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Sesiones

Fine tuning neuromodulation by adenosine and neuroprotection

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ABSTRACT

Adenosine fine tuning neuromodulation is a very subtle change, similar to what *e.g.,* a pianist does, modulating a sound through insertion of another sound; so, modifying the characteristics of the previous sound. This is what adenosine does to modulate neurotransmitter actions. Adenosine neuromodulation is operated through high affinity inhibitory A_1 and excitatory A_{2A} receptors. These receptors have the particularity of interacting with receptors for other neurotransmitters and neuromodulators as well as with adenosine transport systems. This modulation is involved in neuroprotection namely via adenosine A_1 receptors in neurotoxicity during hypoxia, reactive oxygen species insults either inhibiting NMDA receptors or as part of interleukin-6 neuroprotecive effects. On the other hand A_{2A} receptors mainly trigger the action of several promoters of neuroprotection, e.g., BDNF and GDNF.

Key words: Adenosine receptors.—Neuromodulation.—Neuroprotection.

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RESUMEN

Neuromodulación fina por adenosina y neuroprotección

La neuromodulación fina por adenosina supone un cambio muy sutil, similar al que, por ejemplo, realiza un pianista, modulando un sonido a través de la inserción de otro, modificando así las características del sonido previo. Esto es lo que hace la adenosina para modular las acciones de los neurotransmisores. La neuromodulación por adenosina se realiza a través de receptores de alta afinidad inhibitorios A_1 y excitatorios A_2. Estos receptores poseen la particularidad de interaccionar con receptores para otros neurotransmisores y neuromodulares, así como con los sistemas de transporte de adenosina. Esta modulación está implicada en la neuroprotección vía receptores de adenosina A_1 en la neurotoxicidad producida durante la hipoxia, daños producidos por las especies reactivas del oxígeno inhibiendo los receptores NMDA o como parte de los efectos neuroprotectores de la interleukina-6. Por otro lado, los receptores A_{2A} principalmente desencadenan la acción de varios promotores de la neuroprotección, como el BDNF y el GDNF.

Palabras clave: Receptores de adenosina.—Neuromodulación.—Neuroprotección.

INTRODUCTION

At chemical synapses, neuromodulators exert a **«fine tuning»,** a very subtle change, similar to what, for example, a pianist does, modulating a sound (the effect of a neurotransmitter) through insertion of another sound (the effect of a neuromodulator), so, modifying the caracteristics of the previous sound. So far adenosine fills completely the criteria of a neuromodulator (see 26).

In this paper I shall discuss the potential of this neuromodulator as a neuroprotector, which also means, till certain point, to be involved in neuroregeneration and brain repair.

Knowing how neuroregeneration occurs it is possible to conceive processes of protecting the nervous system, in other words to induce and to develop neuroprotection. The cellular elements and the chemical neuromediators involved in brain insults act via interconnections between the cellular elements and their secretions; the immune system and the nervous system are highly regulated in normal physiology, which benefits the organism. When neuronal cells suffer insults the connections in the brain are deprived of

the local control over microcirculation and necessary oxygen, rendering membrane potentials useless to modulate neuronal function. The fact that chemical mediators are already part of normal physiology, whether during development or adulthood, means that their activity can be modified by specific agonists and antagonists to restore homeostasis or to promote the safe pathways that can lead to regeneration. During the past decades considerable experimental and clinical data have been accumulated regarding cellular and biochemical events associated with neuroprotection, neuroregeneration and brain repair.

Three situations related to neuroprotection will be discussed in the context of adenosine-related medicines as therapeutical potential agents. The points I shall emphasize are: first, the insults of neurons caused by hypoxia/ischemia, which is a typical situation that occurs during stroke; secondly, I will discuss the reactive oxygen species (ROS) as a way to induce lesions in neurons and its relation to neurodegenerative diseases and thirdly, how to enhance the putativte neuroprotective actions contained in neurotrophic factors, such as e.g. the brain derived neurotrophic factor (BDNF) and the glial derived neurotrophic factor (GDNF).

Before I go into these aspects I would like to give you briefly some biographical notes about my research context that can explain *why a medical doctor graduated in 1965, who wishes to be a psychiatrist enters into basic neuroscience research.* In fact, when in 1957 I started my studies on Philosophy and in particular Psychology, I was fascinated by Perception and in particular Behaviour. Why that strange behaviour of the main character, Meursault of the Albert Camus novel «L'Etranger»? I decided to go into the medical school to become a Psychiatrist. It was indeed with great joy that in 1977 when the first edition of the book ***From Neuron to Brain by*** Stephen Kuffler and John Nichols appear, I read in the preface: *«We work mostly on the machinary that enables neurons to function. Students who become interested in the nervous system almost always tell us that their curiosity stems from a desire to understand perception, consciousness, behaviour, or other higher functions of the brain. Knowing of our preoccupation with the workings of isolated nerve cells or simple cell systems, they are frequently surprised that we ourselves started with similar motivations, and they are even more surprised that*

we have retained those interests. In fact, we believe we are working toward that goal (and in that respect probably we do not differ from most of our colleagues and predecessors). We hope, to show that we are pointed in the right direction».

However, when in 1964 I did the disciplines of psychiatry and neurology, I have an enormous deception, there was almost no help for psychiatric patients. Psychiatric hospitals were filled with «prisoners-like», more than patients. So, I decided to give up psychiatry. After spending almost four compulsory years as a Medical Doctor of the Portuguese Army, two of them in Mozambique, I started in 1970 my first studies on Neuropharmacology. The scientific question was: how brain interferes with autonomic responses? The idea was to relate the cardiotonic effects of ouabain on the brain, with its cardiovascular actions, in particular blood pressure (BP) and heart rate (HR). As HR is under the influence of acetylcholine (ACh) we also measured by bioassay ACh release (27).

This sort of experimental approach had many limitations resulting from being an *in vivo* investigation with many variables involved and also because it was not possible to measure chemically very tiny amounts of ACh. It was like being almost 2000 years back in the History of Pharmacology. Being in the Real Academia Nacional de Farmacia, I think it is appropriate to recall ***Galen*** *(130-200 ac), and his* ***fluid theory*** of cerebral ventricles and nerves as vessels to circulate fluids.

After two years of research under supervison of Fernando Peres-Gomes, he decided to send me to Edinburgh to learn about the most simple cholinergic synapse —the Neuromuscular Junction— as well as to learn about measuring electrophysiologically ACh. Knowing about this junction (synapse) one could start to know about central nervous system synapses and in consequence to know about brain functions and dysfunctions. This makes me fill closer to my initial interest —psychiatry—. Also my readings of Egas Moniz (*Nobel Prize, 1949*), who based upon Ramon y Cajal (*Nobel Prize, 1906*) observations, wrote in 1953: «*...Os neurónios encadeam-se e as fibrilhas dos cilindraxis não se ligam em soldadura a outras células; formam contactos através dos botões de Held, de forma variada [...] Sobre esta noção anatómica, que devemos a Ramón y Cajal, concluí*

que essas ***sinapses****, repetidas em míriadas de células,* ***são a base orgânica do pensamento****. A vida psíquica normal depende do bom funcionamento sináptico; e* ***as perturbações mentais provêm do desarranjo das sinapses»,*** *In* ***«Como consegui realizar a leucotomia prefrontal». Conferências Médicas Literárias VI,*** strongly support my decision to go into basic research to study synapses.

Two other important reasons that encouraged my interests on the Neuromuscular Junction came from my readings of Claude Bernard, (1813-1878), one of my scientific heroes. In 1849 he wrote the important paper on ***«Action physiologique des venins (curare)»***, published at *C. R. Sceances Soc. Biol. Ses Fil.* 1: 90 where he states that «*Chez lez animaux le système nerveux et musculaire met tous ces organes en sympathie ou en rapport les uns avec les autres», and in the Physiology Course at the Univ. of Paris in the* **Sommaire de la «Vingt et uniéme leçon, delivered on** 28 mai 1856, he wrote: *"le curare est sans action sur les organes actifs de la circulation, et il n'enléve pas au sang ses aptitudes physiologiques". —Action du curare sur le systéme nerveux:* ***il abolit les manifestations du systéme nerveux et laisse intact le systéme musculaire—.*** *On peut prouver par lá que la contractilité musculaire et l'excitabilité des nerfs moteurs sont deux propriétés distinctes»*. The second important reason was the work by the Nobel prize winners, Bernard Katz, Ulf Von Euler and Julius Axelrod awarded jointly in 1970 for their discoveries concerning the humoral transmitters in the nerve terminals and the mechanism for their storage, release and inactivation. It happens that one of the scientific «dilet» sons of Bernard Katz was Bernard Ginsborg just moving in early seventies to the University of Edinburgh, where he was going to be my supervisor.

ADENOSINE AND THE NEUROMUSCULAR JUNCTION

In the late sixties Bernard Katz and Paul Fatt taught Bernard Ginsborg the secrets of Neuromuscular Transmission at the Univ Coll, Lond. When Bernard Ginsborg moved to Edinburgh he started to study the physiology and pharmacology of the Neuromuscular Junction, and together with his post-doc, David Hirst. They were the

first to describe that adenosine inhibits neurotransmitter release and this was done using as a synapse the Rat Neuromuscular Junction (12, 13). When I arrived in Edinburgh, Bernard Ginsborg asked me to continue this work in another model —the Frog Neuromuscular Junction— (32, 33), since in this model there is no margin of safety, making this nicotinic cholinergic synapse more similar to the central excitatory synapses, now known to be the glutamatergic synapses.

ADENOSINE AND THE CENTRAL NERVOUS SYSTEM SYNAPSES

As described for its action on endplate potentials (EPPs), adenosine at the frog neuromuscular junction, inhibited the amplitude of the excitatory post-synaptic potentials (EPSPs) recorded from the Guinea pig Olfactory cortex slices, as first described by Okada & Kuroda (22). This work has been followed by the work of Dunwiddie's group, Stone's group using rat hippocampal slices, as well as by my group, in several publications since 1985 (see list in PubMed), where it was described that the A_1-receptor inhibitory effects of adenosine and adenosine analogues on glutamatergic transmission, possess almost all characteristics of those previously observed using the frog neuromuscular junction as a model of synaptic transmission.

Endogenous Adenosine

Adenosine antagonism, adenosine uptake inhibition, adenosine deaminase, 5'-ecto-nucleotidase inhibition were used to manipulate and to recognize the role of endogenous adenosine (29). In parallel we came across with the existence of A_{2A} excitatory effects at the neuromuscular junction (29) and in the hippocampus, the first description was the effect of an A_{2A} agonist (CGS21680) on rat hippocampal glutamatergic synaptic transmission (35). This was further supported by *in situ* hybridization autoradiograms, which confirmed the co-expression of A_1 and A_{2A} in hippocampal brain slices. The relative importance of A_{2A} receptors *vis à vis* A_1 receptors in the hippocampus was shown in 1996 when A_{2A} receptors prove to

be more effective than A_1 receptors in high frequency stimulation (7), and in 2003 when it was demonstrated that A_{2A} receptors are more effective than A_1 receptors in aged than in young rats (see 31). It appears that whilst the most predominant action of extracellular adenosine in the hippocampus is A_1-receptor mediated inhibition of synaptic transmission, the less abundant A_{2A}-receptor are devoted to: prime, trigger or modulate the action and/or inactivation of other neuromodulators and, therefore, to fine-tune neuronal activity (37). Other interesting observations on A_{2A} effects include stimulation by A_{2A}-receptors of the carotid body arterial chemoreceptors and ventilation. Since hypoxia is the physiological stimuli of carotid body chemoreceptors, the finding that a substance, preferentially released by hypoxia-adenosine, stimulates ventilation seems to close a circle of understanding the mechanisms involved in hypoxic stimulation of ventilation (20, 21).

ADENOSINE AND NEUROPROTECTION

What means neuroprotection?

It is a collective therapeutic approach to provide neuronal survival. In a normal neuron the amount of O_2 supplied is according to the cell needs, ATP production fills the needs of the cell to make the pumps to work and pH is established around 7.0. When hypoxia occurs there is necrosis in some cells and in the *penumbra* involving necrosis, the cells enter into a process of apoptosis where it will be possible to rescue some of the cells that did not enter into the necrosis processes.

How could we protect nerve cells from insults?

By this I mean to protect neurons from ischemia and/or hypoxia, from trauma, from neurodegenerative diseases. What do all these situations have in common? The answer is the death of neurons. So the emergent question is how neurons are killed? Glutamate is now accepted to be the main «killer» of neurons after brain insults. Glutamate acts both as a primary excitatory neurotransmitter and a

potential neurotoxin within the mammalian brain. Experimental evidence suggests that hyperactivity of the glutamate NMDA-receptor mediated current system contributes to neuronal death. Also, this neural injury is followed by gliosis which has been linked to the severity of brain lesions. In this context, investigation of substances that can selectively inhibit the glutamate NMDA receptor subtype in humans, while also therapeutically controlling glial cell responses and/or triggering the release of neuroprotective factors from these cells following brain insults, would be of great help in neuroprotection. This has led to the application of pharmacological strategies to limit the lesions and subsequent neurological deficits. The research on pharmacological agents will help to promote functional rewiring of brain after injury in order to obtain neural repair and neurological rehabilitation.

So, the immediate question is: what occurs at a glutamatergic synapse during insults? The actual *consensus* is that too much glutamate is being released and the NMDA-R «till then silent» is opened and becomes too much permeable to Ca^{2+} ions. Because the cell suffers from energy supply i.e. there is no ATP available for the Ca^{2+} pumps to expel the excess of Ca^{2+}, the neurons die. According to this theory if one inhibits glutamate release during insults and/or inhibits NMDA-R activation one could save neurons from the deleterious effects of glutamate.

How one could inhibit glutamate release during insults?

It is well known that if one measures synaptic transmission activity (e.g EPSPs) from rat hippocampal slices, and hypoxia is applied for 90 minutes, this insult inhibits and eventually completely blocks synaptic transmission, an effect that is completely recovered when hypoxia is terminated. It is well established that the inhibitory neurotransmitter, gamma-amino-butyric acid (GABA), inhibits glutamatergic transmission in normoxic conditions but it does not work during hypoxia (see 19).

If GABA, the main inhibitory transmitter in the brain, is not apparently effective during hypoxia to control glutamate involvement in the hypoxia insult, the question one could ask is: which other

substances, also abundant in the brain, as GABA, will have the ability to inhibit glutamatergic transmission?

In the brain, besides the classical neurotransmitters, there are substances that behave as neuromodulators of neurotransmitters and even of neuromodulators themselves see e.g. (35). These substances, in contrast with classical neurotransmitters, are not contained in vesicles, are not released through exocytosis but through transporters, and can be released post-synaptically to act both pre- and post-synaptically on specific receptors. One of these substances intensively studied in the last 25 years is adenosine.

Adenosine exists in all cells, and is released from apparently all insulted cells, including neurons and glial cells. Once in the extracellular space, adenosine modifies cell functioning by operating G protein-coupled receptors.

Adenosine as a neuromodulator

Adenosine is indeed consensually recognised as a very important substance in the homeostasis of the cells of the nervous system, being in relation to cardio-protection once named «a signal of life» (11). It modulates the activity of the nervous system by acting pre-synaptically (by inhibiting or facilitating transmitter release), post-synaptically, and/or non-synaptically. The way adenosine is doing this is through activation of physiologically relevant high affinity adenosine receptors (A_1, A_{2A}) as well as of a lower affinity receptors (A_{2B}, A_3), which might be relevant in pathological conditions (see 31). Besides affecting directly the neuronal cells, adenosine receptor activation also influences the action of other neurotransmitters as well as of other neuromediators. Because adenosine uses very subtle manners to participate in these interactions, it acts as a *fine tuner*, considering that in this way, adenosine is involved in a very sophisticated interplay between its own receptors and the receptors for other synaptic mediators. From these modulatory actions it is possible to anticipate potential therapeutic applications, especially when these actions are mediated by high-affinity adenosine A_{2A} receptors in regions where clear functions have been identified. The A_{2A} receptor is highly expressed in the striatopallidal GABAergic neurones and expressed in

lower levels in other brain regions. A_{2B} possess low levels of expression in the brain. A_3 has apparently intermediate levels of expression in the human cerebellum and hippocampus and low levels in most of the brain (31). The well known striatal A_{2A}/D_2 receptor interactions may offer new therapeutic leads for basal ganglia disorders, such as Parkinson's disease and Huntington's chorea, as well as for schizophrenia (31). Inhibition of D_2 receptors in the ventral striatum seems to be associated with the antipsychotic effect of neuroleptics, while inhibition of dopamine D_2 receptors in the dorsal striatum is related to their extrapyramidal side effects. In the periphery, the interaction of A_{2A} receptors with other receptors that regulate acetylcholine release from motor nerve terminals could reveal the importance of A_{2A} receptors when deficits in acetylcholine release occur (e.g. myasthenic syndromes). The excitatory effects of A_{2A} receptors on acetylcholine release might prove useful when developing cognitive enhancers that by increasing acetylcholine release could be of therapeutic interest in dementia (e.g. Alzheimer's disease). Since the adenosine A_1 receptors inhibit neurotransmitter release, and inhibit A_{2A} receptor functioning, one can also predict that adenosine-related medicines that combine adenosine A_1 receptor blockade with A_{2A} receptor activation will be useful in situations where an increase in neurotransmitter release is needed. There are also situations where A_{2A} receptor activation might prove to be excitotoxic (e.g. increase in glutamate release) and, therefore, A_{2A} antagonism will be needed (31).

It is worth noting that after several years of concentrating most of the adenosine research efforts on adenosine A_1 receptors, the adenosine A_{2A} prove to be a very promising receptor in terms of understanding the subtle ways used by this nucleoside to implement a harmonic and fine control of synaptic activity.

Although adenosine is not a neurotransmitter on its own, it shares via A_1 activation many properties attributed to the major inhibitory neurotransmitter —GABA, i.e. decreases excitability mediated by glutamate—. So GABA and adenosine constitute key molecules in the control of glutamatergic synaptic transmission in the central nervous system (see above). Adenosine is able to functionally disconnect GABAergic interneurones by inhibiting their glutamatergic input, a process that might be particularly relevant under conditions of intense adenosine release, such as it happens during hypoxia. Thus,

although indirectly, adenosine inhibitory (A_1) receptors might control GABAergic functioning. Direct excitation of GABAergic nerve terminals by adenosine might occur through A_{2A} receptors (31).

Adenosine as a modulator of other neuromediators

Besides its direct pre- and post-synaptic actions on neurons, adenosine is rich in nuances of priming, triggering and braking the action of several neurotransmitters and neuromodulators. Adenosine is also able to «synchronize» or «desynchronise» receptor activation for neuropeptides such as calcitonin gene-related peptide (CGRP) and vasoactive intestinal peptide (VIP), nicotinic autofacilitatory receptors, NMDA receptors, metabotropic glutamate receptors, as well as adenosine own receptors (37). Also the inhibitory action of adenosine A_{2A} and A_{2B} receptors on nitric oxide (NO) production and iNOS expression in glial cells (6) can constitute a partnership aiming neuronal protection after insults. Of particular importance is the recent finding by Chao (6) that TrkB neurotrophin receptors can be activated in the absence of neurotrophins, providing that A_{2A} receptors are activated. The authors emphasize that although there are suggestions proposing the use of neurotrophins in Alzheimer's dementia, amyotrophic lateral sclerosis and peripheral sensory neuropathy, it has been difficult to deal with delivery and pharmacokinetics of these molecules. Thus, the possibility of promoting trophic effects by manipulating the degree of activation of A_{2A} receptors, may prove highly relevant in neurodegenerative disorders where sustained actions on survival signalling pathways are needed.

Selective activation of adenosine A_{2A} instead of A_1 receptors

Adenosine A_1 and A_{2A} receptors can co-exist in the same nerve terminal (35). Evidence for this arrived from the observation that an A_1 receptor agonist acting pre-synaptically inhibits and an A_{2A} receptor agonist acting also presynaptically enhances the amplitude of endplate potentials recorded intracellularly from a single endplate, which in the case of the striated muscle receives input from only one

nerve ending. The coexistence of both subtypes of high affinity adenosine receptors prompted the question on how adenosine chooses to activate A_1 or A_{2A} receptors, and under which conditions does it discriminate between these receptors. It appears that instead of the classical view that endogenous adenosine activates A_1 receptors regardless of whether it is released as such or formed from adenine nucleotides, adenosine formed from adenine nucleotides acts preferentially on A_{2A} receptors, and adenosine released as such acts preferentially on A_1 receptors (9). This may result from different localization of A_1 and A_{2A} receptors in relation to adenosine release sites and ecto-5'-nucleotidase localization. An indication that burst-like adenosine formation from released adenine nucleotides might be, at least in part, responsible for its preferential action on A_{2A} receptors, is the finding that when motor nerve endings are stimulated at low frequency, i.e., under conditions where smaller amounts of adenine nucleotides are released, the ecto-5'-nucleotidase inhibitor, a,b-methylene ADP (AOPCP), enhances (31) rather than inhibits acetylcholine release. Indeed, the pattern of neuronal firing influences the pattern of purine release and extracellular formation, and in consequence, influences the way purines modulate synaptic transmission. High frequency stimulation favours ATP release, and adenosine formation, which preferentially activates adenosine A_{2A} receptors activation, whereas low frequency stimulation favours adenosine release, and preferential adenosine A_1 receptor activation (see 31).

Modulation of NMDA receptors

In the isolated rat hippocampal neurons, A_1 receptor activation inhibits NMDA receptor-mediated currents (34). In the hippocampus A_1 receptors are also inhibitory of glutamate release and A_{2A}-receptors facilitate glutamatergic synaptic transmission (31). Interestingly, the inhibitory action of A_1 receptor agonists on NMDA receptor mediated currents in hippocampal neurons is observed in a concentration range compatible with a tonic inhibitory action of adenosine. Due to the important role played by NMDA receptors in synaptic plasticity phenomena, as well as in neuronal injury after prolonged stimulation/depolarisation conditions, it is conceivable that the

ability of A_1 receptors to inhibit NMDA currents is the basis of the A_1 receptor mediated inhibition of synaptic plasticity (LTP and LTD) as well as contributes to A_1 receptor mediated neuroprotective actions (31).

To summarize this point I would like to stress that adenosine exerts pre-, post- and non-synaptic effects by acting on high affinity receptors: A_1 and A_{2A}. There are also low affinity receptors: A_{2B} and A_3, but so far their activation is not apparently related with neuromodulation with potential implications for neuroprotection.

Adenosine-Receptor interactions with Adenosine transporter

NECA activates adenosine tranport in cultured chromaffin cells (8). This was the first work to making connection between adenosine receptors and adenosine transport. Recently (24), using hippocampal synaptosomes described the nature of this receptor, an A_{2A} receptor, which when activated enhances adenosine uptake, and that A_{2A} receptor activation enhances adenosine release. So, it appears that A_{2A} receptors regulate adenosine transporters influencing the extracellular availability of this nucleoside.

HYPOXIA RELEASES ADENOSINE

It is well established and consistent that after hypoxia/ischemia insults there is release of adenosine into the synaptic cleft. This adenosine is responsible for the inhibition of synaptic transmission during hypoxia, since the A_1-receptor antagonist, a xanthine derivative, DPCPX, attenuates this inhibitory effect of hypoxia by at least 50%. Therefore, the inhibitory effect of hypoxia is a consequence of A_1-receptor activation by huge amounts of adenosine being released (34). One interesting aspect in the adenosine effect is that if one tries to recover from hypoxia in the presence of the A_1 antagonist, DPCPX, the recovery is never complete as it happens during recovery without A_1 blockade. This suggests that adenosine is absolutely needed for complete recovery from hypoxia. This effect of adenosine is a consequence of pre-synaptic inhibition of glutamate

release and subsequent reduction of NMDA-receptor activation (34). So, adenosine A_1-receptor activation by released adenosine depresses hippocampal synaptic transmission during hypoxia but is a process that facilitates recovery upon re-oxygenation.

CEREBRAL ISCHEMIA AND TRAUMA

Cerebral ischemia leads to brain damage caused by pathogenetic mechanisms that are also activated by neurotrauma. These mechanisms include among others excitotoxicity, overproduction of free radicals, inflammation and apoptosis. Furthermore, cerebral ischemia and trauma both trigger similar auto-protective mechanisms including the production of heat shock proteins, anti-inflammatory cytokines and endogenous antioxidants. Neuroprotective therapy aims at minimizing the activation of toxic pathways and at enhancing the activity of endogenous neuroprotective mechanisms. According to (18), the similarities in the damage-producing changes in endogenous substances in the brain may imply that neuroprotective compounds found to be active against one of these conditions may indeed be also protective in the other.

Hypothermia

The use of hypothermia to treat various neurological emergencies, initially introduced into clinical practice in the 1940s and 1950s, had been considered without interest by the 1980s. However, in the early 1990s, there was a revival of its use in the treatment of severe traumatic brain injury. The success of mild hypothermia led to the broadening of its application to many other neurological emergencies. Mild hypothermia has been applied with varying degrees of success in many neurological emergencies, including traumatic brain injury, spinal cord injury, ischemic stroke, subarachnoid haemorrhage out-of-hospital cardiopulmonary arrest, hepatic encephalopathy, perinatal asphyxia (hypoxic-anoxic encephalopathy), and infantile viral encephalopathy. According to (15), there is no total consensus on the efficacy and safety of mild hypothermia though preliminary clinical studies have shown that mild hypothermia can be a feasible and

relatively safe treatment. Recently it has been shown *in vitro* patch clamp studies that in conditions of 32 ºC, adenosine inhibits NMDA receptor currents in hypoxia suggesting that adenosine might exert neuroprotection during hypothermia (34).

In summary, during hypoxia the neuroprotection induced by adenosine operates both a decrease of neurotransmitter release via inhibition of pre-synaptic calcium entry by blocking calcium channels (28) and post-synaptically inhibiting calcium entry via inhibition of NMDA-receptors.

Finally I also would like to mention our recent observations on stimulation with interleukin-6 (IL-6), which increases A_1 receptor-mediated inhibition of synaptic transmission. In WT but not in IL6-KO mice, seizures induce adenosine A_1 receptor expression. These observations reinforce the role of adenosine as an anti-epileptic agent. IL-6-induced amplification of A_1 receptor function enhances the responses to readily released adenosine during hypoxia, enables neuronal rescue from glutamate-induced death, and protects animals from chemically induced convulsing seizures. Taken together, these results suggest that IL-6 minimizes the consequences of excitotoxic episodes on brain function through the enhancement of endogenous adenosinergic signaling (see 2).

REACTIVE OXYGEN SPECIES (ROS)

During brain insults with xanthine-xanthine oxidase there is production of reactive oxygen species (ROS). It was described by Almeida et al (1) that the synaptic inhibition induced by ROS needs adenosine to completely recover in contrast with what happens in the presence of adenosine A_1 receptor blockade with DPCPX (1).

ENHANCEMENT OF NEUROPROTECTIVE EFFECTS OF NEUROTROPHIC FACTORS

Neurotrophic factors have been shown to have potential therapeutic applications in neurodegenerative diseases, and nerve growth factor (NGF) is neuroprotective in models of excitotoxicity.

NGF administration, in the acute, post-traumatic period following fluid-percussion brain injury, apparently improves post-traumatic cognitive deficits (39) and a neurotrophic factor treatment following traumatic brain injury is neuroprotective (16). Brain-derived neurotrophic factor (BDNF) has been shown to be neuroprotective in models of excitotoxicity, axotomy and cerebral ischemia. Blaha et al. (3) demonstrated the therapeutic potential of brain-derived neurotrophic factor following traumatic brain injury in the rat. Neurotrophic factors are involved in neuronal maturation during development, and protect neurons in experimental models of neurodegenerative diseases. However, because of the blood brain barrier it is very difficult to deliver these factors into the brain. A way to overcome this difficulty was implemented by (38) using trial infusions of nerve growth factor (NGF) in three patients with Alzheimer's disease. NGF was infused continuously through a neurosurgically implanted cannula into the lateral ventricles of the brain (38). However, technical difficulties discouraged the continuation of this therapy.

BDNF is a neurotrophin abundant in the brain and recently it has been shown that BDNF applied acutely facilitates synaptic transmission if, before its application one depolarises the preparation with potassium (4). Also in hippocampal synaptosomes BDNF facilitates potassium-evoked glutamate release providing synaptosomes have been previously depolarised by potassium (5).

Another group (17) showed that A_{2A} receptors induce phosphorylation of BDNF-TrkB-receptors. Since depolarisation enhances the release of adenosine our group (see 9) decided to investigate if activating A_{2A} receptors we could selectively facilitate BDNF actions on synaptic transmission. In fact with the activation of A_{2A} receptors with selective agonists or by increasing the adenosine production in the brain with adenosine kinase inhibitors (e.g. iodotubercidin), it is possible to induce facilitatory effects of BDNF on synaptic transmission in hippocampal slices (see Figure 1). In non-A_{2A} pre-conditioned slices there is no BDNF effect on synaptic transmission (9). It therefore appears to exist potential for the use of A_{2A} agonists or substances such as adenosine kinase inhibitors, which through peripheral administration could wake up «sleeping» neurotrophic factors in order to induce neuroprotection. This is also

true in ageing (10) where neuroprotection is hardly needed. There is a relationship between age-related changes in the density of TrkB receptors and adenosine A_{2A} receptors on what concerns BDNF-induced enhancement of synaptic transmission in the hippocampus (10). This should be taken into consideration whenever evaluating BDNF actions in nerve cells, and may prove relevant in the design of BDNF-based therapeutic strategies.

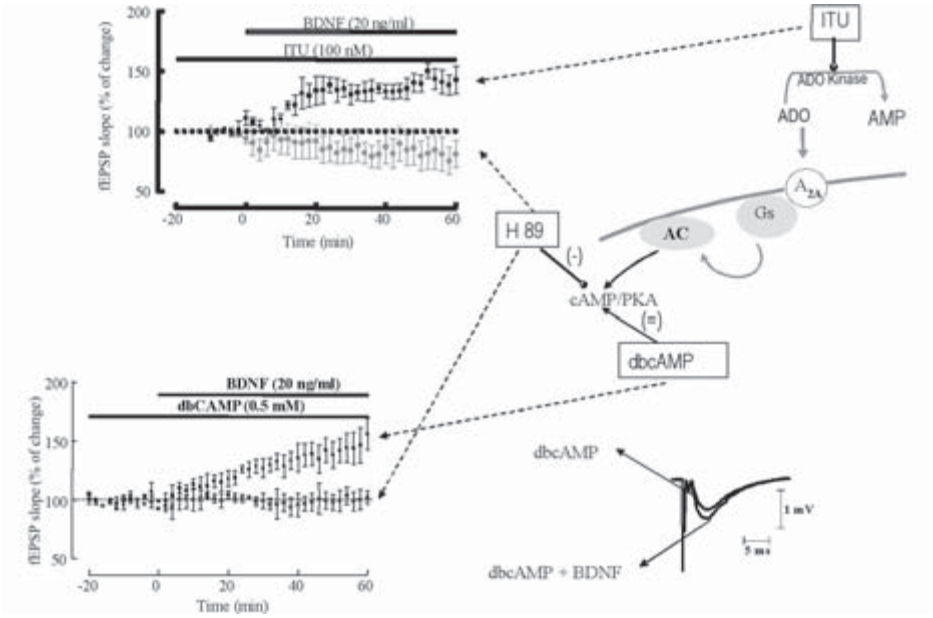

FIGURE 1. ***Mechanisms involved in the activation adenosine A_{2A}-receptors.*** *It is proposed an intracellular cascade involving the cyclic AMP/PKA and its potential relationship with the BDNF enhancement of synaptic transmission.*

Also GDNF (14) in striatum promotes release of dopamine. GDNF has been pointed out as a potential therapeutic approach in the management of Parkinson's disease (25). So, the prevention of GDNF effect by A_{2A} receptor antagonism point towards the need of further studies on the consequences of long-term therapy with A_{2A} receptor blockers in neurodegenerative diseases where neurotrophic factors may play a beneficial role. One issue that should be explored in the future is the optimal time window for combined beneficial effects for GDNF and A_{2A} receptor agonists/antagonists. If, in the late stages

of neurodegenerative diseases, A_{2A} receptor antagonists can be advantageous, in the early stages, where an enhancement of neurotrophic factors should be highly desirable, A_{2A} receptor antagonists should be avoided in order to allow neurotrophic influences.

Perspectives to explore the potential use of adenosine to induce neuroprotection

By using A_{2A} agonists or adenosine kinase inhibitors (which induce the production of considerable amounts of endogenous adenosine) that cross blood brain barrier, it would be possible to promote the facilitation of BDNF or GDNF actions on synaptic transmission (see Figure 2).

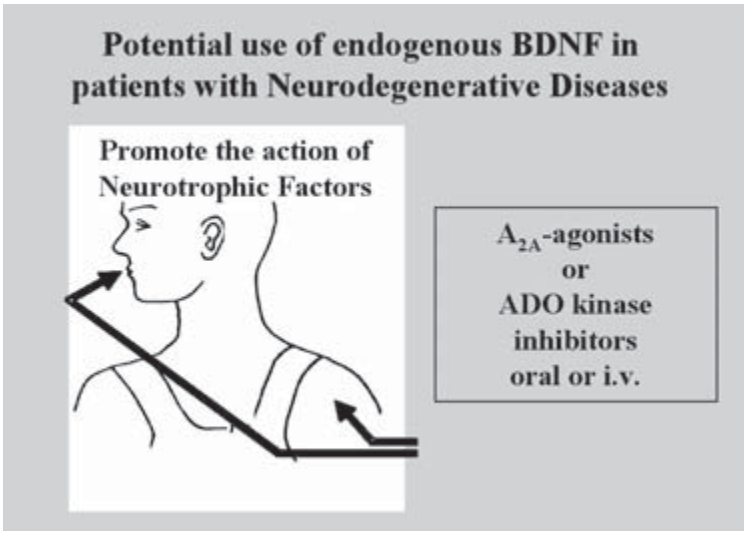

Figure 2. ***Perspectives to explore the potential use of adenosine to induce neuroprotection.*** *By using A_{2A} agonists or adenosine kinase inhibitors (which induce the production of considerable amounts of endogenous adenosine) that cross blood brain barrier, it would be possible to promote the facilitation of BDNF on synaptic transmission.*

CONCLUSIONS

1. Synaptic transmission during neuronal insults is under tight control of endogenous extracellular adenosine.
2. A_1-Receptor minimize the activation of toxic pathways e.g. inhibiting glutamate release, inhibiting NMDA receptor-mediated currents, and IL-6 facilitates A_1-Receptor inhibition, and A_{2A}-Receptor interplays with receptors for other neuromodulators via subtle modifications of fine tuning, «synchronizing» or «desynchronizing» receptor activation.
3. A_{2A}-Receptors enhance the activity of endogenous neuroprotective mechanisms e.g. the action of neurotrophic factors, which are devoted to protect neurons from lesions (e.g. BDNF, GDNF), so, by A_{2A} receptor activation one can overcome difficulties found with the use of neurotrophins in neurodegenerative disorders.

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