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Sesiones

New Perspectives in Ocular Pharmacology: Nucleotides as Therapeutic Agents

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JESÚS PINTOR*
Departamento de Bioquímca, E. U. Óptica.
Universidad Complutense de Madrid

ABSTRACT

Diadenosine polyphosphates are a group of nucleotides which modulate physiological processes in the eye such as tear secretion, corneal wound healing and intraocular pressure. This regulation is carried out by P2 purinergic receptors termed P2X and P2Y as well as dinucleotide receptors. Diadenosine polyphosphates are present in tears and when topically applied they can produce tear secretion in a process mediated by P2Y receptors. P2Y receptors are also present in corneal epithelial cells and they can accelerate the rate of re-epithelialization after superficial injuries. On the other hand, intraocular pressure is reduced by all those nucleotides activating P2X receptors. Due to the importance of the presence of these receptors in ocular structures it is possible to think in the use of dinucleotides, naturally occurring and synthetic ones, for the treatment of dry eye, corneal injuries and glaucoma.

Key words: Corneal wound healing.—Diadenosine polyphosphates.—Dry eye.—Glaucoma.—Intraocular pressure.—Nucleotide receptors.

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* Discurso de ingreso como Académico Correspondiente.

Dirección de contacto: Dep. Bioquímica. E.U. Óptica. Universidad Complutense de Madrid. C/ Arcos de Jalón, s/n. 28037. Madrid. Telf.: +34-91-3946859. Fax: +34-91-3946885.

E-mail: jpintor@vet.ucm.es

Abbreviations: ABC, ATP binding cassette; ApnA, diadenosine polyphosphates; CFTR, the cystic fibrosis transmembrane conductance regulator; HPLC, high performance liquid chromatography; IOP, intraocular pressure.

RESUMEN

Nuevas perspectivas en farmacología ocular: los nucleótidos como agentes terapéuticos

Los diadenosina polifosfatos son un grupo de nucleótidos que modulan procesos relevantes de la fisiología ocular como la secreción corneal, la cicatrización corneal y la presión intraocular. Esta regulación es llevada a cabo a través de receptores purinérgicos del tipo P2 como son los P2X y P2Y, así como por el receptor de dinucleótidos. Los diadenosina polifosfatos están presentes en las lágrimas y cuando son instilados tópicamente pueden inducir la secreción lagrimal en un proceso mediado por receptores del tipo P2Y. También los receptores P2Y están presentes en las células del epitelio corneal que aceleran el proceso de cicatrización corneal tras una lesión superficial. Por otra parte, la presión intraocular se puede reducir aplicando aquellos nucleótidos que activen receptores del tipo P2X. Debido a la presencia de estos receptores en las estructuras oculares es posible pensar en el uso de los dinucleótidos, naturales o sintéticos, para el tratamiento del ojo seco, heridas corneales y el glaucoma.

Palabras clave: Cicatrización corneal.—Diadenosina polifosfato.—Ojo seco.—Glaucoma.—Presión intraocular.—Receptores de nucleótidos.

OCULAR PATHOLOGIES

In spite of the number of ocular pathologies is immense, three pathological states are now important due to their incidence in modern society: *dry eye, corneal wound healing* and *glaucoma*. Apart from the incidence of all of them in the world population, the two first do not have an effective treatment in most of the cases. On the other hand glaucoma has several pharmacological treatments but most of them present wide side effects. In the three cases the development of new pharmacological compounds is necessary.

The strategy of pharmaceutical companies regarding the discovery of new compounds is mainly the variation of well-established pharmaceutical species by modifying their chemical structures in order to obtain better agonists, antagonists or enzyme inhibitors. Also companies, by means of their research and development departments try to find new molecules, receptors and enzymes as pharmacological targets. Nonetheless, the discovery of new attractive compounds is mostly carried out by research centres and universities. Taking into account this point of view, we have

modestly contributed to the discovery of new biological molecules and their implications in the eye physiology and physiopathology. We have investigated the role of extracellular nucleotides and dinucleotides in the eye. Nucleotides and dinucleotides can exert extracellular actions by means of the activation of different membrane receptors termed P2 purinergic receptors, which are described in the following section.

RECEPTORS FOR EXTRACELLULAR NUCLEOTIDES

The effects of nucleotides are due to the existence of receptors in cell membranes termed P2 receptors that transmit the message into the cell producing certain changes in the cell biochemistry. In this sense, nucleotides can bind and activate to two major types of receptors, metabotropic nucleotide receptors or P2Y receptors and ionotropic P2X receptors (1, 2). P2Y receptors are seven transmembrane domain proteins coupled to phospholipase C via G proteins, although some of the newly cloned P2Y receptors can regulate adenylate cyclase and presumably can be coupled to other second messenger system (3-5). The family of P2Y receptor is formed by six cloned receptors termed as $P2Y_1$, $P2Y_2$, $P2Y_3$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and the UDP-glucose receptor (3, 6-9). P2X receptors are receptor-operated ion channels selective to small cations such as Na^+ and Ca^{2+}. These receptors are involved in fast synaptic transmission between neurones and between autonomic nerves and smooth muscle, where ATP is the main transmitter. Molecular biology has permitted the identification of up to seven P2X receptors named $P2X_1$– $P2X_7$. It is generally accepted by the scientific community that these receptors are formed by more than one subunit because each subunit contains only two transmembrane domains. The differences established between the pharmacological experiments performed in expression systems and in native tissues strongly suggests that P2X receptors are heteromeric rather than homomeric receptors (for a full review, see 10).

The eye contains P2 receptors in its structures and thus it is susceptible to be activated by the presence of nucleotides. These changes in eye physiology can include lachrymal apparatus, cornea,

ciliary body, iris, trabecular meshwork, lens, and the retina (11, 12). Not all the structures previously mentioned have been fully investigated; nevertheless it has been possible to understand the role of nucleotides and dinucleotides on corneal surface and intraocular pressure (IOP).

THE OCULAR SURFACE

There are two main pathological states regarding the ocular surface. On the one hand dry eye, and on the other hand corneal wound healing that occurs after superficial injuries. None of them have efficacious treatments and are lacking of the discovery of new substances for their care.

Dry eye and tear secretion

Tear secretion is an important physiological process because tear forms the interface between the air and the ocular tissues. Tears protect the corneal surface with antibacterial systems, such as lysozyme and lactoferrin, provide the epithelium with nutrients, and lubricate the eyelids. Also, they wash away those particles that may damage the cornea. There are situations in which some problems in the volume or in the quality of tears occur, and therefore may produce several negative effects such as corneal function failures, discomfort, and even pain (a sandy feeling that may be accompanied by irritation). If the problem is related to a reduction in the total volume, the mucin layer of the corneal epithelium will not guarantee a uniform film, thus shortening the tear break-up time. Specialists such as optometrists and ophthalmologists, by means of dyes such as fluorescein, Bengal pink, or lissamine green, can follow these changes of the normal tear film to a pathological state. Tear film stability is particularly important for contact lens wearing, because a deficient volume or a poor quality will make the adaptation of contact lenses difficult. Between 20% and 30% of contact lens wearers develop ocular dryness. Extreme conditions such as air conditioning, wind, heaters, or a prolonged read without a regular blinking frequency will facilitate an increase of tear evaporation.

Within the above percentage, between 12% and 20% reduce significantly the use of contact lenses because of the mentioned discomfort. Most of the approaches to solve this problem lie in changing the tear viscosity by the application of compounds based on preparations containing carboxymethylcellulose, hydroxypropylcellulose, or polyvinylalcohol. There are no good pharmacological solutions available to restore normal tear film properties.

Nucleotides are interesting compounds restoring the normality in those individuals presenting ocular dryness. Nucleotides and dinucleotides have been described in human and experimental animal tears. The most representative ones are the diadenosine polyphosphates, which are dinucleotides formed by two adenosines linked by a variable number of phosphates which can fluctuate between 2 and 7 (abbreviated as ApnA, n = 2-7) (13). These dinucleotides have been described by means of high performance liquid chromatography (HPLC) as presented in Figure 1A. Diadenosine triphosphate, Ap_3A, diadenosine tetraphosphate Ap_4A and diadenosine pentaphoshate Ap_5A have been described in human tears while Ap_3A was absent in rabbit tears. The concentration between human and rabbit tears were also different. While in humans the concentrations of the dinucleotides are in the nanomolar range in the animal it was in the micromolar one (14, 15).

One interesting point after demonstrating their presence in tears is to understand where these dinucleotides come from. Experiments performed in healthy human volunteers demonstrate that nucleotides and dinucleotides are released from the ocular surface, probably both cornea and conjunctiva by mechanical stress (16). It has been demonstrated that an increase in the blinking frequency produces an associated increase in the diadenosine polyphosphate tear concentration. Although the molecular mechanism has not been elucidated, it is possible, as occurs in the central nervous system, that nucleotides are liberated from nerve terminals, but there is evidence that nucleotides can be transported out of cells. Epithelial cells (17, 18), and in particular ocular epithelial cells, use different transport mechanisms as a regulated procedure for nucleotide release. The ATP binding cassette (ABC) transporter, the cystic fibrosis transmembrane conductance regulator (CFTR) or

glycoprotein P have been proposed as elements involved in the release of nucleotides (19, 20). It has been described that ATP leaves corneal endothelial cells by means of connexin hemichannels when these cells are stimulated mechanically (21). We think that the latter may be the most plausible mechanism although we cannot discard the other previously suggested.

Since diadenosine polyphosphates are present naturally in tears, experiments were performed in order to investigate whether the topical application of these substances were able to change tear production. Of all the tested dinucleotides (Ap_2A-Ap_6A, at 100 μM), Ap_4A, Ap_5A and Ap_6A were able to increase tear secretion between 20% to 60%, the best being Ap_4A (Figure 1B). Some mononucleotides also were able to increase tear production UTP and ATP being the best ones and increasing tearing 60% and 40% respectively over the control values (14).

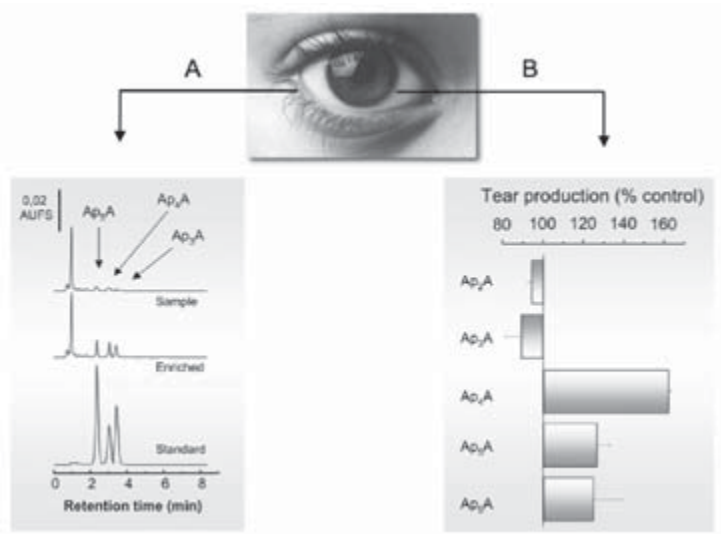

FIGURE 1. ***Presence and effect of diadenosine polyphosphates on tear secretion. A.*** *The presence of diadenosine polyphosphates has been identified by high performance liquid chromatography indicating the presence of diadenosine triphosphate, Ap_3A, diadenosine tetraphosphate, Ap_4A and diadenosine pentaphosphate, Ap_5A in human tears.* ***B.*** *The topical application of 100 μM of the dinucleotides produce a different behaviour on the rate of tear secretion depending on the phosphate chain. Among all, Ap_4A was able to increase tear secretion 60% above the normal tear production.*

When a wide range of concentrations of the dinucleotides were used, it was possible to establish concentration-response curves which permitted to calculate EC_{50} values for the diadenosine polyphosphates. Ap_4A presented an EC_{50} value of 2.76 μM, Ap_5A a value of 1.77 μM and Ap_6A a value of 3.16 μM (14).

The ability of some diadenosine polyphosphates activating tear production, is indicating that they can be considered as constitutive activators of tear release being this mechanism independent from the one that naturally control the main lachrymal glands (16). Moreover, the topical application of some of these compounds such as Ap_4A, may be useful for the treatment of dry eye specially those cases in which this pathology takes place with a lack of tear volume.

The design of new pharmacological compounds with nucleotidic structure such as diquafosol aka INS365, brings interesting expectations for dry eye treatment. This compound is a congener of Ap_4A (diuridine tetraphosphate, Up_4U) and presents a similar behaviour to the one observed for UTP or diadenosine polyphosphates (22). This substance enhanced tear secretion even after consecutive doses for several days as revealed by Schirmer scores. Tear secretion was increased 5-15 min after the compound application, and the effect persisted longer with solution of diquafosol of 8.5%. The clinical trials performed with humans with dry eye disease demonstrated good toleration in all the individuals at all the doses assayed. Applications of 25 μL of 0.5%, 1%, 2%, and 5% w/v produced increased tear secretion in patients with mild to moderate dry eye. All these results suggest the unequivocal utility of this compound as a therapeutic agent for dry eye syndrome (23).

In a model of dry eye developed in rats, the topical application of the dinucleotide Up_4U (diquafosol) increased tear secretion, surface health, and release of glycoproteins from goblet cells. These interesting properties of this dinucleotide indicate the therapeutic benefits of this compound for the treatment of dry eye (24).

Wound healing

The cornea is the most superficial part of the eye. This structure is suitable of having different damages coming from the

environmental medium. Foreign bodies, dirty or defective contact lenses and also refractive surgery, produce injury on the corneal epithelium, the most external part of the cornea.

The corneal wound healing process occurs in three main steps: a lag phase in which after the injury happens cells surrounding the affected area detach from Bowman's membrane and those cells which are affected by the injury are eliminated by polymorphonuclear leukocytes. A second step in which the cells in the periphery of the affected area move centripetally to cover the free space, and finally, a proliferative step in which cells divide to give the corneal epithelium its normal thickness.

Although all these stages are relevant, a critical step is the migration phase since it avoids the invasion of bacteria to inner parts of the eye, and other problems derived from an inadequate corneal thickness.

In vivo experiments

We have investigated the possible effect of mono and dinucleotides on the corneal wound healing process. The presence of these substances in tears may suggest a role of them in the healing process, therefore it was evaluated the possible role of these compounds on corneal wound healing in vivo by means of New Zealand white rabbits which were injured with n-heptanol. In the absence of any added nucleotide (vehicle) the time of re-epithelialisation of a 3 mm corneal wound is of 32.2 hours and the rate of migration is 72.4 μm/hour. When the wounds are treated with 100 μM UTP or Ap_4A the rate of healing was accelerated to 121.6 μm/h and 93.7 μm/h respectively. None of the other mono or dinucleotides did significantly modify the rate of healing (25).

The positive effect of these two compounds is mediated by P2Y receptors and presumably $P2Y_2$ since UTP and Ap_4A are full agonists. Also, the application of antagonists such as suramin, PPADS or reactive blue 2, were able to reverse the effect produced by UTP and Ap_4A. Unfortunately due to the lack of more selective antagonists it was not possible to fully confirm the P2Y receptor subtype (25).

In vitro experiments

In order to understand the molecular mechanism that make corneal epithelial cells to migrate faster after the challenge with some nucleotides and dinucleotides, experiments were performed with corneal epithelial cells in primary cultures (Figure 2A).

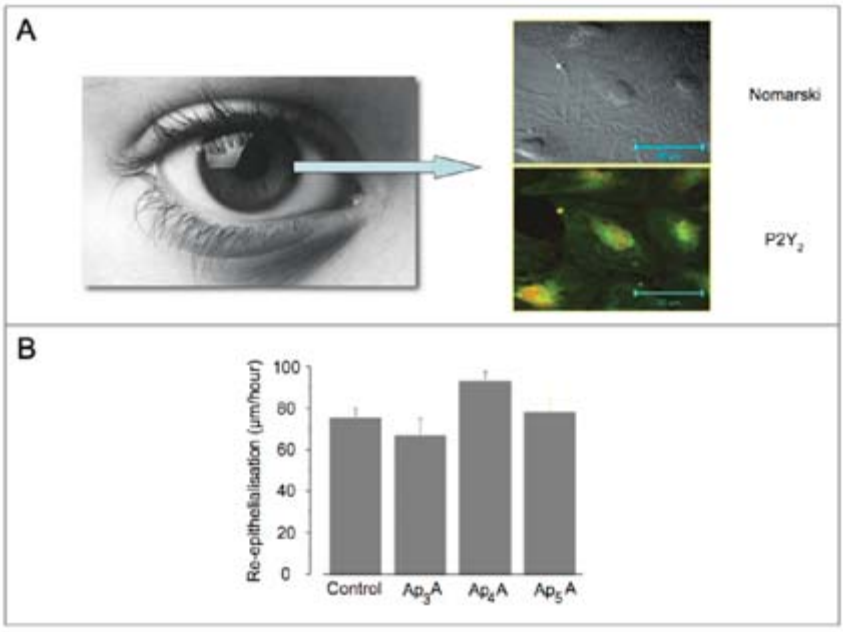

FIGURE 2. ***Effect of dinucleotides on corneal wound healing. A.*** *Corneal epithelial cells were isolated and seeded as primary cultures which can be observed under Normaski optics and provide positive labelling to the $P2Y_2$ receptor antibody.* ***B.*** *Diadenosine polyphosphates were able modify the rate of re-epithlialization, Ap_4A being the best dinucleotide in its ability to accelerate cell migration.*

The treatment of epithelial cells with diadenosine polyphosphates after scratching the monolayer with a pipette tip, demonstrated that as occur in vivo, Ap_4A was able to accelerate the rate of healing while Ap_3A and Ap_5A clearly delayed the rate of healing (Figure 2B). Other nucleotides, such as ADP did not modify the rate of healing when compared to control. In both cases, a positive or negative effect on

re-epithelialisation, the effects may be justified by the activation of different P2Y receptors subtypes, according to our pharmacological studies. It is clear that the pharmacology of P2 receptors is rather complicated due to the lack of selective antagonists. A possible picture of the receptors involved in the acceleration of re-epithelialisation or its delay is maybe possible by studying the behaviour of several purinergic agonists (26).

The increase in the rate of re-epithelialization has been observed when Ap_4A, and UTP were applied. This profile matches quite well with that described by Lazarowski et al. (27), in which Ap_4A and UTP are the best agonists on the cloned $P2Y_2$ receptor. A similar $P2Y_2$ profile has been previously described for corneal wound healing in the *in vivo* experiments described in the previous paragraph. With corneal epithelial cells in culture, it has been possible to detect some dinucleotides which clearly reduce the rate of healing. Together with the study of mononucleotides it was possible to obtain a profile for those nucleotides reducing the rate of re-epithelialisation. A profile with the ranking order $Ap_5A > Ap_3A \cong$ UDP suggests the involvement of a $P2Y_6$ receptor. This fact is altering the idea of the $P2Y_6$ receptor being a pyrimidinoceptor, sensitive to UTP and UDP. Nevertheless, studies performed with diadenosine polyphosphates and $P2Y_6$ receptors heterologously expressed in 1321N1 cells, demonstrate that both Ap_3A and Ap_5A are agonists of the $P2Y_6$ receptor although the concentration required for the receptor stimulation are higher than those of the best agonist, UDP (28). Also recently, the design of novel dinucleoside polyphosphates with uridine as nucleoside moiety (Up_nU), demonstrate that some of them are quite effective activating the $P2Y_6$ receptor (29).

The involvement of metabotropic P2 receptors in corneal wound healing has been reported by other groups (30-32). The presence of $P2Y_2$, $P2Y_4$ and $P2Y_{11}$, on corneal epithelial cells seem to be clear from a pharmacological point of view. Discussion arises when the presence of $P2Y_1$ or $P2Y_6$ is investigated. The assay of UDP together with the enzyme hexokinase suggests the presence of a $P2Y_6$ receptor in these cells (30).

Concerning the second messenger system underlying the activation of those receptors, it seems that both intracellular Ca^{2+}

mobilization (31, 32) the typical P2Y receptor second messenger system, and MAPKinase cascade activation[33] are involved. These intracellular mechanisms which accelerate the rate of healing, seems to be triggered by $P2Y_2$ and $P2Y_4$ membrane receptors (32). These results match well with the effect of Ap_4A, UTP and ATP we have observed in our investigation, which indicates the activation of a $P2Y_2$ receptor.

Ap_4A is effective inducing an increase in the rate of migration by stimulating a $P2Y_2$ receptor, while Ap_3A and Ap_5A do the opposite by activating a $P2Y_6$ receptor. This dual role of diadenosine polyphosphates may have a physiological meaning in the intact tissue. Corneal wound healing is a process that occurs in 3 main steps as previously commented: lag phase, migration and mitosis (33). It could be the case that in the intact corneal epithelium, $P2Y_2$ receptors would be the relevant ones facilitating the migration rate in intact corneas. It is necessary to take into consideration that in primary corneal epithelial cells in culture, the lag phase is reduced. This reduction is because the cells do not need to carry out some pathophysiological processes that occur in the intact cornea after injury. In the whole cornea, polymorphonuclear leukocytes remove necrotic cells from the wound margin. Also, hemidesmosomal attachments between the basament membrane and the basal cells disappear (33). All these phenomena does not occur in our preparation since it only contains a monolayer of epithelial cells.

On the other hand, $P2Y_6$ would be critical stopping migration and starting the third phase (mitosis). It is clear that we do not have any evidence for the role of $P2Y_6$ receptor to confirm this idea, but it would not be strange to think about $P2Y_2$ and $P2Y_6$ as switchers that control the transition from wound healing phase 2 to phase 3.

Nucleotides dinucleotides in the aqueous humour and the control of intraocular pressure

Aqueous humour is a physiologically relevant fluid that permits the nutrient supply to nonvascular structures such as the corneal endothelial cells and the lens. This fluid contains measurable amounts of both mono- and dinucleotides. The most relevant adenine

mononucleotides found in the whole aqueous humour were AMP, ADP, and ATP, which presented concentration values of 10.4 μM, 1.9 μM, and 1.0 μM, respectively (34). Investigations developed by Mitchell et al. (18) describe the concentration of ATP in the ciliary epithelium in particular in the immediate area where it is released. The concentration values for ATP were between 4 and 8 μM. The difference between both values may be as a consequence of either a dilution effect or due to the activity of ecto-nucleotidases (35). Diadenosine polyphosphates were also present in the aqueous humour of the rabbit, at lower concentrations than ATP, their values being 0.34 μM and 0.08 μM for Ap_4A and Ap_5A, respectively (34).

The presence of both mono and dinucleotides in the aqueous humour is suggesting a physiological role of these substances in the control of relevant processes such as intraocular pressure.

The control of intraocular pressure (IOP) is an important process where nucleotides may participate as therapeutic agents. Glaucoma is a pathology that, in many cases, is produced by an increase in the IOP. Pathophysiology of elevated IOP is linked either with the increased production of aqueous humour by ciliary body or an increased outflow resistance. This raise in IOP is transmitted to the retina, producing an occlusion of the posterior ciliary artery, generating ischaemic optic atrophy and gradual retinal degeneration. Moreover, the pressure can collapse optic nerve head structural support and axons can be damaged by mechanical compression. The final result of these complex negative actions is blindness (36).

IOP is a physiological process highly controlled by the nervous system. The sympathetic and parasympathetic nervous systems regulate both the production and drainage of aqueous humour. Part of the pharmacological treatments for ocular hypertension lie in the interference of either the synthesis of the aqueous humour (controlled by the sympathetic system) or of its removal (regulated by the parasympathetic). In this sense, many pharmacological approaches have tried to reduce IOP by altering the functioning of the innervation that controls the dynamics of aqueous humour. The use of beta-blockers in order to reduce the production of aqueous humour such as betaxolol or timolol is currently practised. Also, the application of cholinergic agonists such as carbamylcholine has been

used in order to reduce the resistance to aqueous outflow (37-39). Compounds that do not interfere with the nervous system have been used to reduce IOP. These include carbonic anhydrase inhibitors (decreasing aqueous production) or more recently the use of prostaglandin analogues such as latanoprost (Xalatan).

Until the year 2000, the only purines tested for their ability to modify IOP were adenosine and derivatives that demonstrated relevant physiological effects on IOP (40). Adenosine acting through adenosine A2 receptors produces an increase in IOP when topically applied to rabbit eyes, while A1 produce a decrease in IOP (41, 42).

The application of mono- and dinucleotides depicted a clear pattern of modulators on IOP. Thus, mononucleotides were classified under two main groups: on the one hand those elevating IOP and on the other those reducing it. Among the first, 2-MeSATP, ATP-γ-S and the natural compound ATP presented a clear increase on IOP, which was maximal 2-3 h after the compound instillation. This profile fits well with P2Y receptor pharmacology, although the destruction of these nucleotides by means of ectonucleotidases and the corresponding adenosine formation cannot be discarded (43). In this sense, a recent work by Farahbakhsh and Cilluffo (35) described the presence of $P2Y_1$ and $P2Y_2$ receptors in the rabbit ciliary body epithelial cells, which may be responsible for the action of 2-MeSATP, ATP-γ-S, as well as other P2Y agonists. On the contrary, βγ-meATP and αβ-meATP produced a clear and marked reduction in rabbit IOP. The time course to obtain the maximal effect was similar to the one obtained for the hypertensive compounds (3 h). Concentration-response analysis presented IC_{50} values of 1.52 mg/mL and 0.55 mg/mL and a maximal reduction in IOP of 35.66% and 45.04% for αβ-meATP and βγ-meATP respectively. The hypotensive effect produced by these two mononucleotides were blocked by the P2 antagonist PPADS but were unaffected by the adenosine antagonists DPCPX (43). βγ-meATP and αβ-meATP, activate P2 receptors, presumably $P2X_2$ receptors, present in cholinergic terminals that are in the trabecular meshwork. The activation of this $P2X_2$ receptor would generate an increase in acetylcholine release, which facilitates both the relaxation of trabecular meshwork cells and the elimination of aqueous humour (Figure 3).

Diadenosine polyphosphates (ApnA) present a similar behaviour as that observed for mononucleotides. Of all the tested dinucleotides, Ap_3A and Ap_4A presented a clear reduction in IOP, with the others presenting a hypertensive effect. Ap_4A was a potent agonist and produced a decrease in intraocular pressure (29.6% of decrease), at concentrations 3 orders of magnitude below those at which Ap_2A, Ap_3A, or Ap_5A produced an increase. The dose-response curve for Ap_4A did not appear to inflect at the highest concentrations tested points at which activation of the excitatory receptor might be expected. At the lowest concentrations tested, none of Ap_2A, Ap_3A, and Ap_5A produced a decrease in intraocular pressure, which implies that in addition to there being two separate populations of receptors, one mediating an increase and the other a decrease in intraocular pressure, this latter receptor is specific for Ap_4A. It is possible that Ap_4A also activates the excitatory receptor, but in the mixed

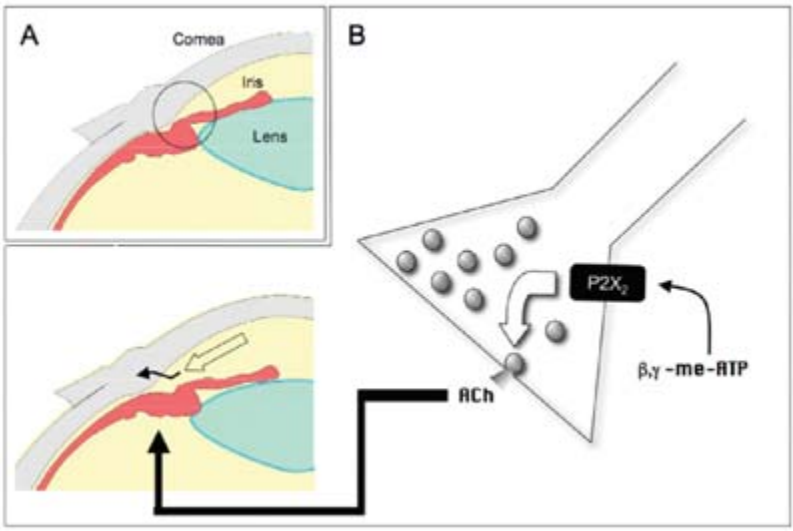

FIGURE 3. ***Effect of nucleotides reducing IOP by acting on cholinergic terminals located in the ciliary muscle. A.*** *Under normal conditions the irido-corneal angle (inside the circle) permits the drainage of the aqueous humour through the trabeculum.* ***B.*** *P2X receptors present in cholinergic terminals from the parasympathetic nervous system innervating the ciliary processes can stimulate the release of more acetylcholine (ACh). This transmitter contracts the ciliary muscle that pulls the scleral spur opening the irido-corneal angle therefore reducing the hydrodynamic resistance of the aqueous humour.*

population the effects of activation of the receptor that mediates a decrease in pressure predominated.

When investigating the place where Ap_4A exert its action and in clear contrast to what happen with mononucleotides, Ap_4A facilitates the drainage of the aqueous humour in the trabecular meshwork. This fact leads us to hypothesize that, at least in part, the hypotensive effect of Ap_4A in eyes is mediated by an increase in aqueous outflow (Figure 4). When instilled topically, dinucleotides are likely to stimulate purinergic receptors present in the trabecular meshwork to increase aqueous humour outflow as well as other purinergic receptors present in the eye, such as those in the ciliary. Nevertheless, the hypotensive effect of Ap_4A seems to be well correlated with the increase in outflow facility found here (44). By means of immunocytochemical and western blot techniques we have described the presence of $P2Y_1$, $P2Y_2$, and $P2Y_4$ in bovine trabecular meshwork cells. In contrast, the immunocytochemistry and Western blot results seem to clearly indicate the absence of $P2Y_6$ and $P2Y_{11}$ purinoceptors. Using the same antibodies, it was possible to identify these receptors in rat ocular structures such as the corneal epithelium ($P2Y_6$) and the retinal pigmented epithelium ($P2Y_{11}$) and to confirm the existence of $P2Y_1$ and $P2Y_2$ in sections containing the trabecular meshwork (44, 45). Other studies have also reported the presence of $P2Y_1$ and $P2Y_2$ receptors in bovine trabecular meshwork cells (46) and $P2Y_1$, $P2Y_4$, and $P2Y_{11}$ in a human trabecular meshwork cell line (47). ApnAs are known to activate several different purinergic receptors: Ap_3A and Ap_4A both activate $P2Y_1$ receptors with different selectivity, whereas Ap_5A is, in general, less effective at this receptor (48).

Furthermore, Ap_4A is a good agonist at $P2Y_2$ and $P2Y_4$ receptors, and although Ap_3A and Ap_5A can also activate these receptors, they do so with less affinity. P2Y receptors act via a Gq/11 protein coupling to activate PLC, IP_3 formation, and mobilization of [Ca2]i, although coupling to adenylyl cyclase, PLA2, PKC, NO synthase, or BKCa channels activation has also been described (1).

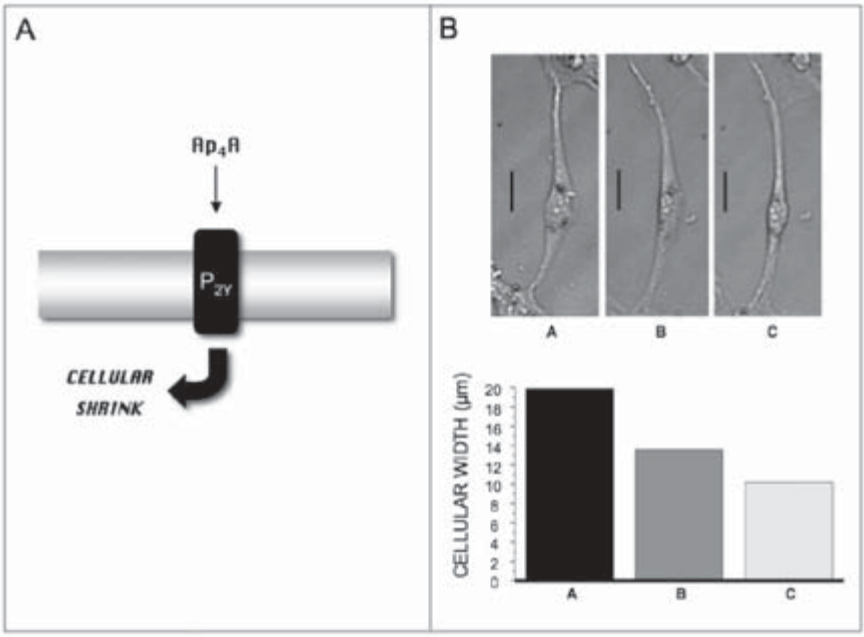

FIGURE 4. ***P2Y receptors present in trabecular meshwork cells. A.*** *P2Y receptors produce the shrink of trabecular meshwork cells this permitting the facilitation of the aqueous humour drainage.* ***B.*** *Visualization of a single trabecular meshwork cell in culture after being challenged with Ap_4A. It can be noticed how the cell shrinks after the superfusion of the dinucleotide.*

CONCLUDING REMARKS

The function of mono and dinucleotides in the eye indicates that these group of substances are developing interesting roles in three important ocular processes: tear production, wound healing and intraocular pressure. They are present in tears and in the aqueous humour, therefore indicating they are naturally activating and/or modulating these physiological processes. In addition, the topical administration of some of these compounds can rescue the eye from some pathological situations.

We are not so far away from finding any of these compounds in the pharmacy. Diquafosol, also termed Up_4U, has passed FDA requirements to soon appear in the USA market as a treatment for

dry eye condition. Other such as denufosol, aka dCp_4U, is on clinical trials. This dinucleotide is useful for the treatment of the retinal detachment since it reabsorbs the liquid that is accumulated between photoreceptors and the retinal pigmented epithelium. Following the same philosophy it will not be strange to see in the near future pharmaceutical compounds based on dinucleotides suitable for the treatment of ocular pathologies such as glaucoma, corneal wound healing or ocular surface infections.

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