BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 157, 1142–1153

0007-1188/09
www.brjpharmacol.org

REVIEW

Dinucleoside polyphosphates: strong endogenous agonists of the purinergic system

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The purinergic system is composed of mononucleosides, mononucleoside polyphosphates and dinucleoside polyphosphates as agonists, as well as the respective purinergic receptors. Interest in the role of the purinergic system in cardiovascular physiology and pathophysiology is on the rise. This review focuses on the overall impact of dinucleoside polyphosphates in the purinergic system. Platelets, adrenal glands, endothelial cells, cardiomyocytes and tubular cells release dinucleoside polyphosphates. Plasma concentrations of dinucleoside polyphosphates are sufficient to cause direct vasoregulatory effects and to induce proliferative effects on vascular smooth muscle cells and mesangial cells. In addition, increased plasma concentrations of a dinucleoside polyphosphate were recently demonstrated in juvenile hypertensive patients. In conclusion, the current literature accentuates the strong physiological and pathophysiological impact of dinucleoside polyphosphates on the cardiovascular system.
British Journal of Pharmacology (2009) **157,** 1142–1153; doi:10.1111/j.1476-5381.2009.00337.x; published online 25 June 2009

Keywords: dinucleoside polyphosphates; purinergic system; cardiovascular system; extracellular mediators; intracellular mediators; vascular tone; proliferation

Abbreviations: ERK, extracellular signal-regulated kinase; H-1152, (S)-(+)-2-methyl-1-[(4-methyl-5-iso-quinolinyl) sulfonyl]-homopiperazine: Rho-kinase inhibitor; Hep-G2, human hepatocellular liver carcinoma cell line; MAPK, mitogen-activated protein kinase; OAG, oleoyl-2-acetyl-sn-glycerol; PI3K, phosphatidylinositol 3-kinase; Raf-1, serine/threonine-specific kinase (EC 2.7.11.1); MAP, kinase kinase kinase (MAP3K); VSMC, vascular smooth muscle cell; Xp_nX, dinucleoside polyphosphates [with X = adenosine, guanosine, inosine or uridine; *n* = number of phosphate groups (p)]

Introduction

Interest in the functional roles of nucleotides and the underlying purinergic system in cardiovascular physiology and pathophysiology continues to grow (Gabriels *et al.*, 2002). The purinergic system consists of mononucleosides, mononucleoside polyphosphates and dinucleoside polyphosphates as agonists, as well as the respective purinergic receptors, which possess very different functions (Ralevic and Burnstock, 1998). The purinergic signalling system that controls vascular regulation displays a high degree of complexity.

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Received 21 July 2008; revised 16 January 2009; accepted 10 March 2009

Purinergic receptor system (purinoceptors)

The complexity of the purinergic signalling system is partially due to the large number of agonists that constitute this system. This is further complicated by the diversity of the purinergic receptors (purinoceptors), including the subtypes of the P1, P2X and P2Y receptors, as well as formation of heteropolymeric P2X ion channels (Nori *et al.*, 1998; Robertson *et al.*, 2001), and P2X splicing variants (Cheewatrakoolpong *et al.*, 2005; Koshimizu *et al.*, 2006). Additionally, there are numerous soluble and membrane-bound ectonucleotidases (Yegutkin *et al.*, 2000; 2007; Linden, 2001; Leipziger, 2003) that transform one active purinoceptor agonist into an active agonist for another purinoceptor. In the last several years, our understanding of the role(s) the purinergic system plays in normal and pathological cardiovascular physiology has been vastly increased by the identification of new purinergic agonists and cloning of several purine receptor subtypes.

The potent actions of purine mononucleoside polyphosphates on cardiovascular system vessels were first described in 1929 (Drury and Szent-Györgyi, 1929). Since then, the impact of mononucleoside polyphosphates in various vasoregulatory processes, like immunomodulatory and prothrombotic responses in the cardiovascular system, have been described in detail elsewhere (Burnstock, 2002; Moore and MacKenzie, 2007).

Dinucleoside polyphosphates as mediators of the purinergic system

In recent years, interest in dinucleoside polyphosphates as strong purinergic agonists has been growing. Dinucleoside polyphosphates mediate vascular tone regulation (Schlüter *et al.*, 1994; Jankowski *et al.*, 2005), as well as vascular smooth muscle and mesangial cell proliferation (McLennan, 1992; Heidenreich *et al.*, 1995).

Occurrence and physiological effects of dinucleoside polyphosphates

Molecular structure of dinucleoside polyphosphates

Dinucleoside polyphosphates (Xp_nX) consist of two nucleotides (ribosylated nucleic acids), which are interconnected by a polyphosphate chain of two to seven phosphates through phosphoester bonds at the 5′-position of two ribose moieties (where X = adenosine and/or guanosine, or uridine; n = number of phosphate groups). Figure 1 shows the molecular structure of P_1,P_4 uridine adenosine tetraphosphate (Up_4A) as an example of a dinucleoside polyphosphates. In comparison with mononucleoside polyphosphates, dinucleoside polyphosphates have relatively long half-lives. Furthermore, metabolites of dinucleoside polyphosphates may serve as potential sources of extracellular ATP and other purines.

Occurrence of dinucleoside polyphosphates

P_1,P_4 diadenosine tetraphosphate (Ap_4A) was the first diadenosine polyphosphate to be identified in mammalian tissue (Rapaport and Zamecnik, 1976), was subsequently also identified in human platelets (Flodgaard and Klenow, 1982; Lüthje and Ogilvie, 1983). Following these early discoveries, numerous different nucleotides have been isolated from human tissues. For example, diadenosine polyphosphates (Ap_nA, with n = 2–7) were isolated from body fluids and cells (Pintor *et al.*, 1992a; Schlüter *et al.*, 1994; 1998; Hoyle *et al.*, 1996; Jankowski *et al.*, 1999; 2001a; 2003a). Dinucleoside polyphosphates are released into the circulation from several cell types, including activated platelets (Flodgaard and Klenow, 1982; Lüthje and Ogilvie, 1983; Jankowski *et al.*, 1999), chromaffin cells of the adrenal glands (Rodriguez del Castillo *et al.*, 1988; Castillo *et al.*, 1992; Pintor *et al.*, 1991; 1992a), tubular cells (Jankowski *et al.*, 2007a; 2008) or from synaptic vesicles (Zimmermann *et al.*, 1993). Ap_nA are important neurotransmitter molecules in the nervous system (Delicado *et al.*, 2006), and the importance of purines for neurotransmission in general was recently reviewed in an excellent manner by G Burnstock (Burnstock, 2007). In addition, Ap_nA stimulate different responses in the cardiovascular system, including control of vascular tone and prevention of platelet aggregation (Flores *et al.*, 1999). Release of dinucleoside polyphosphates may result in local concentrations in the range of 10^{-5} mol·L^{-1} or even higher (Ogilvie, 1992). Diadenosine polyphosphates have a direct effect on the vascular tone (Busse *et al.*, 1988; Schlüter *et al.*, 1994; Ralevic *et al.*, 1995; Hoyle *et al.*, 1996; Inscho *et al.*, 1998; van der Giet *et al.*, 1998; Jankowski *et al.*, 1999; Luo *et al.*, 1999b; Gabriels *et al.*, 2000; Lewis and Evans, 2000). In the vasculature of isolated perfused rat kidney, Ap_5A and Ap_6A were effective at a concentration of 10^{-9} mol·L^{-1}, and contractions in aortic rings were elicited at 10^{-8} mol·L^{-1}. Intra-aortic injection in the rat caused a prolonged increase in blood pressure (Schlüter *et al.*, 1994). The vasoconstrictive action of Ap_7A on the vasculature of the isolated perfused rat kidney Ap_7A is lower than that of Ap_6A. The threshold of the vasoconstrictive action of Ap_7A is in the range of 10^{-5} mol·L^{-1} (Jankowski *et al.*, 1999). Vasoconstriction induced by the diadenosine polyphosphates is mediated by an increase in intracellular free calcium ions, $[Ca^{2+}]_i$ (Tepel *et al.*, 1996; 1997).

Recently, interest in uridine (5′)-adenosine (5′) tetraphosphate (Up_4A) (Figure 1) has increased (Jankowski *et al.*, 2005). Up_4A was isolated from the supernatant of stimulated human endothelium and was identified by mass-spectrometry. Stimulation with adenosine 5′-triphosphate (ATP), uridine 5′-triphosphate (UTP), acetylcholine, endothelin, A23187 and mechanical stress releases Up_4A from endothelium, suggesting that Up_4A contributes to vascular regulation. Up_4A plasma concentrations found in healthy subjects are high enough to cause vasoconstriction. Up_4A is the first endogenous dinucleoside polyphosphate isolated from living organisms, which contains both purine and pyrimidine moieties. Several lines of evidence strongly suggest that Up_4A has a functional role in the cardiovascular system, including its vasoactive effects (Figure 2), plasma concentrations and its release upon endothelial stimulation.

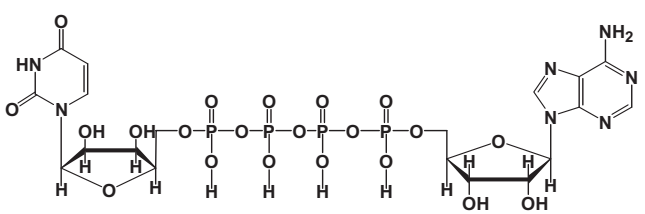

Figure 1 Molecular structure of uridine (5′)-adenosine (5′) tetraphosphate (Up_4A).

Vasodilatory effects of dinucleoside polyphosphates

A small number of dinucleoside polyphosphates have vasodilatory effects, for example, Ap_2A on the tone of isolated mesenteric arterial bed of rats (Ralevic and Burnstock, 1996). Ap_3A and Ap_4A both induce vasodilation upon perfusion of arteries containing endothelium, whereas Ap_4A causes vasoconstriction in arteries from which the endothelium has been removed (Busse *et al.*, 1988). Arterial infusion of Ap_4A produced a dose-dependent decrease of systemic blood pressure and coronary vascular resistance (Nakae *et al.*, 1996). These vasodilatory effects of dinucleoside polyphosphates are

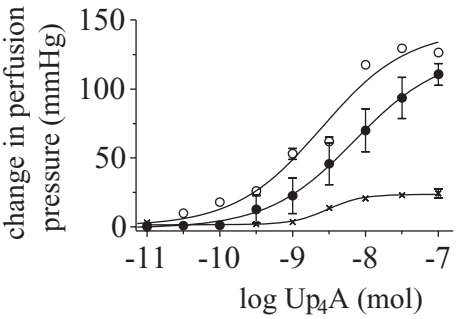

Figure 2 Change in perfusion pressure in the isolated perfused rat kidney induced by Up_4A alone (●), with α,β-methylene ATP (×) and with L-NAME (○) [Figure adapted from Jankowski *et al.* (2005)].

mediated via endothelial A_2 receptors (Ralevic and Burnstock, 1996) and endothelial metabotropic P2Y receptors (Ralevic *et al.*, 1995; Hilderman and Christensen, 1998; Rump *et al.*, 1998; Gabriels *et al.*, 2000; Malmsjo *et al.*, 2000b). Stimulation of endothelial $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors causes endothelium-derived hyperpolarizing factor- and NO-mediated dilatation (Malmsjo *et al.*, 2000a,b; Mombouli and Vanhoutte, 1993; Vanhoutte, 1991). Ohata *et al.* reported that in aortic strips *in situ* $[Ca^{2+}]_i$ waves within endothelial cells are induced by ATP via the $P2Y_1$ purinoceptor, but not by the $P2Y_2$ purinoceptor (Ohata *et al.*, 1997).

In addition to endothelium-dependent dilation effects, Ap_4A and Ap_5A were also demonstrated to directly affect vascular smooth muscle cell (VSMC) dilation. Dilation induced by both dinucleoside polyphosphates was mediated by cAMP, which affects a decrease of $[Ca^{2+}]_i$, possibly through activation of K^+ channels (Sumiyoshi *et al.*, 1997). Direct, endothelium-independent vasodilation induced by purinoceptor agonists was also observed in intrapulmonary arteries isolated from newborn piglets and found to be mediated by a P2Y receptor on the VSMCs (McMillan *et al.*, 1999).

The different functional effects of Ap_4A (constriction in some smooth muscle and relaxation in others) may be explained by the action of a specific Ap_4A hydrolase. This Ap_4A hydrolase hydrolyses Ap_4A in an asymmetric fashion to yield AMP, which is dephosphorylated (Walker and Hilderman, 1993) to yield adenosine, and ATP, which is converted to AMP and inorganic pyrophosphate (Lüthje and Ogilvie,1985; 1988; Ogilvie *et al.*, 1989; Hankin *et al.*, 1995; Thorne *et al.*, 1995). This enzyme is presumed to be involved in the regulation of the intracellular adenosine, AMP, ADP and ATP levels (Guranowski and Sillero, 1992). Thus, it is possible that the application of Ap_4A may produce adenosine, AMP, ADP and ATP, leading to stimulation of multiple receptors such as nucleotide receptors and adenosine receptors. Conversely, there was no significant difference in the potency of Ap_4A and Ap_5A to induce relaxation of the guinea pig left atrium (Hoyle *et al.*, 1996). Sumiyoshi *et al.* (1997) also observed almost equal potency for Ap_4A- and Ap_5A-induced coronary vasorelaxation. However, it is still possible that Ap_4A might stimulate receptors in its intact form (i.e. without degradation) because it has been reported that Ap_nA are substance with long half-lives (Busse *et al.*, 1988; Pohl *et al.*, 1991; Hoyle *et al.*, 1996). In agreement with this speculation, the specific and saturable membrane receptors for Ap_4A have been reported to be present in brain, cardiac, liver, kidney, spleen and adipose tissue (Hilderman *et al.*, 1991; Walker and Hilderman, 1993).

Proliferative effects of dinucleoside polyphosphates on VSMCs

Dinucleoside polyphosphates do not only directly influence the vascular physiology, but also increase the proliferation rate of VSMCs. Growth-stimulating effects of nucleoside polyphosphates have been demonstrated in numerous types of vascular beds (Erlinge, 1998; Jankowski *et al.*, 2001b; Verspohl *et al.*, 2004; Jankowski *et al.*, 2007a,b). The ATP-induced proliferation of VSMCs is coupled to a Gq-protein and triggers phosphoinositide hydrolysis with subsequent activation of protein kinase C, serine/threonine-kinase Raf-1 and mitogen-activated protein kinase (MAPK) (Yu *et al.*, 1996; Wilden *et al.*, 1998). Tu *et al.* observed that $P2Y_2$ receptor stimulation involves the activation of Ras/Raf/MEK/MAPK pathway, which is modulated by $[Ca^{2+}]_i$, protein kinase C and tyrosine kinase (Tu *et al.*, 2000). ATP-stimulated proliferation of coronary artery smooth muscle cells requires independent activation of both the extracellular signal-regulated kinase (ERK/MAPK) cascade and phosphatidylinositol 3-kinase (PI3K) signalling pathways (Wilden *et al.*, 1998). $P2Y_2$ receptor stimulation results in increased c-fos mRNA expression in cultured aortic smooth muscle cells and stimulates proliferation of vascular tissue (Malam-Souley *et al.*, 1996). Ap_4A is equipotent to ATP for induction of these effects (Erlinge *et al.*, 1995).

Vascular smooth muscle cell proliferation and c-fos proto-oncogene expression are induced by Ap_3A, Ap_4A, Ap_5A (Jankowski *et al.*, 2001b), Ap_nG and Gp_nG ($n = 3–6$) (Schlüter *et al.*, 1998) as well as Ap_2A, Ap_2G and Gp_2G (Jankowski *et al.*, 2001a). In vascular tissues, the proliferative effect of the diguanosine polyphosphates Gp_nG (with $n = 3–6$) is significantly stronger than that of ATP (Schlüter *et al.*, 1998). Micromolar concentrations of Ap_3A, Ap_4A, Ap_5A and Ap_6A also stimulate growth in rat glomerular mesangial cells (Heidenreich *et al.*, 1995; Schulze-Lohoff *et al.*, 1995). Moreover, these diadenosine polyphosphates potentiate the growth response to platelet-derived growth factor, but not to insulin-like growth factor-1 (Heidenreich *et al.*, 1995).

Effects of dinucleoside polyphosphates on platelet aggregation

The dinucleoside polyphosphates are potent antagonists of ADP-induced platelet aggregation (Jankowski *et al.*, 1999). These inhibitory effects of dinucleoside polyphosphates on platelet aggregation are mediated by the $P2Y_1$, the $P2Y_{12}$ and the $P2X_1$ receptors and appears to be via competitive inhibition of ADP and ATP, with Ap_4A having a K_i of approximately 0.7 $mmol \cdot L^{-1}$ (Kunapuli and Daniel, 1998). Because plasma Ap_4A concentrations are significantly lower than the K_i, a systemic impact is unlikely. However, the interaction may be caused by local effects as the concentrations close to platelets are significantly higher than plasma concentrations. A comparison of the homologous series of Ap_nA compounds with phosphate chain lengths from two to six revealed Ap_5A as the most potent inhibitor of ADP-induced platelet aggregation,

followed by Ap_6A and Ap_4A, which were more potent than Ap_3A or Ap_2A. Inhibition of platelet aggregation by dinucleoside polyphosphates is proposed to be due to direct competition between the dinucleoside polyphosphates and ADP at a specific receptor site on the platelet membrane (Harrison *et al*., 1975; Kunapuli, 1998). Dinucleoside polyphosphates inhibit release of ADP from blood platelets with a potency that decreases with decreasing chain length. Thus, dinucleoside polyphosphates in platelets may fulfil an anti-aggregatory role. In human neutrophils, Ap_3A, Ap_4A, Ap_5A and Ap_6A produce an increase in intracellular free calcium via a G-protein-coupled receptor (Pintor *et al*., 1997).

Release of dinucleoside polyphosphates

Up to millimolar range concentrations of dinucleoside polyphosphates are stored in secretory granules from platelets, in adrenal chromaffin cells and in central nervous synaptosomes (Flodgaard and Klenow, 1982; Lüthje and Ogilvie, 1983; Rodriguez del Castillo *et al*., 1988; Pintor *et al*., 1991). The intracellular concentration of diadenosine (5′, 5′) tetraphosphate (Ap_4A) during normal growth (Garrison and Barne, 1992) correlates directly with the proliferative state of the cell or tissue (Rapaport and Zamecnik, 1976; Remy, 1992). Ap_4A levels are known to respond to cellular stresses, such as oxidation and heat shock. Ap_4A has been described as an alarmone that signals the onset of cellular and metabolic stress, although its precise role remains unclear (Brevet *et al*., 1985; Garrison *et al*., 1986; Remy, 1992).

Metabolism of dinucleoside polyphosphates

Subsequent inactivation of the released nucleotides is thought to be mainly regulated by vascular endothelial (Marcus *et al*., 2003) and lymphoid (Heptinstall *et al*., 2005) membrane-bound nucleoside triphosphate diphosphohydrolase (NTPDase; also known as ecto-ATPDase, CD39) and ecto-5′-nucleotidase (CD73). Ectohydrolases are present on a broad variety of cell types, including aortic endothelial cells (Mateo *et al*., 1997a,b), chromaffin cells (Gasmi *et al*., 1998), rat mesangial cells, bovine corneal epithelial cells, the human hepatocellular liver carcinoma cell line (Hep-G2) and periodontal cells (von Drygalski and Ogilvie, 2000). The human diphosphorylated inositol phosphate phosphohydrolase was shown to be a candidate for regulating signalling of diadenosine polyphosphates by hydrolysis of Ap_5A and Ap_6A in preference to other diadenosine polyphosphates (Safrany *et al*., 1999). The enzymatic breakdown of dinucleoside polyphosphates leads to generation of mononucleotides and nucleotides that, in turn, are biologically active in vascular tissues. In contrast to these traditional paradigms that focus on nucleotide-inactivating mechanisms, it has now become clear that nucleotide-phosphorylating enzymes adenylate kinase and NDP kinase are also co-expressed on the cell surface and finely control the purinergic signalling cascade via two counter-balancing pathways, ATP-inactivating and ATP-regenerating respectively (Yegutkin *et al*., 2000; Yegutkin and Burnstock, 2000). The identification of a complex mixture of nucleotide pyrophosphatase/phosphodiesterase (NPP), NTPDase, adenylate kinase and other soluble purinergic enzymes freely circulating in the bloodstream adds another level of complexity to the understanding of the regulatory mechanisms of purine homeostasis within the vasculature (Birk *et al*., 2002; Yegutkin *et al*., 2003; 2007; 2008). The agonistic effects of nucleotides are obviously mediated by complex mechanisms, including: (i) dinucleoside polyphosphate receptor pathway; (ii) inhibition of ecto-adenylate kinase activity; and (iii) generation of biologically active ATP and adenosine.

Table 1 gives a characteristic overview of endogenous dinucleoside polyphosphates that have been identified in human tissues and cells.

Purinoceptor system

The physiological and pathophysiological effects of mononucleosides, mononucleoside polyphosphates and dinucleoside polyphosphates are mediated via nucleotide-selective receptors. Essentially two major purine receptors subfamilies have been described based on pharmacological, functional and cloning data (Ralevic, 2000; Burnstock, 2002). P1 receptors are preferably activated by adenosine, while P2 receptors are activated by ATP, ADP, UTP and UDP and also by dinucleoside polyphosphates. Using molecular, biochemical and pharmacological criteria P1 receptors have been further subdivided into four subgroups: A_1, A_{2A}, A_{2B} and A_3 according to molecular, biochemical and pharmacological criteria. The group of P2 receptors is divided into P2X and P2Y (Figure 3) receptors according to their molecular structure and the signal transduction pathways they regulate. Selective agonists or antagonists discriminating adequately between families of P2X and P2Y receptors, or between subtypes of receptors within each of these groups have been discovered. Some selective antagonists include the $P2X_7$ antagonist [^{3}H]-2-cyano -1-[(1*S*)-1-phenylethyl]-3-quinolin-5-ylguanidine (Donnelly-Roberts *et al*., 2008), the $P2Y_1$-selective antagonist N^6-methyl 2′-deoxyadenosine 3′,5′-bisphosphate (Boyer *et al*., 1998) and the potent and relatively selective $P2X_1$ antagonist diinosine pentaphosphate (Ip_5I) (King *et al*., 1999). Purinoceptors are characterized by high plasticity, and they are dynamically regulated during development. The purinoceptor system plays a general role as a sympathetic regulator of vasomotor tone (Kennedy, 1996; Ralevic, 2000). The purinoceptor system that controls vascular homeostasis displays a high degree of complexity.

P2X receptors mediating dinucleoside polyphosphate effects

P2X receptors are ligand-gated ion channels, which are opened by purinergic messengers (Bo *et al*., 2000), thus mediating rapid changes in the membrane permeability of monovalent and divalent cations (Bean, 1992; Dubyak and el-Moatassim, 1993; Burnstock, 2006; Erb *et al*., 2006). Stimulation of ionotropic P2X receptors induces an influx of Na^+-ions and Ca^{2+}-ions into the cytosol. The increase of the concentration of these ions triggers depolarization of the membrane potential, which opens potential operated Ca^{2+}-channels (Usune *et al*., 1996). The resulting Ca^{2+}-influx

Table 1 Isolations, identifications and characterizations of dinucleoside polyphosphates in human tissues and their receptor-mediated vascular effects

Compound	*Isolation from human tissue*	*Known vascular effects and receptor type involved (in brackets)*	*References*
Ap_2A	Myocardium Platelets Placenta Adrenal glands	Vasodilation (A_2) and vasoconstriction (A_1) in coronary arteries and renal vessels Proliferation of vascular smooth muscle cells (P2Y)	Hoyle *et al.* (1996); van der Giet *et al.* (1997a); Luo *et al.* (1999a); Jankowski *et al.* (2001a,b,c; 2003b)
Ap_2G	Platelets	Proliferation of vascular smooth muscle cells (P2Y)	Jankowski *et al.* (2001a)
Gp_2G	Platelets	Proliferation of vascular smooth muscle cells (P2Y)	Jankowski *et al.* (2001a)
Ap_3A	Myocardium Platelets Placenta Adrenal glands Plasma Proximal tubule Epithelial cells	Vasodilation (P2Y and A_2) and vasoconstriction (A_1 and P2X) in coronary arteries, renal vessels and mesenteric vessels Proliferation of vascular smooth muscle cells (P2Y)	Lüthje and Ogilvie (1983); Ogilvie and Jakob (1983); Schlüter *et al.* (1994); van der Giet *et al.* (1997a); Luo *et al.* (1999a); Jankowski *et al.* (2001a,b,c; 2003a,b; 2007a)
Ap_3G	Platelets	Vasoconstriction in renal vessels (P2X) Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998)
Gp_3G	Platelets	Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998)
Ap_4A	Adrenal gland Plasma Platelets Brain Proximal tubule epithelial cells	Vasoconstriction in renal vessels (P2X, A_1) and mesenteric vessels (P2X) Proliferation of vascular smooth muscle cells (P2Y)	Ogilvie *et al.* (1989); Pintor *et al.* (1992a); van der Giet *et al.* (1997a; 1998); Jankowski *et al.* (2001a,b; 2003a,b)
Ap_4G	Platelets	Vasoconstriction in renal vessels (P2X) Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998)
Gp_4G	Platelets	Vasoconstriction of renal vessels (P2X) Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998)
Ap_5A	Adrenal glands Plasma Platelets Placenta Brain Proximal tubule epithelial cells	Proliferation in vascular smooth muscle cells (P2Y) Vasoconstriction in renal vessels (P2X) and coronary arteries (P2X) Vasodilation in coronary arteries (P2Y)	Pintor *et al.* (1992a); Schlüter *et al.* (1994); van der Giet *et al.* (1997a; 1999; 2001; 2002); Jovanovic *et al.* (1998); Jankowski *et al.* (2001a,b,c; 2003a,b; 2007a);
Ap_5G	Platelets	Vasoconstriction in renal vessels (P2X) Proliferation of vascular smooth muscle cells (P2Y) Vasoconstriction in renal vessels (P2X) and coronary arteries (P2X) Vasodilation in coronary arteries (P2Y)	Schlüter *et al.* (1998); van der Giet *et al.* (2001; 2002)
Gp_5G	Platelets	Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998); van der Giet *et al.* (2001; 2002)
Ap_6A	Adrenals Plasma Platelets Red blood cells Placenta Proximal tubule epithelial cells	Proliferation of vascular smooth muscle cells (P2Y) Vasoconstriction in renal vessels (P2X) and coronary arteries (P2X) Vasodilation in coronary arteries (P2Y)	Pintor *et al.* (1992b); Schlüter *et al.* (1994); van der Giet *et al.* (1997a; 1998; 2001; 2002); Jankowski *et al.* (2001a,b.c; 2003a.b; 2007a)
Ap_6G	Platelets	Vasoconstriction in renal vessels (P2X) and coronary vessels (P2X) Vasodilation in coronary arteries (P2Y) Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998); van der Giet *et al.* (2001; 2002)
Gp_6G	Platelets	Proliferation of vascular smooth muscle cells (P2Y)	Schlüter *et al.* (1998); van der Giet *et al.* (2001; 2002)
Ap_7A	Platelets	Vasoconstriction in renal vessels (P2X)	Jankowski *et al.* (1999)
Up_4A	Endothelial cells Tubule cells	Vasoconstriction and proliferation	Jankowski *et al.* (2005; 2007b; 2008); Gui *et al.* (2008)

increases $[Ca^{2+}]_i$, which affects the constriction of the VSMCs (Tepel *et al.*, 1997). There are seven P2X receptor cDNAs currently known, $P2X_1$–$P2X_7$ (Khakh *et al.*, 2001). When expressed individually in heterologous systems, $P2X_1$ and $P2X_3$ subunits form channels activated by ATP or α,β-methylene ATP, whereas $P2X_2$, $P2X_4$ and $P2X_5$ form channels activated by ATP but not α,β-methylene ATP (North and Surprenant, 2000). $P2X_1$ and $P2X_3$ receptors are characterized by strong and rapid desensitization, whereas desensitization of $P2X_2$, $P2X_4$ and $P2X_6$ receptors is relatively modest (Ralevic and Burnstock, 1998). Therefore, $P2X_1$-induced vasoconstrictions are transient, whereas $P2X_2$- or $P2X_4$-mediated vasoconstrictions are permanent as long as the agonist is present.

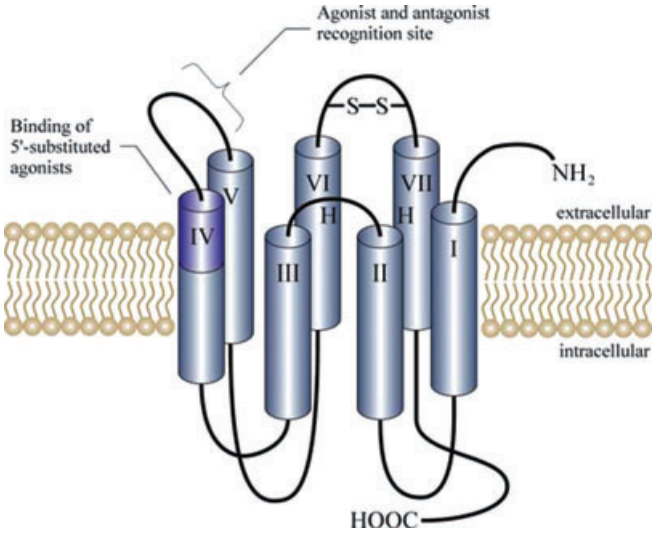

Figure 3 Structure of P2Y membrane receptors. P2Y are G-protein-coupled receptors (Fields and Burnstock, 2006), which act via a downstream signalling cascade including G-proteins and inositol triphosphate among other factors (Barnard and Simon, 2001).

The ion-gating pore is formed by the aggregation of three P2X monomers (Nicke *et al.*, 1998). Co-expression of P2X subtypes in heterologous expression systems can result in the formation of heteropolymeric P2X trimers (Nori *et al.*, 1998). Therefore, it can be assumed that heteropolymeric channel formation is also possible *in vivo*. Heteropolymeric P2X receptors clearly differ from their homomeric relatives (Torres *et al.*, 1998; Bianchi *et al.*, 1999) and may therefore constitute an important mechanism for generating functional diversity of ATP- and dinucleoside polyphosphate-mediated responses. The $P2X_1$ forms large, approximately elliptical clusters on the smooth muscle cells of mesenteric, renal and pulmonary arteries as well as in the aorta and in veins, which are restricted to the adventitial surface of the media. At the adventitial surface, the large clusters are immediately apposed to sympathetic varicosities. In the pulmonary artery, large receptor clusters were found throughout the media of the vessel. Smaller, spherical $P2X_1$ clusters occur throughout the media of arteries of all sizes. The small $P2X_1$ clusters are not associated with varicosities (Hansen *et al.*, 1999). This observation may point to a paracrine role of purinergic agonists. $P2X_1$, $P2X_2$ and $P2X_4$ are co-expressed in smooth muscle cells of coronary vessels as well as in peripheral vessels, including aorta, pulmonary artery, renal artery, femoral artery, internal and external iliac arteries. The co-expression of P2X receptor subtypes substantiates the possibility of a heteropolymeric assembly of the P2X ion channels. In contrast, no mRNA transcripts of $P2X_1$, $P2X_2$ or $P2X_4$ were found in the superior mesenteric artery (Nori *et al.*, 1998). A further aspect contributing to the complexity of the purinergic system is the existence of P2X splice variants. In the human bladder cells, Hardy *et al.* detected a $P2X_1$ receptor splice variant, which lacks part of the second transmembrane domain. It was suggested that isoforms might be potential sites for modifying or regulating putative purinergic activation (Hardy *et al.*, 2000). This view is supported by the observation of Chen *et al.*, who published that ATP-induced currents in cells expressing $P2X_{2/1}$ and $P2X_{2/2}$ variants were large and desensitized rapidly, whereas the current in those cells expressing the $P2X_{2/3}$ variant was much smaller and desensitized more slowly (Chen *et al.*, 2000). Alternatively, spliced $P2X_4$ RNAs in human smooth muscle cells were identified (Dhulipala *et al.*, 1998).

The $P2X_4$ receptor is the most prominent P2X receptor in human vascular endothelial cells from umbilical veins, aorta, pulmonary artery and skin microvessels. Vascular endothelial cells are continuously exposed to variations in blood flow, which plays an important role in vessel growth or regression and in the local development of atherosclerosis. The shear stress that occurs during changes in blood flow leads to substantial release of ATP and dinucleoside polyphosphates from endothelial cells (Burnstock, 1999; Jankowski *et al.*, 2005), and these purines might mediate alterations in the balance between proliferation and apoptosis. This endothelium-dependent response to ATP is absent in atherosclerotic patients. Consequently, $P2X_4$ mRNA expression was much higher in these cells than was the expression of other subtypes, including $P2X_1$, $P2X_3$, $P2X_5$ and $P2X_7$ (Yamamoto *et al.*, 2000). $P2X_2$ receptors are located on neurons and on endothelial cells in rat blood vessels (Hansen *et al.*, 1999; Zemkova *et al.*, 2004).

The calcium-permeable $P2X_1$ receptor is considered the principal mediator of vasoconstriction (Kennedy, 1996), with $P2X_1$ protein clusters on the adventitial surface of blood vessels immediately adjacent to sympathetic nerve varicosities (Hansen *et al.*, 1999). However, $P2X_1$ transcripts co-localize with $P2X_2$, $P2X_4$ and $P2X_5$ mRNA in muscle cells of a number of blood vessels, which alludes the added presence of heteromeric P2X receptors (Lewis and Evans, 2000; 2001; Pulvirenti *et al.*, 2000; Turner *et al.*, 2003). For example, heteromeric $P2X_{1/5}$ receptors have been implicated in vasoconstriction of submucosal arterioles in the guinea pig (Surprenant *et al.*, 2000).

P2Y receptors mediating dinucleoside polyphosphate effects

P2Y receptors are 7-membrane-spanning proteins (King *et al.*, 2000; Abbracchio *et al.*, 2006; Burnstock, 2006; Erb *et al.*, 2006). Some common mechanisms of signal transduction shared by most 7-membrane-spanning receptors include activation of phospholipase C and/or regulation of adenyl cyclase activity (Burnstock, 2006; Erb *et al.*, 2006). P2Y receptors do not act directly by inducing a cation influx, but via a downstream signalling cascade including G-proteins and inositol triphosphate among other factors (Barnard and Simon, 2001).

P2X and G-protein-coupled P2Y receptors expressed in VSMCs were reported to mediate vasoconstriction (Inscho *et al.*, 1998; Fukumitsu *et al.*, 1999; Hillaire-Buys *et al.*, 1999; McMillan *et al.*, 1999; Shen *et al.*, 2004). The vasoconstriction inducing P2Y receptor is probably coupled to PLC-β1 via Gαq/11 and to PLC-β3 via Gβγ3 (Murthy and Makhlouf, 1998a,b). In human coronary arteries, extracellular nucleotides elicit constriction primarily by activation of P2X and $P2Y_2$ receptors, whereas a role for $P2Y_1$ and $P2Y_6$ receptors was excluded (Malmsjo *et al.*, 2000b). UTP- and ATP-induced vasoconstriction in intrapulmonary arteries is consistent with activation of the $P2Y_4$ receptor subtype (McMillan *et al.*, 1999),

which is sensitive to Ap_4A. Dinucleoside polyphosphate vasoconstriction is also mediated by the adenosine A_1 receptor (Vahlensieck *et al.*, 1996; van der Giet *et al.*, 1997b; 1998; 1999; Gabriels *et al.*, 2000). Because the vasoconstrictive effects of Ap_2A and Ap_3A, but not the vasoconstrictive effect of Ap_5A, are mediated by A1 receptors *(*van der Giet *et al.*, 1997b), vasoconstriction is likely due to a direct effect of intact dinucleoside polyphosphates on the A1 receptor rather than effects caused by dinucleoside polyphosphate metabolites.

Purinoceptor heteromerization

These principally different mechanisms led to the terminology metabotropic and ionotropic used for P2Y receptors and P2X receptors respectively. The purine receptor subtypes have been characterized according to their molecular structures and according to their pharmacological characteristics, both of which show considerable differences. Different purinoceptor subtypes may be expressed in the same cell type, and heteromeric receptors are formed among different purinoceptor subtypes (Torres *et al.*, 1998; Le *et al.*, 1999; Barnard and Simon, 2001). These purinoceptor heteromeres may show pharmacological properties quite different from those of purinoceptor homomeres (Torres *et al.*, 1998). The ability to form heteromeres has been demonstrated both in P2X receptors (Le *et al.*, 1999) and in P2Y receptors (Barnard and Simon, 2001) and contributes to the diversity of pharmacological properties manifested by the purinoceptor family.

Distribution of purinoceptor

Characterization of P2X and P2Y expression in vascular endothelium has been an especially active area of investigation in recent years. Different purine nucleotides activate endothelial cells in distinct ways, suggesting that at least two different endothelial purinoceptors exist. Pirotton *et al.* (1996) initially demonstrated and others have since confirmed (Bultmann *et al.*, 1997) that both $P2Y_1$ and $P2Y_2$ receptors are co-expressed in endothelial cells from bovine aorta. P2Y receptors have also been identified in endothelial cells from rat cerebral vessels (Vigne *et al.*, 2000; Mistry *et al.*, 2003) and from mesenteric arteries (Malmsjo *et al.*, 2000a,b). These findings have been confirmed also in rat renal glomeruli (Harada *et al.*, 2000). Currently, it remains unclear whether other purinoceptor subtypes are also expressed in endothelial cells. Undoubtedly, the endothelial $P2Y_1$ receptor subtype mediates vasodilation (Bultmann *et al.*, 1997; Malmsjo *et al.*, 2000b; Vigne *et al.*, 2000). In addition, the $P2Y_2$ and $P2Y_4$ receptor subtypes may also play a role in vasodilation (Malmsjo *et al.*, 2000b).

Specific dinucleoside polyphosphate receptors

Dinucleoside polyphosphates have the capacity to potentiate signalling effects via P2 receptors (primarily, via $P2X_1$, $P2X_3$ and $P2Y_1$ subtypes), although the existence of specific dinucleoside polyphosphate receptors has also been proposed (Flores *et al.*, 1999; Delicado *et al.*, 2006). However, the specificity of dinucleoside polyphosphate receptors and their implication into multiple extracellular signals are still poorly understood, primarily due to the complexity of the purinergic signalling cascade. The effects of the dinucleoside polyphosphates are blocked by desensitization of P2X receptors with α,β-methylene ATP subtype (Bo *et al.*, 1998; Kunapuli and Daniel, 1998; Wang *et al.*, 2002) or blockade by diinosine pentaphosphates (Ip_5I) (Hoyle *et al.*, 1997).

Uridine adenosine tetraphosphate (Up_4A)

Physiological and pathophysiological effects of Up_4A

Mean total plasma Up_4A concentrations are significantly increased in juvenile hypertensives compared with juvenile normotensive subjects (Jankowski *et al.*, 2007b). Accordingly, Up_4A shows a significant association with juvenile hypertension. The plasma Up_4A concentrations correlate with left ventricular mass and intima media wall thickness in hypertensive subjects. The increased intima media thickness may be related to proliferative effects of Up_4A, as Up_4A has been demonstrated to increase human VSMC proliferation. Up_4A is obviously an important risk factor of juvenile hypertension. Furthermore, Up_4A was identified in renal tissue (Jankowski *et al.*, 2008). Stimulation of tubule cells with oleoyl-2-acetyl-sn-glycerol (OAG) increases the release rate of Up_4A from tubule cells about 10-fold. Up_4A acts as a strong vasoconstrictive mediator on afferent arterioles, but has no significant effect on the tone of efferent arterioles, suggesting a functional role of Up_4A as an autocrine hormone for glomerular perfusion. Because of the predominant effect of the Up_4A on afferent arterioles, Up_4A may decrease glomerular perfusion, intra-glomerular pressure and, hence, glomerular filtration rate. The release of Up_4A from renal tubular cells may be an additional mechanism whereby tubular cells could affect renal perfusion. Up_4A release may further contribute to renal vascular autoregulation mechanisms. Up_4A obviously plays a role in renal haemodynamics and blood pressure regulation.

Moreover, Up_4A stimulated contraction of isolated rat pulmonary arteries in a concentration-dependent manner (Gui *et al.*, 2008). Up_4A is potent as UTP and UDP in arteries without endothelium, while much more effective than UTP and UDP in preparations with endothelium. The vasoconstrictor effect of Up_4A is inhibited by suramin, but not by P_1,P_5 diinosine pentaphosphate (Ip_5I) or desensitization of P2X receptors with α,β-methylene-ATP. Up_4A-induced contraction is inhibited by pretreatment with thapsigargin, nitrendipine or EGTA, but unaffected by the specific Rho-kinase inhibitor (S)-(+)-2-methyl-1-[(4-methyl-5-isoquinolinyl) sulfonyl]-homopiperazine (H-1152) (Ikenoya *et al.*, 2002). Furthermore, unlike ATP and UTP, Up_4A does not induce relaxation of preparations with endothelium precontracted with phenylephrine. Up_4A is obviously a potent vasoconstrictor, but not a vasodilator, of the rat pulmonary artery. It is likely that Up_4A acts through a suramin-sensitive P2Y receptor. The contractile effect of Up_4A involves the entry of extracellular Ca^{2+} and release of Ca^{2+} from intracellular stores, but not Ca^{2+} sensitization via the RhoA/Rho-kinase pathway. Up_4A, therefore, may be important for regulation of pulmonary vascular tone.

Plasma concentrations of dinucleoside polyphosphates
Physiologically relevant concentrations of dinucleoside polyphosphates are present in human plasma. The mean total plasma diadenosine polyphosphate concentrations (μmol·L^{-1}; mean ± SEM) in cubital veins of normotensive subjects are 0.89 ± 0.59 for Ap_3A, 0.72 ± 0.72 for Ap_4A, 0.33 ± 0.24 for Ap_5A and 0.18 ± 0.18 for Ap_6A (Jankowski *et al.*, 2003a). In adrenal venous plasma, significantly higher diadenosine polyphosphate concentrations are detectable than in plasma from the infrarenal and suprarenal vena cava. Mean total plasma Up_4A concentrations are in the range of 55.5 ± 15.2 nmol·L^{-1} (Jankowski *et al.*, 2005). Adrenal medulla (Jankowski *et al.*, 2003a) and endothelial cells (Jankowski *et al.*, 2005) are obviously a source of plasma dinucleoside polyphosphates in humans.

Therapeutic aspects of the purinergic system

There have been promising developments concerning purinergic anti-thrombotic drugs (Cattaneo, 2006; Gachet, 2006; Gachet *et al.*, 2006). Platelets are known to express $P2Y_1$, $P2Y_{12}$ and $P2X_1$ receptors (Hollopeter *et al.*, 2001). Clinical trials like CURE (Yusuf *et al.*, 2001) and CREDO (Beinart *et al.*, 2005) have provided clear evidence that the purinergic antithrombotic drugs clopidogrel and ticlopidine reduce the risks of recurrent strokes and heart attacks, especially when combined with aspirin (Kam and Nethery, 2003; Kunapuli *et al.*, 2003).

Moreover, dinucleoside polyphosphates have been shown to possess beneficial properties in the treatment of various diseases, such as chronic obstructive pulmonary disease. Dinucleoside polyphosphates facilitate the clearance of mucous secretions from the lungs of mammals, including humans, being treated for diseases, such as cystic fibrosis (Picher and Boucher, 2000) and chronic bronchitis (Picher and Boucher, 2000). Furthermore, properties of diadenosine polyphosphates may serve to help in the treatment of some ocular pathologies like dry eye (Yerxa *et al.*, 2002; Guzman-Aranguez *et al.*, 2007) and retinal detachment (Guzman-Aranguez *et al.*, 2007). Recent findings showing increased dinucleoside polyphosphate concentration in hypertensive patients (Jankowski *et al.*, 2007b) may provide novel therapeutic approaches for hypertension in the future.

Patented therapeutic effects of dinucleoside polyphosphates
Some potential therapeutic effects of dinucleoside polyphosphates are protected by international patents. For example, Stutts *et al.* claim the rights to the therapeutic effects of dinucleoside polyphosphates in the context of asthma, bronchiectasis, post-operative mucous retention, pneumonia and primary ciliary dyskinesia (Stutts *et al.*, 1995). Moreover, prevention and treatment of pneumonia in immobilized patients using dinucleoside polyphosphates are protected by Jacobus and Leighton (1996). Further patents claim treatment of sinusitis (Jacobus *et al.*, 1998a,b; Jacobus *et al.*, 1996), otitis media (Drutz *et al.*, 1996) and nasolacrimal duct obstruction (Yerxa and Brown, 2003) with dinucleoside polyphosphates.

Acknowledgements

This review was supported by a grant from the German Research Foundation (DFG, Ja-972 /11-1; JJ), Federal Ministry of Education and Research (BMBF; 01GR0807); JJ), by a Rahel-Hirsch-scholarship from the Charité (VJ) and by the Sonnenfeld Foundation (JJ and VJ).

Conflicts of interest

None.

References

Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C *et al.* (2006). International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. *Pharmacol Rev* **58**: 281–341.

Barnard EA, Simon J (2001). An elusive receptor is finally caught: P2Y(12′), an important drug target in platelets. *Trends Pharmacol Sci* **22**: 388–391.

Bean BP (1992). Pharmacology and electrophysiology of ATP-activated ion channels. *Trends Pharmacol Sci* **13**: 87–90.

Beinart SC, Kolm P, Veledar E, Zhang Z, Mahoney EM, Bouin O *et al.* (2005). Long-term cost effectiveness of early and sustained dual oral antiplatelet therapy with clopidogrel given for up to one year after percutaneous coronary intervention results: from the Clopidogrel for the Reduction of Events During Observation (CREDO) trial. *J Am Coll Cardiol* **46**: 761–769.

Bianchi BR, Lynch KJ, Touma E, Niforatos W, Burgard EC, Alexander KM *et al.* (1999). Pharmacological characterization of recombinant human and rat P2X receptor subtypes. *Eur J Pharmacol* **376**: 127–138.

Birk AV, Bubman D, Broekman MJ, Robertson HD, Drosopoulos JH, Marcus AJ *et al.* (2002). Role of a novel soluble nucleotide phosphohydrolase from sheep plasma in inhibition of platelet reactivity: hemostasis, thrombosis, and vascular biology. *J Lab Clin Med* **139**: 116–124.

Bo X, Sexton A, Xiang Z, Nori SL, Burnstock G (1998). Pharmacological and histochemical evidence for P2X receptors in human umbilical vessels. *Eur J Pharmacol* **353**: 59–65.

Bo X, Schoepfer R, Burnstock G (2000). Molecular cloning and characterization of a novel ATP P2X receptor subtype from embryonic chick skeletal muscle. *J Biol Chem* **275**: 14401–14407.

Boyer JL, Mohanram A, Camaioni E, Jacobson KA, Harden TK (1998). Competitive and selective antagonism of P2Y1 receptors by N6-methyl 2′-deoxyadenosine 3′,5′-bisphosphate. *Br J Pharmacol* **124**: 1–3.

Brevet A, Plateau P, Best-Belpomme M, Blanquet S (1985). Variation of Ap4A and other dinucleoside polyphosphates in stressed Drosophila cells. *J Biol Chem* **260**: 15566–15570.

Bultmann R, Hansmann G, Tuluc F, Starke K (1997). Vasoconstrictor and vasodilator effects of guanine nucleotides in the rat aorta. *Naunyn Schmiedebergs Arch Pharmacol* **356**: 653–661.

Burnstock G (1999). Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. *J Anat* **194**: 335–342.

Burnstock G (2002). Purinergic signaling and vascular cell proliferation and death. *Arterioscler Thromb Vasc Biol* **22**: 364–373.

Burnstock G (2006). Purinergic signalling. *Br J Pharmacol* **147**: S172–S181.

Burnstock G (2007). Physiology and pathophysiology of purinergic neurotransmission. *Physiol Rev* **87**: 659–797.

Busse R, Ogilvie A, Pohl U (1988). Vasomotor activity of diadenosine triphosphate and diadenosine tetraphosphate in isolated arteries. *Am J Physiol* **254** (5 Pt 2): H828–H832.

Castillo CJ, Moro MA, Del Valle M, Sillero A, Garcia AG, Sillero MA (1992). Diadenosine tetraphosphate is co-released with ATP and catecholamines from bovine adrenal medulla. *J Neurochem* **59**: 723–732.

Cattaneo M (2006). ADP receptors: inhibitory strategies for antiplatelet therapy. *Drug News Perspect* **19**: 253–259.

Cheewatrakoolpong B, Gilchrest H, Anthes JC, Greenfeder S (2005). Identification and characterization of splice variants of the human P2X7 ATP channel. *Biochem Biophys Res Commun* **332**: 17–27.

Chen C, Parker MS, Barnes AP, Deininger P, Bobbin RP (2000). Functional expression of three P2X(2) receptor splice variants from guinea pig cochlea. *J Neurophysiol* **83**: 1502–1509.

Delicado EG, Miras-Portugal MT, Carrasquero LM, Leon D, Perez-Sen R, Gualix J (2006). Dinucleoside polyphosphates and their interaction with other nucleotide signaling pathways. *Pflugers Arch* **452**: 563–572.

Dhulipala PD, Wang YX, Kotlikoff MI (1998). The human P2X4 receptor gene is alternatively spliced. *Gene* **207**: 259–266.

Donnelly-Roberts DL, Namovic MT, Surber B, Vaidyanathan SX, Perez-Medrano A, Wang Y *et al*. (2008). [(3)H]A-804598 ([(3)H]2-cyano-1-[(1S)-1-phenylethyl]-3-quinolin-5-ylguanidine) is a novel, potent, and selective antagonist radioligand for P2X7 receptors. *Neuropharmacology* **56**: 223–229.

Drury AN, Szent-Györgyi A (1929) The physiological activity of adenine com-pounds with especial reference to their action upon the mammalian heart. *J Physiol* **68**: 213–237.

Drutz D, Rideout J, Jacobus K (1996) Method of treating otitis media with uridine triphosphates and related compounds. USA: US-Pat. 6,423,694, Inspire Pharmaceuticals, Inc.

von Drygalski A, Ogilvie A (2000). Ecto-diadenosine 5′,5‴-P1,P4-tetraphosphate (Ap4A)-hydrolase is expressed as an ectoenzyme in a variety of mammalian and human cells and adds new aspects to the turnover of Ap4A. *Biofactors* **11**: 179–187.

Dubyak GR, el-Moatassim C (1993). Signal transduction via P2-purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol* **265**: C577–C606.

Erb L, Liao Z, Seye CI, Weisman GA (2006). P2 receptors: intracellular signaling. *Pflugers Arch* **452**: 552–562.

Erlinge D (1998). Extracellular ATP: a growth factor for vascular smooth muscle cells. *Gen Pharmacol* **31**: 1–8.

Erlinge D, You J, Wahlestedt C, Edvinsson L (1995). Characterisation of an ATP receptor mediating mitogenesis in vascular smooth muscle cells. *Eur J Pharmacol* **289**: 135–149.

Fields RD, Burnstock G (2006). Purinergic signalling in neuron-glia interactions. *Nat Rev Neurosci* **7**: 423–436.

Flodgaard H, Klenow H (1982). Abundant amounts of diadenosine 5′,5‴-P1,P4-tetraphosphate are present and releasable, but metabolically inactive, in human platelets. *Biochem J* **208**: 737–742.

Flores NA, Stavrou BM, Sheridan DJ (1999). The effects of diadenosine polyphosphates on the cardiovascular system. *Cardiovasc Res* **42**: 15–26.

Fukumitsu A, Takano Y, Iki A, Honda K, Saito R, Katsuragi T *et al*. (1999). Endogenous ATP released by electrical field stimulation causes contraction via P2x- and P2y-purinoceptors in the isolated tail artery of rats. *Jpn J Pharmacol* **81**: 375–380.

Gabriels G, Endlich K, Rahn KH, Schlatter E, Steinhausen M (2000). In vivo effects of diadenosine polyphosphates on rat renal microcirculation. *Kidney Int* **57**: 2476–2484.

Gabriels G, Rahn KH, Schlatter E, Steinmetz M (2002). Mesenteric and renal vascular effects of diadenosine polyphosphates (ApnA). *Cardiovasc Res* **56**: 22–32.

Gachet C (2006). Regulation of platelet functions by P2 receptors. *Annu Rev Pharmacol Toxicol* **46**: 277–300.

Gachet C, Leon C, Hechler B (2006). The platelet P2 receptors in arterial thrombosis. *Blood Cells Mol Dis* **36**: 223–227.

Garrison PN, Barnes LD (1992). Determination of dinucleoside polyphosphates. In: McLennan AG (ed.). *AP4A and Other Dinucleoside Polyphosphates*. CRC Press: Boca Raton, FL, pp. 29–61.

Garrison PN, Mathis SA, Barnes LD (1986). In vivo levels of diadenosine tetraphosphate and adenosine tetraphospho-guanosine in Physarum polycephalum during the cell cycle and oxidative stress. *Mol Cell Biol* **6**: 1179–1186.

Gasmi L, Cartwright JL, McLennan AG (1998). The hydrolytic activity of bovine adrenal medullary plasma membranes towards diadenosine polyphosphates is due to alkaline phosphodiesterase-I. *Biochim Biophys Acta* **1405**: 121–127.

van der Giet M, Khattab M, Borgel J, Schlüter H, Zidek W (1997a). Differential effects of diadenosine phosphates on purinoceptors in the rat isolated perfused kidney. *Br J Pharmacol* **120**: 1453–1460.

van der Giet M, Khattab M, Börgel J, Schlüter H, Zidek W (1997b). Differential effects of diadenosine phosphates on purinoceptors in the rat isolated perfused kidney. *Br J Pharmacol* **120**: 1453–1460.

van der Giet M, Jankowski J, Schlüter H, Zidek W, Tepel M (1998). Mediation of the vasoactive properties of diadenosine tetraphosphate via various purinoceptors. *J Hypertens* **16**: 1939–1943.

van der Giet M, Cinkilic O, Jankowski J, Tepel M, Zidek W, Schlüter H (1999). Evidence for two different P2X-receptors mediating vasoconstriction of Ap_5A and Ap_6A in the isolated perfused rat kidney. *Br J Pharmacol* **127**: 1463–1469.

van der Giet M, Westhoff T, Cinkilic O, Jankowski J, Schlüter H, Zidek W *et al*. (2001). The critical role of adenosine and guanosine in the affinity of dinucleoside polyphosphates to P2X-receptors in the isolated perfused rat kidney. *Br J Pharmacol* **132**: 467–474.

van der Giet M, Schmidt S, Tölle M, Jankowski J, Schlüter H, Zidek W *et al*. (2002). Effects of dinucleoside polyphosphates on regulation of coronary vascular tone. *Eur J Pharmacol* **448**: 207–213.

Gui Y, Walsh MP, Jankowski V, Jankowski J, Zheng XL (2008). Up_4A stimulates endothelium-independent contraction of isolated rat pulmonary artery. *Am J Physiol Lung Cell Mol Physiol* **194**: L733–L738.

Guranowski A, Sillero A (1992). *Enzymes Cleaving Dinucleoside Polyphosphates*. CRC Press: Boca Raton, FL.

Guzman-Aranguez A, Crooke A, Peral A, Hoyle CH, Pintor J (2007). Dinucleoside polyphosphates in the eye: from physiology to therapeutics. *Prog Retin Eye Res* **26**: 674–687.

Hankin S, Matthew N, Thorne H, McLennan AG (1995). Diadenosine 5′,5‴-P1,P4-tetraphosphate hydrolase is present in human erythrocytes, leukocytes and platelets. *Int J Biochem Cell Biol* **27**: 201–206.

Hansen MA, Dutton JL, Balcar VJ, Barden JA, Bennett MR (1999). P2X (purinergic) receptor distributions in rat blood vessels. *J Auton Nerv Syst* **75**: 147–155.

Harada H, Chan CM, Loesch A, Unwin R, Burnstock G (2000). Induction of proliferation and apoptotic cell death via P2Y and P2X receptors, respectively, in rat glomerular mesangial cells. *Kidney Int* **57**: 949–958.

Hardy LA, Harvey IJ, Chambers P, Gillespie JI (2000). A putative alternatively spliced variant of the P2X(1) purinoreceptor in human bladder. *Exp Physiol* **85**: 461–463.

Harrison MJ, Brossmer R, Goody RS (1975). Inhibition of platelet aggregation and the platelet release reaction by alpha, omega diadenosine polyphosphates. *FEBS Lett* **54**: 57–60.

Heidenreich S, Tepel M, Schlüter H, Harrach B, Zidek W (1995). Regulation of rat mesangial cell growth by diadenosine phosphates. *J Clin Invest* **95**: 2862–2867.

Heptinstall S, Johnson A, Glenn JR, White AE (2005). Adenine nucleotide metabolism in human blood – important roles for leukocytes and erythrocytes. *J Thromb Haemost* **3**: 2331–2339.

Hilderman RH, Christensen EF (1998). P1,P4-diadenosine 5′

tetraphosphate induces nitric oxide release from bovine aortic endothelial cells. *FEBS Lett* **427**: 320–324.

Hilderman RH, Martin M, Zimmerman JK, Pivorun EB (1991). Identification of a unique membrane receptor for adenosine 5′,5‴-P1,P4-tetraphosphate. *J Biol Chem* **266**: 6915–6918.

Hillaire-Buys D, Dietz S, Chapal J, Petit P, Loubatieres-Mariani MM (1999). Involvement of P2X and P2U receptors in the constrictor effect of ATP on the pancreatic vascular bed. *J Soc Biol* **193**: 57–61.

Hollopeter G, Jantzen HM, Vincent D, Li G, England L, Ramakrishnan V *et al*. (2001). Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **409**: 202–207.

Hoyle CH, Ziganshin AU, Pintor J, Burnstock G (1996). The activation of P1- and P2-purinoceptors in the guinea-pig left atrium by diadenosine polyphosphates. *Br J Pharmacol* **118**: 1294–1300.

Hoyle CH, Pintor J, Gualix J, Miras-Portugal MT (1997). Antagonism of P2X receptors in guinea-pig vas deferens by diinosine pentaphosphate. *Eur J Pharmacol* **333**: R1–R2.

Ikenoya M, Hidaka H, Hosoya T, Suzuki M, Yamamoto N, Sasaki Y (2002). Inhibition of rho-kinase-induced myristoylated alanine-rich C kinase substrate (MARCKS) phosphorylation in human neuronal cells by H-1152, a novel and specific Rho-kinase inhibitor. *J Neurochem* **81**: 9–16.

Inscho EW, Cook AK, Mui V, Miller J (1998). Direct assessment of renal microvascular responses to P2-purinoceptor agonists. *Am J Physiol* **274**: F718–F727.

Jacobus K, Rideout J, Yerxa B, Pendergast W, Siddiqi S, Drutz D (1996) Method of treating sinusitis with uridine triphosphates and related compounds. USA: US-Pat. 5,789,391, Inspire Pharmaceuticals, Inc.

Jacobus K, Rideout J, Yerxa B, Pendergast W, Siddiqi S, Drutz D (1998a) Method for treating sinusitis with uridine triphosphates and related compounds. USA: US-Pat. 5,981,506, Inspire Pharmaceuticals, Inc.

Jacobus K, Rideout J, Yerxa B, Pendergast W, Siddiqi S, Drutz D (1998b) Method of treating sinusitis with uridine triphosphates and related compounds. USA: US-Pat. 5,958,897, Inspire Pharmaceuticals.

Jacobus KM, Leighton HJ (1996) Method of preventing or treating pneumonia in immobilized patients with uridine triphosphates and related compounds. USA: US-Pat. 5,763,447, Inspire Pharmaceuticals (Durham, NC).

Jankowski J, Tepel M, van der Giet M, Tente IM, Henning L, Junker R *et al*. (1999). Identification and characterization of P_1, P_7-diadenosine-5′-heptaphosphate from human platelets. *J Biol Chem* **274**: 23926–23931.

Jankowski J, Hagemann J, Tepel M, van der Giet M, Stephan N, Henning L *et al*. (2001a). Dinucleotides as growth promoting extracellulary mediators: presence of dinucleoside diphosphates Ap_2A, Ap_2G and Gp_2G in releasable granlues of platelets. *J Biol Chem* **276**: 8904–8909.

Jankowski J, Hagemann J, Yoon MS, van der Giet M, Stephan N, Zidek W *et al*. (2001b). Increased vascular growth in hemodialysis patients induced by platelet-derived diadenosine polyphosphates. *Kidney Int* **59**: 1134–1141.

Jankowski J, Yoon MS, Stephan N, Zidek W, Schlüter H (2001c). Vasoactive diadenosine polyphosphates in human placenta: possible candidates in the pathophysiology of pre-eclampsia? *J Hypertens* **19**: 567–573.

Jankowski J, Jankowski V, Laufer U, van der Giet M, Henning L, Tepel M *et al*. (2003a). Identification and quantification of diadenosine polyphosphate concentrations in human plasma. *Arterioscler Thromb Vasc Biol* **23**: 1231–1238.

Jankowski J, Jankowski V, Seibt B, Henning L, Zidek W, Schlüter H (2003b). Identification of dinucleoside polyphosphates in adrenal glands. *Biochem Biophys Res Commun* **304**: 365–370.

Jankowski V, Tölle M, Vanholder R, Schönfelder G, van der Giet M, Henning L *et al*. (2005). Identification of uridine adenosine tetraphosphate (Up_4A) as an endothelium-derived vasoconstrictive factor. *Nat Med* **11**: 223–227.

Jankowski V, Karadogan S, Vanholder R, Nofer JR, Herget-Rosenthal S, van der Giet M *et al*. (2007a). Paracrine stimulation of vascular smooth muscle proliferation by diadenosine polyphosphates released from proximal tubule epithelial cells. *Kidney Int* **71**: 994–1000.

Jankowski V, Meyer AA, Schlattmann P, Gui Y, Zheng XL, Stamcou I *et al*. (2007b). Increased uridine adenosine tetraphosphate concentrations in plasma of juvenile hypertensives. *Arterioscler Thromb Vasc Biol* **27**: 1776–1781.

Jankowski V, Patzak A, Herget-Rosenthal S, Tran TN, Lai EY, Gunthner T *et al*. (2008). Uridine adenosine tetraphosphate acts as an autocrine hormone affecting glomerular filtration rate. *J Mol Med* **83**: 333–340.

Jovanovic A, Jovanovic S, Mays DC, Lipsky JJ, Terzic A (1998). Diadenosine 5′,5″-P1,P5-pentaphosphate harbors the properties of a signaling molecule in the heart. *FEBS Lett* **423**: 314–318.

Kam PC, Nethery CM (2003). The thienopyridine derivatives (platelet adenosine diphosphate receptor antagonists), pharmacology and clinical developments. *Anaesthesia* **58**: 28–35.

Kennedy C (1996). ATP as a cotransmitter in perivascular sympathetic nerves. *J Auton Pharmacol* **16**: 337–340.

Khakh BS, Burnstock G, Kennedy C, King BF, North RA, Seguela P *et al*. (2001). International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. *Pharmacol Rev* **53**: 107–118.

King BF, Liu M, Pintor J, Gualix J, Miras-Portugal MT, Burnstock G (1999). Diinosine pentaphosphate (IP5I) is a potent antagonist at recombinant rat P2X1 receptors. *Br J Pharmacol* **128**: 981–988.

King BF, Burnstock G, Boyer JL, Boeynaems J, Weisman GA, Kennedy C *et al*. (2000). Nucleotide receptors: P2Y receptors. In: Girdlestone D (ed.). *The IUPHAR Compendium of Receptor Characterization and Classification*. IUPHAR Media: 2000, pp. 306–320.

Koshimizu TA, Kretschmannova K, He ML, Ueno S, Tanoue A, Yanagihara N *et al*. (2006). Carboxyl-terminal splicing enhances physical interactions between the cytoplasmic tails of purinergic P2X receptors. *Mol Pharmacol* **69**: 1588–1598.

Kunapuli SP (1998). Multiple P2 receptor subtypes on platelets: a new interpretation of their function [In Process Citation]. *Trends Pharmacol Sci* **19**: 391–394.

Kunapuli SP, Daniel JL (1998). P2 receptor subtypes in the cardiovascular system. *Biochem J* **336**: 513–523.

Kunapuli SP, Ding Z, Dorsam RT, Kim S, Murugappan S, Quinton TM (2003). ADP receptors – targets for developing antithrombotic agents. *Curr Pharm Des* **9**: 2303–2316.

Le KT, Boue-Grabot E, Archambault V, Seguela P (1999). Functional and biochemical evidence for heteromeric ATP-gated channels composed of P2X1 and P2X5 subunits. *J Biol Chem* **274**: 15415–15419.

Leipziger J (2003). Control of epithelial transport via luminal P2 receptors. *Am J Physiol Renal Physiol* **284**: F419–432.

Lewis CJ, Evans RJ (2000). Lack of run-down of smooth muscle P2X receptor currents recorded with the amphotericin permeabilized patch technique, physiological and pharmacological characterization of the properties of mesenteric artery P2X receptor ion channels. *Br J Pharmacol* **131**: 1659–1666.

Lewis CJ, Evans RJ (2001). P2X receptor immunoreactivity in different arteries from the femoral, pulmonary, cerebral, coronary and renal circulations. *J Vasc Res* **38**: 332–340.

Linden J (2001). Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol* **41**: 775–787.

Luo J, Jankowski J, Knobloch M, van der Giet M, Gardanis K, Russ T *et al*. (1999a). Identification and characterization of diadenosine 5′,5‴-P1,P2-diphosphate and diadenosine 5′,5‴-P1,P3-triphosphate in human myocardial tissue. *FASEB J* **13**: 695–705.

Luo J, Jankowski J, Tepel M, von der Giet M, Zidek W, Schlüter H (1999b). Identification of diadenosine hexaphosphate in human erythrocytes. *Hypertension* **34**: 872–875.

Lüthje J, Ogilvie A (1983). The presence of diadenosine 5′,5‴-P1,P3-triphosphate (Ap_3A) in human platelets. *Biochem Biophys Res Commun* **115**: 253–260.

Lüthje J, Ogilvie A (1985). Catabolism of Ap3A and Ap4A in human plasma. Purification and characterization of a glycoprotein complex with 5′-nucleotide phosphodiesterase activity. *Eur J Biochem* **149**: 119–127.

Lüthje J, Ogilvie A (1988). Catabolism of Ap4A and Ap3A in whole blood. The dinucleotides are long-lived signal molecules in the blood ending up as intracellular ATP in the erythrocytes. *Eur J Biochem* **173**: 241–245.

McLennan AG (1992). *Ap4A and Other Dinucleoside Polyphosphates*. CRC Press, Inc.: Boca Raton, FL.

McMillan MR, Burnstock G, Haworth SG (1999). Vasoconstriction of intrapulmonary arteries to P2-receptor nucleotides in normal and pulmonary hypertensive newborn piglets. *Br J Pharmacol* **128**: 549–555.

Malam-Souley R, Seye C, Gadeau AP, Loirand G, Pillois X, Campan M *et al*. (1996). Nucleotide receptor P2u partially mediates ATP-induced cell cycle progression of aortic smooth muscle cells. *J Cell Physiol* **166**: 57–65.

Malmsjo M, Edvinsson L, Erlinge D (2000a). P2X receptors counteract the vasodilatory effects of endothelium derived hyperpolarising factor. *Eur J Pharmacol* **390**: 173–180.

Malmsjo M, Hou M, Harden TK, Pendergast W, Pantev E, Edvinsson L *et al*. (2000b). Characterization of contractile P2 receptors in human coronary arteries by use of the stable pyrimidines uridine 5′-O-thiodiphosphate and uridine 5′-O-3-thiotriphosphate. *J Pharmacol Exp Ther* **293**: 755–760.

Marcus AJ, Broekman MJ, Drosopoulos JH, Islam N, Pinsky DJ, Sesti C *et al*. (2003). Metabolic control of excessive extracellular nucleotide accumulation by CD39/ecto-nucleotidase-1: implications for ischemic vascular diseases. *J Pharmacol Exp Ther* **305**: 9–16.

Mateo J, Miras-Portugal MT, Rotllan P (1997a). Ecto-enzymatic hydrolysis of diadenosine polyphosphates by cultured adrenomedullary vascular endothelial cells. *Am J Physiol* **273**: C918–C927.

Mateo J, Rotllan P, Marti E, De Aranda G, Solsona I, Miras-Portugal C *et al*. (1997b). Diadenosine polyphosphate hydrolase from presynaptic plasma membranes of Torpedo electric organ. *Biochem J* **323**: 677–684.

Mistry H, Gitlin JM, Mitchell JA, Hiley CR (2003). Endothelium-dependent relaxation and endothelial hyperpolarization by P2Y receptor agonists in rat-isolated mesenteric artery. *Br J Pharmacol* **139**: 661–671.

Mombouli JV, Vanhoutte PM (1993). Purinergic endothelium-dependent and -independent contractions in rat aorta. *Hypertension* **22**: 577–583.

Moore SF, MacKenzie AB (2007). Murine macrophage P2X7 receptors support rapid prothrombotic responses. *Cell Signal* **19**: 855–866.

Murthy KS, Makhlouf GM (1998a). Coexpression of ligand-gated P2X and G protein-coupled P2Y receptors in smooth muscle. Preferential activation of P2Y receptors coupled to phospholipase C (PLC)-beta1 via Galphaq/11 and to PLC-beta3 via Gbetagammai3. *J Biol Chem* **273**: 4695–4704.

Murthy KS, Makhlouf GM (1998b). Differential regulation of phospholipase A2 (PLA2)-dependent Ca^{2+} signaling in smooth muscle by cAMP- and cGMP-dependent protein kinases. Inhibitory phosphorylation of PLA2 by cyclic nucleotide-dependent protein kinases. *J Biol Chem* **273**: 34519–34526.

Nakae I, Takahashi M, Takaoka A, Liu Q, Matsumoto T, Amano M *et al*. (1996). Coronary effects of diadenosine tetraphosphate resemble those of adenosine in anesthetized pigs: involvement of ATP-sensitive potassium channels. *J Cardiovasc Pharmacol* **28**: 124–133.

Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E *et al*. (1998). P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J* **17**: 3016–3028.

Nori S, Fumagalli L, Bo X, Bogdanov Y, Burnstock G (1998). Coexpression of mRNAs for P2X1, P2X2 and P2X4 receptors in rat vascular smooth muscle: an in situ hybridization and RT-PCR study. *J Vasc Res* **35**: 179–185.

North RA, Surprenant A (2000). Pharmacology of cloned P2X receptors. *Annu Rev Pharmacol Toxicol* **40**: 563–580.

Ogilvie A (1992). Extracellular functions of ApnA. In: McLennan AG (ed.). *Ap4A and Other Dinucleoside Polyphosphates*. CRC Press Inc.: Boca Raton, FL, pp. 229–273.

Ogilvie A, Jakob P (1983). Diadenosine 5′,5‴-P1,P3-triphosphate in eukaryotic cells: identification and quantitation. *Anal Biochem* **134**: 382–392.

Ogilvie A, Luthje J, Pohl U, Busse R (1989). Identification and partial characterization of an adenosine(5′)tetraphospho(5′)adenosine hydrolase on intact bovine aortic endothelial cells. *Biochem J* **259**: 97–103.

Ohata H, Ujike Y, Momose K (1997). Confocal imaging analysis of ATP-induced Ca^{2+} response in individual endothelial cells of the artery in situ. *Am J Physiol* **272**: C1980–C1987.

Picher M, Boucher RC (2000). Biochemical evidence for an ecto alkaline phosphodiesterase I in human airways. *Am J Respir Cell Mol Biol* **23**: 255–261.

Pintor J, Torres M, Miras-Portugal MT (1991). Carbachol induced release of diadenosine polyphosphates – Ap4A and Ap5A – from perfused bovine adrenal medulla and isolated chromaffin cells. *Life Sci* **48**: 2317–2324.

Pintor J, Diaz-Rey MA, Torres M, Miras-Portugal MT (1992a). Presence of diadenosine polyphosphates – Ap_4A and Ap_5A – in rat brain synaptic terminals. Ca^{2+} dependent release evoked by 4-aminopyridine and veratridine. *Neurosci Lett* **136**: 141–144.

Pintor J, Rotllan P, Torres M, Miras-Portugal MT (1992b). Characterization and quantification of diadenosine hexaphosphate in chromaffin cells: granular storage and secretagogue-induced release. *Anal Biochem* **200**: 296–300.

Pintor J, Gualix J, Miras-Portugal MT (1997). Dinucleotide receptor modulation by protein kinases (protein kinases A and C) and protein phosphatases in rat brain synaptic terminals. *J Neurochem* **68**: 2552–2557.

Pirotton S, Communi D, Motte S, Janssens R, Boeynaems JM (1996). Endothelial P2-purinoceptors: subtypes and signal transduction. *J Auton Pharmacol* **16**: 353–356.

Pohl U, Ogilvie A, Lamontagne D, Busse R (1991). Potent effects of AP3A and AP4A on coronary resistance and autacoid release of intact rabbit hearts. *Am J Physiol* **260**: H1692–H1697.

Pulvirenti TJ, Yin JL, Chaufour X, McLachlan C, Hambly BD, Bennett MR *et al*. (2000). P2X (purinergic) receptor redistribution in rabbit aorta following injury to endothelial cells and cholesterol feeding. *J Neurocytol* **29**: 623–631.

Ralevic V (2000). P2 receptors in the central and peripheral nervous systems modulating sympathetic vasomotor tone. *J Auton Nerv Syst* **81**: 205–211.

Ralevic V, Burnstock G (1996). Discrimination by PPADS between endothelial P2Y- and P2U-purinoceptors in the rat isolated mesenteric arterial bed. *Br J Pharmacol* **118**: 428–434.

Ralevic V, Burnstock G (1998). Receptors for purines and pyrimidines. *Pharmacol Rev* **50**: 413–492.

Ralevic V, Hoyle CH, Burnstock G (1995). Pivotal role of phosphate chain length in vasoconstrictor versus vasodilator actions of adenine dinucleotides in rat mesenteric arteries. *J Physiol (Lond)* **483**: 703–713.

Rapaport E, Zamecnik PC (1976). Presence of diadenosine 5′,5‴-P1, P_4-tetraphosphate (Ap_4A) in mamalian cells in levels varying widely with proliferative activity of the tissue: a possible positive 'pleiotypic activator'. *Proc Natl Acad Sci USA* **73**: 3984–3988.

Remy P (1992). *Intracellular Functions of ApnN: Eukaryotes*. CRC Press: Boca Raton, FL.

Robertson SJ, Ennion SJ, Evans RJ, Edwards FA (2001). Synaptic P2X receptors. *Curr Opin Neurobiol* **11**: 378–386.

Rodriguez del Castillo A, Torres M, Delicado EG, Miras-Portugal MT (1988). Subcellular distribution studies of diadenosine polyphosphates – Ap_4A and Ap_5A – in bovine adrenal medulla: presence in chromaffin granules. *J Neurochem* **51**: 1696–1703.

Rump LC, Oberhauser V, von Kugelgen I (1998). Purinoceptors mediate renal vasodilation by nitric oxide dependent and independent mechanisms. *Kidney Int* **54**: 473–481.

Safrany ST, Ingram SW, Cartwright JL, Falck JR, McLennan AG, Barnes LD *et al*. (1999). The diadenosine hexaphosphate hydrolases from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* are homologues of the human diphosphoinositol polyphosphate phosphohydrolase. Overlapping substrate specificities in a MutT-type protein. *J Biol Chem* **274**: 21735–21740.

Schlüter H, Offers E, Brüggemann G, van der Giet M, Tepel M, Nordhoff E *et al*. (1994). Diadenosine phosphates and the physiological control of blood pressure. *Nature* **367**: 186–188.

Schlüter H, Gross I, Bachmann J, Kaufmann R, van der Giet M, Tepel M *et al*. (1998). Adenosine(5′) oligophospho-(5′) guanosines and guanosine(5′) oligophospho-(5′) guanosines in human platelets. *J Clin Invest* **101**: 682–688.

Schulze-Lohoff E, Zanner S, Ogilvie A, Sterzel RB (1995). Vasoactive diadenosine polyphosphates promote growth of cultured renal mesangial cells. *Hypertension* **26**: 899–904.

Shen J, Seye CI, Wang M, Weisman GA, Wilden PA, Sturek M (2004). Cloning, up-regulation, and mitogenic role of porcine P2Y2 receptor in coronary artery smooth muscle cells. *Mol Pharmacol* **66**: 1265–1274.

Stutts I, Monroe J, Boucher J, Richard C, Eduardo RAC (1995). Dinucleotides useful for the treatment of cystic fibrosis and for hydrating mucus secretions. USA: US-Pat. 5,635,160, The University of North Carolina at Chapel Hill.

Sumiyoshi R, Nishimura J, Kawasaki J, Kobayashi S, Takahashi S, Kanaide H (1997). Diadenosine polyphosphates directly relax porcine coronary arterial smooth muscle. *J Pharmacol Exp Ther* **283**: 548–556.

Surprenant A, Schneider DA, Wilson HL, Galligan JJ, North RA (2000). Functional properties of heteromeric P2X(1/5) receptors expressed in HEK cells and excitatory junction potentials in guinea-pig submucosal arterioles. *J Auton Nerv Syst* **81**: 249–263.

Tepel M, Lowe S, Nofer JR, Assmann G, Schlüter H, Zidek W (1996). Diadenosine polyphosphates regulate cytosolic calcium in human fibroblast cells by interaction with P2x purinoceptors coupled to phospholipase C. *Biochim Biophys Acta* **1312**: 145–150.

Tepel M, Jankowski J, Schlüter H, Bachmann J, van der Giet M, Ruess C *et al*. (1997). Diadenosine polyphosphates' action on calcium and vessel contraction. *Am J Hypertens* **10**: 1404–1410.

Thorne NM, Hankin S, Wilkinson MC, Nunez C, Barraclough R, McLennan AG (1995). Human diadenosine 5′,5‴-P1,P4-tetraphosphate pyrophosphohydrolase is a member of the MutT family of nucleotide pyrophosphatases. *Biochem J* **311**: 717–721.

Torres GE, Haines WR, Egan TM, Voigt MM (1998). Co-expression of P2X1 and P2X5 receptor subunits reveals a novel ATP-gated ion channel. *Mol Pharmacol* **54**: 989–993.

Tu MT, Luo SF, Wang CC, Chien CS, Chiu CT, Lin CC *et al*. (2000). P2Y(2) receptor-mediated proliferation of C(6) glioma cells via activation of Ras/Raf/MEK/MAPK pathway. *Br J Pharmacol* **129**: 1481–1489.

Turner CM, Vonend O, Chan C, Burnstock G, Unwin RJ (2003). The pattern of distribution of selected ATP-sensitive P2 receptor subtypes in normal rat kidney: an immunohistological study. *Cells Tissues Organs* **175**: 105–117.

Usune S, Katsuragi T, Furukawa T (1996). Effects of PPADS and suramin on contractions and cytoplasmic Ca^{2+} changes evoked by Ap_4A, ATP and alpha, beta-methylene ATP in guinea-pig urinary bladder. *Br J Pharmacol* **117**: 698–702.

Vahlensieck U, Boknik P, Knapp J, Linck B, Muller FU, Neumann J *et al*. (1996). Negative chronotropic and inotropic effects exerted by diadenosine hexaphosphate (Ap_6A) via A1-adenosine receptors. *Br J Pharmacol* **119**: 835–844.

Vanhoutte PM (1991). Platelet-derived serotonin, the endothelium, and cardiovascular disease. *J Cardiovasc Pharmacol* **17**: S6–S12.

Verspohl EJ, Hagemann J, Lempka M (2004). Vascular smooth muscle cells (VSMC) proliferation of streptozotocin-diabetic animals induced by diadenosine polyphosphates. *Exp Clin Endocrinol Diabetes* **112**: 142–147.

Vigne P, Breittmayer JP, Frelin C (2000). Diadenosine polyphosphates as antagonists of the endogenous P2Y1 receptor in rat brain capillary endothelial cells of the B7 and B10 clones. *Br J Pharmacol* **129**: 1506–1512.

Walker J, Hilderman RH (1993). Identification of a serine protease which activates the mouse heart adenosine 5′,5‴,P1,P4-tetraphosphate receptor. *Biochemistry* **32**: 3119–3123.

Wang L, Karlsson L, Moses S, Hultgardh-Nilsson A, Andersson M, Borna C *et al*. (2002). P2 receptor expression profiles in human vascular smooth muscle and endothelial cells. *J Cardiovasc Pharmacol* **40**: 841–853.

Wilden PA, Agazie YM, Kaufman R, Halenda SP (1998). ATP-stimulated smooth muscle cell proliferation requires independent ERK and PI3K signaling pathways. *Am J Physiol* **275**: H1209–H1215.

Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J (2000). P2X4 receptors mediate ATP-induced calcium influx in human vascular endothelial cells. *Am J Physiol Heart Circ Physiol* **279**: H285–H292.

Yegutkin G, Bodin P, Burnstock G (2000). Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5′-nucleotidase along with endogenous ATP from vascular endothelial cells. *Br J Pharmacol* **129**: 921–926.

Yegutkin G, Jankowski J, Jalkanen S, Gunthner T, Zidek W, Jankowski V (2008). Dinucleotide polyphosphates contribute to purinergic signalling via inhibition of adenylate kinase activity. *Biosci Rep* **28**: 189–194.

Yegutkin GG, Burnstock G (2000). Inhibitory effects of some purinergic agents on ecto-ATPase activity and pattern of stepwise ATP hydrolysis in rat liver plasma membranes. *Biochim Biophys Acta* **1466**: 234–244.

Yegutkin GG, Samburski SS, Jalkanen S (2003). Soluble purine-converting enzymes circulate in human blood and regulate extracellular ATP level via counteracting pyrophosphatase and phosphotransfer reactions. *FASEB J* **17**: 1328–1330.

Yegutkin GG, Samburski SS, Mortensen SP, Jalkanen S, Gonzalez-Alonso J (2007). Intravascular ADP and soluble nucleotidases contribute to acute prothrombotic state during vigorous exercise in humans. *J Physiol* **579**: 553–564.

Yerxa B, Brown E (2003) Di(uridine 5′)-tetraphosphate and salts thereof. USA: US-Pat. 20040014713.

Yerxa BR, Mundasad M, Sylvester RN, Garden JC, Cooper M, Kellerman DJ (2002). Ocular safety of INS365 ophthalmic solution, a P2Y2 agonist, in patients with mild to moderate dry eye disease. *Adv Exp Med Biol* **506**: 1251–1257.

Yu SM, Chen SF, Lau YT, Yang CM, Chen JC (1996). Mechanism of extracellular ATP-induced proliferation of vascular smooth muscle cells. *Mol Pharmacol* **50**: 1000–1009.

Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK (2001). Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* **345**: 494–502.

Zemkova H, He ML, Koshimizu TA, Stojilkovic SS (2004). Identification of ectodomain regions contributing to gating, deactivation, and resensitization of purinergic P2X receptors. *J Neurosci* **24**: 6968–6978.

Zimmermann H, Volknandt W, Wittich B, Hausinger A (1993). Synaptic vesicle life cycle and synaptic turnover. *J Physiol Paris* **87**: 159–170.