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Minireview

The neurotransmitter role of diadenosine polyphosphates

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Abstract Diadenosine polyphosphates present at the cytosol can be transported to secretory granules allowing their exocytotic release. Extracellularly, they can act through specific metabotropic or ionotropic receptors, or as analogues of P2X and P2Y nucleotide receptors. The specific ionotropic receptor P4 is present in synaptic terminals, and modulated by protein kinases (PK) A and C and protein phosphatases. Activation of PKA or PKC, directly or through membrane receptors, results in a decrease of affinity or in reduction of the Ca^{2+} transient respectively. Adenosine and ATP, both products of the extracellular destruction of diadenosine polyphosphates, acting through A_1 or P2Y receptors respectively, are important physiological modulators at the P4 receptor.

Key words: Adenine dinucleotide; Ap_nA; Nucleotide receptor; Protein kinase; Protein phosphatase; Purinergic receptor

1. Diadenosine polyphosphates: intracellular roles

α,ω-adenine dinucleotides have emerged as extracellular signalling molecules together with adenosine and ATP in neural and non-neural tissues. Adenine dinucleotides, also termed diadenosine polyphosphates, are made up of two adenosine moieties bridged by a phosphate chain whose length varies between three and six phosphates. It is generally accepted that some aminoacyl-tRNA synthetases and enzymes with adenylyl reaction intermediates produce these compounds as secondary products [1–3].

Diadenosine polyphosphates are present in prokaryotic and eukaryotic cells, their levels, which are below the μM range, increase to μM levels after oxidative or heat shock treatment [1,4,5]. In eukaryotic cells, their concentration also depends on the cell cycle and Ap_4A can activate DNA replication and DNA repair [6,7].

In cell cytoplasm, diadenosine polyphosphates are able to regulate enzymes, ion channels and transporters. They behave as enzyme inhibitors involved in the nucleotide phosphate transfer mediated by adenosine kinase and adenylate kinase, as they are transition state analogues of these enzymes [8,9]. Nevertheless, they behave as activators of the cytosolic 5′-nucleotidase [10]. Plasma membrane proteins can be modulated on the intracellular side by diadenosine polyphosphates. This effect has been demonstrated in the K^+_{ATP} channel present in cardiac cells, where they mimic the effect of ATP [11]. In addition, in pancreatic β-cells, an increase in the extracellular glucose levels produces an enhancement of up to 40 times of Ap_3A and Ap_4A, reaching intracellular concentrations of 11.2 μM an 13.6 μM respectively, activating the K^+_{ATP} channel involved in insulin secretion [12]. Moreover, ATP as well as Ap_4A positively modulate the nucleoside transporter, which is a crucial step in the recovery of extracellular adenosine, on the intracellular side.

Cytosolic hydrolases and phosphorylases, specific for the phosphate chain length, cleave adenine dinucleotides to form adenine mononucleotides in both pro- and eukaryotic cells [13,14]. One of these enzymes, diadenosine triphosphate hydrolase (Ap_3A hydrolase), is the fragile histidine triad protein (FHIT) which is a tumor suppressor [15].

2. Extracellular roles of diadenosine polyphosphates

2.1. Presence and release

Diadenosine polyphosphates are stored in the secretory granules, where the presence of adenine mononucleotides has also been described. Platelets contain significant amounts of Ap_3A, Ap_4A, Ap_5A and Ap_6A together with ADP and serotonin in their dense granules [16,17]. Chromaffin cells from adrenal medulla contain Ap_4A, Ap_5A, and Ap_6A accompanying ATP and catecholamines [18,19]. In the cholinergic model of *Torpedo* the dinucleotides present in synaptic vesicles are Ap_4A and Ap_5A together with acetylcholine and ATP [20].

The transport of adenine dinucleotides and mononucleotides is carried out by the same vesicular transporter, which shows little selectivity, but appears to be highly modulated by the intracellular levels of the substrates, in a kinetic behavior known as mnemonic [21]. In this model, changes in the cytosolic concentration of dinucleotides induce slow conformational transitions between different states of the transporter, which results in drastic variations in transport capacity.

The induced release of adenine dinucleotides from secretory granules maintains the same proportionality as the granular content with respect to mononucleotides and aminergic or cholinergic compounds. Secretion from neural tissues, synaptic terminals and chromaffin cells requires the presence of extracellular Ca^{2+}, and equivalent amounts of Ap_4A, Ap_5A and Ap_6A are released [19,22]. Diadenosine polyphosphates, Ap_4A and Ap_5A, have been identified and quantified by means of in vivo release experiments using the push-pull technique in the caudate putamen area of conscious rats after amphetamine stimulation. The stability of Ap_4A and Ap_5A was better than ATP, since only AMP and adenosine were measured by HPLC together with the dinucleotides [23].

As a result of cellular release the dinucleotide concentration in the vicinity of the secretory event has been calculated to be around 25 μM for chromaffin cells, these levels significantly decreased by dilution when reaching more distant areas [19].

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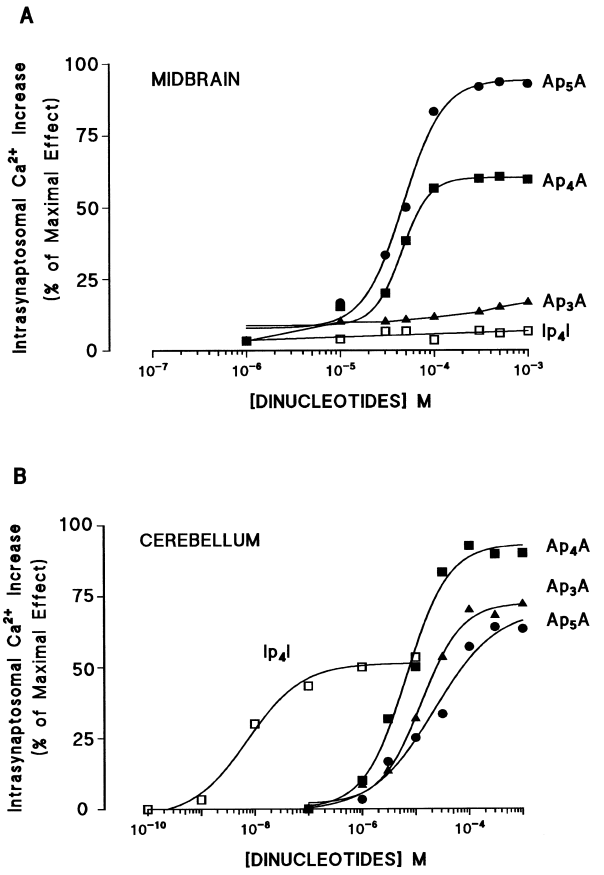


Fig. 1. Heterogeneity of dinucleotide receptors based on the intrasynaptosomal Ca^{2+} response curves induced by different structural analogues. A: Pharmacology of the dinucleotide receptor present in rat midbrain synaptic terminals. The best agonist of this receptor is Ap_5A, Ip_4I being an antagonist. B: Pharmacology of the dinucleotide receptor present in cerebellar guinea pig synaptic terminals. The best full agonist was Ap_4A whereas in terms of affinity the best was Ip_4I.

Concentrations of Ap_4A and Ap_5A close to 60 nM can be detected from brain perfusion samples after strong stimulation. These are below 2 nM in non-stimulated brain perfusates [23]. These data need to be taken into account when studying specific receptors and physiological actions.

2.2. Receptors

Due to the close structural analogy between diadenosine polyphosphates and ATP it is necessary to be aware of the difficulties in clearly identifying their specific physiological receptors and differentiate them from pharmacological actions on other nucleotide receptors [24]. First approaches to the adenine dinucleotide receptors were performed by means of ligand binding studies [25,26]. Several authors described the existence of binding sites for $[^3H]Ap_4A$ in bladder, cardiac cells, chromaffin cells, liver and in mouse and rat brain. Scatchard analyses in most of the cases are not linear plots, as occurs in rat brain synaptosomes. In this neural model, combining Scatchard analysis and competition studies, up to three different binding sites can be found. One is a very highly specific binding site (K_d in the pM range), the second is a highly specific binding site (K_d in the nM range) and the third is in the μM range.

Autoradiographic studies performed in rat brain with $[^3H]Ap_4A$ showed a widespread but heterogeneous distribution. Cerebellar granular layer, superior olive, VIIth cranial nerve and some areas in the olfactory bulb were densely marked [27].

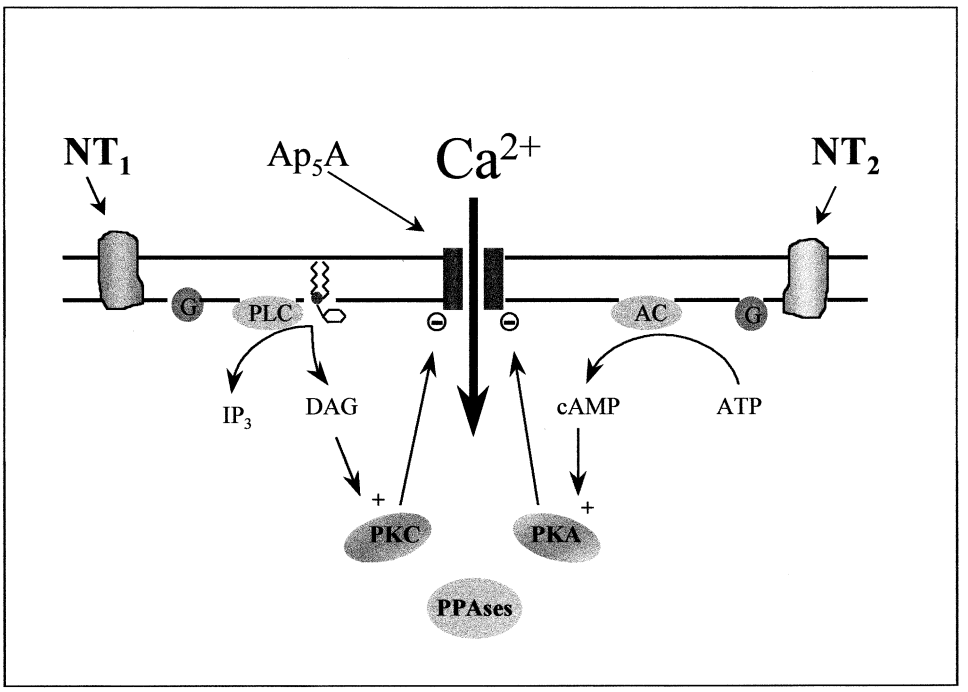
Ap_nA have proven to be agonists of some nucleotide receptors including metabotropic, P2Y, and ionotropic, P2X. Among the best known examples are their actions on $P2X_1$ from vas deferens, and the former P2U (now $P2Y_2$) cloned from human lung tissue [28–30]. Moreover, actions on allosteric sites different from that of ATP have been described on the homomeric $P2X_2$ expressed in oocytes where Ap_5A potentiates the entry of cations at low nM concentrations [31]. This feature is not only restricted to ionotropic P2X receptors since the P2Y receptor present in cerebellar astrocytes is also potentiated by Ap_5A, effect which lasts for up to 6 h [32].

Receptors only stimulated by diadenosine polyphosphates have been described in mouse, rat and guinea pig synaptosomes, and are termed *dinucleotide* receptors or P4 receptors [33–35]. This presynaptic receptor is coupled to Ca^{2+} entry by a mechanism which is voltage independent, nevertheless, the initial Ca^{2+} entry depolarizes the terminal permitting the opening of voltage dependent Ca^{2+} channels (VDCC) sensitive to ω-conotoxin G-VI-A, indicating the participation of an N-type Ca^{2+} channel [33]. The dinucleotide receptor present in mouse brain appears to be coupled to the L-type VDCC since it is blocked with verapamil [34].

From a pharmacological point of view not all adenine dinucleotides present the same maximal effect in rat midbrain synaptosomes. The most effective is diadenosine pentaphosphate, followed by diadenosine tetraphosphate, which is a partial agonist, diadenosine triphosphate having almost no effect. Their EC_{50} values were all similar at around 50 μM (Fig. 1A). It is noteworthy that dinucleotide receptors present in rat midbrain are pharmacologically different from those present in guinea pig [33,35]. Dinucleotide receptors present in both cerebellum and in diencephalon of guinea pig induce Ca^{2+} entry to synaptic terminals similar to that described for rat midbrain; nevertheless, while in rat Ap_5A is the most effective dinucleotide, in guinea pig the even dinucleotides (Ap_4A and Ap_6A) are more effective than Ap_3A and Ap_5A. This probably indicates the existence of different subtypes of dinucleotide receptors (Fig. 1B). This is also supported by the receptor present in mouse where Ap_4A elicits bigger Ca^{2+} transients than in rat and guinea pig but with an EC_{50} that is also higher (214 μM) [34].

The *dinucleotide* receptor is not sensitive to P2 antagonists such as suramin, DIDS (4,4′-diisothiocyanostilbene-2,2′-disulfonic acid) and PPADS (pyridoxalphoshate-6-azophenyl-2′,4′-disulfonic acid). A new series of diadenosine polyphosphates derivatives, the diinosine polyphosphates (Ip_nI), behave as good antagonists of the dinucleotide receptor [36]. These compounds are synthesized by enzymatic deamination of the corresponding diadenosine polyphosphates [37]. Ip_3I and Ip_4I were antagonists at the μM range but diinosine pentaphosphate (Ip_5I) with an IC_{50} value in the nM range was an excellent antagonist of the midbrain dinucleotide receptor. This is especially important because antagonists for nucleotide receptors currently available are poorly specific.

Protein kinases and phosphatases modulate the dinucleotide receptor present in rat brain [38]. Presynaptic neurotransmitter receptors coupled to stimulation of protein kinases (PK) A and C reduce the Ca^{2+} transients elicited by 100 μM Ap_5A.



Compound	Effect on PKC	Effect on P_4 receptor maximal response	Compound	Effect on PKA	Effect on P_4 receptor affinity
PDBu	Activation	Decreases	Forskolin	Activation	Decreases
Stauros.	Inhibition	Increases	DiBucAMP	Activation	Decreases
PKC-IP	Inhibition	Increases	PKA-IP	Inhibition	Increases
ATP	Activation	Decreases	Adenosine	inhibition	Increases

Fig. 2. Dinucleotide receptor modulation by intracellular signalling. Schematic diagram of the dinucleotide receptor modulation by stimulation of second messenger systems coupled to membrane receptors in rat midbrain synaptic terminals. Activation of protein kinases A and C severely diminishes the affinity and the maximal effect of the receptor respectively. The action of different effectors which produce changes in the affinity and maximal effects on the dinucleotide receptor. The adenosine effect is mediated in this model through an A_1 receptor which inhibits adenylate cyclase (AC), while ATP stimulates a P2Y receptor positively coupling to phospholipase C (PLC). PKA-IP and PKC-IP are respectively protein kinases A and C inhibitory peptides. Staurosp. corresponds to staurosporine, PDBu to phorbol 12,13-dibutyrate and DiBucAMP is dibutyryl-cAMP.

Activators of PKA, such as forskolin (which stimulates adenylate cyclase), inhibited the response elicited by Ap_5A. PDBu, which activates PKC, also inhibited the Ca^{2+} transients elicited by Ap_5A. Inhibitors of PKA and PKC, such as the PKA inhibitory peptide or staurosporine respectively, favored the Ca^{2+} transients induced by Ap_5A (Fig. 2).

The effect of protein kinases is reversed by protein phosphatases which allow a better functioning of the dinucleotide receptor when stimulated by 100 μM Ap_5A. This has been demonstrated by blocking the activity of phosphatases with okadaic acid (general inhibitor of PPases), microcystin (inhibitor of PP2A) and cyclosporin (an inhibitor of calcineurin).

The existence of a battery of ecto-nucleotidases in the synaptic cleft permits the cleavage of diadenosine polyphosphates and ATP to adenosine (see below) [39]. To see whether adenosine and ATP may produce any change in diadenosine polyphosphate responses, alkaline phosphatase was added to the synaptosomal preparation transforming the ATP present at the extracellular space into adenosine (Fig. 3). Disappearance of ATP and appearance of adenosine produced a dual effect on the Ap_5A dose-response curve. Firstly, the sigmoidal dose-response curve for Ap_5A was transformed in a biphasic curve, which presented a component in the nanomolar range plus another in the micromolar range. Secondly, the maximal effect of the μM step was higher than in control. In the presence of alkaline phosphatase, the nM component of the dose-response curve returned to μM values when the adenosine A_1 receptor was blocked with the specific antagonist DPCPX [40]. This indicates that adenosine through an A_1 receptor (negatively coupled to adenylate cyclase and reducing the PKA activity) permits the dinucleotide receptor to reach a higher affinity state than in the absence of the nucleoside. The change in the maximal effect was not due to adenosine but to ATP degradation, since in the absence of alkaline phosphatase but in the presence of the P2 antagonist PPADS, the maximal response was increased to the same extent as with

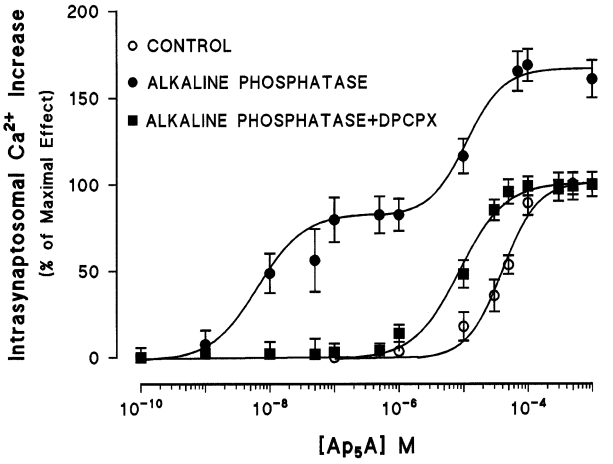


Fig. 3. Diadenosine polyphosphate receptor modulation by nucleotides and adenosine. Dose-response curves for diadenosine pentaphosphate (Ap_5A) in rat midbrain synaptic terminals. Control Ap_5A (alone ○), in the presence of alkaline phosphatase employed to destroy the extracellular nucleotides present in the preparation to form adenosine (●). The same as the former with addition of the adenosine A_1 receptor antagonist DPCPX (8-cyclopentyl-1,3-dipropylxanthine) (■).

the enzyme [41]. A P2Y type receptor, coupled to PLC and activating PKC, would be responsible for a reduction in the activity of the dinucleotide receptor. These results correlate well with the action of protein kinases and phosphatases.

Stimulation of rat brain synaptic terminals releases diadenosine polyphosphates as well as ATP. The amount of ATP is approximately 25 times higher than that of dinucleotides [22]. In this initial situation, the dinucleotide receptor would be sensitive to Ap_5A with an EC_{50} in the μM range and a maximal effect of about 30 nM, because ATP activates a P2 receptor. As soon as ecto-nucleotidases start cleaving ATP and adenosine appears, this nucleoside acting through the adenosine A_1 receptor produces a change in the dinucleotide receptor that makes it more sensitive to Ap_5A, the EC_{50} thus being in the nM range.

2.3. Ectoenzymatic degradation of dinucleotides

The existence of ecto-enzymes able to hydrolyze diadenosine polyphosphates with high affinity has been reported in endothelial cells from blood vessels, cultured chromaffin cells and plasma membranes from *Torpedo* synaptic terminals [42–45]. Their K_m values for Ap_nA (n=3–5) are around 0.5–5 μM. All these enzymes hydrolyse the Ap_nA in an asymmetric way, producing AMP and the corresponding $Ap_{(n-1)}$ nucleotide, these compounds being substrates of a cascade of ectonucleotidase activities, including ecto-ATPase (or apyrase) and 5′-nucleotidase, producing adenosine as final product [39].

Differences between the effect of ions on diadenosine polyphosphate hydrolases present in neural tissues and endothelial cells have been described, suggesting the presence of distinct forms of the enzyme in these two types of tissue. The enzymes of neural origin were stimulated by Ca^{2+}, Mg^{2+} and Mn^{2+} in a similar way, whereas vascular endothelial enzymes were inhibited by Ca^{2+}, Mn^{2+} being a much more potent activator of this activity than Mg^{2+} [43,45,46].

Finally, it is worth noting that the existence of high affinity ecto-enzymes able to hydrolyze diadenosine polyphosphates and terminate their actions through purinergic receptors contributes to confirming the role of these dinucleotides as extracellular messengers (Figs. 1–3).

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References

[1] McLennan, A.G. (1992) Ap_4A and Other Dinucleoside Polyphosphates, CRC Press, Boca Raton, FL.
[2] Zamecnik, P.C., Stephenson, M.L., Janeway, C.M. and Randerath, K. (1966) Biochem. Biophys. Res. Commun. 24, 91–97.
[3] Sillero, M.A.G., Guranowski, A. and Sillero, A. (1991) Eur. J. Biochem. 202, 507–513.
[4] Rapaport, E. and Zamecnik, P.C. (1976) Proc. Natl. Acad. Sci. USA 73, 3984–3988.
[5] Baker, J.C. and Jacobson, M. (1986) Proc. Natl. Acad. Sci. USA 83, 2350–2352.
[6] Grummt, F., Waltl, G., Jantzen, H.M., Hamprecht, K., Huebscher, U. and Kuenzle, C.C. (1979) Proc. Natl. Acad. Sci. USA 76, 6081–6085.
[7] Baxi, M.D. and Vishwanatha, J.K. (1995) Biochemistry 34, 9700–9707.
[8] Leinhardt, G.E. and Secemsky, I.I. (1973) J. Biol. Chem. 248, 1121–1123.
[9] Rotllán, P. and Miras-Portugal, M.T. (1985) Eur. J. Biochem. 151, 365–371.
[10] Pinto, R.M., Canales, J., Sillero, M.A.G. and Sillero, A. (1986) Biochem. Biophys. Res. Commun. 138, 261–267.
[11] Jovanovic, A. and Terzic, A. (1996) Br. J. Pharmacol. 117, 233–235.
[12] Ripoll, C., Martin, F., Rovira, J.M., Pintor, J., Miras-Portugal, M.T. and Soria, B. (1996) Diabetes 45, 1431–1434.
[13] Ramos, A. and Rotllán, P. (1995) Biochim. Biophys. Acta 1253, 103–111.
[14] Lévêque, F., Blanchin-Roland, S., Fayat, G., Plateau, P. and Blanquet, S.J. (1990) Mol. Biol. 212, 319–329.
[15] Barnes, L.D., Garrison, P.N., Siprashvile, Z., Guranowski, A., Robinson, A.K., Ingram, S.W., Croce, C.M., Ohta, M. and Huebner, C. (1996) Biochemistry 35, 11529–11535.
[16] Lüthje, J. and Ogilvie, A. (1983) Biochem. Biophys. Res. Commun. 115, 253–260.
[17] Schlüter, H., Offers, E., Brüggemann, G., van der Giet, M., Tepel, M., Nordhoff, E., Karas, M., Spieker, C., Witzel, H. and Zidek, W. (1994) Nature 367, 186–188.
[18] Rodriguez del Castillo, A., Torres, M., Delicado, E.G. and Miras-Portugal, M.T. (1988) J. Neurochem. 51, 1696–1703.
[19] Pintor, J., Rotllán, P., Torres, M. and Miras-Portugal, M.T. (1992) Anal. Biochem. 200, 296–300.
[20] Pintor, J., Kowalewski, H.J., Torres, M., Miras-Portugal, M.T. and Zimmerman, H. (1992) Neurosci. Res. Commun. 10, 9–14.
[21] Gualix, J., Fideu, M.D., Pintor, J., Rotllán, P., García-Carmona, F. and Miras-Portugal, M.T. (1997) FASEB J. 11, 981–990.
[22] Pintor, J., Diaz-Rey, M.A., Torres, M. and Miras-Portugal, M.T. (1992) Neurosci. Lett. 136, 141–144.
[23] Pintor, J., Porras, A., Mora, F. and Miras-Portugal, M.T. (1995) J. Neurochem. 64, 670–676.
[24] Hoyle, C.H.V. (1990) Gen. Pharmacol. 6, 827–831.
[25] Hilderman, R.H., Martin, M., Zimmerman, J.K. and Pivorun, E.B. (1991) J. Biol. Chem. 266, 6915–6918.
[26] Pintor, J., Diaz-Rey, M.A. and Miras-Portugal, M.T. (1993) Br. J. Pharmacol. 108, 1094–1099.
[27] Rodriguez-Pascual, F., Cortes, R., Torres, M., Palacios, J.M. and Miras-Portugal, M.T. (1997) Neuroscience 77, 247–255.
[28] Westfall, T.D., McIntyre, C.A., Obeid, S., Bowes, J., Kennedy, C. and Sneddon, P. (1997) Br. J. Pharmacol. 121, 57–62.
[29] Lazarowski, E.R., Watt, W.C., Stutts, M.J., Boucher, R. and Harden, T.K. (1995) Br. J. Pharmacol. 115, 1619–1627.
[30] Mateo, J., Miras-Portugal, M.T. and Castro, E. (1996) Br. J. Pharmacol. 119, 1223–1232.
[31] Pintor, J., King, B.F., Miras-Portugal, M.T. and Burnstock, G. (1996) Br. J. Pharmacol. 119, 1006–1012.

[32] Jimenez, A.I., Castro, E., Delicado, E.G. and Miras-Portugal, M.T. (1998) Neurosci. Lett. 246, 1–3.
[33] Pintor, J. and Miras-Portugal, M.T. (1995) Br. J. Pharmacol. 115, 895–902.
[34] Pivorun, E.B. and Nordone, A. (1996) J. Neurosci. Res. 44, 478–489.
[35] Pintor, J., Puche, J.A., Gualix, J., Hoyle, C.H.V. and Miras-Portugal, M.T. (1997) J. Physiol. 504, 327–335.
[36] Pintor, J., Gualix, J. and Miras-Portugal, M.T. (1997) Mol. Pharmacol. 51, 277–284.
[37] Guranowski, A., Starzynska, E., Günther Sillero, M.A. and Sillero, A. (1995) Biochim. Biophys. Acta 1243, 78–84.
[38] Pintor, J., Gualix, J. and Miras-Portugal, M.T. (1997) J. Neurochem. 68, 2552–2557.
[39] Zimmermann, H. (1996) Drug Dev. Res. 39, 337–352.
[40] Williams, M. (1987) Annu. Rev. Pharmacol. Toxicol. 27, 315–345.
[41] Turner, J.T., Weisman, G.A. and Fedan, J.S. (1998) The P2 Nucleotide Receptors, Humana Press, Englewood Cliffs, NJ.
[42] Goldman, S.J., Gordon, E.L. and Slakey, L. (1986) Circ. Res. 59, 362–366.
[43] Ogilvie, A., Luthje, J., Pohl, U. and Busse, R. (1989) Biochem. J. 259, 97–103.
[44] Rotllan, P., Ramos, A., Pintor, J. and Miras-Portugal, M.T. (1991) FEBS Lett. 280, 371–374.
[45] Mateo, J., Rotllan, P., Marti, E., Gomez de Aranda, I., Solsona, C. and Miras-Portugal, M.T. (1997) Biochem. J. 323, 677–684.
[46] Mateo, J., Miras-Portugal, M.T. and Rotllan, P. (1997) Am. J. Physiol. 273, C918–C927.