patients suffering from neurodegenerative disorders known as tauopathies, including Alzheimer's disease (AD) and Frontotemporal lobar degeneration (FTLD). In AD, the progression of Tau pathology corresponds to the progression of clinical symptoms, suggesting a central role in the development of cognitive deficits. However, pathways underlying Tau pathology-induced cognitive deficits remain ill-defined. Previous epidemiological and experimental studies pointed out that chronic caffeine consumption reduces AD risk and associated cognitive deficits. These protective effects were ascribed to the blockade of adenosine A_{2A} receptors (A_{2A}Rs), which are found upregulated in AD patient's brains, notably at the neuronal level, in correlation with Tau pathology development and cognitive deficits. These observations suggest a link between A_{2A}R dysregulation, Tau pathology and memory in AD. Here, we evaluated the expression of A_{2A}R on human brain samples from FTLD patients with Tau mutation. We also developed a conditional model (Tet-Off) allowing A_{2A}R overexpression in CAMKII-positive neurons, crossed with THY-Tau22 mice, who develop a progressive hippocampal Tau pathology associated with cognitive decline. Animals were evaluated at 5-6 months of age, when pathology is expressed but not maximal. We demonstrated for the first time an association between neuronal upregulation of A_{2A}R and Tau pathology in the cortex of FTLD patients. Promoting neuronal A_{2A}R upregulation in a tauopathy mouse model led to a hippocampal upregulation of C1q associated with a loss of glutamatergic synapses and a potentiation of spatial memory deficits, suggesting an instrumental role of neuronal A_{2A}R dysregulation towards Tau pathology-induced synaptic loss and cognitive alterations. Altogether, these data suggest that neuronal A_{2A}R dysregulation seen in the brain of AD patients contributes to the development of Tau-induced cognitive impairments. Limiting A_{2A}R dysregulation may therefore represent a new therapeutic approach in AD and other tauopathies.

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BLOOD PURINE CONCENTRATION AS A NOVEL DIAGNOSTIC FOR SEIZURES AND EPILEPSY

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A simple method for diagnosing seizures and epilepsy using the detection of biomarkers from blood offers advantages over currently available methods. Principally: lower cost, less necessary expertise, potential for detecting seizures retrospectively, and fast results. Criteria for a clinically useful biomarker include sensitivity, specificity, minimal invasiveness, ease of analysis and robustness to artefacts. We used an enzyme-based detection system, which can reliably measure concentrations of adenosine in the blood. This method involves the immediate analysis of a small amount of fresh blood (finger prick), with results obtained within 5 minutes. In an intra-amygdala kainic acid mouse model of status epilepticus, blood adenosine concentrations increased, 40 minutes following kainic acid injection and remained elevated for 4 hours following seizure termination with lorazepam. Concentrations of adenosine in the blood correlated with the severity of seizures, as indicated by the total power of EEG ($R^2 = 0.4892$, $p < 0.0001$) and with markers of neuronal death in the CA3 region of the hippocampus three days following injection ($R^2 = 0.2805$, $p = 0.0006$). Data obtained from the epilepsy monitoring unit, Beaumont Hospital, indicate that baseline (>24h seizure free) blood adenosine concentrations are elevated in epilepsy patients compared with controls ($t_{34} = 2.907$, $p = 0.0064$) and that blood adenosine increases sharply immediately following generalized tonic-clonic seizures. Further, patients with non-epileptic attack disorder showed blood adenosine concentrations no different from controls. These results indicate that blood adenosine concentrations are elevated following seizures in both mice and patients, and that in epilepsy patients; blood adenosine is both chronically elevated and acutely elevated following seizures.

P7. PURINERGIC SIGNALING IN THE TUMOR GROWTH AND METASTASIS

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CHARACTIZATION OF CD39 EXPRESSION ON T CELLS IN ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is the most prevalent acute leukemia among adults. Even after initially successful therapy most patients experience relapses that might be due to immune escape. In order to overcome immune escape in AML it is pivotal to identify relevant immune cell subsets and checkpoints.

We performed multicolor flow cytometry on peripheral blood mononuclear cells (PBMCs) and AML blasts of 14 patients with newly diagnosed AML and 9 healthy donors (HDs), focusing on differentiation, exhaustion markers (e.g. PD-1) and the two ectoenzymes CD39 and CD73.

We observed an increased frequency of PD1+CD8+ T cells in AML patients compared to HD (11.2% vs. 3.9%; $p=0.08$). Similarly, in the CD4-compartment we found on both subsets, conventional (CD4+CD25^{lo}, CD4^{cons}) and regulatory (CD4+CD127^{lo}CD25^{hi}) CD4+ T cells, an increase of PD1-expressing cells from AML patients as compared to HD (CD4^{cons}: 11.1% vs. 2.3%; $p=0.05$; Tregs: 24.4% vs. 11.7%; $p=0.05$). Concomitantly, the expression of CD39 was also increased on CD4^{cons} and Tregs from AML patients as compared to HD (T^{cons}: 11.1% vs 2.3%; $p=0.05$; Tregs: 67.9% vs 39.2%; $p=0.01$). Of note, the overall frequency of Tregs did not differ between PBMCs from AML and HD.

Based on these data we aim to investigate the role of CD39 on the inhibitory capacity of Tregs, activation status and the proliferative capacity of T effector cells. Currently, we are investigating further functional assays to assess the role of CD39 and other purinergic mediators in the progression of AML.

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RESVERATROL ACTS THROUGH ADENOSINE RECEPTORS IN TUMORAL CELL LINES

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Extracellular adenosine is one of the major constituents of the tumor microenvironment and plays a crucial role in proliferation, angiogenesis and metastasis in cancer. The effects of this purine are triggered through four G-protein coupled receptors: A₁, A_{2A}, A_{2B} and A₃. A₁ and A₃ receptors inhibit adenylyl cyclase activity through G_{i/o} protein whereas A_{2A} and A_{2B} receptors activate this enzymatic system through G_s protein. Current efforts are focused on resveratrol (RSV) action, a diet polyphenolic phytoalexin found in many plant species such as grapes, peanuts and red wine. This molecule has shown promising effects in inhibiting proliferation and cancer progression in several tumoral models. However, its molecular mechanisms are poorly understood. Recently, we have described that RSV acts as a non-selective adenosine receptor agonist. Therefore, the aim of the present work was to study the antitumoral effect of RSV and the possible mechanism involving adenosine receptors. To this end, two tumoral cell lines were used, rat C6 glioma and human HeLa epithelioma cervix cells. Cell viability by XTT method and adenosine receptors quantification by Western-blotting and real time PCR were assayed. Results show that RSV was able to cause cell death in a time and concentration dependent manner in both cell lines. The treatment with this polyphenol caused a modulation of several adenosine receptor types. In addition, 5'-nucleotidase activity and adenosine levels were determined in HeLa cells and it was observed that RSV alters these parameters. As RSV has been shown to be a non-selective adenosine receptors agonist, our results suggest that a possible mechanism underlying antitumoral effect of RSV could be through adenosine receptor binding.

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EXTRACELLULAR ADENOSINE PROMOTES GLIOBLASTOMA INVASIVENESS THROUGH THE MODULATION OF MESENCHYMAL STEM CELL SECRETOME

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Glioblastoma (GBM) is an aggressive, fast-growing brain tumor. Mesenchymal stem cells (MSCs) exhibit tropism for tumor microenvironment, influencing tumor progression. Adenosine (ADO), a purine nucleoside, reaches high concentrations and activates signaling pathways involved in cancer cell proliferation and invasiveness. The activity of specific adenosine receptor (AR) subtypes on glioma cells have been widely explored. However, the effects of extracellular ADO on glioma aggressive traits is still unclear as well as its role on cancer cells-MSC communication. Herein, human U343MG cells were used as a model to investigate the cell proliferation, motility and the expression of epithelial-mesenchymal transition (EMT)-related genes in response to ADO.

ADO did not alter U343MG growth rate and migratory capacity. However, ADO was able to induce the transcription of EMT master-gene (Snail, Slug, and ZEB1) without promoting a complete transition. These effects were related to the ability of ADO to significantly modify the equilibrium of different intracellular signaling pathways.

High extracellular ADO concentration may also affect MSC modifying their interplay with glioma cells. In these respect, the modification of glioma cell proliferation, migration and expression of EMT-related genes were analyzed after the treatment with MSC-conditioned medium (CM). ADO modified the secretion of pro-inflammatory cytokine from BM-MSC. The CM promoted the increase of glioma motility and induced a partial phenotypic change of glioblastoma cells.

In conclusion, these results demonstrate that ADO may affect glioma biology directly and through the modulation of the paracrine factors released by BM-MSCs. The modification of pivotal intracellular signaling pathways after the ADO chronic exposure may promote EMT highlighting the importance of the extracellular ADO levels in the control of glioma aggressiveness. Supported by PRIN 2015, 2015E8EMCM_007

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INHIBITION OF DUAL-SPECIFICITY PHOSPHATASES PROMOTES P2X7 RECEPTOR EXPRESSION IN NEUROBLASTOMA CELLS IN A p38-DEPENDENT MANNER

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The expression of the purinergic receptor P2X7 (P2X7R) in neuroblastoma cells is associated with accelerated growth rate, angiogenesis, metastasis and poor prognosis. Also, an increase in P2X7R expression prolongs the survival of neuroblastoma cells under restrictive conditions, including serum or glucose deprivation. Previously, we identified the specificity protein 1 (Sp1) as the main transcription factor involved in basal expression of *P2rx7* gene in N2a neuroblastoma cells. Here, we show a new regulatory mechanism of *P2rx7* gene expression mediated by dual specificity phosphatases (DUSPs). DUSPs tightly modulate the activity of mitogen activated kinases (MAPKs) by dephosphorylation of the threonine-X-tyrosine motifs in their activation domains. Treatment of neuroblastoma cells with (E)-2-benzylidene-3-(cyclohexylamino)-2,3-dihydro-1H-inden-1-one (BCI), an allosteric inhibitor of DUSP1 and DUSP6 phosphatases, increases the expression of P2X7R at both transcriptional and protein levels, and this effect is significantly reduced by p38/ERK inhibition. DUSP6 is a constitutive cytosolic phosphatase that mainly dephosphorylates ERK1/2, while DUSP1 belongs to the subgroup of mitogen- or stress-inducible nuclear phosphatases capable of mostly dephosphorylate p38 and JNK. Remarkably, BCI treatment strongly enhances p38 and JNK phosphorylation, while ERK1/2 phosphorylation is reduced, suggesting that BCI mainly inhibits DUSP1 activity in neuroblastoma cells. The observed decrease in ERK phosphorylation is mediated by activation of protein phosphatase 2 following p38 phosphorylation. We also demonstrate that the effect of BCI on P2X7R expression is independent of Sp1, as well as other transcription factors such as AP-1, CREB and Myc. Taken together, these results show that neuroblastoma cells have an alternative mechanism independent of Sp1 and dependent on the phosphorylation state of p38/ERK kinases involved in the transcriptional regulation of P2X7R expression.

- García-Huerta P, Díaz-Hernandez M, Delicado EG, Pimentel-Santillana M, Miras-Portugal MT, Gómez-Villafuertes R. J Biol Chem. 2012;287(53):44628-44.
- Gómez-Villafuertes R, García-Huerta P, Díaz-Hernández JI, Miras-Portugal MT. Sci Rep. 2015;5:18417.

EFFECT OF WEAK BASE DRUGS ON LYSOSOMAL CAPACITY OF CANCER CELLS: POSSIBLE INVOLVEMENT OF P2X4 RECEPTOR.

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It is believed that lysosomal sequestration of hydrophobic weak base chemotherapeutic drugs prevents these drugs to reach their target sites, resulting in a significantly reduced cytotoxic effect. In addition, recent results suggested that lysosomal sequestration of hydrophobic weak base drugs triggers lysosomal biogenesis mediated by activation of transcription factor EB (TFEB), which in turn further increases lysosomal sequestration of hydrophobic weak base anticancer drugs and thus enhance resistance against these drugs.

Here we addressed the question whether lysosomal biogenesis is the only mechanism that increases lysosomal sequestration capacity. We observed that lysosomal sequestration of some tyrosine kinase inhibitors (TKIs) induced expansion of lysosomal compartment. However, expression analysis of lysosomal genes, including LAMP1, LAMP2, vacuolar ATPase subunit B2, ACP, and GLB controlled by TFEB did not indicate increased expression. Instead, we found that high concentrations of TKIs induced lysosomal fusion. Our results further suggested that lysosomal fusion is Ca^{2+} dependent and that P2X4 receptor contributed to this process significantly. In conclusion, we demonstrated that lysosomal sequestration capacity can be significantly enlarged due to the lysosomal fusion with apparent participation of P2X4 receptor in cancer cells.

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P2X7 RECEPTOR INFLUENCES METASTATIC POTENTIAL AND VESICULAR RELEASE IN MELANOMA

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Metastatic melanoma is still a clinical challenge and new targeted drugs and therapeutic approaches are needed. It was already demonstrated that the administration of P2X7 antagonists in an *in vivo* model of melanoma reduced cancer growth suggesting P2X7 as a potential therapeutic target for this disease. We investigated the expression of P2X7 in commercially available melanoma specimens by immune-histochemical and RT-PCR analysis. Moreover, the metastatic potential of SK-MEL-28 and MA-MEL-19, two human melanoma cell lines expressing P2X7, was tested in *in vitro* scratch and soft agar assays after treatment with P2X7 antagonists AZ10606120 and A740003. Finally, in an effort to identify the mechanisms activated by P2X7 in metastatic melanoma, we studied release of vesicles from SK-MEL-28 and MA-MEL-19 following stimulation with the agonists ATP and BzATP. Data obtained from immunohistochemistry show increased P2X7 expression in metastatic melanoma patients. Moreover, mRNA expression of P2X7A and B was higher in stage IV melanomas that spread in lung and distant organs than stage III and stage IV with skin metastasis. These data suggest a role for P2X7 isoforms in melanoma dissemination. P2X7 antagonists treatment reduced *in vitro* migration and invasion capacity of SK-MEL-28 and MA-MEL-19. Interestingly, these cell lines released vesicles when stimulated with P2X7 agonists ATP and BZATP. To our knowledge this is the first demonstration of P2X7 mediated vesicles released from cancer cells. These preliminary data suggest a possible role of P2X7 in melanoma metastasis possibly due to vesicles release and make P2X7 a potential pharmacological target for advanced melanoma treatment.

P8. PURINERGIC SIGNALING IN IMMUNOLOGY AND INFLAMMATION

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THE ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF AR170, A POTENT AND SELECTIVE A₃ RECEPTOR AGONIST, IN A RAT MODEL OF COLITIS

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Introduction. The pharmacological activation of adenosine A₃ receptors has shown potential usefulness in the management of bowel inflammation. However, the role of these receptors in the control of visceral hypersensitivity in the presence of intestinal inflammation has not been investigated.

Methods. In a first set of experiments, the effects of AR170, a potent and selective A₃ receptor agonist, and dexamethasone (DEX, a standard anti-inflammatory comparator) were tested in male rats (n=6 for each group) with colitis induced by intrarectal administration of 2,4-dinitrobenzenesulfonic acid (DNBS, 30 mg/rat), to assess tissue inflammatory parameters [macroscopic damage, tumor necrosis factor (TNF), interleukin-1 β (IL-1 β), and myeloperoxidase (MPO)]. Animals received AR170 (3 mg/kg/day), DEX (1 mg/kg/day) or vehicle intraperitoneally for 6 days, starting 1 day before the induction of colitis. In a separate set of experiments, visceral pain was assessed by recording somatic motor responses to colo-rectal distension (CRD) in animal with colitis. The effects of AR170 were evaluated after both acute (0.5-4.5 mg/kg i.p.) and repeated (1.5 mg/kg/day i.p.) administration.

Results. Colitis was associated with a decrease in body weight, and increase in spleen weight. Macroscopic damage score as well as tissue TNF, IL-1 β and MPO levels were enhanced as well. In the first series of experiments, AR170, but not DEX, improved body weight. Both drugs counteracted the increase in spleen weight, ameliorated the macroscopic colonic damage and decreased TNF, IL-1 β and MPO tissue levels were observed also in rats treated with both test drugs. The second set of experiments displayed an enhanced visceromotor response to CRD in animals with colitis. Visceral hypersensitivity was decreased by acute administration of AR170 in a dose-dependent fashion, and it was attenuated also with repeated administration of this ligand. The inhibitory effect of AR170 was fully reversed by the selective antagonist MRS1523 (8 mg/kg i.p.).

Conclusions. AR170 exerts beneficial effects on bowel inflammation, counteracting inflammatory cell infiltration and decreasing pro-inflammatory cytokine levels. In parallel, the activation of A₃ receptors by AR170 was associated with a significant relief of visceral hypersensitivity under bowel inflammation.

P2X7R PLAY AN IMPORTANT ROLE IN ANTIDEPRESSANT EFFECT OF TAVNS

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Background: Depression is a common affective disorder. As a new method of combining acupuncture and neuromodulation technology, transcutaneous vagus nerve stimulation (taVNS) is currently used in the treatment of many diseases. We've previously confirmed through clinical trials that taVNS can be effective in treating depression, and the data from animal experiments suggest that the antidepressant mechanism is associated with inflammation and purine receptor receptors in the brain. This study was designed to assess the effectiveness of taVNS on depressive-like behavior caused by chronic restraint stress (CRS) in rats, and explore its potential mechanism of interaction between inflammation and purine receptor.

Material and methods: 18 male SD rats were equally divided into control group, model group and model+taVNS group, used open-field test, sucrose preference test and forced swimming test to ascertain whether taVNS affects CRS induced depression-like behaviors of rats, and the expression of P2X7R, NLRP3 and NF- κ B in the hippocampus and prefrontal cortex was detected by real-time quantitative polymerase chain reaction (Real-time Q-PCR).

Results: The data showed that taVNS can significantly reverse stress-induced Depression-like behavior in rats, and the antidepressant effect of taVNS was accompanied by markedly decreased the mRNA expression of P2X7R, NLRP3 and NF- κ B in the hippocampus and prefrontal cortex ($p < 0.05$).

Conclusions: taVNS may play an antidepressant role by reducing inflammation in the hippocampus and prefrontal cortex, and this anti-inflammatory effect may be negatively correlated with the mRNA expression of P2X7R.

Keywords: taVNS, depression, inflammation, purine receptor, rats

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ADENOSINE A2A RECEPTOR MODULATION OF MICROGLIA MORPHOLOGIC REMODELLING IN EARLY NEURODEVELOPMENT - GENDER BIAS IN PHYSIOLOGY AND PSYCHIATRIC DISORDERS

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Sexual differentiation in rodents occurs in the late gestational period, promoting gender asymmetries. Microglia (innate immune cells of the central nervous system) exert important functions from development onwards, fundamentally supported by their highly dynamic cellular processes, in constant expansion and retraction in order to monitor brain homeostasis. We previously described sex differences in microglia morphology in the adult prefrontal cortex (PFC). Prenatal anxiogenic stimulus are associated with anxious-like phenotypes at adulthood, in parallel with a gender-specific morphological remodelling of microglia in the PFC. The chronic blockade of adenosine A2A receptors (A2AR), modulators of microglia morphology, recovered this phenotype only in males. These receptors emerge as potential therapeutic targets in anxiety, due to its ability to modulate microglia.

To clarify the organizational role of A2AR in microglia morphological differentiation, we quantitatively characterized microglial processes (number and length) in wild type and A2AR KO mice in the PFC and in the dorsal hippocampus (dHIP) (key brain regions in mood disorders), in both sexes by immunohistochemistry followed by manual 3D reconstruction using Neurolucida software.

We observed physiological interregional and sex differences: male microglia were more complex in the dHIP, while female microglia were more complex in the PFC. The genetic deletion of A2AR promoted a hypertrophy of PFC microglia only in females.

Concluding, there are microglial subpopulations characterized by regional- and sex-specific morphological asymmetries. The impact of A2AR genetic deletion anticipates an organizational effect of these receptors upon the segregation of microglia subpopulations with sex and regional specificities.

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INTERACTION OF P2X7 WITH THE ER STRESS/UNFOLDED PROTEIN RESPONSE PATHWAYS

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Some chemotherapeutic agents such as doxorubicin (DOX) can activate the immune system by eliciting immunogenic cell death (ICD) of cancer cells. ICD is induced by triggering of the ER stress/unfolded protein response (UPR) pathways, and is characterised by the translocation of calreticulin (CRT) from the ER to the cell surface accompanied by the release of other danger signals such as ATP. In this context, P2X7 plays an important role as a receptor on antigen-presenting cells (APCs) that mediates their pro-inflammatory maturation. Complementary to its role on APCs we investigated the role of P2X7 on tumour cells with regard to the induction of ICD.

In murine Yac-1 lymphoma cells low doses of extracellular ATP (eATP) synergistically enhanced the cytotoxic effects of DOX. This effect was blocked by an antagonistic and further enhanced by an agonistic nanobody to P2X7. Gating of P2X7 augmented the initial uptake of DOX into cells, but enhanced cell death was also observed when DOX was washed away before exposure to eATP, suggesting an interaction of the downstream signalling pathways. Sub-threshold doses of eATP also synergistically enhanced the cytotoxicity of the proteasome inhibitor Bortezomib, as well as DOX-mediated CRT translocation, suggesting that P2X7 might interact with the UPR. Incubation of Yac-1 cells with eATP alone for 10 min induced CRT translocation, phosphatidylserine surface exposure, and DAPI uptake. Interestingly, all these effects were blocked in the presence of GSK2606414, an inhibitor of the PKR-like ER kinase (PERK).

Our results suggest that P2X7 signalling is intimately linked with the ER and provides an early input into the ER stress/UPR pathway that may modify the outcome of this signaling pathway when it is induced by other agents.

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ADENOSINE A_{2B} RECEPTOR ACTIVATION IMPROVES EPIDERMAL BARRIER INTEGRITY DAMPENED BY INFLAMMATION

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The integrity of the skin barrier is compromised in psoriasis due to the increased proliferation and aberrant differentiation of keratinocytes, the main cellular type in epidermis. Recent studies have shown that A_{2B} is the most expressed adenosine receptor in human keratinocytes, being able to regulate epidermal proliferation and inflammatory response. Proteins like filaggrin, loricrin and involucrin play an important role in the maintenance of barrier function. In the present study we determine the involvement of A_{2B} receptors regarding epidermis integrity in the mouse skin hyperplasia induced by 12-O-tetradecanoylphorbol-13-acetate (TPA).

The A_{2B}R agonist BAY60-6583 (BAY) (1 µg/site), the A_{2B}R antagonist PSB-1115 (5µg/site), and both together were applied on the shaved backs of female Swiss mice 30 minutes before TPA (2nmol/site) for three consecutive days. On day four, animals were sacrificed and 1 cm² punch biopsies were collected and processed for immunochemistry assays.

Treatment with the A_{2B} antagonist PSB worsened the severity of TPA-induced skin lesions whereas BAY markedly ameliorated them. The immunohistochemical analysis showed that the expression of both, filaggrin and loricrin, was decreased after treatment with either PSB, the stimulus TPA, or their combination, compared to naïve mice. In contrast, application of BAY enhanced the expression of these proteins within the epidermis. Besides, BAY improved the TPA-induced aberrant expression of involucrin in stratum corneum, effect that was abrogated by the antagonist PSB.

These results demonstrate the beneficial role of A_{2B} receptor in regulating epidermal integrity and show the interest of this receptor as a potential pharmacological target in the treatment of pathologies where excessive inflammatory response may affect to the skin's architecture such as psoriasis.

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COLLABORATIVE RESEARCH CENTER SFB 1328: ADENINE NUCLEOTIDES IN IMMUNITY AND INFLAMMATION

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Extracellular and intracellular adenine nucleotides (AN) impact on all central processes in biology and medicine. AN are essential and ubiquitous signaling molecules involved in regulating universal cellular processes, including (i) cell-cell communication and (ii) intracellular signaling. Unresolved issues regarding the signaling function of extracellular AN in inflammation, e.g. adenosine triphosphate (ATP) or nicotinamide adenine dinucleotide (NAD), relate to the timing and location of their release, their conversion by ecto-enzymes, and their biological role within the balance of inflammatory processes. Likewise, the precise role of intracellular AN second messengers, e.g. nicotinic acid adenine dinucleotide phosphate (NAADP) or 3',5'-cyclic adenosine monophosphate (cAMP), in the spatio-temporal control of signaling processes by forming or modulating microdomains with their metabolizing enzymes, specific binding proteins or receptors, or target ion channels remains largely unknown. The central goal of the research consortium is to further our understanding of the regulatory roles of AN and their kinetics in the context of inflammatory diseases. Specific aims relate to (i) modulation of the balance between pro- and anti-inflammatory processes by AN converting ecto-nucleotidases and purinergic receptors, and to (ii) AN-driven intracellular calcium signaling and cAMP signaling in inflammation. Based on the local Research Network "Regulatory Adenine Nucleotides at Membranes" (Landesforschungsförderung Hamburg), the collaborative research center SFB 1328 has been formed, centrally located in Hamburg. National and international experts in the AN research field from Bonn, Genova (Italy), Göttingen und Munich significantly strengthen our initiative. By interdisciplinary integration we will develop an integral view of AN biology and pathophysiology, providing the basis for novel diagnostic methods and innovative treatment strategies, focusing on inflammatory diseases in immune, adipose and nervous systems. The poster will present the aims, structure and job opportunities of the SFB 1328.

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INVESTIGATION ON THE ROLE OF THE P2X7R-NLRP3-IL-1 β AXIS IN THE PATHOGENESIS OF HIDRADENITIS SUPPURATIVA

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Hidradenitis Suppurativa (HS) is a chronic cutaneous inflammatory pathology of the terminal hair follicle characterised by painful, inflamed, deep lesions in the axillae, groin and anogenital region. HS pathogenesis is multifactorial involving genetic, hormonal, immunological, microbial, environmental and dietary factors. In HS lesional skin, the NLRP3-inflammasome inflammatory pathway has been found over-expressed [1]. Until now, no research has been dedicated to explore in HS the role of the pro-inflammatory P2X7R, the main activator of the NLRP3 inflammasome and IL-1 β production [2].

The aim of this study was to evaluate whether P2X7R is involved in HS pathogenesis. For this, thirty HS patients and thirty healthy control (HC) subjects were studied. Immuno-histochemical (IHC) analysis of skin samples was performed using anti-P2X7R, anti-NLRP3 and anti-IL-1 β antibodies. Ficoll gradient isolated peripheral blood mononuclear cells (PBMCs) were used to detect Benzoyl-ATP (BzATP)-stimulated IL-1 β secretion following lipopolysaccharide (LPS) incubation. IL-1 β in plasma and PBMCs supernatants was measured using the human IL-1 β /IL-1F2 Quantikine ELISA kit (R&D System).

P2X7R IHC staining scored from 2+ to 4+ in lesional skin of 21 out of 30 HS patients, whereas it ranged from - to 2+ in HC subjects ($p < 0.001$). A variable degree of positivity was detected in the dermis, especially in the inflammatory infiltrate, of HS patients. NLRP3 and IL-1 β staining was analogously higher in HS than in HC skin samples ($p < 0.001$). IL-1 β plasma levels were more elevated in HS patients than in HC subjects ($p = 0.02$). Unexpectedly, BzATP-stimulated PBMCs of HS patients released lower levels of IL-1 β than PBMCs of HC subjects ($p < 0.05$).

Our data indicate P2X7R-NLRP3- IL-1 β axis involvement in the pathogenesis of HS. Respect to HC subjects, HS patients showed higher P2X7R expression in the lesional skin and higher plasma IL-1 β levels. Since, as found elsewhere [3], PBMCs of HS patients appeared either inhibited or exhausted in IL-1 β release, a role for P2X7R-NLRP3 inflammasome in boosting IL-1 β production at HS skin level is suggested. These results open new perspectives in finding novel efficacious therapies for HS.

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ROLE OF CD73 IN THE DEVELOPMENT OF MURINE ALLERGIC ASTHMA

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Background: Allergic asthma is the most frequent respiratory disease. CD73 is the key enzyme in the extracellular adenosine production from adenosine monophosphate (AMP) [1]. To date, very little is known about the role of CD73 enzyme in the development of allergic asthma [2]. Our study was aimed to investigate the role of CD73 in the development of allergic sensitization and in the tissue response to allergen exposure.

Material and methods: Experiments were performed on female wild type (wt) and CD73-deficient (CD73^{-/-}) mice. Animals were sensitized by subcutaneous injection with 100 µg of ovalbumin (OVA) on day 1 and day 8. 21 days thereafter, mice were twice challenged with 5% aerosolized OVA or with saline as control. All mice were sacrificed 24 h after the last challenge to take main bronchi, pulmonary tissue and blood for functional, molecular and cellular studies.

Results: OVA sensitization induced a significant increase in CD73 bronchi expression and plasma activity (n=7, ***p<0.001). In sensitized-allergen challenged mice, plasma IgE levels tended to be higher in CD73^{-/-} mice compared to wt mice. In addition, in sensitized-allergen challenged mice, we observed elevated peribronchial inflammation, goblet cell metaplasia and mucus production, that were further increased in lung sections of CD73^{-/-} mice.

On the other hand, in vitro bronchial reactivity to carbachol was not increased by exposure to allergen in CD73^{-/-} mice (n=8, **p<0.01).

Conclusions: Our data suggest that CD73 enzyme plays a dual role in the development of allergic asthma.

References:

- [1] Cicala C, Ialenti A. Eur. J. Pharmacol., 2013, 714: 522-525.
- [2] Bentley JK et al., Respir Res., 2010, 11:127.

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THERAPEUTIC POTENTIAL OF ADENOSINE IN INFLAMMATORY SKIN DISEASES

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Inflammatory skin diseases are characterized by an increased epidermal turnover and altered immune and inflammatory responses. Since adenosine, through the interaction of its receptors, regulates inflammation and immunity, we sought to study the expression and function of adenosine receptors in keratinocytes and in an inflammatory model of epidermal hyperplasia.

We observed that A_{2B} subtype is the most prominently expressed in epidermal cells from healthy donors, followed by the A_{2A} receptor; neither mRNA levels for A₁ nor A₃ receptors were detected. Activation of A_{2B} receptors induced cell-cycle arrest through the increase of intracellular calcium. In contrast, A_{2A} activation induced keratinocyte proliferation via p38-mitogen-activated protein kinase activation. We found an altered pattern of Adenosine receptors in epidermis from psoriatic patients, consisting in decreased A_{2B} and increased A_{2A} receptor expression, which was reproduced in keratinocytes exposed to diverse inflammatory cytokines. In the murine model of skin hyperplasia induced by of 12-O-tetradecanoylphorbol-13-acetate (TPA, 2 nmol/site) for three consecutive days, topical pretreatment with the A_{2A} agonist CGS-21680 (5 µg/site) had a profound anti-inflammatory response and prevented the epidermal hyperplasia, while promoting collagen deposition in dermis. The A_{2B} agonist BAY60-6583 (1-10 µg/site) also improved the severity of skin lesions and diminished in a dose-dependent manner IL-1β, CXCL-1 and IL-6 levels determined in skin homogenates. Interestingly, the A_{2B} antagonist PSB-1115 (10-50 µg/site) not only reversed the beneficial effect of BAY on the skin, but also worsened the lesions, enhancing the inflammatory response and inducing a clear degradation of epidermal layer.

Our results suggest that adenosine plays an important role regulating epidermal homeostasis, and thus may constitute an interesting therapeutic strategy in inflammatory hyperproliferative skin diseases such as psoriasis.

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NEW 4'-TETRAZOLYL ADENOSINE DERIVATIVES AS POTENTIAL AGENTS FOR WOUND HEALING

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Ligands for A_{2A} adenosine receptors (ARs) are potential therapeutic candidates for many disorders and seem to regulate the wound healing process. The introduction of substituents with different length in the 2-position of adenosine (Ado) and 5'-N-ethylcarboxamidoAdo (NECA) favours the interaction of the resulted nucleosides with the A_{2A}AR. Known examples of such compounds are 2-phenylethylthioNECA (VT 7) and CGS21680 that possess high A_{2A}AR affinity but moderate selectivity. Furthermore, it has been reported that a tetrazolyl moiety in 4'-position of 2-alkylaminoAdo derivatives led to compounds endowed with a dual effect, resulting A_{2A}AR agonists and A₃AR antagonists. Starting from these observations, a new series 4'-tetrazolyl Ado derivatives bearing different arylalkyl-thio, -amino, and -oxy chains in 2-position were designed and synthesized. All the new compounds were tested in binding and functional assays at human ARs and their ability to facilitate wound healing has been evaluated by determining their efficacy to improve human fibroblast migration. Binding data showed that the substitution of the 5'-N-ethylcarboxamido group of VT 7 with the 4'-ethyltetrazolyl substituent favours the interaction with the A_{2A}AR (2-phenylethylthio-4'-ethyltetrazolylAdo, K_i = 5.8 nM *versus* VT 7, K_i = 24 nM). The functional study confirms the dual behaviour at ARs of the new derivatives (2-phenylethylthio-4'-ethyltetrazolylAdo, hA_{2A}AR EC₅₀ = 160 nM and hA₃AR IC₅₀ = 80 nM). Moreover, from the in vitro evaluation of fibroblast migration emerged that most of the new compounds were able to improve cell migration better than epidermal growth factor (EGF), which was used as the positive control. Hence, the new 4'-ethyltetrazolyl-2-substituted Ado derivatives could be as new tools useful in wound healing.

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INTERACTION OF THE SPHINGOSINE-1-PHOSPHATE PATHWAY WITH PURINERGIC RECEPTORS

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Abstract: Cells release ATP in many ways. One of them is via the cell volume-sensitive anion channel VRAC (also referred to as VSOR, VSOAC) induced by activation of S1P receptors. We have previously reported about this S1P-induced ATP release measured by voltage clamp and luciferase assay. Here, we investigated whether the S1P-induced ATP release can affect cell functions like cell migration by activating purinergic P2X or P2Y receptors. The microglia cell line BV-2 has been used to conduct the experiments. In order to assess the effects of a S1P-induced ATP release we used scratch assays (also 'wound healing assay'). S1P, like ATP and ADP, stimulates cell migration into the scratch area. The inhibition of S1P receptors and of the downstream G_i proteins reduced the cell migration. Antagonists of VRAC, which lead to reduced ATP release, were also able to diminish the cell migration. Furthermore, direct inhibition of ATP-gated P2X4 or P2X7 receptors or ADP-stimulated P2Y12 receptors blocked the stimulating effects of S1P on cell migration. We conclude that there is an interaction between S1P receptors and purinergic receptors mediated by a S1P-induced ATP release via VRAC and that the amount of released ATP is capable to stimulate cell migration of BV-2 microglia cells via activation of P2X4, P2X7 and P2Y12 receptors.

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THE P2X7 RECEPTOR IS SHED INTO CIRCULATION AND CORRELATES WITH C-REACTIVE PROTEIN LEVELS

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The P2X7 receptor (P2X7R) is an ATP-gated cation channel involved in several disease conditions, inflammation included (1). P2X7R activation determines NLRP3-inflammasome-mediated IL-1 β release, T lymphocytes proliferation and differentiation, and release of microvesicles/microparticles (MVs/MPs) loaded with different molecules, P2X7 subunits too. Some inflammatory membrane receptors (e.g., IL-1R, TNFR, IL-2R) shed under various pathophysiological conditions. To date, there are no measurements of a shed form of P2X7R (sP2X7R) which might be present in blood and whose levels might be associated to inflammation.

Here we tested sP2X7R using an ELISA kit (Cusabio) (expressed as means \pm SE) or SDS-PAGE and Western Blot (WB). Serum samples were obtained from 26 healthy controls and from 87 patients admitted at our Hospital and stratified into four sub-groups according to diagnosis at admission: infective diseases (n=42), cancer (n=16), ischemic diseases (n=10) and others (trauma and autoimmune diseases) (n=19). Serum or plasma samples were analyzed by ELISA. MVs/MPs-enriched pellets, obtained by ultracentrifugation of serum or plasma samples, and MVs/MPs-deprived plasma/serum were analyzed by ELISA or SDS-PAGE and WB. Supernatants from BzATP-stimulated Ficoll gradient-separated peripheral blood monocytes from 4 healthy subjects were tested by ELISA.

In healthy subjects, blood sP2X7R ranged from 16.74 to 82.17 ng/L (40.97 \pm 3.82). In patients, blood sP2X7R levels correlated to those of the inflammatory marker C reactive protein (CRP). sP2X7R ranged from 33.1 to 484.0 ng/L (114.78 \pm 12.22, n=45) in patients with CRP<3 mg/L, and from 63.65 to 1092.3 ng/L (204.2 \pm 30.94, n=42) in patients with CRP>3 mg/L. In the four sub-groups, a positive CRP-sP2X7R correlation was found. In addition, both CRP and sP2X7R were significantly higher in the infective disease patients versus the other three sub-groups. In plasma/serum, sP2X7R was partially associated to MVs/MPs. sP2X7R was present in supernatants of peripheral blood monocytes stimulated with BzATP.

In conclusion, the P2X7R has been found shed into circulation, and its blood levels are increased in various inflammatory disease conditions (2). A possible application of sP2X7R as diagnostic/prognostic inflammatory marker is therefore suggested.

P9. PURINERGIC SIGNALING IN THE CARDIOVASCULAR SYSTEM

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REGULATION OF IL-6 FORMATION IN THE HEART BY CD73-DERIVED ADENOSINE

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Interleukin-6 (IL-6) is secreted by the infarcted heart showing a dual response: in the acute phase of myocardial infarction (MI) IL-6 is cardio-protective but can turn to be harmful when the IL-6 secretion becomes chronic. A previous study suggested the presence of a targetable adenosine–A2bR–IL-6-axis triggered by adenosine formed by the ischemic heart. We therefore studied A2bR signaling und functional relevance in monocytes/macrophages in the infarcted murine heart.

IL-6 expression and secretion was analyzed in murine peritoneal macrophages as well as monocytes from healthy human donors after A2bR activation (BAY60-6583). Protein expression/secretion of several cytokines or regulatory proteins was analyzed by qPCR and Bioplex. We found that IL-6 expression and secretion was strongly induced by A2bR stimulation in murine macrophages and human monocytes. This effect was mediated by Gq-signaling, since a specific Gq-inhibitor (FR-900359(UBO-QIC)) strongly inhibited A2bR-induced IL-6 expression/secretion. We further analyzed the expression of IL-6 mRNA regulatory factors and found that in A2bR^{-/-} macrophages, the expression of Regnase-1, an IL-6-mRNA destabilizing protein was significantly increased. To analyze the functional impact of T-cell derived adenosine on IL-6 formation in the infarcted heart, we analyzed the release of various cytokines into the effluent perfusate from isolated hearts (Langendorff) from mice lacking CD73 on T-cells (CD4 T-cells are known to be the main adenosine producers under these conditions). We found that IL6, MCP and MIP1 β , were significantly reduced in CD4CD73^{-/-} mice.

In summary this study shows that adenosine formed by CD73 on T-cells controls IL-6 secretion post MI, most likely involving A2bR stimulation of macrophages in a Gq-mediated manner. This opens the possibility that inhibitors of A2bR may be therapeutically beneficial in limiting cardiac IL-6 formation.

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THE ENDOTHELIAL P2Y2 RECEPTOR MODULATES SPROUTING.

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Purinergic P2Y receptors are G protein-coupled surface receptors that respond to extracellular nucleotides and are critical regulators of several functions within the vascular system, including platelet aggregation and vascular inflammation. While endothelial P2Y receptors in particular have been shown to be required for vasodilatation and barrier function, little is known about their role in angiogenesis and endothelial sprouting. Here, we demonstrate that the purinergic receptor P2Y2 is a key regulator of endothelial sprouting. Enhanced expression of P2Y2 in HUVEC and in human iPSC-derived endothelial colony forming cells leads to increased sprouting through an autocrine mechanism in a VEGF-independent manner and to tubulogenesis in fibrin matrices in absence of supporting cells. Overexpression of P2Y11 on HUVEC as a control did not influence sprouting. Conversely, the use of a selective antagonist or siRNA-mediated knock-down of P2Y2 impairs sprouting and tubulogenesis. Mechanistically, overexpression of P2Y2 in endothelial cells results in a pro-angiogenic phenotype, confirmed by the induced expression of the pro-angiogenic genes ANGPT2, CXCR4 and CD34 and constitutive phosphorylation of ERK1/2 and VEGFR-2, suggesting that P2Y2-overexpressing cells acquire an angiogenic tip-cell phenotype. Moreover, stimulation of P2Y2 with its ligand uracil triphosphate does not influence sprouting in wild type endothelial cells unless P2Y2 was constitutively expressed, indicating that a ligand-independent mechanism may be responsible for the observed effects. Additionally, spontaneous vascular network formation induced by P2Y2 overexpression is impaired upon VEGFR-2 inhibition. Our data suggest an essential function of P2Y2 in blood vessel growth, presumably through a cross-talk of P2Y2 with VEGFR-2.

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POLYPHENOLS INCREASE ATP RELEASE AND NO PRODUCTION BY SEPARATE MECHANISMS IN MESENTERIC ENDOTHELIAL CELL CULTURES.

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Endothelial cells participate in vascular wall regulation through the release of multiple vasoactive chemicals such as ATP and nitric oxide (NO) the most potent endogenous dilator. In view that diet polyphenols have vasodilator effects, we assessed whether 30 μ M quercetin (Q, flavonol) or 100 nM conjugated delphinidin (D3G, anthocyanin), modified ATP released and NO production in endothelial cells derived from rat mesenteric bed with and without (basal) mechanical stimulation. Cells were cultured in control media or with 25 mM glucose (mimicking diabetes) or 10 mM KCl supplement. Extracellular ATP was monitored following chloroacetaldehyde derivatization; extracellular NO was quantified by chemiluminescence while intracellular NO was determined by DAF fluorescence. Q increased basal, ATP overflow from 50 ± 16 to 102 ± 24 pmol/mg protein (n=28 well, p<0.05), 5 min following mechanical stimulation, extracellular ATP rose from 162 ± 32 to 591 ± 169 pmol/mg protein (n=16 well, p<0.05), without modifying basal or NO production 5 min post stimuli. In contrast, D3G doubled extracellular NO from 14.3 ± 2.2 to 26.8 ± 1.9 nmol/mg protein (n=6, p<0.01), without modifying extracellular ATP. Not all NO produced is due to ATP release, since apyrase (4 U/mL) only partially annulled both extracellular and intracellular NO production, consistent with two targets of polyphenols in these cells. In KCl enriched medium, basal ATP overflow increased 163 ± 50 pmol/mg protein (n=12 well, p<0.01); while that evoked 1 min post mechanical stimuli caused a rise of 815 ± 255 pmol/mg protein (n=6 well, p<0.05). Addition of Q to KCl supplemented cultures increased extracellular ATP 743 ± 158 pmol/mg protein (n=6 well, p<0.05) 5 min post stimuli. Moreover, in glucose enriched medium, Q did not change extracellular ATP outflow. In sum, polyphenols activate separate mechanisms to increase ATP and NO overflow, consonant with vasodilator mechanism and beneficial effect of high KCl diets. Funded by FONDECYT 117-0842 and grant 21141226 to CC and CEDENNA FB0807

THE ACTIVATION OF ADENOSINE A_{2A} BUT NOT A_{2B} RECEPTORS IS INVOLVED IN URIDINE ADENOSINE TETRAPHOSPHATE-MEDIATED CORONARY SMOOTH MUSCLE RELAXATION

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Activation of both adenosine A_{2A} and A_{2B} receptors contributes to coronary vasodilation. We previously demonstrated that uridine adenosine tetraphosphate (Up₄A) is a novel vasodilator in the porcine coronary microcirculation, acting mainly on A_{2A} receptors and partially on P2 receptors. A major part of vasodilation produced by Up₄A is mediated by smooth muscle cell (SMC) relaxation and activation of SMC A_{2A} receptors. Here, we further investigated whether activation of SMC A_{2B} or P2 receptors is involved in Up₄A-mediated coronary relaxation. Both A_{2A}R and A_{2B}R may stimulate H₂O₂ production leading to activation of K_{ATP} channels in SMCs, we also studied the involvement of H₂O₂ and K_{ATP} channels in Up₄A-mediated porcine coronary SMC relaxation. Coronary small arteries dissected from the apex of porcine hearts were mounted on wire myograph for Up₄A concentration responses (10⁻⁹-10⁻⁵ M). Up₄A-mediated relaxation was significantly blunted in isolated porcine coronary small arteries without endothelium (E_{max}: 70±5%) vs. arteries with intact endothelium (E_{max}: 93±4%). Up₄A-induced coronary SMC relaxation was attenuated by the A_{2A} receptor antagonist (SCH58261) (E_{max}: 24±5%) but not the A_{2B} receptor antagonists (MRS1754, E_{max}: 80±8%; CVT6883, E_{max}: 78±9%) or the non-selective P2R antagonist (PPADS, E_{max}: 76±10%). Despite more abundant endogenous A_{2B} receptor expression vs. A_{2A} receptors (~20-fold difference), Up₄A affected neither A_{2A} nor A_{2B} receptor mRNA level, as assessed with real-time PCR, in primary cultured porcine SMCs. Up₄A-induced coronary SMC relaxation was blunted by H₂O₂ catabolism with catalase (E_{max}: 61±10%). This effect was not altered by K_{ATP} channel blockade glibenclamide (E_{max}: 75±5%). Finally, SCH58261 (E_{max}: 18±8%) attenuated Up₄A-induced porcine coronary SMC relaxation to the similar extent as the combination of SCH58261 and catalase (E_{max}: 20±8%). In conclusion, Up₄A-induced porcine coronary SMC relaxation is mediated by activation of A_{2A}-H₂O₂ axis. This process does not involve A_{2B} receptors, P2 receptors or K_{ATP} channels.

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PURINE METABOLISM IN CARDIAC FIBROBLASTS AND EPICARDIUM-DERIVED CELLS, SIMULTANEOUSLY ISOLATED FROM THE INFARCTED MOUSE HEART

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Release of nucleotides from the infarcted heart and their extracellular conversion to adenosine play an important role in the process of post-infarct tissue remodeling. Purines are known to have impact on the differentiation of cardiac fibroblasts (CF) into myofibroblasts (= activated cardiac fibroblasts (aCF)). However, the CF/aCF population is most likely heterogeneous and hard to capture by conventionally used markers. Another issue is the contamination of the CF/aCF population with epicardium-derived cells (EPDC), which are formed on the epicardial surface above the infarcted tissue. To discriminate between aCF and EPDC, we established a fast (8 min) and efficient protocol for the simultaneous isolation of aCF and EPDC from the infarcted mouse heart. Quantitative PCR analysis of genes involved in the extracellular purine metabolism revealed that in all cell fractions Enpp1 and Enpp3 is more highly expressed than CD39. In functional assays, however, ATP breakdown was largely inhibited when cells lacked CD39, suggesting an only minor role of Enpp1/3 in ATP breakdown. Concerning the four adenosine receptors, EPDC highly expressed Adora2b, while CF/aCF showed lower expression levels. Analysis of the kinetics of extracellular ATP metabolism in short term cultured aCF and EPDC showed that the main degradation product is AMP with only negligible adenosine formation. This finding supports the hypothesis that AMP generated by aCF and EPDC diffuses to surrounding immune cells to serve as a substrate for CD73 mainly localized on T cells, thereby producing anti-inflammatory adenosine in the infarcted heart.

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IMPACT OF CD73-DERIVED ADENOSINE ON THE CELL-TYPE SPECIFIC CYTOKINE RESPONSE IN THE INFARCTED MURINE HEART

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In previous work we have shown that adenosine derived from CD73, expressed on T-cells, orchestrates the healing process after myocardial infarction, and that A2bR is specifically up-regulated on cardiomyocytes as well as on immune cell after infiltrating the infarcted heart (Borg et al. (2017)). Here, we are exploring the cellular mechanisms by which CD73-derived adenosine is linked to cardiac cytokine response in the post infarct phase.

CD4-CD73^{-/-} and A2bR^{-/-} mice were subjected to 50 min occlusion of the left descending coronary artery with subsequent reperfusion. Three days post MI, coronary effluent perfusate was collected from isolated perfused hearts (Langendorff) and the cytokine profile (Bioplex) was determined. In CD4-CD73^{-/-} mice, cardiac IL-6 production was significantly reduced when compared to WT controls, suggesting that T-cell derived adenosine controls IL-6 in the heart. Since the A2bR was reported to also impact on IL-6 secretion e.g. in fibroblasts, we analyzed IL-6 expression (qPCR) in FACS-sorted cardiomyocytes, cardiac B-cells, T-cells, granulocytes and macrophages three days post MI in A2bR^{-/-} mice. In all cell types analyzed – besides T-cells – IL-6 expression was significantly reduced compared to WT controls. Cardiac T-cells lacking A2bR showed an increase in pro-inflammatory cytokines (IL-2, TNF α and IFN γ) in the post-MI heart. Finally, we stimulated human CD11b⁺CD14⁺CD15⁻ monocytes with lipopolysaccharides in the presence or absence of the A2bR agonist BAY60-6583. Stimulation of A2bR strongly induced IL-6 secretion thereby emphasizing the importance of the adenosine - A2bR – IL-6 axis in the human system.

Collectively our data indicate that CD73-derived adenosine acts via A2bR on the cytokine secretion of different cells types of the infarcted heart in a cell-type specific manner.

P10. PURINERGIC SIGNALING IN RENAL, GASTROINTESTINAL AND MUSCULOSKELETAL SYSTEM

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ADP-INDUCED P2Y₁-MEDIATED OSTEOGENESIS IS DOWNREGULATED BY OVEREXPRESSED P2Y₁₂ AND P2Y₁₃ RECEPTORS IN POSTMENOPAUSAL MESENCHYMAL STEM CELLS

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While ATP is a known promoter of the osteogenic differentiation of human bone marrow mesenchymal stem cells (BM-MSCs), the osteogenic role of ADP-sensitive P2Y₁, P2Y₁₂ and P2Y₁₃ receptors has been a matter of debate. The P2Y₁R sensitizes human BM-MSCs to parathormone, while the P2Y₁₃R has been associated to bone remodelling only in rodents. Blockage of P2Y₁₂R with the anti-thrombotic drug, clopidogrel, causes a dual role on bone homeostasis depending on dosage. To clarify the role of ADP-sensitive P2Y receptors in human osteogenesis, BM-MSCs were isolated from young females and postmenopausal (Pm) women, which were cultured for 35 days with an osteogenic-inducing medium. Cells growth (MTT assay), osteogenic commitment (alkaline phosphatase activity, ALP), and bone nodule formation (Alizarin red assay) were significantly decreased in BM-MSC cultures from Pm women compared to younger females. Selective activation of P2Y₁R with MRS 2365 (0.1 μ M) significantly increased proliferation of BM-MSCs from young females by 64 \pm 6% and 35 \pm 4% at culture days 7 and 14, respectively; increases were also observed on ALP activity (1073 \pm 368%, at day 21) and on culture mineralization (185 \pm 28%, at day 35). The P2Y₁R agonist was devoid of effect on Pm BM-MSCs unless P2Y₁₂ and P2Y₁₃ receptors activity were blocked with AR-C66096 (0.1 μ M) and MRS 2211 (10 μ M), respectively. Readmission of P2Y₁R-induced osteogenesis by these blockers is more likely in Pm BM-MSCs overexpressing P2Y₁₂R and P2Y₁₃R *vis a vis* P2Y₁R. Data suggest that co-activation of P2Y₁₂R and/or P2Y₁₃R downregulates ADP-induced P2Y₁-mediated osteogenic differentiation of BM-MSCs in Pm women. This leads us to propose that P2Y₁R agonists used in combination with P2Y₁₂R and/or P2Y₁₃R antagonists may be a useful strategy for the treatment of bone defects in Pm women.

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ADENOSINE A_{2A} RECEPTOR ACTIVATION PROMOTES FIBROSIS BY FAVORING PANNEXIN-1 OVEREXPRESSION IN HUMAN SUBCUTANEOUS FIBROBLASTS

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Hemichannels containing pannexin-1 (Panx1) and connexin43 (Cx43) have an important role in fibrogenesis associated with chronic diseases (Cronstein & Sitkovsky, 2017, Nature Rev Rheumatol. 13:41; Cogliati et al., 2016, J Membr Biol. 249:199). The adenosine A_{2A} receptor (A_{2A}AR) has a pro-fibrotic role in human subcutaneous tissue fibroblasts (Pinheiro, 2014, PhD Thesis (ICBAS) University of Porto). Here, we set out to investigate if there is a link between A_{2A}AR activation and the expression of hemichannels containing Panx1 and Cx3 in human subcutaneous fibroblasts. Human fibroblasts were isolated from the subcutaneous tissue of organ donors with no clinical history of connective tissue disorders after appropriate Ethical approval. We analyzed the expression of A_{2A}AR, Panx-1 and Cx43 in human subcutaneous fibroblasts by immunofluorescence confocal microscopy and investigated their role in cell proliferation (MTT test) and type I collagen production (Sirius Red assay) by cells kept in culture for 28 days (first subculture). Prolonged exposure (during 28 days) of human subcutaneous fibroblasts to adenosine or its stable analogues, NECA (300 nM) and CGS 21680 (10 nM), significantly increased type I collagen production. Blockade of Panx1 hemichannels with probenecid (100µM) or carbenoxolone (300 µM) decreased type I collagen production by 30-60% compared to the control situation. Panx1 immunoreactivity of cultured human subcutaneous fibroblasts increased significantly in the presence of NECA (300 nM); an effect that was fully prevented by the selective A_{2A}AR antagonist, SCH 442416 (10 nM). Conversely, NECA (300 nM) reduced Cx43 immunoreactivity in these cells. Data show here for the first time that adenosine A_{2A}AR activation causes Panx1 overexpression in human subcutaneous fibroblasts, while decreasing the amount of Cx43 hemichannels.

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URINARY ATP CONCENTRATION IS RELATED TO DETRUSOR PRESSURE DURING VOIDING

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The bladder urothelium releases huge amounts of ATP into the bladder lumen in response to mechanical stress, both stretch and increase in the local pressure (Ferguson et al., 1997, J Physiol 505: 503-11). The urinary ATP concentration is directly correlated to the voided volume, suggesting that urothelium stretch impacts on urinary ATP levels (Silva-Ramos et al., 2013, PLoS One 8:e64696). Here, we investigated if bladder internal pressure during voiding also influences urinary ATP concentrations. Male patients scheduled for urodynamic investigation of LUTS, were recruited. Patients were asked to void at normal desire to an uroflowmetry apparatus. Then, the bladder was catheterized with a 7Fr double lumen catheter and the urine residual volume

| Correlation between urinary ATP (nM) | Pearson's <i>r</i> | P |
|--------------------------------------|--------------------|--------|
| Voided volume | 0,238 | 0,096 |
| Pdet@Qmax | 0,689 | <0,001 |
| Bladder outlet obstruction index | 0,640 | <0,001 |
| Bladder contractility index | 0,561 | <0,001 |

measured. Next, standard filling cystometry and pressure-flow studies were performed. Urine samples collected during the uroflowmetry were snap frozen in liquid nitrogen and preserved at -80°C until ATP determination using the luciferin-luciferase method. A total of 50 patients were included in the study. The patients' average age was 59.8 [27-80] years old and the mean urinary ATP concentration was 2.95 [0.45-12.53] nM.

Correlation between urinary ATP and Pdet@Qmax was independent of the voided volume ($p < 0,001$). The urinary ATP content was not different among patients with and without detrusor overactivity: 3.05 ± 0.37 and 2.87 ± 0.62 nM, respectively. Data suggest that the voiding dynamics significantly affects urinary ATP concentrations. Higher detrusor pressures during voiding are correlated to higher urinary ATP concentrations, an effect that is independent of the voided volume. Detrusor overactivity had no impact on urinary ATP concentrations. Thus, both stretch and pressure induced urothelial ATP release impacting on urinary ATP concentrations. Urinary ATP may be a surrogate of detrusor pressure during voiding.

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β_3 -ADRENOCEPTOR COUPLING TO EPAC1 AND PKC PROMOTES ADENOSINE RELEASE VIA ENT1 LEADING TO INHIBITION OF CHOLINERGIC NEUROTRANSMISSION IN THE HUMAN BLADDER

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Selective β_3 -adrenoceptor agonists, such as mirabegron, are increasingly prescribed for the treatment of overactive bladder. Our group demonstrated that activation of G_s-protein-coupled β_3 -adrenoceptors favors the release of adenosine in urothelium-denuded human and rat detrusor strips, via equilibrative nucleoside transporters 1 (ENT1; Silva et al., 2017, *Am J Physiol* 313: F388-F403). Thus, cholinergic inhibition by β_3 -adrenoceptor agonists may be indirectly mediated by adenosine released from the detrusor leading to retrograde activation of inhibitory A₁ receptors on cholinergic nerve terminals (Silva-Ramos et al., 2015, *Purinergic Signal* 11: 595-606). Here, we investigated the cyclic AMP responsive element, protein kinase A (PKA) and the exchange protein directly activated by cAMP (EPAC), most likely participating in β_3 -induced cholinergic inhibition of the urinary bladder obtained from human organ donors and Wistar rats. Using immunofluorescence confocal microscopy and Western blot analysis, we show that the human and the rat detrusor exhibit β_3 -adrenoceptor, EPAC1 and ENT1 immunoreactivity, but express very small amounts of β_2 -adrenoceptor and EPAC2 proteins. The EPAC inhibitor, ESI-09, prevented β_3 -induced adenosine release from human and rat detrusor strips caused by mirabegron and isoprenaline, respectively. ESI-09, but not the PKA inhibitor, H-89, attenuated inhibition of [³H]ACh release from stimulated (10 Hz) detrusor strips caused by drugs activating β_3 -adrenoceptors, adenylylcyclase (forskolin), and EPAC1 (8-CTP-2Me-cAMP). Isoprenaline-induced inhibition of [³H]ACh release was also prevented by inhibitors of protein kinase C (chelerythrine and Go6976) and ENT1 (dipyridamole and NBTI). Pretreatment with ESI-09, but not with H-89, prevented the reduction of the voiding frequency caused by isoprenaline and forskolin in urethane-anaesthetized rats. Data suggest that β_3 -adrenoceptor-induced inhibition of cholinergic neurotransmission in human and rat urinary bladders involves activation of an EPAC1/PKC pathway downstream cyclic AMP production resulting in adenosine outflow via ENT1.

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PANNEXIN1 KO MICE ARE IRRESPONSIVE TO TENOFOVIR INDUCED BONE LOSS

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Introduction: Tenofovir is anti-retroviral agent prescribed to human immunodeficiency virus (HIV) patients as part of the drug regimen known as highly active anti-retroviral therapy (HAART). Tenofovir also causes osteopenia resulting in pathological fractures and hospitalisation in these patients. This is now increasingly significant since tenofovir is one of the components of HIV pre-exposure prophylaxis, which is available for the population at risk. Currently there are not many studies in this regard but several authors have reported that this population also suffers a decrease in BMD.

A recently published study suggests that this is caused by a reduction of extracellular adenosine produced by Tenofovir mediated blockage of Pannexin-1 ATP transporter and this effect can be reversed by dipyridamole that blocks adenosine re-uptake. To further confirm this study Pannexin-1 KO (PANX1KO) mice were studied and compared to C57BL/6J (WT) mice.

Methods: PANX1KO animals were treated daily with 75mg/Kg of tenofovir, 25mg/kg tenofovir or both during 4 weeks, after that bone mineral density (BMD) was measured. Additionally primary osteoclasts were differentiated and treated with different concentrations of Tenofovir and dipyridamole. The differentiation stage and extracellular ATP levels were studied.

Results: In WT mice Tenofovir caused a reduction bone mineral density and this was reversed in the animals who received both drugs. However PANX1KO mice that receive Tenofovir did not present a lower BMD. In WT mice Dipyridamole inhibited osteoclast differentiation ($p=0.0068$). The dipyridamole induced inhibition was reverted with Tenofovir in a dose dependent manner ($p=0.0055$). PANX1KO mice osteoclast differentiation was also inhibited by dipyridamole ($p=0.0005$), however Tenofovir was unable to revert dipyridamole induced inhibition ($p=0.9756$). Additionally, opposed to the WT osteoclasts, PANX1KO mice did not decrease ATP release when treated with Tenofovir ($p=0.3292$).

Conclusion: In the absence of pannexin-1 transporter tenofovir is not able to influence BMD or osteoclast differentiation.

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ADENOSINE A_{2B} RECEPTORS ON ENTERIC GLIA MEDIATE COLONIC DYSMOTILITY ASSOCIATED WITH HIGH FAT DIET-INDUCED OBESITY

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At present, the role played by A_{2B} receptors (A_{2B}Rs) in the modulation of enteric glial cell (EGC) functions, and thereby their involvement in the pathogenesis of intestinal dysmotility associated with high-fat diet (HFD)-induced obesity remains unknown. We evaluated the *in vitro* effects of A_{2B}R ligands on colonic contractions in HFD-fed mice, also investigating the role of EGCs in this context.

C57BL/6 mice were fed with standard diet (SD, 18% from fat) or HFD (60% from fat) for 8 weeks. Tachykininergic contractions were examined on colonic preparations in presence of BAY60-6583 (A_{2B}Rs agonist, 1μM) or MRS1754 (A_{2B}Rs antagonist, 0.01μM) with or without the gliotoxin fluorocitrate (FC, 50μM). A_{2B}R, glial fibrillary acid protein (GFAP) and substance P (SP) expression were examined by immunohistochemistry. To mimic an HFD, EGCs were incubated with palmitate (PA, 400μM) and/or lipopolysaccharide (LPS, 10μg/ml), with or without BAY60-6583 (0.05μM) and MRS1754 (0.25μM). The expression of toll-like receptors (TLR)-4 and SP release were evaluated in EGCs.

The electrically-evoked colonic tachykininergic contractions were enhanced in HFD mice as compared to SD mice. MRS1754 enhanced these electrically-induced contractions in HFD mice, while it was less effective in SD mice. BAY60-6583 decreased tachykininergic contractions, with higher efficacy in HFD mice. The effects of A_{2B}R ligands on colonic contractions were blunted upon incubation with FC. HFD mice showed an increased expression of colonic A_{2B}Rs, GFAP and SP. In cultured EGCs, co-incubation with LPS and PA increased the TLR-4 expression and SP release, as compared with control cells. Under co-incubation with LPS and PA, BAY60-6583 decreased TLR-4 expression and SP release. Such effects were antagonized by MRS1754.

HFD is associated with a hyperactivation of EGCs, likely through TLR-4, contributing to colonic tachykininergic motor dysfunction. In this setting, A_{2B}Rs expressed on EGCs modulate both the TLR-4 expression and the activity of excitatory tachykininergic nerves.

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RENAL PAPILLA EXPRESSES HIGH LEVELS OF THE UDP-GLUCOSE RECEPTOR P2RY14

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Among the metabotropic P2Y receptors, P2RY14 (GPR105) is activated by UDP-sugars. Some physiological relevance of P2RY14 has been shown in the immune system and insulin secretion but its broader expression suggests involvement in additional functions. Using a knockout/knockin reporter (LacZ) mouse model and quantitative real-time PCR we found P2RY14 expression in kidney, most prominent in the renal papilla. General assessment of kidney function revealed no difference between wildtype and knockout mice regarding urine excretion, kidney weight, microscopic morphology and zonation of the kidney. However, immunofluorescence staining for the principal cell marker aquaporin 2 showed colocalization with β -galactosidase expressing cells in our knockout mice. To investigate P2RY14 function in renal papilla at the molecular level, we performed RNA sequencing of wildtype and knockout papillae. It revealed 409 differentially expressed genes. Highest expression differences between WT and KO papillae were found for GPR171 and the serine palmitoyl CoA-transferase small subunit B (Sptssb). GPR171 neighbors P2RY14 on chromosome 3 and may compensate for P2RY14 deficiency. Sptssb is a regulatory subunit of the serine palmitoyl CoA-transferase (SPT). SPT catalyzes the condensation of serine and palmitoyl-CoA which is the first and rate-limiting step in the biosynthesis of sphingolipids. Moreover, expression of additional genes related to sphingolipid metabolism and signaling differed significantly. Analyses of sphingolipid composition of the kidney papilla using thin-layer chromatography and ESI-MS showed a significant decrease in the amount of 16:0 sphingomyelin, a product generated downstream of SPT. Our data suggests that P2RY14 in renal papilla may have protective functions against the concentrated urine and modulates sphingomyelin metabolism.

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ROLE OF UDP-GLUCOSE RECEPTOR P2Y14 IN OSTEOBLASTS

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Purinergic receptor P2Y14 is uniquely responsive to UDP-glucose (UDPG). P2Y14 expression was reported in all bone-residing cells, including mesenchymal stem cells and osteoblasts, however its role in bone is unknown. We confirmed gene and protein P2Y14 expression in C2C12-BMP2 osteoblastic cell line (C2-OB) and primary murine osteoblasts (P-OB). To study P2Y14 function, we generated CRISPR-Cas9-mediated P2Y14 knockout C2-OB clones (KO), or used P2Y14-antagonist PPTN. In P-OB, UDPG induced changes in the cytosolic free calcium concentration $[Ca^{2+}]_i$, which were potentiated by PPTN. While C2-OB did not respond to UDPG with an increase in $[Ca^{2+}]_i$, KO cells demonstrated a response that was similar to PPTN-treated P-OB. UDPG also stimulated PPTN-sensitive stress-fiber formation in P-OB cells, and PPTN- or KO-sensitive ERK1/2 phosphorylation in C2-OB cells. P2Y14 knockout or inhibition reduced proliferation in C2-OB and CB-OB cells, and was associated with higher AMPK α phosphorylation. Osteogenic differentiation was induced by treating P-OB with ascorbic acid and β -glycerol phosphate in the presence of vehicle or PPTN for 28 days. Alkaline phosphatase activity, collagen deposition and mineralization were reduced in PPTN-treated cells at day 14 of differentiation, however normalized by day 28. Expression of OSX, RUNX2, COLA1, DMP1 and SOST significantly and similarly increased with osteogenic differentiation of vehicle-and PPTN-treated cells for the first 14 days, however at day 28 expression of these genes was significantly higher in PPTN-treated cultures compared to vehicle-treated. Of interest, while P2Y14^{-/-} mice from the International Mouse Phenotyping Consortium were similar to wild-type in bone mineral density, their tibia length was significantly increased. Taken together, these findings position P2Y14 as a modulator of osteoblast differentiation and further our understanding of the function of purinergic system in bone.

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TENOFOVIR CAUSES BONE LOSS VIA DECREASED BONE FORMATION AND INCREASED BONE RESORPTION, WHICH CAN BE COUNTERACTED BY ADENOSINE A2A RECEPTOR IN MICE

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Background or Introduction: Osteopenia and fragility fractures have been associated with HIV infection and Tenofovir, a common antiviral in HIV treatment. In murine models and human cell lines, tenofovir inhibits ATP release and decreases extracellular adenosine levels. Adenosine, and adenosine A2A receptor, inhibits osteoclast formation, and increasing local adenosine concentration with dipyridamole, an agent that blocks adenosine cellular uptake, stimulates new bone formation as well as rhBMP-2. We hypothesized that tenofovir regulates bone resorption by diminishing endogenous adenosine levels and determined whether dipyridamole and A2A receptor may counteract the effects.

Material and methods: M-CSF/RANKL-induced-primary murine osteoclasts were studied as the number of TRAP-positive-cells after challenge with tenofovir alone or in combination with dipyridamole. Differentiation markers were studied by RT-PCR, and MAPK/NFkB expression by Western Blot. Male C57Bl/6 mice and A2AKO littermates were treated as follows: saline 0.9% (control), tenofovir 75mg/Kg/day, dipyridamole 25mg/Kg/day, combination tenofovir/dipyridamole (n=10, 4 weeks). Calcein/AlizarinRed-labelling of newly formed bone was used, and long bones were prepared for microCT/histology.

Results: Tenofovir produced a dose-dependent increase in osteoclast differentiation ($EC_{50}=44.5nM$) that was reversed by dipyridamole ($IC_{50}=0.3\mu M$). Tenofovir increased Cathepsin K and NFATc1 mRNA levels and dipyridamole reversed the effect. Dipyridamole reversed the effect of tenofovir on pERK1/2, pp38 and NFkB nuclear translocation. WT mice treated with tenofovir lost nearly 10% of body weight ($p<0.001$). MicroCT revealed decreased BMD and altered trabecular bone in tenofovir-treated mice, reversed by dipyridamole. TRAP-staining showed increased osteoclasts in tenofovir-treated mice ($p<0.005$) an effect reversed by dipyridamole. Similar results were obtained for Cathepsin K and CD68 and RANKL-positive-cells, whereas OPG-positive-cells decreased with tenofovir. Similar results were obtained for tenofovir in A2AKO mice, but dipyridamole was not able to reverse the effect.

Conclusions: These results suggest that treatment with agents that increase local adenosine concentrations, like dipyridamole, and activate adenosine A2A receptor might prevent bone loss following tenofovir treatment.

P11. PURINERGIC SIGNALING IN OTHER FUNCTIONS

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HETERODIMERIZATION OF ADENOSINE RECEPTORS IN BROWN ADIPOSE TISSUE

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Background: the purine nucleoside adenosine regulates brown adipose tissue (BAT) physiology by signalling via the abundantly expressed Gs-coupled A2A and A2B adenosine receptors. BAT is specialized in burning energy by uncoupling ATP synthesis due to its unique uncoupling protein 1 (UCP1) and adenosine activates human and murine brown adipocytes (BA) at low nanomolar concentrations (Gnad et al.; 2014). An increasing number of evidence suggests adenosine receptor homo- and heterodimer formation. Therefore, we analysed the role of A2B signalling and heterodimerization in the context of adenosine-mediated BA activation.

Material and methods: to study the role and consequence of A2B heterodimerization, we used pharmacological inhibition as well BA/BAT deficient of A2B. Moreover, we applied biological resonance energy transfer (BRET) assays and proximity ligation assays (PLA).

Results: adenosine-mediated BA activation was suppressed after pre-treatment with an A2B-specific antagonist. This complete dependence of adenosine signalling on A2B was not expected since adenosine can enhance murine and human BAT activity via the A2A receptor (Gnad et al.; 2014; Ruan et al.; 2018) and recent work showed that heterologously overexpressed A2B inhibits signalling of A2A (Hinz et al.; 2018). In line with the repressed adenosine signalling, A2A-mediated energy expenditure was fully blunted in adipose-specific A2B knockout mice. On a molecular level, BRET analysis showed specific molecular A2B/A2A interaction in murine and human BA. In situ PLA revealed co-localization of endogenous A2B and A2A receptors in murine BAT. To disrupt A2B/A2A heterodimerization, peptides derived from TM regions 5 and 6 of both receptors were applied, which abrogated adenosine-induced BA activation demonstrating the functional relevance of these regions/interactions. Vice versa, no differences in A2B-activated BA activation of A2A+/+ and A2A-/- BA could be detected.

Conclusion: we describe the adenosine receptor A2B as pivotal centrepiece for adenosine-mediated brown adipocyte activation forming heterodimers with A2A receptors. Moreover, A2B signals independently of A2A, but is crucially required for A2A receptor signalling in BA/BAT.

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SIGNIFICANCE OF ADENOSINE A_{2A} RECEPTORS IN THE COMMENSAL-PATHOGEN SWITCH IN THE ELDERLY GUT

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Immunosenescence and inflammaging of the elderly is a public health issue, associated with increased morbidity and mortality due to infections and chronic inflammatory gut diseases.^{1,2} The adenosine A_{2A} receptor (A_{2A}R) contributes to fine-tuning inflammatory and immune responses, prompting an efficient elimination of threats while minimizing tissue damage.³

We studied the gut A_{2A}R distribution/density in an *in vivo* mice model of aged, adult and young individuals; assessed the relative intestinal over-colonization by *Candida albicans*, a gut commensal that can turn into an aggressive agent of opportunistic infections^{1,2}, and the correlation between this, tissue damage and gut microbiota diversity.

We showed that elderly mice are more prone to over-colonization by *C.albicans* than adults and young. This seems to be related with higher growth rate in intestinal lumen, independent of gut tissues invasion, with higher inflammation of the elderly gut. These mice have a higher stomach colonization and increased yeast-to-hypha transition, a virulent trait. When compared with young and adults, aged mice have lower gut A_{2A}R density and *C.albicans* overgrowth failed to increase it.⁴

These results indicate that aged mice have lower ability to cope with inflammation due to *C.albicans* over-colonization and phenotypic switch, related with the inability to adaptively adjust A_{2A}R density. In conclusion, gut over-colonization/dysbiosis control and microbiota homeostasis is likely to be regulated by the purinergic system, particularly A_{2A}R.

1) Bischoff. Curr Opin Clin Nutr Metab Care.2016;19:26.

2) Köhler et al. Microbiol Spectrum.2017;5:FUNK-0014.

3) Hasko et al. Nat Rev Drug Discov.2008;7:759.

4) Rodrigues et al. Oncotarget.2016;7:62862.

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**POSTNATAL SUBVENTRICULAR ZONE NEUROGENESIS:
ADENOSINE A_{2A} RECEPTORS AS REGULATORS OF BRAIN-
DERIVED NEUROTROPHIC FACTOR**

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Constitutive neurogenesis takes place in both adult mammalian subventricular zone of the lateral ventricle and in the subgranular zone of the dentate gyrus (DG) in the hippocampus. This study evaluated whether adenosine A_{2A} receptors (A_{2A}Rs) have a role in postnatal SVZ neurogenesis and if they are required for brain-derived neurotrophic factor (BDNF)-induced neurogenesis, namely in cell proliferation and neuronal differentiation, and for the capacity of progenitor cells to divide and self-renew within the SVZ.

Results using SVZ-derived neurosphere cultures demonstrated that neither A_{2A}R agonist (CGS21680, 30 nM), A_{2A}R antagonist (ZM241385, 50 nM) nor BDNF (30ng/ml), altered cell viability (measured by propidium iodide staining). A cell-fate study was also performed using an immunocytochemistry against Sox2 (a marker of neural stem cells with the ability to self-renew). Neither A_{2A}R activation nor blockade changed the number of Sox2+/+ SVZ cell-pairs derived from a progenitor cell division. Furthermore, neither proliferation (measured by BrdU staining) nor neuronal differentiation (measured by NeuN staining) of cultured cells were affected by either A_{2A}R agonist or antagonist incubation alone. Importantly, the *in vitro* data was corroborated in an *in vivo* 6-week-old rat model, where CGS 21680 (100 nM) was intraventricularly delivered for 28 days and BrdU was administered in the first 3 days of the treatment. In fact, A_{2A}R activation did not change proliferation nor neuronal differentiation (measured by BrdU and NeuN double-staining) *in vivo*. Nevertheless, BDNF enhancement of cell proliferation and neuronal differentiation *in vitro* was completely prevented by A_{2A}R antagonist. Conversely, A_{2A}R agonist enhanced axonal and dendritic length and branching of SVZ-derived neurons.

Taken together, data here described reveal a novel role for A_{2A}Rs as modulators of SVZ neurogenesis. Contrary to hippocampal neurogenesis (S. Xapelli – oral session), A_{2A}R activation in the SVZ does not increase neurogenesis, only promotes axonal and dendritic growth. However, A_{2A}R endogenous activation is crucial for BDNF-mediated actions.

ROLE OF ADENOSINE IN ADIPOSE TISSUE FIBROSIS

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Two types of adipose tissue can be distinguished: white and brown adipose tissue. White adipose tissue (WAT) plays an important role as energy storage. Brown adipose tissue (BAT) is not primarily used for energy storage, but is characterized by energy dissipation and thermogenic function. BAT generates heat via the numerous mitochondria that contain the uncoupling protein 1 (UCP1) by uncoupling ATP synthesis (non-shivering-thermogenesis).

It has been shown that obesity is associated with fibrosis in WAT. The resulting deposition of collagen fibers causes insulin resistance [1]. Interestingly, during obesity, BAT adapts a white fat-like phenotype. This process is also known as whitening, and fibrosis in the tissue can be observed [2]. However, the mechanisms underlying fibrosis in BAT are not well understood.

We established a method to isolate primary fibroblasts of different fat depots. The preliminary data show an increased number of fibroblasts in BAT after feeding mice a high fat diet (60% kcal from fat) compared to a control diet (13% kcal from fat). Moreover, the adenosine receptor A2B (ADORA2b) is highly expressed in BAT-derived fibroblasts compared to the other adenosine-receptor-subtypes and its expression is also increased after feeding mice a high fat diet.

Therefore, we focused on the role of the ADORA2b in BAT fibrosis, using ADORA2b deficient (ADORA2b^{-/-}) mice. The ADORA2b^{-/-} mice have a higher expression of profibrotic genes, such as collagens and TGFβ in BAT compared to ADORA2b^{+/+} mice. Furthermore, the Sirius Red Stainings of BAT and WAT of ADORA2b^{-/-} mice show a higher amount of collagen fibers than in the wild-type mice.

Our data indicate that ADORA2b plays a role in fibrotic processes in adipose tissue.

References:

- 1) Marcelin et al. A PDGF α -Mediated Switch toward CD9^{high} Adipocyte Progenitors Controls Obesity-Induced Adipose Tissue Fibrosis. *Cell Metabolism* 2017.25, 1–13
- 2) Halberg et al. Hypoxia-Inducible Factor 1 α Induces Fibrosis and Insulin Resistance in White Adipose Tissue. *Mol Cell Biol.* 2009. 29(16): 4467–4483

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P2X7R INDIRECTLY REGULATES THE JAM-A PROTEIN CONTENT VIA MODULATION OF THE GSK-3 β AND PKC- β 1

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The alveolar epithelial cells represent an important part of the alveolar barrier, which is maintained by tight junction proteins, particularly JAM-A, occludin and claudin-18 that regulate paracellular permeability.

Precision-cut lung slices of wildtype and knockout lungs and immortal epithelial lung E10 cells were treated with bleomycin, the P2X7R inhibitor oxATP and the agonist BzATP, respectively, to evaluate early changes in JAM-A expression. Immunohistochemical and biochemical methods were used. In order to examine the role of GSK-3 β and PKC- β 1 in the expression of JAM-A in alveolar epithelial cells, we used LiCl for GSK-3 β and an specific inhibitor for PKC- β 1 inhibiting experiments, respectively.

In this study, we report in the P2X7R knockout mice a strong increase in epithelial JAM-A expression as compared to the wildtype. Data showed evidence for a P2X7R dependent JAM-A expression *in vitro*. Inhibition of the P2X7R using oxATP increased JAM-A, whereas activation of the receptor decreased JAM-A protein level. Inhibiting experiments with LiCl showed a modulating effect on BLM-induced alterations in JAM-A levels. PKC- β 1 inhibition normalized the BLM-induced increase in protein level of JAM-A. In addition, BLM treated precision-cut lung slices from *P2rx7^{-/-}* mice responded with a lower increase in mRNA expression of JAM-A than BLM-treated precision-cut lung slices from WT mice.

Our data suggest that increased constitutive JAM-A protein level in *P2rx7^{-/-}* mice may have a protective effect against BLM-induced lung injury.

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