Purinergic Signaling and Blood Vessels in Health and Disease

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Abstract—Purinergic signaling plays important roles in control of vascular tone and remodeling. There is dual control of vascular tone by ATP released as a cotransmitter with noradrenaline from perivascular sympathetic nerves to cause vasoconstriction via P2X receptors, whereas ATP released from endothelial cells in response to changes in blood flow (producing shear stress) or hypoxia acts on P2X and P2Y receptors on endothelial cells to produce nitric oxide and endothelium-derived hyperpolarizing factor, which dilates vessels. ATP is also released from sensory-motor nerves during antidromic reflex activity to produce relaxation of some blood vessels. In this review, we stress the differences in neural and

ABBREVIATIONS: $[Ca^{2+}]_{i}$, intracellular Ca$^{2+}$ concentration; 2-MeSADP, 2-methylthio ADP; 2-MeSATP, 2-methylthio ATP; 5-HT, 5-hydroxytryptamine; α,β-meATP, α,β-methylene ATP; Ach, acetylcholine; ATL146e, 4-[3-[6-amino-9-(5-ethylcarbamoyl)-3,4-dihydroxy-tetrahydrofuran-2-yl]-9H-purin-2-yl]-prop-2-ynyl]-cyclohexane-carboxylic acid methyl ester; AMPK, AMP-activated protein kinase; Ang-II, angiotensin II; ANP, atrial natriuretic peptide; ApoA, diadenosine triphosphate; ApoA, diadenosine tetraphosphate; ApoA, diadenosine pentaphosphate; ApoA, diadenosine hexaphosphate; ATP;S, adenosine-5′-(γ-thio)-triphosphate; AZD6140, 3-[2-[3-[4-difluorophenyl]cyclopropyl]amino]-5-propylsulfanilimethyltetrazolo[1,5-a]pyrimidine-3-yl-5-(2-hydroxyethoxy)cyclopentane-1,2-diol; BBB, blood-brain barrier; BX 667, (4S)-(4-ethoxycarbonylpirazin-1-yl)-1-[(4-ethyl-1-oxidopropyl-2-yl)oxy]-7-methyl[quinoline-2-carbonyl]aminol-5-oxopentonic acid; CORD, calctinin gene-related peptide; COX, cyclooxygenase; DOCA, deoxycorticosterone acetate; EDCF, endothelium-derived contracting factor; EDHF, endothelium-derived hyperpolarizing factor; EJPs, excitatory junction potentials; ET, endothelin; HIF, hypoxia-inducible transcription factors; HUVECs, human umbilical vein endothelial cells; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NA, noradrenaline; NANC, nonadrenergic, noncholinergic; NF, nuclear factor; NGF, nerve growth factor; NK, natural killer; NO, nitric oxide; NOS, nitric-oxide synthase; NPY, neuropeptide Y; PAF-1, plasminogen activator inhibitor-1; PD-81723, 2-amino-4,5-dimethyl-3-thienyl-3(trifluoromethyl)phenylmethanone; PEDF, pigment epithelium-derived factor; PGI, prostacyclin; PK, protein kinase; PPDAS, pyridoxalphosphate-6-azophenyl-2,4-disulfonic acid; PSB 0739, 1-amino-9,10-dihydro-9,10-dioxo-4-[[(phenylmethyl)-3-sulfophenyl]amino]-2-anthracenesulfonic acid; ROS, reactive oxygen species; RT-PCR, reverse transcriptase-polymerase chain reaction; SHH, spontaneously hypertensive rats; siRNA, small interfering RNA; TNF-α, tumor necrosis factor-α; TRPC, transient receptor potential channel; Tp4, thymosin β-4; UpA, uridine adenosine tetraphosphate; VEFG, vascular endothelial growth factor; WKY, Wistar-Kyoto.
endothelial factors in purinergic control of different blood vessels. The long-term (trophic) actions of purine and pyrimidine nucleosides and nucleotides in promoting migration and proliferation of both vascular smooth muscle and endothelial cells via P1 and P2Y receptors during angiogenesis and vessel remodeling during restenosis after angioplasty are described. The pathophysiology of blood vessels and therapeutic potential of purinergic agents in diseases, including hypertension, atherosclerosis, ischemia, thrombosis and stroke, diabetes, and migraine, is discussed.

I. Introduction

Although it is clear that purinergic signaling plays a pivotal role in the control of vascular tone and remodeling, there has been a tendency to extrapolate the purinergic mechanisms involved in a particular vessel to operate in all vessels. However, it has become clear that there are significant differences in the purinergic regulatory mechanisms in different blood vessels and in different species related to the specific physiologic roles of the particular vessel. Therefore, a detailed analysis of the purinergic control mechanisms in different vessels follows. Common to most vessels is dual control of vascular tone by ATP released as a cotransmitter with noradrenaline (NA) from sympathetic perivascular nerves causing constriction, whereas ATP released from endothelial cells by shear stress (produced by changes in blood flow) and hypoxia acting on endothelial cells releases endothelium-derived relaxing factor(s) (EDRF) (see Burnstock, 1999a, 2007). The long-term (trophic) actions of purines in promoting migration of endothelial cells and proliferation of both smooth muscle and endothelial cells during angiogenesis also appears to be a common feature of blood vessels (see Teuscher and Weidlich, 1985; Burnstock, 2002).

The field lacks selective ligands for many of the different P2 receptor subtypes, but the use of multiple experimental approaches (e.g., RT-PCR, Western blotting) and commercially available ligands has, in general, provided good predictions of the P2 receptor subtypes present in blood vessels. Where studies with P2 receptor knockouts have been carried out, these have largely confirmed and/or refined our existing interpretations of P2 receptor expression. For example, mesenteric arteries from P2X1 receptor knockout mice confirmed the involvement of P2X1 receptors in ATP-mediated vasoconstriction and transient inward currents and in sympathetic neurotransgenic vasoconstriction (Vial and Evans, 2002). P2Y6 receptor knockout mice confirmed the involvement of these receptors in aortic endothelium-dependent relaxation to UDP and smooth muscle contraction to UDP (and UTP) (Bar et al., 2008). P2Y2 knockout mice have confirmed an endothelium-dependent vasorelaxant function of P2Y2 receptors (although unusually these were sensitive to ATP but not UTP) (Guns et al., 2006). Native P2 receptor characterization is an area that would benefit from the further use of molecular biology techniques. The main challenges with regard to P2 receptor characterization are the development of potent and selective ligands to discriminate between different P2X receptors and between P2Y2 and P2Y4 receptors (see Table 1). There is considerable evidence showing the expression of P2X mRNA and protein in endothelial cells, but little is known about their function; the more widespread application of molecular biology techniques is clearly needed to identify the possible roles of P2X subtypes in the endothelium, as is discussed in section II.B.2). Commercially available ligands do not discriminate well between P2Y2 and P2Y4 receptors, and the default position is that one or both are expressed in tissues where responses to UTP (P2Y2/4 agonist, weak P2Y6 agonist) are demonstrated. However, the use of P2Y6 receptor knockouts showed that the contractions induced by ATP in mouse aorta was mediated via P2Y6 receptors and not via P2Y2 receptors, which were shown by RT-PCR to be expressed in the smooth muscle (Bar et al., 2008; Kauffenstein et al., 2010a). Similarly, the use of knockouts and antisense oligonucleotides showed the functional expression of vasocontractile P2Y4 and P2Y6 but not P2Y2 receptors in mice mesenteric arteries and cerebral arterioles (Vial and Evans, 2002; Bar et al., 2008; Kauffenstein et al., 2010a; Brayden et al., 2013). In contrast, in rat mesenteric arteries, small interfering RNA (siRNA) knockdown of P2Y2 mRNA demonstrated the almost exclusive involvement of P2Y2 receptors in contraction to UTP (Morris et al., 2011). This demonstrates pronounced species differences in the expression of vasocontractile P2Y receptors, which were not detected through the use of pharmacology alone. This is an area where the more widespread application of molecular biology techniques across species will help to clarify our interpretation of the expression of vasocontractile P2Y receptors.

For information about purinergic peripheral and central nervous control of the vascular system, several articles are recommended (Spyer et al., 1997; Burnstock, 2007; Gourine et al., 2009; Cruz et al., 2010; Passamani et al., 2011; Burnstock and Verkhratsky, 2012; Ichinose et al., 2012). Finally, the pathophysiology and therapeutic potential of purinergic agents to treat vascular diseases will be reviewed.

II. Dual Control of Vascular Tone by Perivascular Nerves and Endothelial Cells

For many years, control of vascular tone was considered to be antagonistic sympathetic noradrenergic constrictor nerves and parasympathetic cholinergic dilator nerves. However, after recognition of the conceptual
advances about cotransmission (Burnstock, 1976) and EDRFs (Furchgott, 1984), it is now clear that vascular tone is under the dual control of cotransmitters released from perivascular nerves and various factors released from endothelial cells (see Burnstock, 1987, 1988, 1989a, 1990a, 1993a, 2002, 2008c; Ralevic and Burnstock, 1996a).

The possibility that ATP is released together with NA from sympathetic nerves supplying the rabbit portal vein was raised early by Che Su (see Su, 1977, 1985), although the potent effects of ATP on venous blood vessels was also recognized early (see Vanhoutte, 1978) and also the roles of adenosine and ATP in exercise hyperemia of skeletal and cardiac muscle (see Forrester, 1981). Two types of receptors for purines were identified: P1 receptors activated by adenosine and P2 receptors activated by ATP and ADP (Burnstock, 1978), with P2 receptors further subdivided into P2X and P2Y (Burnstock and Kennedy, 1985). P2 purinoceptors were later identified on both vascular smooth muscle and endothelial cells (see Pearson and Gordon, 1989).

Release of ATP from endothelial cells in response to changes in blood flow (due to shear stress) and hypoxia has been demonstrated (Bodin et al., 1991; Burnstock, 1999; see Bodin and Burnstock, 2001b). Evidence has been presented for the mechanism of ATP transport from endothelial cells being vesicular exocytosis (Bodin and Burnstock, 2001a), but also via connexin 43 hemichannels (Faigle et al., 2008). Pannexin hemichannels and calcium homeostasis modulator 1 ion channels are structurally and functionally related to connexins, and there is increasing evidence for a widespread involvement of these other ATP-conducting anion channels and ATP-binding cassette transporters in ATP release from cells (Dubyak, 2009; Lazarowski, 2012; Lohman et al., 2012a; Taruno et al., 2013). Further studies using molecular approaches are warranted to investigate further their potential role in purinergic control of the vasculature. Reviews describing dual control of local blood flow by nucleotides released by nerves and endothelial cells are available (Burnstock and Kennedy, 1986; Burnstock, 1990a, 2008a, 2010).

Ectonucleotidases are ectoenzymes associated with the cell membrane that have an important role in determining extracellular concentrations of nucleotides and nucleosides (Yegutkin, 2008; Zimmermann et al., 2012). They include ectonucleotidase 5'-triphosphate diphosphohydrolases (E-NTPDases), including E-NTPDase1/ecto-ATPase/CD39, which hydrolyzes nucleoside tri- and diphosphates (found at the surface of endothelial and vascular smooth muscle cells), and E-NTPDase2 (CD39L1) (expressed on the adventitial surface of blood vessels), which hydrolyzes nucleoside triphosphates. Ecto-5'-nucleotidases (CD73) and alkaline phosphatases catalyze the extracellular formation of adenosine from AMP. Nucleotide sugars are resistant to hydrolysis by ectonucleoside di- and triphosphohydrolases, but they and other nucleotides are hydrolyzed by nucleotide pyrophosphatase. Extracellular levels of nucleotides, and consequently their actions at cell surface purine receptors, are a balance between their release from cells by the mechanisms discussed above and hydrolysis by ectonucleotidases (Fig. 1). Studies on isolated vessels from E-NTPDase1 knockouts and vessels in the presence of ecto-ATPase inhibitors have shown that ectonucleotidases can profoundly influence agonist potencies (Crack et al., 1994; Kauffenstein et al., 2010a). This has pharmacological and physiologic relevance for P1 and P2 receptor activities in health and disease, which is discussed in subsequent sections.

### Table 1

<table>
<thead>
<tr>
<th>Ion Channel/Principal Transduction</th>
<th>Agonists</th>
<th>Selective Agonists</th>
<th>Selective Antagonists</th>
</tr>
</thead>
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<tr>
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<td>a,β-meATP, 1,β,γ-meATP</td>
<td>NF023, NF449</td>
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<td>A317491</td>
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<td>MRS2179, MRS2500</td>
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<td>UDP &gt; UTP</td>
<td>UDP, PSB 0474 (= 3- phenacylUDP)</td>
<td>MRS2957</td>
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<td>ATP &gt; UTP</td>
<td>N546, NAD&lt;sup&gt;+&lt;/sup&gt; NAADP&lt;sup&gt;+&lt;/sup&gt;</td>
<td>NF517, NF340</td>
</tr>
<tr>
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<td>ADP, 2-MeSADP</td>
<td>MRS2211</td>
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<td>PPTN</td>
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### Purinergic Signaling and Blood Vessels
Sympathetic nerves in isolated blood vessel preparations can be selectively stimulated causing ATP (and NA) release, and the pharmacological tools with which to investigate P2X<sub>1</sub> receptors are relatively good (a number of selective antagonists are commercially available, see Table 1). P2X<sub>1</sub> receptor knockout mice have confirmed, in mesenteric arteries, the involvement of P2X<sub>1</sub> receptors in ATP-mediated vasoconstriction and transient inward currents and in sympathetic neurogenic vasoconstriction (Vial and Evans, 2002). Moreover, P2X<sub>1</sub> receptor clusters have been described on vascular smooth muscle in regions adjacent to sympathetic nerve varicosities (Hansen et al., 1999; Vial and Evans, 2005) and provide an explanation for why smooth muscle P2X receptors, and not P2Y receptors, are involved in ATP cotransmission.

P2X receptors are ionotropic receptors, and their activation by ATP leads to a rapid response involving calcium entry directly through the P2X cation channels, membrane depolarization, and calcium influx through voltage-activated calcium channels, whereas NA acts more slowly due to adrenoceptor coupling to G proteins and the involvement of second messengers. ATP and NA can act synergistically, resulting in a rapid onset augmented contraction (Kennedy and Burnstock, 1986; Ralevic and Burnstock, 1990; Johnson et al., 2001). In recent articles, evidence was presented that activation of α<sub>1</sub>-adrenoceptors on vascular smooth muscle cells open pannexin 1 channels to release ATP, leading to enhanced vasoconstriction, providing new insights into the regulation of peripheral resistance and blood pressure by sympathetic nervous stimulation (Billaud et al., 2011, 2012). The use of pannexin knockout animals would be valuable to investigate the extent of involvement of pannexins and smooth muscle ATP release in sympathetic signaling throughout the cardiovascular system and also in the myogenic response, because pannexin channels are activated by distension.

By use of calcium confocal imaging in rat mesenteric arteries, neurally released ATP was shown to produce an early junctional calcium transient and this was followed by calcium waves developing later due to the actions of neurally released NA (Lamont et al., 2003; Wier et al., 2009). Nifedipine, an L-type calcium channel blocker, inhibits the purinergic component of sympathetic vasoconstriction in dog (Omote et al., 1989) and rat (Rummery et al., 2007) mesenteric arteries and in rabbit ileocolic and saphenous arteries (Bulloch et al., 2011, 2012), suggesting that ATP activation of P2X receptors on vascular smooth muscle evokes calcium entry through voltage-activated L-type calcium channels. However, in submucosal arterioles of guinea pig ileum, P2X receptor-mediated vasoconstriction is nifedipine insensitive (Galligan et al., 1995).

There is a substantial ATP component of sympathetic neurotransmission in the rabbit saphenous artery

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**Fig. 1.** Extracellular levels of nucleotides and consequent actions at cell surface purine receptors are a balance between their release from cells and hydrolysis by extracellular ectonucleotidases. Nucleotides can be released from cells by connexin hemichannels, pannexin hemichannels, ATP-binding cassette transporters (ABC), and anion channels. Sympathetic nerves release ATP (together with noradrenaline) by vesicular exocytosis. Ectonucleotidase 5'-triphosphohydrolases (ENTPDase) at the surface of cells hydrolyze ATP, ADP, and UTP, whereas ecto-5'-nucleotidase (CD73) further hydrolyzes the AMP formed to adenosine. The nucleotides and adenosine can have auto- and paracrine effects at cell surface P2X, P2Y, and adenosine A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub> receptors.

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**A. Perivascular Nerves**

1. **Sympathetic Nerves.** In many arteries, the mechanical and electrical responses to perivascular sympathetic nerve stimulation are partially resistant to α-adrenoceptor antagonists. Many reports of sympathetic purinergic excitatory cotransmission to various blood vessels are available, although there is considerable variation in the proportions of ATP and NA used (Burnstock, 1988, 1990b, 1995; Ralevic and Burnstock, 1998; Hill et al., 2001; Lewis and Evans, 2001; Ralevic, 2009; Macarthur et al., 2011). There appear to be separate stores of NA and ATP in sympathetic nerve terminals, because N and P/Q type calcium channels control NA release, whereas only N-type channels control ATP release (Demel et al., 2010; see also Ellis and Burnstock, 1989).

The vasoconstrictive actions of ATP released from sympathetic nerves appear to be mediated principally via P2X<sub>1</sub> receptors (Fig. 2). There is evidence for the expression of both P2X and P2Y receptors in vascular smooth muscle from detection of mRNA, antibody recognition, electrophysiological and pharmacological studies, and the use of knockouts and siRNA knockdown. The focus on P2X receptors in vascular smooth muscle, at least with regard to control of vascular tone, is because it has been possible to identify a clear physiologic role for these receptors as mediators of the response to ATP released as a fast excitatory neurotransmitter from sympathetic perivascular nerves (described below).
The depolarizations of smooth muscle arising from the actions of excitatory neurotransmitter are known as excitatory junction potentials (EJPs). Intra- and extracellular recordings in the rat femoral artery have been used to demonstrate the intermittent release of single quanta of ATP responsible for EJPs and to show the similarity to the events occurring during sympathetic cotransmission in the vas deferens (Astrand and Stjärne, 1989). In the rat tail artery, EJPs are resistant to prazosin (Cheung, 1982) but are blocked by α,β-methylene ATP (α,β-meATP) (Sneddon and Burnstock, 1984a; Sedaa et al., 1986; Bao et al., 1989; Fig. 3, A–C) and the P2 receptor antagonist suramin (Jobling and McLachlan, 1992a; Fig. 3D). However, the ATP component appears to be smaller relative to NA in the sympathetic nerves supplying this vessel, so it is more difficult to demonstrate a mechanical prazosin-resistant (purinergic) component. Evidence has also been presented for prazosin-resistant EJPs (Holman and Surprenant, 1980; Suzuki and Kou, 1983) and for cotransmission in the rabbit ear artery involving ATP and NA (Head et al., 1977; Saville and Burnstock, 1988; Leff et al., 1990), but similar to the rat tail artery, it is difficult to demonstrate a prazosin-resistant (purinergic) mechanical component of the response to perivascular nerve stimulation, except with short bursts of pulses lasting approximately a second, which appears to favor the ATP component (Evans and Cunnane, 1992; Kennedy et al., 1986; Bulloch and Starke, 1990). It has been suggested that this might mean that NA may be the most important component of sympathetic cotransmission during activities such as gentle exercise, whereas ATP might be the more important component during stress when short burst frequencies occur in sympathetic nerves (Burnstock, 1988). In the rat mesenteric artery, EJPs and release of NA (measured by continuous amperometry) could be differentially modulated, consistent with the possibility that ATP and NA are nonuniformly stored in different classes of vesicles.
within sympathetic varicosities (Dunn et al., 1999; Brock et al., 2000) and, hence, could be released in different proportions according to the patterns of stimulation.

The most direct way to demonstrate ATP sympathetic cotransmission is to measure and show its release from sympathetic nerves. Su (1975, 1978b) used tritium-labeled adenosine and NA to indicate that ATP is released together with NA from sympathetic nerves supplying the rabbit aorta. The vessels were preloaded with [3H]adenosine (which can be incorporated into new ATP), and subsequent electrical stimulation of perivascular nerves caused neurogenic [3H]purine release, including [3H]ATP (Su, 1975, 1978b). A similar approach was used to demonstrate sympathetic cotransmission of NA and ATP in the dog basilar artery (Muramatsu et al., 1979, 1981) and rabbit pulmonary artery (Katsuragi and Su, 1980, 1982). In a follow-up study in the dog basilar artery, desensitization of P2X receptors with α,β-meATP was used in experiments to further demonstrate sympathetic cotransmission of ATP and NA (Muramatsu and Kigoshi, 1987). Release of ATP from sympathetic nerves has been measured directly, using the luciferin luciferase assay in guinea pig vas deferens, and the ATP was shown to be coreleased with NA (Kirkpatrick and Burnstock, 1987); however, this assay has had limited success in blood vessels, probably because of the lower levels of ATP released. More recently, overflow of ATP has been measured using ATP biosensors from nerves in rat skeletal muscle 1A arterioles (Kluess et al., 2010). The fact that the bioprobes measure ATP in real time is a particular advantage because ATP released from sympathetic nerves is rapidly metabolized by E-NTPDases 1 (CD39) expressed on vascular smooth muscle cells and is also potentially metabolized by soluble ATPase released from the sympathetic nerves themselves (Westfall et al., 2000).

Evidence for adrenergic-purinergic cotransmission has also been obtained from the vascular beds and whole animals. Mean arterial pressure fluctuations in conscious rats are evoked by NA and ATP released from sympathetic nerves, but can be distinguished by their frequency characteristics (Golubinskaya et al., 1999). A contribution of ATP to sympathetic vasopressor responses of the pithed rat has also been demonstrated (Grant et al., 1985; Bulloch and McGrath, 1988a; Schlicher et al., 1989). As with the purinergic neurogenic response in isolated mesenteric arteries from rat and dog (Omote et al., 1989; Rummery et al., 2007), the purinergic component of the vasopressor response to stimulation of the sympathetic outflow of the rat can be blocked by nifedipine, indicating an involvement of L-type calcium channels, whereas the α-adrenoceptor-mediated responses to the cotransmitter NA are relatively resistant to nifedipine (Bulloch and McGrath, 1988b). In urethane-anesthetized rats, purinergic cotransmission was shown to play a major role in the pressor sinocarotid reflex (Tarasova and Rodionov, 1992).

Hypothalamic stimulation in anesthetized rabbits produced skeletal muscle vasodilation, which has been claimed to be mediated by ATP released from sympathetic nerves (Shimada and Stitt, 1984). Adenosine was not involved in vasodilatation produced by exogenous ATP or hypothalamic stimulation. Stimulation of the lumbar sympathetic nerve chains in the ganglion-blocked rabbit produces hindlimb constriction that apparently has no α-adrenergic component (Hirst and

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**Fig. 3.** (A–C) Intracellular recording of the electrical responses of single smooth muscle cells of the rat tail artery to field stimulation of the sympathetic motor nerves (the pulse width was 0.1 ms at 0.5 Hz, indicated by ). (Ai) Control response of the muscle. Note that to each individual stimulus there was a rapid depolarization, and as the train of pulses progressed, a slow depolarization developed. Similar responses were obtained in (Bi) and (Ci), which are also control responses in Krebs solution. (Aii) and (Aiii) show the effect of phentolamine (2 × 10^-6 M, added to the bathing solution). The fast depolarizations produced by each stimulus were not reduced, but there was a progressive reduction in the size of the slow depolarization, which was almost abolished after 6 minutes. (Bii) The tissues have been in the presence of 10^-6 M α,β-methylene ATP for over 15 minutes. The fast depolarizations produced by each stimulus were greatly reduced, but the slow depolarization persisted. (Cii) shows the effect of a higher concentration of α,β-methylene ATP, Here the fast depolarization was totally abolished, whereas the slow depolarization persisted, although reduced to some extent. Subsequent addition of phentolamine (2 × 10^-6 M), together with α,β-methylene ATP, abolished the neurogenic response completely (Ciii). (Reproduced from Sneddon and Burnstock, 1984b, with permission.) (D) Excitatory junction potentials (EJPs) in the main tail artery of the rat. Inhibition of the fast EJPs by suramin (1 mM). (Reproduced from Jobling and McLachlan, 1992a, with permission of the Australian Physiological and Pharmacological Society Inc.)
Lew, 1987). A study of autoperfused intestinal circulation of anesthetized cats treated with atropine and propanolol showed that the initial rapid phase of prazosin-resistant vasoconstriction was abolished after desensitization of P2X purinoceptors with α,β-meATP (Taylor and Parsons, 1989). Schwartz and Malik (1989) concluded from a study of rat isolated kidney that renal vasoconstriction elicited by periarterial nerve stimulation was mainly due to release of a purinergic transmitter, probably ATP, and to a lesser extent NA. In the rat mesenteric arterial bed, a purinergic (α,β-meATP-sensitive) component of the contractile response to sympathetic nerve stimulation was revealed when the perfusion pressure was raised to a level closer to that found in vivo, likely due to postjunctional enhancement of the purinergic contractile response (Pakdeecho et al., 2007b). This has important implications, suggesting that the purinergic component of sympathetic cotransmission may have been underestimated in in vitro vascular preparations studied at low tone/pressure, a suggestion supported by the increase in ATP sympathetic cotransmission observed in the vasculature in hypertension (see section V.A).

With exposure to a cold environment, blood flow to the skin is reduced, preventing excessive heat loss. In the dog, this is achieved by reflex increase in sympathetic tone of cutaneous veins, which is resistant to adrenoceptor antagonism; it is, however, inhibited by desensitization of P2X purinoceptors with α,β-meATP (Flavahan and Vanhoutte, 1986). The possibility is raised that this may be important for thermoregulation and may explain why purinergic cotransmission is more prominent in cutaneous compared with deep blood vessels. NA and ATP act as vasoconstrictors to most blood vessels via α-adrenoceptors and P2X1 purinoceptors; studies carried out on the rabbit coronary artery, where the predominant effect of NA is vasodilation via β-adrenoceptors, have shown that the cotransmitter ATP causes vasodilation via muscle P2Y purinoceptors (Corr and Burnstock, 1991; Keef et al., 1992).

2. Parasympathetic Nerves. Most blood vessels, with the exception of those supplying salivary glands and some cerebral blood vessels are not innervated by parasympathetic nerves (Lundberg, 1996). It is not known yet whether ATP is a cotransmitter in perivascular parasympathetic nerves.

3. Sensory-Motor Nerves. Many blood vessels are innervated by sensory-motor nerves, both unmyelinated C fibers and myelinated Aδ fibers. The principal neurotransmitter in perivascular sensory-motor nerves is calcitonin gene-related peptide (CGRP), which mediates vasorelaxation. There was early evidence that ATP can be released during antidromic stimulation of sensory nerves in the rabbit ear artery causing vasodilation (Holton, 1959). Reviews describing other examples where ATP appears to act as a cotransmitter in sensory-motor nerves during vascular axon reflex activity are available (Rubino and Burnstock, 1996; Burnstock, 1993a, 2009b).

B. Endothelial Cells

Endothelial cells express P1 and P2 receptors, they are a source of purines (which can activate P1 and P2 receptors in an autocrine manner), and they contribute to the metabolism of purines through the actions of cell surface ectonucleotidases. A fundamental role of endothelial cells is the mediation of vasodilatation and thus they counterbalance the vasocontractile effects mediated by ATP and NA released from perivascular sympathetic nerves (Fig. 4). In addition to their autocrine activation by purines, endothelial cells are also a target of nucleotides and nucleosides released from myocytes and blood cells by various stimuli, which act at endothelial P2 receptors to elicit vasodilatation (Fig. 5).

The dominant purine receptors on both animal and human endothelial cells are A2A and A2B adenosine receptors and P2Y1, P2Y2, and P2X4 nucleotide receptors (see Burnstock, 2009a), but vessel- and species-specific differences in receptor subtype expression exist (discussed in the sections on individual blood vessels). Endothelial cell activation by purines leads to release of nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI2) (Van Coevorden and Boeynaems, 1984; Lückhoff and Busse, 1986; Bogle et al., 1991; Kelm et al., 1991), which can cause vasorelaxation and inhibition of platelet aggregation. A recent study using the human forearm claims that in addition to the vasodilator action of ATP via endothelial cell release of NO and prostaglandins, ATP-mediated relaxation also occurs via activation of inwardly rectifying potassium channels (Crecelius et al., 2011, 2012). The importance of the endothelium is evident from the fact that endothelial cell dysfunction promotes platelet activation and leukocyte accumulation at the site of injury; the activated platelets and leukocytes can release ATP, ADP, and UTP. With an intact endothelium these nucleotides can elicit vasodilatation, but when there is endothelial damage they may act as vasoconstrictors via P2 receptors on the vascular smooth muscle, which may lead to local vasoconstriction (Figs. 5 and 6).

ATP is released during changes in flow (involving shear stress) from primary cultures of endothelial cells derived from animal and human blood vessels ( Bodin et al., 1991; see Burnstock, 1999; Bodin and Burnstock, 1996, 2001a), and ATP release was stimulated by hypotonic challenge (Schwiebert et al., 2002). Long-term treatment with transforming growth factor β1 impairs ATP release from bovine aortic endothelial cells in response to hypotonic stress (Watanabe et al., 2007). Uptake of adenosine by endothelial cells and its conversion to ATP was described early (Pearson et al., 1978) and ATP was released by neutrophil elastase...
Increased shear stress leads to release of ATP and endothelin (ET) from freshly isolated endothelial cells from 12-month, but not from 4-month-old rabbits (Milner et al., 1992). UTP and UDP can also be released from endothelial cells of rabbit thoracic aorta in response to shear stress (Saïag et al., 1995). It has been reported that polyphenolic compounds contained in red wine induce release of ATP, ADP, and UTP from endothelial cells in rat isolated aorta to activate P2Y1 and P2Y2 receptors, leading to vasodilation (Mendes et al., 2003).

The functional significance of ATP released from endothelial cells by fluid shear stress is that it acts, via P2 receptors, to permit local or spreading changes in blood vessel contractility (vasodilatation) in response to this stimulus. There is ATP-stimulated release of ATP by human endothelial cells (Bodin and Burnstock, 1996), which would conduct the endothelial cells in a coordinated response via paracrine signaling, via actions at endothelial P2Y and P2X receptors (discussed in subsequent sections). ATP released from rat adrenomedullary endothelial cells after shear stress increases intracellular Ca$^{2+}$ concentration Ca$^{2+}$ and leads to spread to neighboring cells of a Ca$^{2+}$ wave (Hong et al., 2006). The Ca$^{2+}$ wave was blocked by suramin or apyrase, which degrades extracellular ATP, suggesting

**Fig. 4.** Purinergic control of blood vessel tone is a balance between actions of ATP released from sympathetic nerves at the outer surface of the vessel and purines released at its innermost layer, the endothelium. ATP released from sympathetic nerves (together with NA) acts at smooth muscle P2X1 receptors to cause vasoconstriction. Inside the blood vessel, nucleotides released principally from the endothelium and erythrocytes by shear stress and hypoxia act at endothelial P2X4 and P2Y receptors to cause the release of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) leading to vasorelaxation. Cell surface ectonucleotidase 5'-triphosphohydrolases (ENTPDase) hydrolyze ATP, ADP, and UTP, whereas ecto-5'-nucleotidase (CD73) further hydrolyzes AMP to adenosine, which acts at endothelial and smooth muscle A2A and A2B receptors to elicit vasorelaxation.

**Fig. 5.** Cellular sources of nucleotides and nucleosides relevant to the control of blood vessel contractility. In healthy blood vessels, vasoconstriction by ATP released from sympathetic nerves and vasodilatation produced by purines and pyrimidines released from endothelial cells, myocytes, and erythrocytes act in concert to regulate vascular tone. In healthy blood vessels, aggregating platelets release nucleotides that elicit endothelium-dependent vasodilatation. In vascular disease, platelets and white blood cells (WBC) adhere to the dysfunctional/damaged endothelium or the underlying smooth muscle, and the released nucleotides may contribute to a shift in the balance toward vasoconstriction (as well as inducing vascular remodeling).
that ATP released from endothelial cells is the mediator of the spreading Ca\textsuperscript{2+} responses of adjacent endothelial cells (Hong et al., 2006). The mechanism by which ATP is released from endothelial cells is not fully understood. Evidence that ATP release from endothelial cells during shear stress is vesicular was presented (Bodin and Burnstock, 2001a). Later studies have suggested that connexin and pannexin 1 channels are also involved in the mechanism of ATP release (Härtel and Noll, 2011; Lohman et al., 2012a, 2012b). Localized release of ATP from endothelial cells was blocked by caveolin-1 knockdown with siRNA, and the released ATP triggers Ca\textsuperscript{2+} waves at caveolae in vascular endothelial cells (Yamamoto et al., 2011).

Adenosine 5'-tetraphosphate is a highly potent purinergic endothelium-derived vasoconstrictor in human microvessels acting predominantly through activation of smooth muscle P2X\textsubscript{1} receptors (Tölle et al., 2008). Uridine adenosine tetraphosphate (Up\textsubscript{4}A) has also been claimed to be an endothelium-derived vasoconstricting factor (EDCF) in cultured endothelial cells from human dermal microvessels (Jankowski et al., 2005) and rat pulmonary artery (Gui et al., 2008).

Extracellular levels of nucleotides are tightly regulated by the actions of ectonucleotidases. Early studies of the hydrolysis of ATP by ATPases on endothelial cells of pig aorta showed rapid breakdown to ADP and AMP but delay before further breakdown by 5'-nucleotidase to adenosine (Gordon et al., 1986). Ectonucleoside triphosphate diphosphohydrolase 1 (E-NTPDase 1/CD39) is expressed by vascular endothelial cells (Robson et al., 1997; Banz et al., 2008) and is the major member of the E-NTPase family involved in hydrolyzing extracellular nucleotides at the endothelial cell surface. Soluble forms of adenylyl kinase 1 and NTPDase1 (CD39) contribute in the active cycling of circulating nucleotides (Yegutkin et al., 2012). E-NTPDase 1/CD39 regulates P2Y\textsubscript{1} and P2Y\textsubscript{12} receptor-dependent vasorelaxation and in Entpd1 knockout mice (lacking E-NTPDase 1/CD39) aortic relaxations mediated by these receptors were augmented (Kauffenstein et al., 2010b). Conversely, increased E-NTPDase activity within human umbilical vein endothelial cells (HUVECs) inhibited the P2Y receptor-mediated endothelial response (secretion of von Willebrand factor) and the extent of apoptosis triggered by high concentrations of ATP (likely via P2X\textsubscript{7} receptors) (Goepfert et al., 2000). Systemic administration of E-NTPDase 1/CD39 minimized injury-induced platelet deposition and leukocyte recruitment and abrogated neointimal hyperplasia (Drosopoulos et al., 2010). The authors suggest that human E-NTPDase 1/CD39 can play an active role in suppressing thrombotic, inflammatory, and proliferative cellular responses after vascular injury.

Fig. 6. In blood vessels with a healthy endothelium, platelet aggregation is inhibited, but where there is endothelium damage or dysfunction, platelet aggregation can occur, promoting smooth muscle constriction and proliferation. Activated platelets and endothelial cells release ATP, ADP, and UTP. When the endothelium is healthy, these nucleotides act at endothelial P2Y receptors to release nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF), leading to vasodilatation. Activation of endothelial P2Y receptors also leads to the release of prostacyclin (PGI\textsubscript{2}), which, together with NO, inhibits platelet aggregation. Cell surface ectonucleotidase 5'-triphosphohydrolases (ENTPDase) hydrolyze ATP, ADP, and UTP, whereas ecto-5'-nucleotidase (CD73) further hydrolyzes AMP to adenosine, which acts at platelet adenosine receptors to further inhibit aggregation. Where there is endothelium damage or dysfunction, nucleotides released from platelets promote platelet aggregation via platelet P2Y\textsubscript{1}, P2Y\textsubscript{12}, and P2X\textsubscript{1} receptors. ATP, ADP, and UTP released from platelets may cause vascular smooth muscle constriction via P2Y and P2X\textsubscript{1} receptors and smooth muscle proliferation via P2Y receptors. Activation of smooth muscle P2X\textsubscript{1} receptors may inhibit proliferation. Activation of endothelial P2Y\textsubscript{2} receptors increases endothelial expression of tissue factor (TF), a promoter of platelet aggregation. Activation of endothelial P2Y receptors can stimulate secretion of endothelial von Willebrand factor, which would also lead to promotion of platelet aggregation.
Ecto-5'-nucleotidase (CD73) catalyzes the hydrolysis of extracellular AMP to adenosine and is highly expressed on the vascular endothelium. Interferon-α is an upregulator of ecto-5'-nucleotidase leading to adenosine production in HUVECs (Niemelä et al., 2004). Atorvastatin, a cholesterol-lowering drug acting through inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, has been shown to accelerate extracellular nucleotide degradation in human endothelial cells, which may have a protective role in the early steps of endothelial dysfunction, which precedes the development of atherosclerosis (Osman et al., 2008). There is a loss of ecto-5'-nucleotidase from porcine endothelial cells after exposure to human blood, which may have implications in xenograft rejection (Khalpey et al., 2005). Two opposite ATP-generating (involving ecto-adenylate kinase) and ATP-consuming pathways have been identified in human cell-free serum, the balance controlling the duration and magnitude of purinergic signaling in the blood (Yegutkin et al., 2003; Quillen et al., 2006).

A number of in vivo studies have shown cardiovascular effects of purines that are likely to involve the endothelium. In the absence of selective antagonists that can be used in vivo, the receptor subtypes involved in these responses is not entirely clear. ATP introduced into dogs or rats in vivo induced hypotension (Fukunaga et al., 1982; Boarini et al., 1984; Ribeiro and Lima, 1985; Kien et al., 1987). ATP-MgCl₂ introduced intravenously into anesthetized horses reduced systemic and colonic vascular resistance (Tetens et al., 2001a). Regional differences in effects of P2 receptor agonists were described in renal, mesenteric, cerebral, and coronary vascular beds in anesthetized rats (Cox and Smits, 1996). Intravenous injection of ATP or UTP in the anesthetized mouse caused a decrease in systemic arterial pressure via a cAMP pathway, but it was claimed that P2X₁, P2Y₁, and P2Y₄ receptors played little or no role in these effects (Shah and Kadowitz, 2002). Conducted dilations initiated by purines in arterioles in hamster cheek pouch are endothelium dependent and require endothelial Ca²⁺ (Duza and Sarelius, 2003).

In cat in vivo it was shown that α,β-meATP, a P2X receptor agonist, had potent pressor activity in the pulmonary circulation [100-fold more potent than angiotensin II (Ang-II)], whereas it had minor pressor activity in the systemic bed [1000-fold less potent than Ang-II] (Bivalacqua et al., 2002b). It is most likely that these pressor effects are mediated by the contractile actions of purines on the vascular smooth muscle. However, there was a report that after block of NO production, ATP released an EDCF; neither ET nor superoxide contributed to this EDCF, but prostanooids appeared to be involved (Dominiczak et al., 1991).

1. **P2Y Receptors.** Most blood vessels express on their vascular endothelium P2Y₁ receptors, activated by ADP and ATP, and P2Y₂ receptors, activated equipotently by UTP and ATP. P2Y₄ receptors, activated by UTP, and P2Y₆ receptors, activated by UDP, are also expressed on the endothelium of some blood vessels (Wang et al., 2002). Early studies were carried out on bovine aortic endothelial cells and showed distinct effects of ADP and UTP/ATP consistent with actions at different P2 receptors, then called P2Y and P2U receptors; it is now known that these correspond to P2Y₁ receptors and P2Y₂ and/or P2Y₄ receptors, respectively (Kitazono et al., 1992; Wilkinson et al., 1993; Motte et al., 1995). Coexpression of P2Y₁ receptors and P2Y₂ and/or P2Y₄ receptors on the endothelium of different blood vessels has now been widely reported (see sections on individual blood vessels). However, only P2Y₁ receptors were identified in rat brain microvascular endothelial cells (Webb et al., 1996).

Occupation of purinergic receptors on endothelial cells leads to an increase in [Ca²⁺], triggering the co-release of NO, EDHF, and PGI₂ (Van Coeverden and Boeynaems, 1984; Lückhoff and Busse, 1986, 1990; Robertson et al., 1990; Kelm et al., 1991; Marrelli, 2001; Wei et al., 2003). The generation of NO and EDHF by endothelial P2Y₁ and P2Y₂ and/or P2Y₄ receptors causes vasorelaxation (Malmşjö et al., 2000b; You et al., 2005). The involvement of PGI₂ in purinergic, endothelium-dependent, vasorelaxation is variable and appears to depend on both the vessel and the purine receptor subtype. For example, indomethacin, a cyclooxygenase inhibitor, blocked vasorelaxation to UTP (P2Y₂/4 receptors) in canine epicardial coronary artery (Matsumoto et al., 1997a), blocked relaxations to ADP (P2Y₁ receptor), but not those to UTP (P2Y₂/4 receptors) in bovine aortic collateral artery rings (Wilkinson et al., 1994), but had no effect on relaxations to 2-methylthio ADP (2-MeSADP) (P2Y₁ receptor) in rat thoracic aorta (Dol-Gliezes et al., 1999) or relaxations to ADP, ATP, and UTP (P2Y₁ and P2Y₂/4 receptors) in the rat mesenteric arterial bed (Ralevic and Burnstock, 1996c). Endothelial NO and PGI₂ inhibit platelet aggregation. However, ATP and ADP can also stimulate release of von Willebrand factor from cultured human endothelial cells (Palmer et al., 1994; Vischer and Wollheim, 1998), which may promote hemostasis. Moreover, activation of P2Y₂ receptors in human coronary artery endothelial cells has been shown to increase the expression within the endothelial cells of tissue factor, an initiator of the coagulation cascade (Ding et al., 2011). Hence, the actions of purines at their endothelial receptors include pro- and anticoagulant effects, in addition to the important direct effects of ADP and ATP in stimulating platelet aggregation via platelet P2Y₁, P2Y₁₂ and P2X₁ receptors (Gachet, 2008; Hechler and Gachet, 2011).

The initial rise in [Ca²⁺], in response to ATP in bovine cultured endothelial cells caused mobilization of Ca²⁺ from intracellular stores (indicating that a P2Y receptor was involved), which activated K⁺ channels,
that extracellular nucleotide-mediated NOS phosphor-
ylation and Boarder, 1992). A more recent study has shown
that they act as novel endogenous regulators of
protein kinase (PKC) (Pirotton et al., 1990; Purkiss et al., 2003). AMPK plays a key role in the regulation of energy ho-
meostasis and is activated by cellular stress, including
hypoxia/ischemia and hyperglycemia; the stress events are
accompanied by rapid release of ATP from damaged tissues, endothelial cells, and platelets. ADP signaling to bovine aortic endothelial cells via P2Y receptors has shown that expression of AMPK and calcium/calcmodulin-dependent kinase kinase-β is necessary for signaling to endothelial nitric oxide synthase (NOS)
and NO release (Hess et al., 2009). ATP, ADP, and UTP (but not UDP) induced phosphorylation and activation of AMPK in HUVECs via P2Y1, P2Y2, and/or P2Y4 receptors (da Silva et al., 2006). In aortic endothelial cells, ATP activation of P2Y receptors produces a sustained activation of phospholipase D involving protein kinase (PK) C (Pirotton et al., 1990; Purkiss and Boarder, 1992). A more recent study has shown that extracellular nucleotide-mediated NOS phosphor-
ylation involving P2Y1, P2Y2, and possibly P2Y4 recep-
tors is calcium- and PKCδ-dependent, and it was suggested that the signaling pathway may offer new thermo-
peutic avenues for the treatment of endothelial dysfunc-
tion (Da Silva et al., 2009).

FAD is released from cells during inflammation and protects against ischemia-reperfusion injury in rat myocardium. FAD elicits endothelium-dependent vasodi-
lolation in rat perfused mesenteric beds by activation of P2Y receptors (Ralevic et al., 1995b), perhaps by an endothelium-derived relaxing or hyperpolarizing factor other than NO and PGI2 (Hashmi-Hill et al., 2007). Functional P2Y receptors are present on murine aortic endothelium, but endothelium-dependent puri-
nergetic relaxation declines after plaque development,
which involves decreased availability of NO (Korda et al., 2005). Endothelium-dependent vasodilation is reduced with advancing age, but ATP-mediated, in contrast to acetylcholine (ACH)-induced vasodilation is not impaired with age in healthy humans (Kirby et al., 2010). Acyl derivatives of coenzyme A were shown to act as antagonists of ADP-mediated vasodi-
lilation at endothelial P2Y1 receptors and were selective versus vasodilation mediated by UTP acting at endothelial P2Y2 receptors; coenzyme A may be released from cells during certain conditions such as ischemia and diabetes, raising the possibility that they act as novel endogenous regulators of purinergic signaling (Alefishat et al., 2013).

A trophic role for P2Y receptors was suggested when it was proposed that the size of endothelial cells is regulated by P2Y receptors (Tanaka et al., 2003, 2004). A comparative study of P2Y receptor endothelium-mediated responses of single endothelial cells from bovine retina and HUVECs showed different patterns of P2Y2 receptor desensitization and roles of growth factors (Sanabria et al., 2008). It has been reported that ATP-mediated endothelial cell barrier enhancement is associated with cytoskeletal activation and is dependent on both Rac activation and cortactin (Jacobson et al., 2006). Extracellular ATP causes activation of myosin light chain phosphatase in endothelial cells, which is involved in regulation of endothelial barrier function (Härter et al., 2007). β-NAD has been shown to promote the barrier function of endothelial cells cultured from human pulmonary artery, and this involved P2Y1 and P2Y11 receptors and PKA- and EPAC1/Rac1-dependent actin cytoskeleton rearrangement (Umapathy et al., 2010).

2. P2X Receptors. Although the presence of P2Y receptors in vascular endothelial cells is well estab-
lished, there have also been reports about the presence of P2X receptors on the endothelium of a number of blood vessels. P2X1, P2X4, and P2X7, as well as P2Y2 receptors were identified using immunohistochemistry on endothelial cells of the human internal mammary artery, radial artery, and saphenous vein; P2X4 receptors were strongly expressed on saphenous vein compared with the arteries (Ray et al., 2002). In a study of P2 receptor expression in human mammary arteries and HUVECs, the P2X1 receptor was dominant in smooth muscle, but P2X4, P2Y1, and P2Y11 receptors were the most abundant receptors on the endothelium; P2Y2 and P2Y6 receptors were also expressed at lower levels on both smooth muscle and endothelial cells (Wang et al., 2002). Another study of endothelial cell cultures from human coronary and pulmonary arteries and aorta, as well as HUVECs, showed that these expressed P2X4 and P2X5 receptors (Schwiebert et al., 2002). Evidence was presented to show that P2X4 and P2X6 receptors located on HUVECs are restricted to areas of cell-cell contact, and it was suggested that these receptors were associated with VE-cadherin, a marker for cell adhesion, at HUVEC adherens junc-
tions (Glass et al., 2002). P2X4 receptor expression has been shown in endothelial cells of guinea pig cochlear spiral ligament capillaries (Wu et al., 2011). Based on P2X receptor immunostaining, it was claimed that P2X receptors (P2X1, P2X3, and P2X4 subtypes) were present on the endothelium of rat mesenteric artery, aorta, and middle cerebral and coronary arteries (Nori et al., 1998; Hansen et al., 1999). Electron microscopic immunolabeling showed P2X2 receptors localized on endothelial cells in rat brain (Loesch and Burnstock, 2000). P2X3 receptors were shown to be localized on endothelial cells in blood vessels of the rat thymus...
(Glass et al., 2000). Endothelial cells in the rat thyroid immunostained for P2X4, P2X4, and P2X7 receptors, and it was suggested that this unusually rich expression of endothelial P2X receptors may reflect the constant dynamic changes in thyroid activities and the special need for regulation of vascular tone in endothelial barrier formation (Glass and Burnstock, 2001).

Relatively little is known about the function of endothelial P2X receptors. The most comprehensive evidence exists for P2X4 receptors, identifying them as mediators of vasodilatation acting as mechanotransducers of the response to ATP released from endothelial cells. It was shown that P2X4 receptor knockout mice do not have normal endothelial responses to changes in flow, with reduced dilation; the animals have higher blood pressure and excrete smaller amounts of NO (Yamamoto et al., 2006). It was further shown that adaptive remodeling (decrease in vessel size in response to a decrease in blood flow) was also decreased. It was suggested that hypertension in the P2X4 receptor knockouts may have been caused by the removal of ATP-induced vasodilation involving P2X4 receptors and NO and by removal of the negative influence of NO on smooth muscle proliferation (Yamamoto et al., 2006). P2X4 receptors were claimed to mediate the ATP-induced vasodilatation of guinea pig cochlear spiral ligament (Wu et al., 2011). P2X4 receptors were shown, using antisense oligonucleotides designed to knockout P2X4 expression, to mediate ATP-induced Ca\(^{2+}\) influx in primary cultures of HUVECs (Yamamoto et al., 2000). However, a recent study measuring ATP- and UTP-induced Ca\(^{2+}\) mobilization, membrane hyperpolarization, and NO production in HUVECs failed to find any evidence of functional P2X receptors and concluded that P2Y2 receptors are the predominant isomorph underlying these responses in HUVECs (Raqeeb et al., 2011).

mRNA was shown to be expressed for P2X4, P2X5, and P2X7 receptors on bovine aortic endothelium, and whole-cell and outside-out patch electrophysiological studies suggested that there are different roles for these P2X receptor subtypes (Ramirez and Kunze, 2002). \(\alpha,\beta\)-MeATP, presumably acting via P2X1 or P2X3 receptors, caused production of NO by microvascular endothelial cells from rat cremaster muscle; unlike the actions of 2-methylthio ATP (2-MeSATP) at coexpressed endothelial P2Y1 receptors, NO production by \(\alpha,\beta\)-meATP was unaffected by melatonin, indicating distinct sites of action (Silva et al., 2007). It was reported that P2X1 receptors are expressed in the endothelium of rat mesenteric arteries and that their activation leads to vasodilation largely via EDHF (Harrington and Mitchell, 2004). Experiments with P2X1 receptor knockout mice showed that endothelial P2X1 receptors may mediate vasodilation of mesenteric arteries, and it was suggested that this may be of particular importance at the sites of inflammation or vascular insult (Harrington et al., 2007). The more widespread application of molecular biology techniques is clearly needed to identify the possible roles of other P2X subtypes in the endothelium.

When HUVECs were subjected to laminar shear stress, P2X4 receptor mRNA levels began to decrease within 1 hour and further decreased in time, reaching 60% at 24 hours (Korenaga et al., 2001). HUVEC expression of P2X4 and P2X7 receptors was upregulated under inflammatory conditions (Wilson et al., 2007); activation of the P2X7 receptors resulted in release of both pro- and anti-inflammatory interleukin (IL)-1 receptor ligands, the balance of which may provide a means for altering the inflammatory state of the vessel wall. Selective upregulation of P2X4 receptor gene expression by interferon-\(\gamma\) in endothelial cells of human umbilical vein and aorta and microvascular endothelial cells has been reported (Tang et al., 2008). The density of expression of the P2X4 receptor on endothelial cells in rabbit aorta was increased by approximately 10-fold after balloon injury, resulting in intima proliferation (Pulvirenti et al., 2000). Expression levels of P2X4 were significantly higher in endothelium of the human saphenous vein than in the radial and internal mammary arteries, and given that P2X4 modulates vascular contractility and is upregulated in situations involving intima proliferation, it was suggested that vein grafts used in coronary bypass surgery are more susceptible to developing atherosclerosis (Ray et al., 2002). Stimulation of P2X7 receptors on bovine pulmonary artery endothelial cells enhances lipopolysaccharide (LPS)-induced apoptosis via caspase-8 activation (Sylte et al., 2005).

3. P1 (Adenosine) Receptors. There is a review of the early literature describing the role of adenosine in local regulation of blood flow after its release during hypoxia or ischemia (Berne et al., 1983). One role for adenosine is to inhibit the release of sympathetic neurotransmitters via prejunctional receptors (Sollevi and Fredholm, 1983); another is to dilate vessels via the endothelium and smooth muscle (Sparks and Gorman, 1987; Collis, 1989; Tabrizchi and Bedi, 2001). These actions of adenosine would lead to an increase in blood flow to the hypoxic or ischemic tissue. All four subtypes of P1 adenosine receptors, A1, A2A, A2B, and A3, have been identified on the vascular endothelium (and smooth muscle), with A2A and A2B receptors being most commonly expressed.

There was early evidence of the functional expression of P1 (adenosine) receptors in endothelial cells. Removal of the endothelium decreased relaxation of canine coronary arteries to adenosine (Rubanyi and Vanhoutte, 1985). The vasodilator effect of adenosine in rat aorta was similarly shown to be partly endothelium dependent (Yen et al., 1988). Endothelial release of NO was claimed to contribute to the vasodilator effect of adenosine in humans (Smits et al., 1995) and
in human iliac artery (Li et al., 1998) and pig carotid artery (Li et al., 1995). A2 receptors were identified on cultures of guinea pig coronary endothelial cells (Nees et al., 1987), bovine pulmonary artery endothelial cells (Legrand et al., 1989), and rat renal artery endothelial cells (Martin and Potts, 1994). The adenosine receptor on guinea pig microvascular coronary endothelial cells was identified as the A2A subtype (Schiele and Schwabe, 1994). Both A2A and A2B receptors were described on human aortic endothelial cells (Iwamoto et al., 1994). A2A and A2B receptors were also shown to be present on porcine coronary artery endothelial cells (Olanrewaju et al., 2000). An A1 receptor on bovine pulmonary artery endothelial cells was shown to mediate Cl− efflux (Arima et al., 1994), and an A1 receptor was shown to mediate release of NO from the endothelium of rat aorta (Ray and Marshall, 2006). An A3 receptor was shown to mediate endothelium-dependent contractions in mouse aorta; contractions to an A2 selective agonist were abolished by endothelium removal, were absent in A3 receptor knockout mice, and appeared to involve cyclooxygenase-1 (Ansari et al., 2007b).

Analysis of the mechanisms underlying adenosine-evoked release of NO from rat aortic endothelium suggested that A1 receptor-mediated NO release requires extracellular Ca2+, phospholipase A2, and ATP-sensitive K+ channels, whereas A2A receptor-mediated NO release requires extracellular Ca2+ and Ca2+-activated K+ channels (Ray and Marshall, 2006). Cyclic nucleotide-gated channels are claimed to play a key role in A2B receptor-induced endothelial Ca2+ influx in vasorelaxation of mouse aorta strips (Cheng et al., 2008).

In addition to a role of adenosine in hypoxic vasodilation there is evidence that adenosine may promote angiogenesis during ischemia/hypoxia, with the new blood vessels helping to maintain tissue oxygenation (see Auchampach, 2007; see also section IV). A2B receptors on human microvascular endothelial cells modulate expression of angiogenic factors (Feoktistov et al., 2002). Activation of A2B receptors on human and bovine retinal endothelial cells increases vascular endothelial growth factor (VEGF) production and cell proliferation, and A2B receptors may be involved in hypoxic induction of VEGF (Takagi et al., 1996; Grant et al., 1999). Given this important role of A2B receptors, it seems fitting that hypoxia modulates adenosine receptor expression in human endothelial cells by shifting expression of A2A receptors to A2B receptors (Feoktistov et al., 2003). Differences between human micro- and macrovessel endothelial cell responses to adenosine receptors and β-adrenoceptors in hypoxia have been reported (Wiktorowska-Owczarek et al., 2007).

It has been suggested that adenosine may exert anticoagulant activity on vascular endothelial cells via A2A and A3 receptors by downregulating endothelial cell tissue factor expression, particularly during ischemic and atherosclerotic processes, known to be associated with increased purine levels (Deguchi et al., 1998). It has also been suggested that cytokines modulate the expression of A2A and A2B receptors in human microvascular endothelial cells (Khoa et al., 2003). Regulation of Ang-II-induced reactive oxygen species (ROS) production and redox-signaling by A2A receptors on endothelial cells has been reported; inhibition of A2A receptors protects endothelial cells from acute Ang-II-induced oxidative stress and endothelium dysfunction (Thakur et al., 2010). Adherent leukocytes prevent adenosine formation and consequently impair endothelial barrier function by an ecto 5′-nucleotidase (CD73)-dependent mechanism (Henttinen et al., 2003).

Adenosine has been shown to have important vasoactive effects in vivo, some of which appear to involve the endothelium. Adenosine introduced into artificially ventilated dogs caused a profound decrease in systemic vascular resistance (Lagerkranser et al., 1984). NO participates in the hypotensive effect induced by A2 receptor stimulation in anesthetized rats (Stella et al., 1995). Femoral vasodilation induced by intra-arterial adenosine in rabbits was mediated by A1 and A2 receptors (Sakai et al., 1998). A3 receptors mediate hypertension in the pithed rat (Fozard and Carruthers, 1993), but this was later shown to be due to activation of A3 receptors on mast cells, leading to mast cell degranulation (Hannon et al., 1995; Fozard et al., 1996). Intravenous infusion of adenosine or a selective A2A receptor agonist in conscious mice lacking endothelial NOS (knockouts for eNOS) produced a reduction in mean arterial blood pressure (Andersen et al., 2011). Intravenous injection of uridine in conscious rats decreases arterial pressure by activating A1 receptors (Yilmaz et al., 2008).

A recent report claims that adenosine is a major regulator of endothelial progenitor cells (Devaux et al., 2012). Adenosine upregulates the chemokine receptor CXCR4 and stimulates their recruitment in the infarcted heart and enhances repair. Another group has identified A2A and A3 receptors as mediators of human endothelial progenitor cell migration (Fernandez et al., 2012).

There have been reviews concerned with purinergic signaling in endothelial cells over the years (Nees and Gerlach, 1983; Pearson and Gordon, 1985; Boeynaems and Pearson, 1990; Boeynaems et al., 1990; Pirotton et al., 1993; Adair, 2005; Burnstock, 2008a).

A schematic is shown in Fig. 7, which illustrates the receptors involved in the dual control of vascular tone.

III. Purinergic Signaling in Different Vessels

The distribution of purinoceptor subtypes on both smooth muscle and endothelial cells mediating vasoconstriction and vasodilation in different vessels in the body are summarized in Tables 2 and 3.
A. Aorta

The aorta has been the target vessel of many studies of purinergic signaling following the original discovery of EDRF in this vessel (Furchgot and Zawadzki, 1980). Studies carried out in the aorta have contributed to our understanding of endothelial P2 receptors, of the role of the endothelium as a source of purines, which can be released by various stimuli including hypoxia and shear stress, as well as the role of the endothelium in regulating extracellular levels of purines through the actions of cell surface ectonucleotidases.

ATP was shown to be released from endothelial cells, as well as nerves, after transmural stimulation and in response to methoxamine (α-adrenoceptor agonist) in the rabbit aorta (Sedaa et al., 1990). Hypotonic stress induces ATP release from bovine aortic endothelial cells, and this may involve the volume-regulated anion channel, a nucleotide-permeable channel (Hisadome et al., 2002). Polyphenolic compounds contained in red wine may also induce release of ATP, ADP, and UTP from endothelial cells in rat isolated aorta to activate P2Y₁ and P2Y₂ receptors, leading to vasodilation (Mendes et al., 2003). Extracellular levels of purines are tightly regulated by ATP-dephosphohydrolase (apyrase), localized in both intima and media of bovine aorta (Côte et al., 1991, 1992), and by E-NTPDase 1/CD39, also localized on both the endothelium and smooth muscle. E-NTPDase 1/CD39 was shown to be the major ecto-enzyme regulating nucleotide metabolism at the surface of mouse aortic smooth muscle (Kauffenstein et al., 2010a). Gadolinium, which blocks stretch-activated calcium channels, blocked ATP and ADP hydrolysis by ectonucleotidases and thereby increased vascular reactivity of rat aortic rings (Angeli et al., 2011). After release from endothelial cells, purines can act on P2 receptors on the same or adjacent endothelial cells in an

Fig. 7. Schematic diagram illustrating the main receptor subtypes for purines and pyrimidines present in blood vessels involved in control of vascular tone. ATP is released as a cotransmitter with NA and NPY from sympathetic nerves in the adventitia to act at smooth muscle P2X receptors and, in some vessels, P2X₃, P2X₇, P2Y₁, P2Y₂, and P2Y₄ receptors, resulting in vasodilation (and rarely vasoconstriction); ATP is released with calcitonin-gene-related peptide (CGRP) and substance P (SP) from sensory-motor nerves during “axon reflex” activity to act on smooth muscle P2Y receptors, resulting in either vasodilatation or vasoconstriction. P1 (A₁) receptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (arising from ectoenzymatic breakdown of ATP) modulation of transmitter release. P2X₃ receptors are present on a subpopulation of sensory nerve terminals. P1 (A₂) receptors on vascular smooth muscle mediate vasodilatation. Endothelial cells release ATP and UTP during shear stress and hypoxia to act on P2Y₁, P2Y₂, and sometimes P2Y₄, P2Y₁₁, P2X₁, P2X₃, P2X₇, and P2X₄ receptors, leading to the production of nitric oxide (NO) and subsequent vasodilatation. Adenosine tetraphosphate (AP₄) activates P2X₁ receptors to excite smooth muscle. ATP, after its release from aggregating platelets, also acts, together with its breakdown product ADP, on these endothelial receptors. Blood-borne platelets possess P2Y₁ and P2Y₁₂ ADP-selective receptors as well as P2X₇ receptors. Immune cells of various kinds possess P2X₇ as well as P1, P2X₁, P2Y₁, and P2Y₂ receptors. ATP released from red blood cells, which express P2X₇ and P2Y₁₃ receptors, is also involved in some circumstances. The additional involvements of uridine adenosine tetraphosphate (Up₄A) are indicated. (Figure is modified from Burnstock, 1996b, with permission from Blackwell Science Ltd, UK).
auto- and paracrine manner. Purines released locally from erythrocytes or platelets can also regulate function via endothelial purine receptors.

Early articles reported that ATP relaxed porcine isolated aorta, provided the endothelial cells were present (Gordon and Martin, 1982). Endothelium-dependent relaxation of porcine aorta by ATP was shown to be mimicked by ADP, but AMP and adenosine were approximately 120 times less potent (Gordon and Martin, 1983). 2-MeSATP was shown to be 50 times more potent than ATP, whereas UTP was 100 times less potent in relaxing the isolated aorta of newborn pig (Martin et al., 1985), suggesting in retrospect that P2Y\textsubscript{1}, but not P2Y\textsubscript{2} and/or P2Y\textsubscript{4} receptors were involved. It was claimed that ATP mediates endothelial PGI\textsubscript{2} release via a P2Y receptor in porcine aortic endothelial cells (Needham and Boeynaems, 1984). The first evidence that there was more than one P2Y receptor subtype mediating NO and PGI\textsubscript{2} release from bovine aortic endothelial cells (Motte et al., 1993) was followed by the proposal that P2Y\textsubscript{2} and P2U receptors were coexpressed on bovine aortic endothelial cells (Communi et al., 1995).

### Table 2

Summary of the P1 receptors on smooth muscle and endothelial cells mediating vasoconstriction (+) and vasodilatation (−) in different vessels and species

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Smooth Muscle</th>
<th>Endothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R References</td>
<td>R References</td>
</tr>
<tr>
<td>Aorta</td>
<td>A\textsubscript{1} (guinea pig) \textsuperscript{a} + Stogall and Shaw, 1990</td>
<td>A\textsubscript{1} (rat) − Nakhostine and Lamontagne, 1993; Ray et al., 2002</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{2} (rat) − Grbović and Radenković, 2003</td>
<td>A\textsubscript{1} (SHR) + Fahim and Mustafa, 2001</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{2B} (guinea pig) − Balwierzak et al., 1991</td>
<td>A\textsubscript{1} (mouse) + Prentice et al., 2001</td>
</tr>
<tr>
<td></td>
<td>P1 (frog) − Martin, 1992; Martin et al., 1993; Pozzard et al., 2003</td>
<td>A\textsubscript{2A} (rat) − Lewis et al., 1994; Prentice and Hourani, 1996</td>
</tr>
<tr>
<td></td>
<td>− Knight and Burnstock, 1996</td>
<td>A\textsubscript{2A} (mouse) − Ponnoth et al., 2009 (see also Prentice et al., 2002)</td>
</tr>
<tr>
<td>Rat Tail Artery</td>
<td>P1 (rabbit) − Kennedy and Burnstock, 1985\textsuperscript{a}; Headrick et al., 1992</td>
<td>A\textsubscript{2A} (rat) − Prentice and Hourani, 2000</td>
</tr>
<tr>
<td>Ear Artery</td>
<td>A\textsubscript{2A} (rat) − Hiley et al., 1995; Radenković et al., 2005</td>
<td>A\textsubscript{2A} (rat) − Prentice and Hourani, 1996</td>
</tr>
<tr>
<td>Mesenteric artery</td>
<td>A\textsubscript{0} (rat) − Rubino et al., 1995; Prentice et al., 1997</td>
<td>A\textsubscript{2B} (mouse) − Talukder et al., 2003; Ansari et al., 2007a (see also Prentice et al., 2002)</td>
</tr>
<tr>
<td>Coronary Vessels</td>
<td>A\textsubscript{1} (porcine) − Merkel et al., 1992</td>
<td>A\textsubscript{3} (mouse) + Ansari et al., 2007b; El-Awady et al., 2011</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{2A} (human) − Sato et al., 2005</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{3} (porcine) − Arif Hasan et al., 2000; Teng et al., 2005; Rayment et al., 2007b</td>
<td></td>
</tr>
<tr>
<td>Coronary Vessels</td>
<td>A\textsubscript{2B} (human) − Kemp and Cocks, 1999</td>
<td>A\textsubscript{1} (guinea pig) − Rubio and Ceballos, 2003</td>
</tr>
<tr>
<td>Cerebral Vessels</td>
<td>A\textsubscript{2B} (porcine) − Teng et al., 2005</td>
<td>A\textsubscript{2A} (porcine) − Hansen et al., 2000</td>
</tr>
<tr>
<td></td>
<td>P1 (rabbit) − Kennedy and Burnstock, 1985\textsuperscript{a}; Headrick et al., 1992</td>
<td>A\textsubscript{2A} (guinea pig) − Rubio and Ceballos, 2003</td>
</tr>
<tr>
<td>Skeletal Muscle</td>
<td>A\textsubscript{2B} (rat) − Nai and Wima, 1993; Coney and Marshall, 1998; Shin et al., 2000a</td>
<td>−</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>A\textsubscript{1} (dog) − De Mey and Vanhoupte, 1981</td>
<td>A\textsubscript{2B} (rat) − Shin et al., 2000b; Ngai et al., 2001</td>
</tr>
<tr>
<td>Pulmonary Vessels</td>
<td>A\textsubscript{3} (rabbit) − Cassis et al., 1987</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{1} (cat) \textsuperscript{b} + Neely et al., 1991; Cheng et al., 1996</td>
<td>A\textsubscript{2A} (rat) − Mortensen et al., 2009c</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{1} (guinea pig) − Wiklund et al., 1987; Szentmiklós et al., 1995</td>
<td>A\textsubscript{3} (rat) − Danialou et al., 1997</td>
</tr>
<tr>
<td></td>
<td>A\textsubscript{2A} (guinea pig) − Szentmiklós et al., 1995</td>
<td>A\textsubscript{3} (young rabbit) − Steinhorn et al., 1994</td>
</tr>
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<td></td>
<td>A\textsubscript{2} (rabbit) − Steinhorn et al., 1994; El-Kashef et al., 1999</td>
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<tr>
<td>Renal artery</td>
<td>A\textsubscript{3} (cat) − Cheng et al., 1996</td>
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</tr>
<tr>
<td>Afferent arteriole</td>
<td>A\textsubscript{1} (mouse) − Hansen et al., 2003, 2007</td>
<td>A\textsubscript{2A} (rat) − Grbović et al., 2000</td>
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<tr>
<td>Arcuate artery</td>
<td>A\textsubscript{1} (rabbit) \textsuperscript{a} + Weihprecht et al., 1992</td>
<td>A\textsubscript{2A} (rat) − Carroll et al., 2006</td>
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<tr>
<td>Hepatic Portal Vein</td>
<td>A\textsubscript{2} (rat) − Telang et al., 2003</td>
<td>A\textsubscript{2A} (rabbit) − Prior et al., 1999</td>
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<tr>
<td>Chorionic artery</td>
<td>A\textsubscript{2A} (human) + Donoso et al., 2005</td>
<td>A\textsubscript{2B} (human) + Donoso et al., 2005</td>
</tr>
<tr>
<td>and vein</td>
<td>A\textsubscript{2B} (human) − Donoso et al., 2005</td>
<td>−</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Effects of endothelium removal not investigated.

R, response.
### TABLE 3

Summary of the P2 receptors on smooth muscle and endothelial cells mediating vasoconstriction (+) and vasodilatation (−) in different vessels and species

Note that the P2X receptor subtype identification in this table is based on agonist selectivity (usually α,β-mesATP) and thus does not exclude the possible involvement of other homomeric or heteromeric P2X receptors.

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Smooth Muscle</th>
<th>Endothelial Cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Receptor R References</td>
<td>Receptor R References</td>
</tr>
<tr>
<td>Aorta</td>
<td>P2X1 (rat) + White et al., 1985; Li et al., 2011b</td>
<td>P2Y1 (pig) + Martin et al., 1985</td>
</tr>
<tr>
<td></td>
<td>P2X1 (mouse) + Bény, 2004</td>
<td>P2Y1 (rabbit) + Payne et al., 2002</td>
</tr>
<tr>
<td></td>
<td>P2X1/5 + Chin and Chueh, 2000</td>
<td>P2Y1 (guinea pig) + Kaiser and Buxton, 2002</td>
</tr>
<tr>
<td></td>
<td>P2Y1 (rabbit) − Payne et al., 2002</td>
<td>P2Y1 (mouse) + Bény, 2004; Guns et al., 2005, 2006; Kauffenstein et al., 2010b; Payne et al., 2002</td>
</tr>
<tr>
<td>P2Y24 (mouse)</td>
<td>+ Bény, 2004</td>
<td>P2Y2 (mouse) + Büttmann et al., 1997; Dol-Gleizes et al., 1999</td>
</tr>
<tr>
<td>P2Y6 (mouse)</td>
<td>+ Bar et al., 2008; Kauffenstein et al., 2010a</td>
<td>P2Y2 (guinea pig) + Simonsen et al., 1997</td>
</tr>
<tr>
<td>Rat tail artery</td>
<td>P2X1 + Evans and Kennedy, 1994; McLaren et al., 1998; Fukumitsu et al., 1999; Wallace et al., 2006; Li et al., 2011b</td>
<td>P2X1 (rat) + Harrington and Mitchell, 2004</td>
</tr>
<tr>
<td></td>
<td>P2X1 (in early development) + Wallace et al., 2006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2Y1 + Wallace et al., 2006</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2Y4 + Saig et al., 1990</td>
<td></td>
</tr>
<tr>
<td>Ear artery</td>
<td>P2X1 (rabbit) + O’Connor et al., 1990; Leff et al., 1990; von Kugelgen and Starke, 1990a; Zigan et al., 1994; Ren and Zhang, 2002</td>
<td>P2X1 (rabbit) + Mutafova-Yambolieva et al., 2000</td>
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<td></td>
<td>P2X1 (dog) + Mutafova-Yambolieva et al., 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P2X1 (guinea pig) + Mutafova-Yambolieva et al., 2000</td>
<td></td>
</tr>
<tr>
<td>Coronary vessels</td>
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<td>P2X1 (pig) + Olivecrona et al., 2004</td>
</tr>
<tr>
<td></td>
<td>P2X1 (dog) + Mutafova-Yambolieva et al., 2000</td>
<td>P2X1 (rat) + Vials and Burnstock, 1994b; Korczyn et al., 1999; van der Giet et al., 2002</td>
</tr>
<tr>
<td>Cerebral vessels</td>
<td>P2X1 (human) + Mutafova-Yambolieva et al., 2000</td>
<td>P2X1 (pig) + Vials and Burnstock, 1994b; Korczyn et al., 1999; van der Giet et al., 2002</td>
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<tr>
<td></td>
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<td>P2X1 (rabbit) + Vials and Burnstock, 1994b; Korczyn et al., 1999; van der Giet et al., 2002</td>
</tr>
<tr>
<td></td>
<td>P2X1 (mouse) + Mutafova-Yambolieva et al., 2000</td>
<td>P2X1 (rabbit) + Vials and Burnstock, 1994b; Korczyn et al., 1999; van der Giet et al., 2002</td>
</tr>
</tbody>
</table>

(continued)
The P2Y and P2U receptors involved were shown to be pharmacologically similar to cloned P2Y1 and P2Y2 receptors, respectively (Hansmann et al., 1997). It is now known that UTP activates P2Y4 receptors as well as P2Y2 receptors (and is a weak agonist of P2Y6 receptors). Hence, in the absence of selective antagonists, the subtype identity of UTP-sensitive endothelial P2Y receptors is not always apparent. Early studies showed that there is a requirement for PKC in the stimulation of PGI₂ production by bovine aortic endothelial P2Y (P2Y₁) and P2U (P2Y₂ and/or P2Y₄) receptors (Patel et al., 1996).

### TABLE 3—Continued

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Smooth Muscle</th>
<th>Endothelial Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Receptor</td>
<td>R</td>
</tr>
<tr>
<td>P2X₁ (goat)</td>
<td>+ Torregrosa et al., 1990a</td>
<td></td>
</tr>
<tr>
<td>P2X (rabbit)</td>
<td>+ von Kügelgen and Starke, 1990b</td>
<td></td>
</tr>
<tr>
<td>P2Y₂ (rat)</td>
<td>+ Lewis et al., 2000a; Malmjö et al., 2003b</td>
<td></td>
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<tr>
<td>P2Y₂ (bovine)</td>
<td>+ Miyagi et al., 1996</td>
<td></td>
</tr>
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<td>P2Y₂ (rabbit)</td>
<td>+ von Kügelgen and Starke, 1990b</td>
<td></td>
</tr>
<tr>
<td>P2Y₆ (rat)</td>
<td>+ Buckwalter et al., 2003, 2004</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>+ Dézi et al., 1990</td>
<td></td>
</tr>
<tr>
<td>Femoral artery</td>
<td>+ Li et al., 2011b</td>
<td></td>
</tr>
<tr>
<td>Pulmonary vessels</td>
<td>P2X₁ (rat)</td>
<td>+ Liu et al., 1989; McCormack et al., 1989a; Li et al., 2011b</td>
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<td>Pulmonary vessels</td>
<td>P2Y₂ (rat)</td>
<td>+ Rubino and Burnstock, 1996</td>
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<td>P2X₂ (mouse)</td>
<td>+ Vonend et al., 2005b</td>
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<td>P2X₁ (rat)</td>
<td>+ von Kügelgen et al., 1995</td>
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<td>Renal vessels</td>
<td>P2X₁ (rat)</td>
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<td>Renal vessels</td>
<td>P2Y (rabbit)</td>
<td>− Qasabian et al., 1997</td>
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<td>Hepatic artery/bed</td>
<td>P2X₁ (rabbit)</td>
<td>+ Brizzolara and Burnstock, 1991; Mathie et al., 1991b; Ralevic et al., 1991</td>
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<td>Portal vein</td>
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<td>− Brizzolara and Burnstock, 1991</td>
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<td>P2X₁ (rabbit)</td>
<td>+ Kennedy and Burnstock, 1985b</td>
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<td>P2X₁ (rat)</td>
<td>+ Reilly et al., 1987; Reilly and Burnstock, 1987</td>
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<td>+ Taylor and Parsons, 1991</td>
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<td>Carotid artery</td>
<td>P2X₂ (rat)</td>
<td>+ Malmjö et al., 1998</td>
</tr>
</tbody>
</table>

aPatch clamp studies.
bMeasurement of changes in cytosolic calcium concentration.
cMeasurement of hyperpolarization.
dIsolated perfused kidney (not renal artery).
eCotyledons (not umbilical cord and superficial chorionic vessels).
fHepatic bed (P2Y relaxations are endothelium-independent in hepatic artery).
Different P2 receptors on endothelial cells mediating vasodilation and on smooth muscle mediating vasocostriction of rat aorta were described in 1985 (White et al., 1985). Subsequent studies showed that vasorelaxant P2Y1, P2Y2/4, and more rarely P2Y6 receptors are variously expressed on the endothelium in the aortae of different species (Table 2). 2-MeSADP was very effective in relaxing the rat thoracic aorta, indicating the presence of P2Y1 receptors, but not P2Y12 receptors (since the relaxant response was not affected by clopidogrel); UTP also evoked relaxations, likely via P2Y2 and/or P2Y4 receptors (Dol-Gleizes et al., 1999). ADP and ATP-mediated endothelium-dependent relaxations in guinea pig aorta were inhibited by the P2Y1 receptor-selective antagonist MRS2179; UTP relaxations were insensitive to MRS2179 and UDP was inactive, suggesting an involvement of P2Y2 receptors (Kaiser and Buxton, 2002). Ca^2+ waves within endothelial cells are induced by ATP, but not UTP, via P2Y receptors in guinea pig aortic strips (Ohata et al., 1997), consistent with a likely expression of P2Y1 and P2Y4 but not P2Y2 receptors in this vessel. In mouse thoracic aorta, P2Y1, P2Y2, and P2Y6 receptors are present on endothelial cells and mediate vasorelaxation (Bény, 2004; Guns et al., 2005). In a study of mouse aorta, using P2Y2 knockout animals, it was concluded that ATP-evoked relaxation was mainly mediated by the P2Y2 receptor, but that the vasodilator effect of UTP was probably mediated partly via a P2Y6 receptor because it was not different in aortae from P2Y2- or P2Y4-knockout mice (Guns et al., 2005, 2006). It was claimed that GTP and guanosine 5′-O-(3-thiotriphosphate) elicit endothelium-dependent relaxations in rat aorta via P2U (P2Y2 and/or P2Y4) receptors (Bültmann et al., 1997).

Cold storage of rabbit thoracic aorta attenuates P2Y2/4 receptor-mediated endothelium-dependent dilation, whereas P2Y1 receptor-mediated endothelium-independent receptors were only partially reduced (Payne et al., 2002). A decrease in ATP-induced endothelium-dependent relaxation of thoracic aorta in aging rats was reported (Ueda and Moritoki, 1991).

There is some evidence for endothelium-independent purinergic vasorelaxations in aortae. In mouse freshly isolated aortic smooth muscle cells, ATP activated a delayed Ca^{2+}-independent K^+ current (this followed an initial phase involving activation of Ca^{2+}-dependent K^+ and Cl^- currents), and it was suggested that this might represent a novel vasorelaxant pathway in vascular smooth muscle cells (Serir et al., 2006). Diadenosine polyphosphates cause hyperpolarization and activate a Ca^{2+}-dependent K^+ conductance in porcine aortic smooth muscle, probably via P2Y receptors (Schlatter et al., 2000).

ATP contracts the smooth muscle of rat aorta (White et al., 1985), and in patch-clamp studies of rat cultured aortic smooth muscle cells, it was shown that ATP increased [Ca^{2+}], with subsequent activation of both Ca^{2+}-dependent K^+ and Cl^- currents (von der Weid et al., 1993) and L-type and non-L-type Ca^{2+} channels (Kitajima et al., 1993). ATP caused release of Ca^{2+} from intracellular stores in pig cultured aortic smooth muscle cells to increase [Ca^{2+}]_i, which activated Cl^- currents, leading to depolarization and contraction (Droogmans et al., 1991). ATP can initiate Ca^{2+} waves in pig cultured aortic smooth muscle cells (Mahoney et al., 1993). ATP triggered cytosolic [Ca^{2+}]_i oscillations, accompanied with K^+ and Cl^- oscillations, via gap junctions in mouse freshly isolated aortic smooth muscle cells (Fanchaouy et al., 2005). UTP was also able to elicit oscillatory currents due to activation of Cl^- channels in rat aortic myocytes (Muraki et al., 1998). P2Y receptor subtypes P2Y1, P2Y2, P2Y4, and P2Y6 evoke different Ca^{2+} signals in cultured rat aortic smooth muscle cells (Govindan and Taylor, 2012).

In rat aorta, α,β-meATP was more potent than ATP in eliciting vasoconstriction, and these responses were antagonized by arylazido amino propyl-ATP, suggesting an involvement of P2X receptors (White et al., 1985). Studies of isolated smooth muscle cells from the rat aorta (as well as cerebral and mesenteric arteries) indicated that the heteromeric P2X1,2,5 receptor, as well as the P2X7 receptor, was functionally expressed (Sugihara et al., 2009). P2X5 receptors on porcine aortic smooth muscle cells were claimed to be responsible for ATP-induced Ca^{2+} influx (Chin and Chueh, 2000). Evidence for both P2X receptors (mediating Ca^{2+} influx) and P2Y (P2Y2 and/or P2Y4) receptors (mediating release of Ca^{2+} from intracellular stores) in rat aortic smooth muscle was presented (Kitajima et al., 1994; Muraki et al., 1998). Sauzeau et al. (2000) and Govindan et al. (2010) claimed that rat aortic myocytes express P2Y1, P2Y2, P2Y4, and P2Y6 receptors that are coupled to activation of Rho and Rho kinase and consequent stimulation of actin cytoskeleton reorganization. Northern blot analysis showed that rat aortic smooth muscle cells express mRNA for P2Y1, P2Y2, and P2Y5 receptors (Bouchie et al., 2000). It was suggested that during the course of cultivating rat aortic myocytes there was a loss of P2X receptors, which were replaced by P2Y2 receptors (Pacaud et al., 1995; Kumari et al., 2003). In a study of purine receptors in mouse thoracic aorta, it was concluded that, as in rat thoracic aorta, P2X and P2Y receptors (sensitive to UTP) can mediate contraction of the smooth muscle (Bény, 2004). H2O2-induced phasic contractions of rat aorta involve, at least in part, activation of smooth muscle P2 receptors (Shen et al., 2000).

NADP-induced contractions of mouse aorta appear to be mediated by two P2X receptor populations, one located on the endothelium and the other on smooth muscle, both of which lead to contraction (Judkins et al., 2006). UpA, reported to be an endothelium-derived vasoconstrictor, caused a moderate endothelium-dependent relaxation of rat aortic rings contracted with
phenylephrine, but in preparations that had not been preconstricted caused contraction that was potentiated by endothelium removal or NOS inhibition and was mediated by P1 and P2X receptor activation (Linder et al., 2008). Hansen et al. (2010) claimed that Up4A had biphasic effects on mouse aortic vascular smooth muscle, but vasoconstriction dominated at low concentrations. In the same study it was shown further that, in conscious rodents, step-up infusions of Up4A elicited hypotension and electrolyte retention.

ATP stimulates release of matrix metalloproteinase-2 from human aortic smooth muscle. Matrix metalloproteinase-2 degrades elastin and type IV collagen and has been implicated in the development of abdominal aortic aneurysms (Robinson et al., 2006). Extracellular inorganic pyrophosphate (Pi) is a potent suppressor of pathologic calcification in blood vessels. Ectonucleotide pyrophosphatase/phosphodiesterases generate Pi via the hydrolysis of ATP released from rat cultured aortic smooth muscle (Prosdocimo et al., 2009). Plasminogen activator inhibitor-1 (PAI-1) is the primary regulator of plasminogen activation, and elevated levels shift the fibrolytic balance toward thrombosis. Treatment of rat aortic vascular smooth muscle cells with UTP increased PAI-1 mRNA expression and extracellular PAI-1 protein levels by 21- and 7-fold, respectively, whereas UDP stimulated a 5-fold increase in PAI-1 mRNA expression, suggesting that P2Y2 and P2Y6 receptors might be involved (Bouchie et al., 2000). They showed further that ADP produced an inhibitory effect, probably via P2Y1 receptors. Nonadrenergic, noncholinergic (NANC) nerve vasodilation mediated by ATP in rat thoracic aorta has been shown, which was blocked by capsaicin, suggesting that the ATP was released from sensory nerves (Park et al., 2000). It was claimed that the ATP acted on P2Y receptors on the endothelium to mediate vasodilatation via release of NO (Park et al., 2000), although it is hard to see how ATP released from perivascular nerves can reach the endothelial cells without being inactivated during its passage through hundreds of micrometers of smooth muscle and connective tissue. It would rapidly be metabolized by ectonucleotidases present on the endothelium and vascular smooth muscle (Côte et al., 1991, 1992; Kauffenstein et al., 2010a).

P1 (adenosine) receptors have been shown to be present on rabbit aortic smooth muscle (Ghai and Mustafa, 1982). In guinea pig aorta, both A1 and A2 receptor subtypes were identified (Stogall and Shaw, 1990), with some evidence for the expression of A2B receptors (Martin, 1992). A2 receptors are involved in endothelium-dependent relaxation of rat thoracic aorta (Moritoki et al., 1990; Rose’Meyer and Hope, 1990). Later articles have identified adenosine A2A receptors as the mediators of rat aortic endothelium-dependent vasodilation (Lewis et al., 1994; Ray and Marshall, 2006; Nayeem et al., 2008), although A2B receptors have also been implicated (Ansari et al., 2007a). A2A receptor endothelium-dependent relaxation was significantly reduced in A2A receptor knockout mice (Ponnoth et al., 2009). High-salt diet enhances mouse aortic relaxation through A2A receptors (Nayeem et al., 2009).

Endothelium-mediated contraction via A2 adenosine receptors has been described in mouse aorta, probably involving prostaglandins (Ansari et al., 2007b). Immunohistochemical studies of the localization of P1 receptor subtypes revealed differences in the localization of A1, A2A, A2B, and A3 receptors in rat aorta (elastic) and tail artery (muscular) (Leal et al., 2008).

B. Rat Tail Artery

Stimulation of sympathetic nerves supplying the rat tail artery produced EJPs and a slow depolarization; the slow depolarization was due to NA, whereas the EJPs were not blocked by adrenoceptor antagonists (Cheung, 1982). The EJPs were later shown to be due to ATP released as a cotransmitter from sympathetic nerves, because they were blocked by desensitization of P2X receptors by a,β-meATP (Sneddon and Burnstock, 1984b; Neild and Kotecha, 1986). Suramin, a P2 receptor antagonist, was also shown to block the ATP component of sympathetic neurotransmission of the rat tail artery (Bao and Stjärne, 1993). Coreleased NA and ATP from sympathetic nerves supplying the tail artery have synergistic postjunctional contractile actions on the smooth muscle (Johnson et al., 2001; Johnson, 2010).

Presynaptic α2-adrenoceptors mediate autoinhibition of the release of both NA and ATP from sympathetic nerves supplying the rat tail artery (Bao et al., 1991; Msghina et al., 1999), whereas presynaptic β-adrenoceptors mediate facilitation of NA and ATP release (Brock et al., 1997). Differential modulation of NA and ATP release by α2-adrenoceptors has been reported (Brock and Tan, 2004). There is modulation of NA release from sympathetic nerves in rat tail artery by inhibitory P2Y1 and P2Y12 receptors and facilitatory A2A receptors (Gonçalves and Queiroz, 1996; Fresco et al., 2007; Quintas et al., 2009). ET-1 inhibited NA and ATP release from sympathetic nerves in the rat tail artery, whereas high concentrations of ET-3 potentiated the release of both transmitters (Mutafova-Yambolieva and Westfall, 1998). Ca2+ channel blockers reduced the release of both NA and ATP from sympathetic nerves in the rat tail artery (Brock and Cunnane, 1999).

α,β-MeATP had potent contractile actions on the smooth muscle of the rat tail artery (Evans and Kennedy, 1994), suggesting that P2X1 and/or P2X3 receptors were involved. Evidence that ATP acts at two sites to evoke contraction of rat tail artery was presented, i.e., P2X1 receptors and G protein–coupled P2Y receptors (McLaren et al., 1998; Fukumitsu et al., 1999). Contraction of the smooth muscle of rat tail arteries was induced by UTP as well as ATP (Sańag et al., 1990),
suggested that P2Y2 and/or P2Y4 receptors were present as were P2X receptors. Decentralization of the sympathetic outflow to the rat tail artery led to hypersensitivity to α,β-meATP, and it was suggested that the enhanced response of the tail artery after spinal cord transection may contribute to the hypertensive episodes of autonomic dysreflexia in spinaly injured patients (Yeoh et al., 2004).

The amplitude of purinergic EJPs and α-adrenoceptor-mediated depolarizations declined significantly with age, the former to a greater degree than the latter (Jobling and McLachlan, 1992b). Long-term supplementation with a high cholesterol diet decreases the release of ATP from the tail artery of aged rats (Hashimoto et al., 1998b). Both purinergic and adrenergic components are present in reinnervating sympathetic fibers after denervation of rat tail arteries (Tripovic et al., 2011). The rat tail artery is important for both balance and temperature control. In a study of the changes in purinergic signaling in developing and aging rat tail arteries (Wallace et al., 2006), it was shown that functional (contractile) expression of P2X1, P2Y1, and P2Y2 and some P2Y4 receptors on smooth muscle appears postnatally but then decreases with age. P2X4 immunoreactivity was seen on both muscle and endothelial cells at 4–6 weeks, but not later in development; P2Y1 receptors on endothelial cells also decreased with age (Wallace et al., 2006). It was suggested that the presence of a large component of purinergic vasoconstrictor control of vascular tone in the tail artery, compared with mesenteric arteries, was associated with the need for efficient thermoregulation in young rats.

ATP and shear stress (known to release ATP from endothelial cells, see Burnstock, 1999) release NO from rat tail artery endothelial cells, resulting in vasodilation (Kwon et al., 1999). Hypotonic stress increased the [Ca2+]i and outflow of ATP from endothelial cells of the rat tail artery, as well as increasing cell volume (Shinozuka et al., 2001). It has been claimed that stimulation of adrenoceptors on endothelial cells of the rat tail artery leads to release of adenine nucleotides (Hashimoto et al., 1997). Inhibition of an antispasmodicogenic response to hydralazine (a smooth muscle relaxant) in tail arteries by ATP and adenosine was reported in 1981 (Chevillard et al., 1981). Adenosine, acting via A2A receptors, caused a concentration-dependent dilation of rat tail arteries precontracted with NA (Manickavasagar et al., 2009). The contractile responses of the rat tail artery to adenosine is probably mediated by endogenous 5-hydroxtryptamine (5-HT) (Brown and Collis, 1981).

C. Ear Artery

A seminal article showing release of ATP during antidromic stimulation of sensory nerves to produce vasodilatation of rabbit ear artery was published in 1951 (Holton and Perry, 1951; see Holton, 1959). The first hint of sympathetic cotransmission to rabbit ear artery came from experiments that showed that long-term denervation decreased both NA and ATP contents of the artery (Head et al., 1977). The use of reserpine and 6-hydroxydopamine (to deplete catecholamines and damage sympathetic nerve endings, respectively) produced supporting evidence for purinergic cotransmission in the rabbit ear artery (Saville and Burnstock, 1988). Short pulse bursts at low frequency (2–5 Hz) favor the purinergic component of sympathetic nervous control of rabbit ear artery, whereas higher frequencies favor the adrenergic component (Kennedy et al., 1986). Electrical field stimulation of the rabbit ear artery produced release of NA and ATP in a ratio of 1:180, but the ATP release was significantly reduced in endothelium-denuded preparations (Ishii et al., 1996).

EJPs were also recorded in the smooth muscle of guinea pig ear artery and phentolamine (α-adrenoceptor antagonist) enhanced their amplitude (Kajiwara et al., 1981), perhaps indicating block of prejunctional adrenoceptors that inhibit the release of ATP. A combination of prazosin blockade of α1-adrenoceptors and α,β-meATP desensitization of P2X receptors blocked the constrictor responses of guinea pig ear artery to sympathetic nerve stimulation (Morris, 1994). Regional differences in sympathetic cotransmission in guinea pig proximal and distal ear artery vessels were revealed, with the purinergic component greater in the proximal compared with the distal segments (Morris et al., 1998). It was suggested that these differences are related to the responses to thermoregulatory stimuli, which occur predominantly in the distal segments.

α,β-MeATP caused constriction of the rabbit perfused ear artery via actions at P2X receptors, but vasodilatation was elicited by ATP, presumably via P2Y receptors expressed on endothelial cells because they could be blocked by reactive blue 2 (Taylor et al., 1989; Xie et al., 1997). Although there is a lack of consensus about the P2Y versus P2X selectivity of reactive blue 2, it consistently does not block vasorelaxations to adenosine (Reilly et al., 1987; Rump et al., 1998; Glänzel et al., 2003). α,β-MeATP was the most potent agonist of a series of analogs producing contractions of the rabbit ear artery (O’Connor et al., 1990), suggesting that the receptor involved is the P2X1 (or P2X3) subtype. The responses to α,β-meATP were antagonized by the P2 selective antagonists suramin (Leff et al., 1990; von Kügelgen and Starke, 1990a) and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) (Ziganshin et al., 1994). Ionophoretic application of ATP to single smooth muscle cells dispersed from rabbit ear artery induced cation conductance (Benham et al., 1987; Benham, 1989). UTP, equipotent with ATP, produced vasoconstriction of the rabbit ear artery (von Kügelgen et al., 1987), indicating the presence of P2Y2 and/or P2Y4 receptors on the smooth muscle.
ATP produces vasodilatation via P1 receptors located on both smooth muscle and endothelial cells and vasoconstriction via P2 receptors on the smooth muscle of the rabbit ear artery (Kennedy and Burnstock, 1985a). A significantly greater dilator response to adenosine applied luminally compared with that applied at the adventitial surface in experiments with rabbit perfused ear artery was reported (Headrick et al., 1992). Adenosine inhibited nerve-mediated contractions of both rabbit ear and mesenteric arteries; however, the amplitude of EJPs was increased by adenosine in the ear artery, but decreased in the mesenteric artery, perhaps indicating postjunctional events in the ear artery and prejunctional events in the mesenteric artery (Zhang et al., 1989). CGRP, a neurotransmitter in sensory-motor nerves, attenuated sympathetic vasoconstrictor cotransmission in the rabbit isolated ear artery, and this involved a postjunctional mechanism (Maynard et al., 1990). ET-1 enhances vasoconstrictor responses to exogenously administered and neurally released ATP in rabbit perfused ear arteries (La and Rand, 1993).

### D. Mesenteric Vessels

#### 1. Mesenteric Veins

EJPs have been recorded in the dog mesenteric vein, and isoprenaline, acting prejunctionally, increased the amplitude of the EJP but decreased the evoked released of NA (Seki et al., 1990), suggesting that ATP may be released as a cotransmitter from sympathetic nerves. The different effects of isoprenaline on the ATP and NA components of sympathetic neurotransmission is consistent with the suggestion that ATP and NA are nonuniformly stored in different classes of vessels within sympathetic varicosities (Dunn et al., 1999; Brock et al., 2000). The purinergic component of sympathetic vasoconstriction of guinea pig mesenteric veins was greater than that of mesenteric arteries (Smyth et al., 2000). However, release of ATP from sympathetic nerves in canine mesenteric vessels was equal in arteries and veins (Bobalova and Mutafova-Yambolieva, 2001). In a study using the perfused mesenteric bed of the rat, it was claimed that although ATP elicited arterial constriction predominantly via P2X receptors, venous constriction was via P2U (P2Y2 and/or P2Y4) receptors (Ohara et al., 1998).

It has been claimed that multiple P2Y receptors (P2Y1 and P2Y2/4) mediate contraction of the guinea pig mesenteric vein in addition to P2X1 receptors (Mutafova-Yambolieva et al., 2000). NTPDase activity has been described in guinea pig mesenteric veins as well as arteries (Duval et al., 2003). Modulation of transmitter release from sympathetic nerves is mediated by prejunctional P2Y as well as P1 receptors in mesenteric veins, in contrast to mesenteric arteries, where prejunctional modulation is mediated largely by P1 receptors (Talaia et al., 2011).

#### 2. Mesenteric Arteries

In early studies, EJPs were recorded in the dog mesenteric artery in response to field stimulation, although these were considered erroneously to be due to release of NA (Inoue et al., 1983). Studies of rabbit and guinea pig mesenteric arteries led to the conclusion that ATP was likely to be responsible for the generation of EJPs and fast initial transient contraction, whereas NA produced the later sustained contraction (Ishikawa, 1985; Rhee and Wier, 2007). EJPs were shown to be blocked by α, β-meATP desensitization of P2X receptors in guinea pig mesenteric artery (Angus et al., 1988; Nagao and Suzuki, 1988; Meehan et al., 1991). Corelease of NA and ATP from sympathetic nerves supplying the rabbit and dog mesenteric artery was reported (Illes and Nörenberg, 1987; Muramatsu, 1987). Bradykinin facilitates sympathetic purinergic neurotransmission of rat mesenteric arteries (Kansui et al., 2012).

An elegant study of purinergic signaling in small and larger diameter hamster mesenteric arteries confirmed that EJPs in response to nerve stimulation were mediated by P2X receptors (Thapaliya et al., 1999). They also showed that ATP released from perivascular sympathetic nerves activated P2Y2 receptors on the endothelium of the small (but not larger) arteries to act via EDHF. The relaxant action of ATP released by electrical field stimulation of rabbit small mesenteric arteries was similarly claimed to be partially endothelium dependent, but was resistant to inhibition of NOS (Kakuyama et al., 1998). An electrophysiological study of the longitudinal smooth muscle of the chicken anterior mesenteric artery showed that electrical field stimulation produced excitatory membrane responses mediated by ATP acting largely via P2Y receptors (Khalifa et al., 2005).

β-NAD has been claimed to be a sympathetic cotransmitter in the canine mesenteric artery (Smyth et al., 2006). N-type and P/Q-type calcium channels regulate differentially the release of NA, ATP, and β-NAD from sympathetic nerves supplying canine mesenteric blood vessels; N-type channels are equally expressed by arteries and veins, whereas P/Q-type channels are more pronounced in veins (Smyth et al., 2009).

Prejunctional modulation of NA release from sympathetic nerves supplying rabbit and guinea pig mesenteric arteries by ATP and adenosine was reported (Kuriyama and Makita, 1984; Lautt et al., 1988), largely involving A1 receptors (Illes et al., 1988). Prejunctional α2-adrenoceptor activation inhibits sympathetic cotransmitter release from rabbit and dog mesenteric arteries (Illes and Nörenberg, 1987; Muramatsu et al., 1989). Prejunctional α2-adrenoceptors inhibit ATP release during low-frequency, brief duration sympathetic nerve stimulation in guinea pig mesenteric artery (Mutafova-Yambolieva and Keef, 2001). Exogenous and locally generated Ang-II enhanced the purinergic component of sympathetic neurotransmission in the guinea pig mesenteric artery (Onaka et al., 1997).
Pressure is an important determinant of the size of the purinergic component of the response to sympathetic neurotransmission, and it has been shown that ATP is the predominant sympathetic neurotransmitter in rat mesenteric arteries at physiologic pressure; at low pressure the ATP component was significantly reduced (Rummery et al., 2007). An important implication is that the purinergic component of sympathetic neurotransmission may have been underestimated in experimental studies in which the pressure has been kept relatively low. Low- and high-frequency stimulation of sympathetic nerves in rat mesenteric arteries favors the purinergic and noradrenergic components respectively (Sjöblom-Widfeldt and Nilsson, 1990; Lamont and Wier, 2002). Nerve-evoked P2X1 receptor-mediated contractions dominated in rat small and medium sized mesenteric arteries, whereas the noradrenergic component dominated in large (first order) mesenteric arteries (Gitterman and Evans, 2001). Spontaneous release of ATP and NA from sympathetic nerve terminals in rat mesenteric arteries appears to be from different vesicles (Brock et al., 2000). It was claimed recently that breast feeding increases the contractile response of rat mesenteric arteries to sympathetic nerve stimulation, due mainly to increased release of the cotransmitter ATP (Blanco-Rivero et al., 2013).

The main P2 receptor mediating EJPs and contractile responses to ATP is the P2X1 receptor expressed on the vascular smooth muscle. RT-PCR studies of P2X purinoceptor mRNA expression in rat mesenteric artery showed strong expression of P2X1 and P2X4 receptors, less of P2X7, and only weak expression of P2X2, P2X3, and P2X5 receptors (Phillips and Hill, 1999). Immunohistochemical studies showed a dominance of P2X1 receptors on rat mesenteric arterial smooth muscle but also some expression of P2X4 and P2X5 receptor proteins in small and medium-sized arteries (Gitterman and Evans, 2000). The dominance of P2X1 receptors in mediating currents in smooth muscle cells of the rat mesenteric artery was confirmed, and no evidence for an involvement of P2X4, P2X5, or P2X1/5 receptors was found (Lewis and Evans, 2000). 2’3’O-(2,4,6-Trinitrophenyl)-ATP blocked the responses of rat mesenteric arteries to α,β-meATP (Lewis et al., 1998), whereas in mouse mesenteric arteries, the contractile response to α,β-meATP was blocked by the P2X1 receptor antagonist NF023 (1,3,5-trisodium 8-[(3-([3-([4,6,8-tris[(sodioxy)sulfonyl]naphthalen-1-yl]carbamoyl)phenyl]carbamoyl)amino)benzene]amido[naphthalene-1,3,5-trisulfonate]) (Koltsova et al., 2009), suggesting that P2X1 receptors were involved. Studies with P2X1 receptor knockout mice established that P2X1 and P2Y6-like receptors mediate sympathetic neurogenic vasoconstriction of mouse mesenteric arteries (Vial and Evans, 2002). A spliced isoform of the P2X1 receptor, P2X1α, was cloned from rat mesenteric arteries (Okkubo et al., 2000).

Contractile P2Y receptors (P2Y2 and P2Y6) are co-expressed with P2X receptors on the mesenteric arterial smooth muscle. Both ATP and UTP increase [Ca2+]i, in smooth muscle cells of rat small mesenteric arteries (Juul et al., 1992). Low concentrations of ATP were shown to activate P2X receptors, whereas high concentrations additionally activated P2Y receptors (Lagaud et al., 1996). Contractile responses to UTP, mediated by P2Y2 receptors, were significantly more potent in small compared with medium and larger rat mesenteric arteries (Gitterman and Evans, 2000). Contractile responses of the smooth muscle of the rat mesenteric artery were elicited by stimulation of P2Y6 as well as P2X1 receptors (Malmsjö et al., 2000a). Similarly, in mouse mesenteric arteries, there was evidence for contractile responses mediated by both P2Y6 and P2X1 receptors, because responses to UTP and UDP were reduced by a selective P2Y6 antagonist, and a contractile response to α,β-meATP was blocked by the P2X1 receptor antagonist NF023 (Koltsova et al., 2009). G protein-coupled receptor kinase 2 and arrestin-2 regulate UTP-stimulated P2Y2 receptors mediating constriction of rat mesenteric artery smooth muscle (Morris et al., 2011). Whole-cell voltage clamp studies of guinea pig mesenteric terminal arterioles have suggested that ATP activation of Ca2+ currents was mediated by P2Y1- and P2Y11-like receptors (Morita et al., 2002).

In the developing rat mesenteric artery, contractile responses to UTP were greatest at 4–6 weeks postnatal and then declined (Wallace et al., 2006). In rat mesenteric arteries, ATP-induced contraction decreased with age (Konishi et al., 1999). Excitatory innervation of the longitudinal smooth muscle of the chicken anterior mesenteric artery develops during the early postnatal period; cholinergic excitatory neurotransmission precedes purinergic neurotransmission, although purinoceptors are already expressed in the smooth muscle (Alkayed et al., 2011).

An early study showed that ATP dilated the rabbit perforated mesenteric artery (Krishnamurty and Kadowitz, 1983). In this vessel, ATP acts on purinergic receptors on smooth muscle to produce contraction via P2X receptors and additionally endothelium-independent relaxation via P2Y receptors (Mathieson and Burnstock, 1985; Burnstock and Warland, 1987b). Striking evidence for species differences between rabbit and rat mesenteric arterial purinergic signaling was presented in later studies, with the observation that ATP relaxation of rat mesenteric arteries was elicited largely via the endothelium (Vuorinen et al., 1992). Similarly, hyperpolarizations to ATP in hamster mesenteric arteries were endothelium dependent (Thapaliya et al., 1999), as were relaxations to ATP, ADP, and UTP in the hamster mesenteric arterial bed (Ralevic and Burnstock, 1996b).

ATP and UTP both elicit endothelium-dependent vasodilation of rat mesenteric arteries; the ATP effects
are via P2Y<sub>1</sub> receptors and release of NO and EDHF, whereas the UTP effects are via P2U (P2Y<sub>2</sub> and/or P2Y<sub>4</sub>) receptors by a non-NO mechanism, perhaps involving EDHF (Malmsjö et al., 1998, 1999b). Subsequent studies showed that endothelium-dependent relaxation of the rat mesenteric artery was mediated by P2Y<sub>1</sub>, P2Y<sub>2</sub>, and P2Y<sub>4</sub> (but not P2Y<sub>6</sub>) receptors (Malmsjö et al., 2000a). Direct luminal perfusion of ATP and UTP in rat small mesenteric arteries leads to spreading dilatation via P2Y receptor activation of EDHF (Winter and Dora, 2007). Luminal application of the P2Y<sub>1</sub> receptor agonists ADP and ADPβS to rat pressurized small mesenteric arteries induced EDHF-type dilations and endothelial cell Ca<sup>2+</sup> increases, which rapidly desensitize by mechanisms involving PKC (Rodriguez-Rodriguez et al., 2009). In contrast, in hamster mesenteric arteries only ATP and UTP, but not 2-MeSATP (P2Y<sub>1</sub> receptor agonist), elicited endothelial cell hyperpolarization, suggesting an involvement of P2Y<sub>2</sub> receptors (Thapaliya et al., 1999). Diadenosine pentaphosphate, Ap5A, caused constriction of rat small mesenteric arteries via P2X<sub>1</sub> receptors and vasodilation via P2Y<sub>1</sub> receptors (Steinmetz et al., 2000; Lewis et al., 2000b).

It has been claimed that P2X<sub>1</sub> receptors on the endothelium of rat mesenteric arteries mediate vasodilation, which is unaffected by the NOS inhibitor l-N<sup>ω</sup>-nitroarginine methyl ester or the prostaglandin inhibitor indomethacin (Harrington and Mitchell, 2004). Vasorelaxant P2X<sub>1</sub> receptors mediating endothelium-dependent vasorelaxation to ATP have also been claimed on mouse mesenteric arteries (Harrington et al., 2007). In chicken small mesenteric vessels, ATP mediates endothelium-dependent hyperpolarizations of circular smooth muscle cells via P2Y<sub>1</sub> receptors (Draid et al., 2005) and endothelium-dependent hyperpolarization of longitudinal smooth muscle via P2X receptors (Alkayed et al., 2009). Endothelium-dependent and -independent relaxations to GTP and guanosine in rat mesenteric arteries do not appear to involve P1 or P2 receptors (Vuorinen et al., 1994).

In rat isolated mesenteric artery, adenosine receptor agonists elicit vasodilatation via A<sub>2B</sub> receptors located on the smooth muscle and via a second undefined site (possibly intracellular) (Prentice et al., 1997). In contrast, studies carried out in rat small mesenteric arteries showed that smooth muscle relaxation by adenosine may involve A<sub>2A</sub> receptors that inhibit store-operated Ca<sup>2+</sup> entry-mediated increases in cytosolic Ca<sup>2+</sup> levels enhanced by the emptying of the stores (Wang et al., 2009c). A<sub>2B</sub> receptors are the prominent P1 receptor subtype in mouse mesenteric arterial smooth muscle, whereas A<sub>1</sub> receptors play a modulatory role (Teng et al., 2013).

Congestive heart failure is accompanied by enhanced peripheral sympathetic nerve activity, increased vascular resistance, and impaired peripheral blood flow. Congestive heart failure induces downregulation of P2X<sub>1</sub> receptor-stimulated contraction in the mesenteric artery, whereas P2Y receptor-mediated contractile activity remains unchanged (Malmsjö et al., 1999a).

3. Mesenteric Arterial Bed. ATP acts as an important functional sympathetic neurotransmitter constricting the rat perfused mesenteric vascular bed under raised tone conditions, where the perfusion pressure is closer to that found in vivo (Pakdeechote et al., 2007b). The purinergic neurogenic responses were characterized using α,β-meATP, indicating a likely involvement of P2X<sub>1</sub> receptors; at basal tone (unconstricted preparations), responses to nerve stimulation were mediated solely by activation of α<sub>1</sub>-adrenoceptors, but at a raised tone, the responses were partly sensitive to α,β-meATP (Pakdeechote et al., 2007b). As with rat isolated mesenteric arteries at physiologic pressures (Rummery et al., 2007), this suggests that the purinergic component of sympathetic neurotransmission may have been underestimated in studies in which cotransmission has been investigated under relatively low pressures. In the rat perfused mesenteric bed, neuropeptide Y (NPY), released together with NA and ATP from sympathetic nerves (Donoso et al., 1997), enhanced the postjunctional actions of both cotransmitters (Westfall et al., 1995). Cannabinoids inhibit both noradrenergic and purinergic components of sympathetic cotransmission in the rat mesenteric arterial bed (Pakdeechote et al., 2007a). Prejunctional inhibition of sympathetic vasoconstriction of the hamster mesenteric arterial bed is mediated by A<sub>1</sub> (but not A<sub>2</sub>) receptors (Ralevic, 2000a).

Smooth muscle P2X receptors mediating contractile responses to ATP have been described in mesenteric arterial beds of both the rat (Ralevic and Burnstock, 1988, 1991) and golden hamster (Ralevic and Burnstock, 1996b). Contractile responses were also evoked by α,β-meATP, which blocked responses to purine nucleotides, indicating likely actions at P2X<sub>1</sub> receptors (Ralevic and Burnstock, 1988, 1991, 1996b). Selective inhibition by PPADS of contraction mediated by P2X receptors in the perfused rat mesenteric bed was reported (Windscheif et al., 1994). P2X<sub>1</sub> receptor-mediated vasoconstriction of the rat mesenteric arterial bed was unaffected by reduction of pH (to 6.9), but contractions to NA were greatly attenuated (Ralevic, 2000b).

The effects of purines and pyrimidines were studied on the rat mesenteric arterial bed, and it was shown that, in addition to ATP acting via P2X receptors to mediate vasoconstriction, receptors responsive to UTP mediated both vasoconstriction of smooth muscle and vasodilation via endothelial cells (Ralevic and Burnstock, 1991), possibly involving P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors. mRNA encoding for P2Y<sub>1</sub>, P2Y<sub>2</sub>, and P2Y<sub>6</sub> receptors, but not P2Y<sub>4</sub> receptors, was detected in the rat arterial mesenteric bed and, after endothelium removal, which abolished nucleotide-evoked relaxations,
only P2Y<sub>6</sub> mRNA was found; this shows a possible involvement of the P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors in endothelium-mediated vasodilation, whereas the P2Y<sub>6</sub> receptors expressed on the smooth muscle may be involved in vasocostriction (Buvinic et al., 2002). PPADS antagonized P2Y<sub>1</sub>-mediated ADP vasodilation of the rat mesenteric arterial bed, but not UTP-evoked vasodilatation at P2U (P2Y<sub>2</sub> and/or P2Y<sub>4</sub>) receptors (Ralevic and Burnstock, 1996c). A selective P2Y<sub>1</sub> receptor antagonist, MRS2179 ([2-[(hydroxy-oxidophosphoryl)oxy-methyl]-5-(6-methylaminopurin-9-yl)oxolan-3-yl] hydrogen phosphate), also inhibited endothelium-dependent relaxations to 2-MeSATP but not those to UTP in the rat mesenteric arterial bed, showing distinct sites of action of the two nucleotides (Buvinic et al., 2002). It has been shown that diadenosine triphosphate (Ap<sub>3</sub>A) and NADP mediate vasodilation via P2Y<sub>1</sub> receptors, whereas vasodilation to diadenosine diphosphate is mediated partly via P1 and possibly also via P2U (P2Y<sub>2</sub> and/or P2Y<sub>4</sub>) receptors (Ralevic et al., 1995b). Nucleotide vasodilation of the rat mesenteric arterial bed via P2<sub>1</sub> and P2<sub>2</sub>/P2Y<sub>2</sub> receptors on endothelium is mediated primarily by EDHF, with only a small involvement of NO (Malmsjö et al., 2002). In the mesenteric arterial bed of the golden hamster, ATP elicited vasoconstriction via P2X receptors on smooth muscle and vasodilation via P2U (P2Y<sub>2</sub> and/or P2Y<sub>4</sub>) receptors located on endothelium; in contrast to the rat mesenteric arterial bed, there was minimal expression of vasorelaxant endothelial P2Y<sub>1</sub> receptors (Ralevic and Burnstock, 1996b).

ATP has been shown to stimulate vasodilation in the rat mesenteric arterial bed by two different mechanisms producing a biphasic vasorelaxant response (Ralevic, 2001; Stanford et al., 2001). The first, rapid, phase involves the endothelium and NO (Stanford et al., 2001; Ralevic, 2002) and likely involves endothelial P2Y<sub>1</sub>, P2Y<sub>2</sub>, and/or P2Y<sub>4</sub> receptors (described above). The second, prolonged, relaxation was blocked by a<sub>β</sub>-meATP, suggesting an involvement of P2X receptors (Ralevic, 2002). It has been claimed that the second phase involves P2X<sub>1</sub> receptors expressed on the endothelium and generation of EDHF (Stanford et al., 2001; Harrington and Mitchell, 2004). However, in rat isolated mesenteric arteries, EDHF-mediated relaxations to ATP were enhanced after a<sub>β</sub>-meATP desensitization of P2X<sub>1</sub> receptors, and it was suggested that depolarization produced by ATP activation of smooth muscle P2X<sub>1</sub> receptors normally counteracts EDHF produced by concomitant ATP activation of endothelial P2 (P2Y) receptors (Malmsjö et al., 2000b).

In addition to P2 receptor control of mesenteric microvascular tone, adenosine elicits mesenteric vasodilator actions via A<sub>2B</sub> receptors located on the smooth muscle cells (Rubino et al., 1995), although another study showed that vasodilation produced by adenosine in the rat perfused mesenteric arterial bed is mediated partly via the endothelium (Tabrizchi and Lupichuk, 1995). There is a more recent review of purinergic receptors in the splanchnic microcirculation (Morato et al., 2008).

### E. Coronary Vessels

AMP has been known to be a potent dilator of coronary vessels since 1929 (Drury and Szent-Györgyi, 1929), and ATP, ADP and adenosine were later also shown to relax coronary vessels (Green and Stoner, 1950; Berne, 1963; Toda et al., 1982).

Berne (1963) hypothesized that adenosine was the physiologic regulator of blood flow during reactive hyperemia, and this hypothesis dominated the field for the next decade, although clear supporting evidence was lacking (see Nees et al., 1985; Zucchi et al., 1989). For example, an adenosine receptor antagonist, theophylline, failed to block reactive hyperemia, although it blocked the vasodilator actions of exogenously applied adenosine (Afonso et al., 1972; Bünger et al., 1975). Intracoronary adenosine and ATP increased coronary blood flow, but reactive hyperemia in the dog heart after occlusion was not blocked by the adenosine receptor antagonist aminophylline (Eikens and Wilcken, 1973a,b; Giles and Wilcken, 1977; Clemens et al., 1985a). ATP appeared early in the coronary sinus effluent from isolated working rat hearts in response to hypoxia (Clemens and Forrester, 1981). Also, adenosine was not collected in the effluent until long after the hyperemic response occurred, because ADP, after breakdown of released ATP, acts as an inhibitor of 5'-nucleotidase breakdown of AMP to adenosine (Ishibashi et al., 1985). Years later, after the seminal studies of Robert Furchgott and others showed that ATP acted on endothelial P2 receptors to release NO, resulting in vasodilation, and it was shown that ATP was released from endothelial cells by hypoxia and increased flow (Bodin et al., 1991; Vials and Burnstock, 1996), Burnstock (1993b) proposed that reactive hyperemia was due to ATP release from endothelial cells during hypoxia acting to release NO. This took place rapidly, and only later, after ATP was broken down by ectoenzymes to adenosine, did coronary vasodilation of vascular smooth muscle via P1 receptors contribute to the hyperemic response.

In addition to hypoxia, ATP release from cardiac endothelial cells has been shown in response to ACh, bradykinin, 5-HT, and ADP (Yang et al., 1994). ATP, and especially ADP (after breakdown of ATP by ectoenzymes) released from aggregating platelets, causes endothelium-dependent relaxation of canine coronary arteries (Houston et al., 1985). Maximal coronary vasodilatation can be obtained safely with intracoronary ATP (Kato et al., 1999) and also adenosine (Wilson et al., 1990) administration in humans. Indeed, ATP and sodium nitroprusside have been administered to patients to induce and control hypotension during anesthesia (Hashimoto et al., 1982).
Intracoronary adenosine, ATP, and ADP elicit their dilating effects largely via the endothelium (Nees, 1989), implying that both P1 and P2 receptors are expressed by coronary endothelial cells. ATP and adenosine hyperpolarized guinea pig cultured coronary endothelial cells; the adenosine hyperpolarization, but not that to ATP, was antagonized by theophylline (Mehrke and Daut, 1990), indicating that both P1 and P2 receptors are present. Keef et al. (1992) presented evidence for the presence of P2Y receptors on endothelial cells mediating hyperpolarization and P1 and P2 receptors on smooth muscle mediating relaxation in guinea pig and rabbit coronary arteries. It was suggested that ATP may be a more significant relaxant of canine large coronary arteries than adenosine, but that adenosine may be a more significant relaxant than ATP in canine small coronary arteries (White and Angus, 1987). Endothelium-dependent vasodilation evoked by ATP has also been demonstrated in human isolated coronary arteries (Hansmann et al., 1998; Kato et al., 1999). However, direct smooth muscle (endothelium-independent) relaxation to ATP and UTP of human epicardial coronary arteries was also reported (Saetrum Opgaard and Edvinsson, 1997), and ATP was shown to hyperpolarize smooth muscle cells of the guinea pig coronary artery (Takata and Kuriyama, 1980).

In perfused guinea pig heart, vasodilatation by ATP was reported to involve NO (Vials and Burnstock, 1994a; Matsumoto et al., 1997b), although another study concluded that ATP-induced vasodilation of guinea pig heart did not depend on NO production but may be partially dependent on the production of vasodilator prostaglandins (Brown et al., 1992). Adenosine contributes very little to coronary vasodilation in guinea pig hearts from bolus injection of ATP and ADP (Brown et al., 1992; Gorman et al., 2003). ATP infusion in rat heart was associated with large increases in myocardial blood flow (Hoffman et al., 1982). P2Y1 and P1 receptors were identified as the receptor subtypes involved in purinergic endothelium-dependent vasodilation in rat (Vials and Burnstock, 1994b; Korchazhkina et al., 1999) and dog (Bender et al., 2011) heart microvessels. P2Y1 and P2Y2 receptors were described in primary cultures of rat cardiac microvascular endothelial cells (Moccia et al., 2001). There is evidence for UTP-sensitive P2U receptors (P2Y2 and/or P2Y4), as well as P2Y receptors, on human, canine, and guinea pig cardiac endothelial cells (Yang et al., 1996; Matsumoto et al., 1997a; Zünkler et al., 1999). ATP and adenosine have opposing effects on barrier function of the rat coronary microvasculature (Gündüz et al., 2012).

Studies of purine receptors in human, porcine, rabbit, and rat hearts have shown that, in addition to the vasorelaxant, predominantly endothelial P2Y and P1 receptors described above, P2X and P2Y receptors mediating vasoconstriction are expressed on the coronary artery smooth muscle (Fleetwood and Gordon, 1987; Hopwood and Burnstock, 1987; Corr and Burnstock, 1994; Malmşjö et al., 2000c; Rayment et al., 2007a). Evidence was presented that human coronary artery smooth muscle cells express P2Y and P2U (P2Y2 and/or P2Y4) receptors, leading to increases in [Ca2+]i by 2-MeSATP and UTP, respectively (Strøbaek et al., 1996). RT-PCR studies of P2 receptors in human coronary arteries showed strong bands for P2X1 and P2Y2 receptors, with weaker expression of P2Y1, P2Y4, and P2Y6 receptors (Malmşjö et al., 2000c). In the same study, contractile responses of human coronary arteries to α,β-meATP and the stable pyrimidine analog, uridine 5’-O-3-thiotriphosphate, were consistent with activation of P2X1 and P2Y2 receptors, respectively (Malmşjö et al., 2000c). Similarly, UTP-evoked contractions of porcine coronary artery smooth muscle appear to be predominantly P2Y2 receptor-mediated (Rayment et al., 2007a). Coexpression of mRNAs for P2X, P2X2, and P2X4 receptors was shown on the smooth muscle of rat coronary arteries (Nori et al., 1998). UTP was shown to elicit depolarization of rat coronary artery smooth muscle (Welsh and Brayden, 2001). The dinucleoside polyphosphates [Ap5A and diadenosine hexaphosphate (Ap6A)] vasodilate or vasoconstrict rat coronary vessels via P2Y1 receptors on endothelial cells and P2X receptors on smooth muscle, respectively (van der Giet et al., 2002). Up4A has been identified as a novel vasodilator of the coronary microcirculation of swine hearts, acting, possibly through a breakdown product, via P1 but not P2 receptors (Zhou et al., 2013). Human epicardial coronary veins have been shown to contract in response to ATP (Saetrum Opgaard and Edvinsson, 1997).

Transient depolarizations comparable to EJPs were evoked in the smooth muscle of guinea pig circumflex coronary artery by transmural stimulation (Keef and Kreulen, 1988), suggesting that there may be a purinergic component of sympathetic nerve transmission. ATP has been claimed to be the mediator of NANC inhibitory transmission in lamb coronary small arteries via actions at P2Y receptors (Simonsen et al., 1997).

Adenosine is a potent coronary dilator in all species studied, including humans (Cobbín et al., 1974; Olsson et al., 1979; Watt et al., 1987). It can arise directly from cardiomyocytes after intracellular breakdown of ATP and after extracellular breakdown of ATP released from endothelial cells (Pekka Raatikainen et al., 1991). It was suggested that the vasodilator actions of adenosine were mediated by A2 receptors (Leung et al., 1985; Daly et al., 1986; Odawara et al., 1986; see Mustafa et al., 2009), probably located on endothelial cells (Kroll et al., 1987; Des Rosiers and Nees, 1987; Ramagopal et al., 1988a; Ballell et al., 1992; King et al., 1990), perhaps together with another P1 receptor subtype (Nees et al., 1987). Methylxanthines antagonized A2 receptor-mediated coronary vasodilation (Ramagopal
et al., 1988b). Evidence was presented that A2B receptors mediate relaxation to adenosine in human small coronary arteries, which is independent of the endothelium and NO (Kemp and Cocks, 1999). However, Sato et al. (2005) claimed that, in human coronary arteriolar smooth muscle, it is A2A receptors that mediate vasodilation. A review discusses the role of adenosine in dilation of human coronary vessels (Heuschat, 2010). A2A receptors also mediate relaxation of porcine isolated coronary arteries (Rayment et al., 2007b); although A2A receptors are the predominant subtype, A2B receptors may play a role, possibly through the p38 mitogen-activated protein kinase (MAPK) pathway (Teng et al., 2005). Adenosine A2A and A2B receptors mediate NO production in porcine coronary artery endothelial cells (Olanrewaju and Mustafa, 2000). Both A2A and A2B Receptors mediate coronary vasodilation in mice (Flood and Headrick, 2001; Talukder et al., 2003) and an involvement of KATP channels was demonstrated (Sanjani et al., 2011). Upregulation of A2B receptors in A2A receptor knockout mouse coronary artery has been reported (Teng et al., 2008; Sanjani et al., 2011).

An A1-selective agonist, N6-cyclopentyladenosine, was shown to have vasorelaxant activity in porcine and canine coronary arteries (Merkel et al., 1992; Cox et al., 1994). A1 receptors were identified on smooth muscle cells isolated from the porcine coronary artery (Dart and Standen, 1993) as well as A2 receptors (Abebe et al., 1994). Evidence for A3 receptors in the rat coronary circulation has also been presented (Hinschen et al., 2003). In the guinea pig heart, endothelium-dependent coronary vasodilation was shown to be due to multiple P1 receptor subtypes: A1 receptors releasing both NO and PGI2 and A2A and A3 receptors acting mainly via NO (Rubio and Ceballos, 2003). There is evidence for adenosine receptor-mediated hyperpolarization of bovine and porcine coronary artery smooth muscle (Sabouni et al., 1989; Olanrewaju et al., 1995) and of guinea pig cultured coronary endothelial cells (Seiss-Geuder et al., 1992). Ticagrelor, a P2Y12 receptor antagonist, enhances adenosine-induced coronary vasodilatory responses in humans (Wittfeldt et al., 2013). NAD was shown to be a coronary vasodilator in 1971 (Afonso et al., 1971). Inosine transiently decreases coronary flow, but potentiates vasodilation by adenosine (van der Meer and de Jong, 1990). Diadenosine tetraphosphate (Ap4A) elicited coronary vasodilation, probably via P1 receptors (Nakae et al., 1996). Diadenosine polyphosphates are potent constituents of human coronary artery, radial artery, and saphenous vein bypass grafts, and it was suggested that this may play a role in postoperative contraction in these grafts (Conant et al., 2008).

The rabbit heart requires a period of maturation after birth before demonstrating an adult level of coronary responsiveness to exogenous adenosine (Buss et al., 1987). It was suggested that several adenosine receptor subtypes mediate coronary vasodilation in mature rats, but a reduction in the response to adenosine with age may be due to changes in the high-affinity receptor site (Rose et al., 1999) and/or a reduction in adenosine receptor transduction (Hinschen et al., 2001) and/or a reduction of A3 receptor-mediated activity (Hinschen et al., 2003; Jenner and Rose, 2006).

Long-term signaling by adenosine in cardiac microvascular cells has been described in vasculogenesis (Ryzhov et al., 2008). Myocardial blood flow during adenosine-mediated hyperemia was reduced in male endurance athletes compared with untrained men, and the fitter the athlete was, the lower was adenosine-induced myocardial blood flow, although A2A receptors density was unchanged (Heinonen et al., 2008). ATP appears to be one of the factors controlling coronary blood flow during exercise (Farias et al., 2005; Gorman et al., 2010, Gorman and Feigl, 2012). ATP (via P2Y receptors) and insulin act synergistically to promote proliferation of porcine cultured coronary artery smooth muscle cells (Agazie et al., 2001). Sexual dimorphism in the permeability response of coronary microvessels to adenosine has been demonstrated (Huxley et al., 2005).

F. Cerebral Vessels

Adenosine dilates cerebral blood vessels (Berne et al., 1974; Boisvert et al., 1978; Gregory et al., 1980) via P1 receptors (Beck et al., 1984; Palmer and Ghai, 1982; Schütz et al., 1982). Adenosine caused dilation of rabbit hypothalamic blood vessel smooth muscle at low concentrations, but constriction at high concentrations (Livemore and Mitchell, 1983). Adenosine receptors were described on capillaries in the rat brain (Palmer and Ghai, 1982). A2 receptors were identified, mediating relaxation of cat cerebral arteries (Edvinsson and Fredholm, 1983) and rabbit cerebral microvessels (Li and Fredholm, 1985). It has been claimed that human cerebral microvessels express A2, but not A1, receptors (Kalaria and Harik, 1988). A2 receptors have also been described in porcine basilar arteries (McBeen et al., 1988), goat cerebral vessels (Torregrosa et al., 1990), and rat pial arterioles (Ibayashi et al., 1991). Both A2A and A2B receptors were later identified on vascular smooth muscle, mediating vasodilation in rat cerebral cortex (Coney and Marshall, 1998; Shin et al., 2000a). In rat pial arteries, A2B receptors were present on endothelial cells, mediating vasodilation via NO (Shin et al., 2000b; Ngai et al., 2001). A2A receptors mediate glutamate-evoked arteriolar dilation in the rat cerebral cortex (Illff et al., 2003).

A1 and A3 receptor subtypes have been identified on endothelial cells in rat pial and intracerebral arteries (Di Tullio et al., 2004) and human cerebral arteries (Mills et al., 2011). An A1 receptor agonist was shown to attenuate subarachnoid hemorrhage-induced cerebral vasospasm in rats (Lin et al., 2006). According to
Chang et al. (2007), the A2a receptor agonist ATL-146e was shown to attenuate experimental posthemorrhagic cerebral vasospasm in rats. Inosine potentiates adenosine-evoked vasodilation in rat pial arterioles (Ngai et al., 1989). Adenosine is taken up by bovine cortex capillaries by a high-affinity uptake system (Wu and Phillis, 1982; Stefanovich, 1983).

The blood-brain barrier (BBB) is composed of endothelial cells, pericytes, and astrocytes. mRNA for all the P2Y receptor subtypes was present in astrocytes and pericytes, but endothelial cells only expressed P2Y1, P2Y2, and P2Y4 subtypes; NTPDase 1 and 2 were expressed by all 3 cell types (Kittel et al., 2010). Efflux transport of adenosine at the BBB has been described (Isakovic et al., 2004; Murakami et al., 2005). Adenosine receptor-mediated endothelial cell signaling modulates the permeability of the BBB in vivo to facilitate the entry of therapeutic compounds into the central nervous system (Carman et al., 2011). Both mRNA and protein for P2Y12 and GPR17 receptors are expressed on endothelial cells of the BBB (Ceruti et al., 2010). P2Y2 and P2Y4 receptors were strongly expressed at endothelial cells of the BBB (Ceruti et al., 2010). Efflux transport of adenosine at the BBB has been described (Isakovic et al., 2004; Murakami et al., 2005). Adenosine receptor-mediated endothelial cell signaling modulates the permeability of the BBB in vivo to facilitate the entry of therapeutic compounds into the central nervous system (Carman et al., 2011). Both mRNA and protein for P2Y12 and GPR17 receptors are expressed on endothelial cells of the BBB (Ceruti et al., 2010). P2Y2 and P2Y4 receptors were strongly expressed at the glovascular interface and colocalized with glial fibrillary acidic protein around larger vessels in cortex (Simard et al., 2003). P2Y2 receptors were later identified as the main P2 receptor subtype involved in Ca2+ signaling in the human BBB endothelial cell line hCMEC/D3 (Bintig et al., 2012). Tumor necrosis factor-α (TNF-α) inhibits purinergic calcium signaling in BBB endothelial cells by reducing gap junction coupling and inhibiting ATP release (Vandamme et al., 2004). ATP-binding cassette transporters, which are localized at the surface of endothelial cells, play important roles in the maintenance of BBB integrity (ElAli and Hermann, 2011).

ATP was shown early to be more potent than adenosine as a dilator of cerebral vessels in the baboon and cat (Forrester et al., 1975, 1979) and goat (Alborch and Martin, 1980). Intracarotid infusion of ATP increased cerebral blood flow in anesthetized baboons by about 90%, whereas adenosine increased it by less than 10%; further application of ATP and adenosine produced pial arteriolar dilations (Harper, 1985). In an important early study of pial arteries from the rabbit, cat, and man, it was shown that ATP acting on P2 receptors on smooth muscle elicited contraction, whereas adenosine acting via smooth muscle P1 receptors caused relaxation; furthermore ADP and ATP acting on P2 receptors on endothelial cells caused relaxations (Hardebo et al., 1987a).

Constriction of the rabbit basilar artery by ATP and UTP occurred via actions at two distinct receptors (von Kügelgen and Starke, 1990b), in retrospect by P2X and P2Y2 and/or P2Y4 receptors. α,β-MeATP produced potent contractions of goat middle cerebral artery (Torregrosa et al., 1990a), suggesting mediation via P2X receptors. UTP and UDP induced a long-lasting contraction of isolated brain arteries in humans (Urquilla et al., 1978) and dogs (Shirasawa et al., 1983). UTP also induced a sustained contraction of the rat middle cerebral artery (Laing et al., 1995). These early data suggested the functional expression of contractile P2Y2 and/or P2Y4 receptors (sensitive to UTP) and contractile P2Y6 receptors (sensitive to UDP). A later study showed that constriction of rat cerebral (pial) microvasculature is mediated by smooth muscle P2X1, P2Y2, and P2Y6 receptors, a finding supported by both functional and RT-PCR studies (Lewis et al., 2000a). P2X4 receptor transcripts were not detected (Lewis et al., 2000a). The P2Y6 receptor was described as the most potent receptor mediating constriction of the rat basilar artery, with lesser contributions from P2X1 and P2Y2 receptors (Malmşjö et al., 2003b). P2Y4 and P2Y6 receptors contribute substantially to myogenic mechanisms of vasocostriction of rat intraparenchymal cerebral arteries (Brayden et al., 2013). Potent P2Y6 receptor-mediated constriction of human cerebral arteries has also been demonstrated (Malmşjö et al., 2003a). Amyloid-β, which may contribute to cerebrovascular dysfunction in Alzheimer’s disease, enhanced ATP-induced cerebral arteriolar contractions (Dietrich et al., 2010).

The mechanism of UTP-induced cerebrovascular constriction in the rat appears to involve release of prostanoids (Lacza et al., 2001). All inositol trisphosphate (InsP3) receptor isoforms are expressed in rat cerebral artery smooth muscle cells, with inositol trisphosphate type 1 dominant, which contributes to UTP-induced vasoconstriction (Zhao et al., 2008). UTP released Ca2+ waves in smooth muscle cells of rat basilar artery; this may underlie the tonic contractions produced by repetitive cycles of regenerative Ca2+ release from sarcoplasmic reticulum through InsP3-sensitive receptors (Syyong et al., 2009). Mammalian homologs of the Drosophila transient receptor potential channel (TRPC) have been identified in vascular smooth muscle cells. It has been shown that TRPC3 mediates UTP-induced depolarization of smooth muscle cells in rat cerebral arteries (Reading et al., 2005). ATP activated potassium channels and enhanced [Ca2+]i in cultured smooth muscle cells of bovine cerebral arteries (Ikeuchi and Nishizaki, 1995). [Ca2+]i increase and membrane depolarization caused by P2X receptor-mediated currents in rat cerebral artery smooth muscle cells may regulate the subsequent P2Y receptor responses (Kamishima and Quayle, 2004).

Most small arteries respond to an increase in intraluminal pressure by constricting, i.e., the “myogenic” response. It was suggested that mechanical activation may be a common feature of many Gq/11-coupled receptors, which may act as mechanosensors via subsequent signaling to TRPC channels (Mederos y Schnitzler et al., 2008). An involvement of direct mechanical activation of UTP- and UDP-sensitive P2Y4 and P2Y6 receptors (Gq/11 coupled, see Table 1) in the myogenic response
was shown in rat parenchymal arteriolar smooth muscle cells (Brayden et al., 2013). The involvement of TRPC channels in this effect is unclear because the same group showed that TRPC3 channels (but not TRPC6 channels) are involved in UTP-induced depolarization and constriction of rat cerebral arteries; however, antisense oligodeoxynucleotide suppression of TRPC3 had no effect on pressure-induced depolarization or the development of myogenic tone by cerebral artery smooth muscle cells, but suppression of TRPC6 decreased pressure-induced depolarization of cerebral artery smooth muscle cells (Reading et al., 2009). It has been suggested that in female rat pial arterioles, ADP-induced dilation is the result of additive contributions from P2Y1 receptors present on endothelium but also on the glia limitans, the underlying layer of astrocytic glial processes (Xu et al., 2005). ATP interacts with astrocytes to induce enhanced arteriolar dilation (Dietrich, 2012). The purinergic mechanisms involved in gliovascular coupling have been reviewed (Pelligrino et al., 2011).

Endothelium-dependent cerebral artery vasodilatation via P2 receptors involves release of NO and EDHF as in other blood vessels, with most of the evidence coming from studies in rat blood vessels (see below). Interestingly, endothelial TRPC1 and TRPC3 contribute to ATP-mediated relaxation in mouse cerebral arteries (Kochukov et al., 2011), a mechanism thus far not reported for P2 receptor-mediated vasodilatation in other blood vessels. ADP-mediated dilatation of rat cerebral arterioles was endothelium-dependent and caused NO release (Mayhan, 1992), suggesting an involvement of P2Y1 receptors. ATP dilated rat middle cerebral arteries via endothelial P2Y1 and P2Y2 receptors, involving release of NO (You et al., 1997). EDHF, in addition to NO, mediates vasodilation of rat middle cerebral arteries via endothelial P2Y2 receptors by opening an atypical calcium-activated K+ channel (You et al., 1999b). In a more recent article (Geddawy et al., 2010), it was shown that 2-MeSADP elicited EDHF-type relaxation via stimulation of endothelial P2Y1 receptors in monkey cerebral artery. In a comparative study of rat middle cerebral arteries, third-order branches, and penetrating arterioles, it was concluded that the role of NO in purinoceptor-evoked dilations diminishes along the cerebrovascular tree (artery to arterioles), whereas the role of EDHF becomes more prominent (You et al., 1999a). Horiiuchi et al. (2003) showed that dilation of rat intracerebral arterioles by low concentrations of ATP was via P2Y1 receptors, whereas high concentrations activated P2Y2 receptors; NO was involved in P2Y1 but not P2Y2 receptor-mediated dilation.

Rat cultured brain capillary endothelial cells show an increase in [Ca2+]i in response to ATP (Revest et al., 1991). According to Frélin et al. (1993), two subtypes of receptors were identified in these cells: an ADP-specific receptor leading to release of intracellular Ca2+ (in retrospect P2Y1 receptor) and a receptor that recognizes ATP and UTP that is positively coupled to phospholipase C (in retrospect a P2Y2 and/or P2Y4 receptor). P2Y1 receptors were also identified on B10 cells, a cloned cell line of rat brain capillary endothelial cells (Webb et al., 1996). Another article claimed that in rat brain capillary endothelial cells ATP activated P2Y2 receptors coupled to phospholipase C, Ca2+, and MAPK and activated P2Y1 receptors linked to Ca2+ mobilization and a further unidentified receptor linked to an increase in cAMP levels (Albert et al., 1997; Sipos et al., 2000). ATP and UTP caused an increase in [Ca2+]i in the immortalized rat brain endothelial cell line, RBE4, perhaps via P2Y2 and/or P2Y4 receptors (Nobles et al., 1995). P2Y6 receptor proteins were shown to be located on endothelial, but not epithelial, cells in the lateral ventricular choroid plexus of the rat (Johansson et al., 2007). P2Y12 receptors were claimed to be present on rat
brain capillary endothelial cells as well as P2Y<sub>1</sub> receptors (Simon et al., 2001, 2002). P2X<sub>2</sub> receptors were also localized with electron microscopy on vascular endothelial cells in rat brain (Loesch and Burnstock, 2000). Diadenosine polyphosphates are antagonists at P2Y<sub>1</sub> receptors on rat brain capillary endothelial cells (Vigne et al., 2000). P2Y<sub>1</sub> and P2Y<sub>2</sub> receptors were identified on endothelial cells from bovine cerebral arteries (Zhang et al., 1997).

Sympathetic nerve stimulation constricted rabbit basilar arteries via two components, one NA, the other unknown at the time (Lee et al., 1980). ATP produced contraction of canine basilar artery (Muramatsu et al., 1980) and was later proposed to be released as a cotransmitter with NA from sympathetic nerve terminals (Muramatsu et al., 1981). Perivascular nerve stimulation of the guinea pig basilar artery elicited EJPs (Karashima and Kuriyama, 1981; Fujiwara et al., 1982). Receptors for ATP and adenosine were found at both pre- and postjunctional sites in canine cerebral artery (Muramatsu et al., 1983). Prejunctional inhibitory effects of adenosine and AMP on neuromuscular transmission in cat cerebral arteries are mediated by A<sub>1</sub> receptors (Rivilla et al., 1990).

Surprisingly, high concentrations of ATP were shown to be released from brain tissue to affect local blood flow (Forrester, 1978). ATP release from rat brain endothelial cells was claimed to be via connexin hemichannels (Braet et al., 2003). Its levels can be regulated by ADPases present on rat brain capillary cells, which degrade ATP to ADP, and ADP to adenosine (Vigne et al., 1998). Indeed, adenosine, acting via A<sub>2</sub> receptors, was shown to contribute significantly to ATP-mediated pial arteriolar dilations in vivo in rats (Vetri et al., 2009, 2011). Ca<sup>2+</sup>-ATPase was shown with electron microscopy to be localized on rat cerebral endothelium (Nag, 1987).

In aging rat cerebral arteries, there is downregulation of P2X<sub>1</sub> and upregulation of P2Y<sub>1</sub> and P2Y<sub>2</sub> receptor mRNA in smooth muscle cells and downregulation of P2Y<sub>1</sub> and P2Y<sub>2</sub> receptor mRNA in endothelial cells (Miao et al., 2001). Aging improves NOS-dependent reactions of endothelial cells in rat cerebral arteries produced by ADP (Mayhan et al., 2008).

In summary, vasoconstriction to ATP, UTP, and UDP of cerebral arteries is mediated by smooth muscle P2X<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, and P2Y<sub>6</sub> receptors. Adenosine mediates vasodilatation predominantly by actions at A<sub>1A</sub> and A<sub>2B</sub> receptors on the smooth muscle and endothelium. Endothelium-dependent vasodilatation of cerebral arteries is elicited by ADP via P2Y<sub>1</sub> receptors, UTP via P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors, and UDP via P2Y<sub>6</sub> receptors. Long-term effects of UTP include chemotactic, mitogenic, and angiogenic actions on vascular endothelial cells (Satterwhite et al., 1999).

G. Skin Vessels

Thermally induced reflex changes in blood flow through arteriovenous anastomoses in rabbit ear arteries were shown to be mediated by sympathetic nerves, and the possibility was raised that purinergic neurotransmission might be involved in these arteries (Hales et al., 1978) and also in control of arteriovenous anastomoses in chicken foot (Hillman et al., 1982). Deep lingual arteries in the monkey tongue are innervated by adrenergic and purinergic components of sympathetic nerves, both producing vasoconstriction (Toda et al., 1997). Transmural nerve stimulation caused contraction followed by vasodilatation of the labial artery close to the skin of the upper lip of dogs; the contraction was abolished by a combination of prazosin and a,b-meATP, indicating sympathetic cotransmission involving NA and ATP, whereas the relaxation was abolished by the NOS inhibitor L-N<sup>G</sup>-nitroarginine methyl ester (Okamura et al., 1999).

Cold-induced neurogenic vasoconstriction of canine cutaneous veins appears to be mediated largely by an increase in the purinergic component of sympathetic transmission (Flavahan and Vanhoutte, 1986). According to Koganezawa et al. (2006), it was suggested that the cooling-induced reduction in skin blood flow was due to the ATP released from sympathetic nerves stimulating presynaptic P2 receptors on sympathetic nerve terminals to facilitate release of NA, resulting in constriction. Arteriolar contractile responses to nanomolar concentrations of topical adenosine acting at A<sub>1</sub> receptors in the skin microcirculation of hamsters were enhanced when local skin temperature was increased; vasodilator responses evoked by micromolar concentrations of adenosine were temperature insensitive (Stojanov and Proctor, 1990). Adenosine receptor inhibition with theophylline attenuated the skin blood flow response to local heating in humans (Fieger and Wong, 2010). Pharmacological evidence has been presented for both high-affinity A<sub>1</sub> receptors mediating vasoconstriction and low affinity A<sub>2</sub> receptors mediating vasodilatation in hamster skin circulation (Stojanov and Proctor, 1989).

The isolated perfused foot of ducks is a useful preparation for the study of skin circulation, and NANC vasodilator nerves with responses mimicked by low concentrations of ATP were identified early (McGregor, 1979). In cats, after pretreatment with guanethidine to abolish the vasoconstrictor effects of sympathetic nerve stimulation, a NANC vasodilator response to nerve stimulation was revealed in the skin of the paw pad (Bell et al., 1985); it remains to be determined whether this involves ATP. There is evidence that degranulation of mast cells in the skin is produced by ATP released during antidromic impulses from sensory nerve endings during axon reflexes, leading to vasodilatation of skin vessels (Kiernan, 1975). Human isolated subcutaneous resistance arteries were shown to dilate via P2Y and P1 receptors and constrict via P2X receptors (Martin et al., 1991). Intra-arterial infusion of ATP, ADP, and AMP in canine facial and nasal vascular
beds caused vasodilation (Bari et al., 1993). Purinergic agonists [ATP and adenosine-5′-[(γ-thio)-triphosphate (ATPγS)] induce release of inflammatory mediators from human dermal microvascular endothelial cells (Bender et al., 2008). ATP and NA synergize in inducing IL-6 production by human dermal microvascular endothelial cells (Stohl et al., 2010).

Intradermal administration of ATPγS results in an enhanced contact hypersensitivity response in mice, and it was suggested that ATP, when released after trauma or infections, may act as an endogenous adjuvant to enhance the immunoresponse and that P2 receptor agonists may augment the efficacy of vaccines (Granstein et al., 2005). Intradermal administration of ATP does not attenuate tyramine-stimulated vasoconstriction in human skin, in contrast to skeletal muscle vessels (Wingo et al., 2010). Changes in purine receptor expression (increase in P2Y1, P2Y2, and P2X5, decrease in P2X7) on human lower leg epidermal keratinocytes were shown in chronic venous insufficiency (Metcalfe et al., 2006). Improvement of ischemic skin flap survival used in surgery when exposed to ATP has been described (Boss et al., 1984; Cikrit et al., 1984; Zimmerman et al., 1987; Knight et al., 1989), perhaps because of its vasodilator effects (Kuzon et al., 1985). Direct Mg-ATP delivery to cutaneous wounds accelerates healing, perhaps by increasing synthesis of vascular endothelial growth factor (Chiang et al., 2007).

H. Intestinal Vessels

EJPs were recorded from smooth muscle in submucosal arterioles in the guinea pig small intestine (Hirst and Neild, 1978), and these were later shown to be produced by ATP released from sympathetic nerves acting via P2X receptors. In this vessel, vasoconstriction was produced solely by ATP or a related purine nucleotide acting through P2X receptors, whereas the colocalized transmitter NA acted solely as a prejunctional modulator of ATP release (Evans and Surprenant, 1992; Vanner and Surprenant, 1996). ATP and NA released from sympathetic nerve terminals mediate contraction of rabbit isolated jejunal arteries (Evans and Cunnane, 1992) and ileocolonic artery (Bulloch and Starke, 1990). In general, the smaller the blood vessel, the larger the purinergic component (McGrath et al., 1990). Both substance P and CGRP decreased the amplitude of neurally evoked EJPs in arterioles of guinea pig small intestine (Coffa and Kotecha, 1999), suggesting that purinergic neurotransmission can be modulated by neurotransmitter released from sensory nerves. Conversely, ATP released from sympathetic nerve varicosities acts on prejunctional adenosine receptors (after ATP breakdown) on both extrinsic sensory and submucosal vasodilator nerves to inhibit transmitter release (Lomax and Vanner, 2010). Reviews describing neural purinergic control of intestinal vessels are available (Burnstock, 2001a; Kotecha, 2002).

P2X receptors were shown to be present on both arterial and venous blood vessels of the cat intestinal circulation (Taylor and Parsons, 1991) and submucosal arteries in the guinea pig ileum (Galligan et al., 1995). ATP applied to equine colonic arterial and venous rings induced a biphasic response, contraction followed by sustained relaxation that was attenuated but not eliminated by endothelium removal and did not involve NO (Tetens et al., 2001b). Infusion of ATP into stomach, small intestine, and colon of cats caused vasodilation (Sjöqvist et al., 1985). Ca2+ signaling in rat gastric microvascular endothelial cells is mediated by purinergic receptors, probably P2Y receptors, because increases in [Ca2+]i were due to release from intracellular stores (Ehring et al., 2000). Superfusion of mouse colonic submucosal vessels with ATP mediated large vasoconstrictions in controls, but not in mice with 2,4,6-trinitrobenzene sulfonic acid-induced colitis, and it was concluded that reduced purinergic signaling to submucosal arterioles may be due to increased degradation of ATP during colitis (Lomax et al., 2007).

Adenosine acting primarily via A1 receptors (but also via A2A and A2B receptors) produces vasodilation of arteries in rat jejunum (Li et al., 2007). Adenosine, acting via A2 receptors, increases blood flow to the esophageal mucosa, antral mucosa, and small intestine of rabbit (Pennanen et al., 1994). Adenosine is a vasodilator in the canine intestinal mucosa (Sawmiller and Chou, 1991). Microvascular vasodilations to adenosine were significantly greater in the duodenum than in the terminal ileum of the rat; also, adenosine-induced vasodilation was greater in the smaller compared with the larger microvessels (Li et al., 2004). A study of ethanol-induced arteriolar dilation in rat stomach led to the conclusion that ethanol increases gastric mucosal blood flow via A2 receptors in submucosal arterioles (Nagata et al., 1996).

The beneficial effects of ATP on the treatment of intestinal ischemia were claimed to be relatively small (Van der Meer et al., 1982). However, adenosine is effective in protecting the intestinal microvasculature from the injurious effects of ischemia-reperfusion (Grisham et al., 1989), perhaps via A1 receptors (Özçağmak and Sayan, 2007). It was concluded in another study that adenosine acts by modulating the inflammatory response evoked by intestinal reperfusion (Kaminski and Proctor, 1992). Furthermore, gene deletion of 5′-nucleotidase/CD73 significantly enhanced intestinal ischemia-reperfusion injury (Hart et al., 2008a). Beneficial effects of E-NTPDase1/CD39 against murine intestinal ischemia-reperfusion injury have been reported (Guckelberger et al., 2004).

I. Skeletal Muscle Microvasculature and Femoral Artery

Adenosine was considered initially to be the major purine to increase skeletal muscle blood flow in the dog
hindlimb (Dobson et al., 1971; Cotterrell and Karim, 1982). Later studies showed that ATP and ADP cause strong vasoconstriction followed by vasodilation in the rat hindlimb vascular bed (Sakai, 1978) as did UTP and GTP (Sakai et al., 1979a), although it was postulated that they acted via release of 5-HT. Another early study showed the relative vasodilator potency of purines in the cat hindlimb as ADP > ATP >> AMP (Gangarosa et al., 1979), suggesting activation of P2Y<sub>1</sub> receptors.

An important article by Forrester (1981) reported that ATP was the mediator of exercise hyperemia in skeletal muscle. ATP release was investigated in pig skeletal muscle during exercise (Wulf et al., 1984) and release shown during exercise of human thigh muscle, increasing both arterial and venous plasma ATP concentration (Mortensen et al., 2011). During exercise, sympathetic nervous system activity increases and this contributes to an increase in blood pressure, the exercise pressor reflex. Data were presented to suggest that P2 receptors on sensory nerves contribute to this exercise pressor reflex in humans (Cui et al., 2011). ATP released during cat skeletal muscle contraction acts on afferent C fibers to modulate cardiovascular responses (Li et al., 2008a). Short-term exercise training augments sympathetic vasoconstrictor responsiveness and endothelium-dependent vasodilation in resting skeletal muscle, perhaps related to enhanced ATP release (Jendzrowsky and DeLorey, 2012). A review that includes discussion of the involvement of purinergic signaling in control of muscle blood flow during exercise is available (Sarelius and Pohl, 2010).

The ability of contracting muscle and circulating ATP to modulate sympathetic vasoconstriction is impaired in aging humans, and it was suggested that this may be due to a reduction in circulating ATP with advancing age (Kirby et al., 2011), perhaps due to reductions in erythrocyte- and endothelium-mediated ATP release (Kirby et al., 2012, 2013). A recent report claims that aging does not alter the responsiveness of postjunctional P2X<sub>1</sub> and P2X<sub>4</sub> receptors in the skeletal muscle vasculature of the canine hindlimb at rest or during exercise (DeLorey et al., 2012). Femoral artery occlusion significantly increases the level of P2X<sub>3</sub> receptors in lumbar dorsal root ganglion neurons, leading to augmentation of the exercise pressor reflex pathways when blood supply to hindlimb muscles is insufficient, as seen in peripheral artery disease (Liu et al., 2011). Functional sympatholysis refers to attenuation of sympathetic vasoconstrictor activity. Muscle immobilization for two weeks impairs functional sympatholysis but increases exercise hyperemia and the vasodilator responses to infused ATP (Mortensen et al., 2012). In sympathetically denervated humans, this vasodilation is facilitated (Puvi-Rajasingham et al., 1997), suggesting involvement of the cotransmitters NA and ATP.

ATP can be released from nerves in rabbit skeletal muscle to cause skeletal muscle vasodilation via actions on P2 receptors; the ATP release was evoked after hypothalamic stimulation (Shimada and Stitt, 1984). Field stimulation of 1A arterioles from the red portion of rat gastrocnemius muscle was shown to release ATP in two phases from nerves, measured with real-time biosensors (Kluess et al., 2010). They showed further that endothelium removal resulted in an increase in ATP overflow, indicating that endothelial cells attenuate ATP overflow from nerves and that changes in ATP flow occurred during development and aging. Plasma levels of ADP, as well as ATP, increase in strenuous exercise in humans; the ADP significantly augmented platelet activity but this prothrombotic response was partially counteracted by concurrent upregulation of soluble nucleotide-inactivating enzymes, providing insight into the mechanisms underlying the enhanced risk of occlusive thrombus formation in exercising conditions (Yegutkin et al., 2007). Heat stress increases limb blood flow in humans. The increased limb muscle vasodilatation in these conditions of elevated muscle sympathetic vasoconstrictor activity is closely related to the rise in arterial plasma ATP and local tissue temperature (Pearson et al., 2011).

ATP, infused intravenously, can induce pronounced skeletal muscle vasodilatation, as shown in a number of studies in humans. The combined inhibition of NO, prostaglandins, and EDHFs was shown to inhibit ATP-induced vasodilatation in the human leg (Mortensen et al., 2007). Endothelial P2Y<sub>2</sub> receptors are involved in ATP-induced vasodilatation of human leg skeletal muscle and are coupled to release of NO and prostaglandins; adenosine does not appear to be involved (Mortensen et al., 2009a). In human contracting forearm and leg skeletal muscle, activation of ATP/UTP-selective P2Y<sub>2</sub> receptors on endothelial cells increases blood flow and blunts sympathetic vasoconstriction elicited via P2X and α<sub>1</sub> receptors (Kirby et al., 2008; Rosenmeier et al., 2008; Mortensen et al., 2009b). ATP evokes vasodilation of human forearm skeletal microvessels (van Ginneken et al., 2004), but perhaps not via NO (Shiramoto et al., 1997). A role of the endothelium in mediating the dilator actions of ATP via P2Y receptors on microvessels in the rabbit hindlimb was suggested, and in addition there was direct vasconstriction via P2X receptors and vasodilation via P1 receptors (Dézsi et al., 1990). ADP hyperpolarized and relaxed rabbit skeletal muscle resistance arteries; the response was abolished by removal of the endothelium (Brayden, 1991). ATP dilated arterioles of skeletal muscle via the endothelium in rats (Koller et al., 1991) and rabbits (Pohl et al., 1987). ATP and UTP dilate the cat hindlimb vascular bed, but these effects were not blocked by NOS or prostaglandin inhibitors or by inhibition of K<sub>a</sub>ATP channels (Champion and Kadowitz, 2000; Shah et al., 2001).
In the hamster cremaster microcirculatory preparation, ATP-mediated release of arachidonic acid metabolites from the vascular endothelium causes arteriolar dilation (Hammer et al., 2001). ATP induces an increase in [Ca\textsuperscript{2+}]\textsubscript{i}, which stimulates PGI\textsubscript{2} synthesis in endothelial cells of veins isolated from the hamster hindlimb (Choi et al., 2002). In a subsequent study, these authors showed that ATP, but not adenosine, stimulates the release of PGI\textsubscript{2} from the endothelium of perfused vessels isolated from the hamster hindlimb (Hammer et al., 2003).

α,β-MeATP evokes reflex cardiovascular responses in muscle microvessels, via P2X receptors, when injected into the arterial supply of the cat hindlimb vasculature (Li and Sinoway, 2002; Hanna et al., 2002; Gao et al., 2006). P2X receptors can produce vasoconstriction in exercising canine skeletal muscle (Buckwalter et al., 2003, 2004). Muscle acidosis was shown to attenuate P2X receptor pressor responses but enhance TRPV1 pressor responses induced by femoral artery injection of α,β-meATP and capsaicin, respectively, in rats (Gao et al., 2007).

Adenosine and probably ATP contribute to blood flow regulation in the exercising human leg by increasing prostaglandin and NO formation (Mortensen et al., 2009c; see Hellsten et al., 2012). Adenosine produced prolonged vasodilatation of arterioles after contraction or exercise of canine skeletal muscle (Belloni et al., 1979; Kille and Klabunde, 1984; Proctor, 1984). In addition, adenosine acts on presynaptic receptors to inhibit sympathetic nerve-mediated vasoconstriction (Fuglsang and Crone, 1987). However, in studies of canine gracilis muscle, it was concluded that, although released adenosine could contribute to exercise hyperemia, it is likely not to be the main factor, particularly in the initial stage (Ballard et al., 1987; Koch et al., 1990). Adenosine, acting via A\textsubscript{2A} receptors, contributes up to 30% of the functional hyperemic response in the hindlimb of anesthetized cats (Poucher, 1996). Both A\textsubscript{1} and A\textsubscript{2A} receptors were claimed to mediate adenosine-induced vasodilatation of skeletal muscle of rat (Bryan and Marshall, 1999) and cat (Bivalacqua et al., 2002a). It was proposed that, although twitch contractions in rat extensor digitorum long muscle produce a large hyperemia in femoral vessels, adenosine, acting via A\textsubscript{2A} receptors, plays a greater role in the hyperemia associated with tetanic contractions (Ray and Marshall, 2009). A\textsubscript{1} receptors play the predominant role in mediating adenosine-induced vasodilatation of rat diaphragmatic arterioles via NO and ATP-dependent K\textsuperscript{+} channels (Danialou et al., 1997). A\textsubscript{2A} receptors were shown to modulate juvenile female rat skeletal muscle microvascular permeability (Wang and Huxley, 2006).

In femoral arteries of dog and rabbit, vasodilatation evoked by ATP and ADP was endothelium dependent in contrast to the dilatation produced by adenosine, which acted directly on the vascular smooth muscle via A\textsubscript{2} receptors (De Mey and Vanhoutte, 1981; Cassis et al., 1987). Säiag et al. (1990) showed vasoconstriction of femoral artery by ATP and UTP, suggesting that both P2X and P2Y\textsubscript{2} and/or P2Y\textsubscript{4} receptors were present. Sympathetic nervous control of femoral vasoconstriction involves ATP, released as a cotransmitter with NA, playing the dominant role in response to low-frequency stimulation (Bradley and Johnson, 2002; Kluess et al., 2006). Acidosis attenuates P2X receptor vasoconstriction in rat isolated femoral (and iliac) arteries (Kluess et al., 2005a), similar to the attenuation of P2X receptor-mediated pressor responses observed with acidosis in hindlimb skeletal muscle (Gao et al., 2007). Elevated temperature decreases sensitivity of P2X receptors in femoral arteries (Kluess et al., 2005b).

In human and rabbit skeletal muscle, erythrocytes function as an O\textsubscript{2} sensor, contributing to the regulation of blood flow by releasing ATP (González-Alonso et al., 2002; Ellsworth, 2004; Sprague et al., 2009; Ellsworth and Sprague, 2012; González-Alonso, 2012). It was suggested that the hemolysis of erythrocytes that occurs after subarachnoid hemorrhage releases ATP in concentrations that cause vasospasm in the rat femoral artery (Macdonald et al., 1998). ADP is involved in photochemically induced thrombosis of the guinea pig femoral artery (Hirata et al., 1993).

Adenosine appears to mediate fetal femoral arterial vasoconstriction by its involvement in fetal carotid chemoreflexes during hypoxemia (Giussani et al., 2001). A\textsubscript{2} receptor agonists were more potent at eliciting vasodilatation of the femoral vein than the femoral artery of rats; A\textsubscript{2} receptor-induced vasodilatation is partially endothelium dependent in the femoral artery but not in the vein (Abiru et al., 1995).

**J. Pulmonary Vessels**

Evidence for release of NA and ATP as cotransmitters from sympathetic nerves supplying rabbit pulmonary arteries was recognized (Katsuragi and Su, 1982; Mohri et al., 1993). An important advance was made when it was shown that EJPs recorded in the smooth muscle of rat intrapulmonary arteries in response to sympathetic nerve stimulation were mediated by ATP (Inoue and Kannan, 1988). Adenosine, acting prejunctionally, inhibits release of transmitter from sympathetic nerves supplying the rabbit pulmonary artery (Husted and Nedergaard, 1985). In guinea pig pulmonary vessels, inhibitory purinergic neurotransmission has been described, and it was claimed that this was endothelium dependent (Liu et al., 1992).

In the isolated, blood-perfused and ventilated rat lung, P2X receptors activated by α,β-meATP caused vasoconstriction, whereas P2Y receptors, probably largely located on the endothelium, mediated a small vasodilator response (Liu et al., 1989; McCormack et al., 1989a). Vasoconstriction of the rat pulmonary
vascular bed was elicited equipotently by ATP, UTP, and UDP; contractions to UTP and UDP were resistant to suramin antagonism, whereas those to ATP were blocked (Rubino and Burnstock, 1996) and so were probably mediated by P2Y4 and P2Y6 receptors on smooth muscle. In endothelium-denuded rat pulmonary arteries, P2Y2 receptors and a UDP-sensitive receptor (presumably P2Y6) were identified on myocytes (Hartley et al., 1998). In a recent article (Mitchell et al., 2012), P2Y1, P2Y6, and P2Y12 receptors were reported to mediate contraction of rat intrapulmonary arteries. An ATP-induced increase in [Ca^{2+}]_{i} in rat pulmonary artery myocytes was mediated by P2X and P2U (P2Y2 and/or P2Y4) receptors (Guibert et al., 1996; Hartley and Kozlowski, 1997). Regional variation in P2 receptor expression mediating constriction in the rat pulmonary arterial circulation was reported and, although ATP was shown to be a potent constrictor in rat perfused lung (Rubino and Burnstock, 1996), it was only a weak agonist in rat isolated intrapulmonary arteries; in these arteries UTP and UDP were more potent than ATP and were equipotent in inducing vasoconstriction (Rubino et al., 1999). However, another report found that P2Y receptor agonist potencies were similar in large and small rat pulmonary vessels, but P2X receptor agonists were more potent in small arteries (Chootip et al., 2002). However, in a more recent article from this laboratory, it was reported that the pharmacological properties and mRNA and protein expression profiles of P2X receptors in rat small and large pulmonary arteries were very similar and that P2X1 receptors appeared to be the predominant subunit involved (Syed et al., 2010). Rho kinase and PKC play a role in P2X1-evoked contractions of small pulmonary artery (Syed and Kennedy, 2010). In feline pulmonary arteries ATP appears to elicit contraction in part through A1 receptors after its breakdown to adenosine (Neely et al., 1991). ATP and α,β-meATP induce contraction of rabbit pulmonary artery (Baek et al., 2008). Up4A has been identified as a novel endothelium-derived vasoconstrictor (Jankowski et al., 2005) and has been shown to stimulate endothelium-independent contraction of rat isolated pulmonary artery (Gui et al., 2008).

Endothelium-dependent relaxation of human pulmonary arteries by ATP has been demonstrated (Greenberg et al., 1987; Dinh Xuan et al., 1990). In rabbit pulmonary arteries, relaxation to UTP was endothelium dependent, whereas relaxation to ATP was only partially inhibited by removing the endothelium (Qasabian et al., 1997). In contrast, ATP and 2-MeSATP evoked endothelium-dependent vasodilatation in the rat isolated pulmonary circulation, suggesting an involvement of P2Y1 receptors; this involved NO but there was no evidence for a role of prostanooids (Hassésson and Burnstock, 1995; Hakim et al., 1997). Similarly, ATP and 2-MeSATP evoked endothelium-dependent vasodilatation in rat isolated intrapulmonary arteries, also indicating a likely action at P2Y1 receptors; UTP and UDP lacked vasodilator activity (Rubino et al., 1999), showing further species differences compared with the rabbit pulmonary arteries described above. ATP and UTP, acting via P2Y1 and P2Y3 and/or P2Y4 receptors, cause mobilization of intracellular Ca^{2+} and subsequent PGI2 release in bovine pulmonary artery endothelial cells (Lustig et al., 1992; Chen et al., 1996). PKCβI mediates inhibition of P2Y1 and P2Y2 receptor-mediated phosphoinositide turnover in bovine pulmonary artery endothelial cells (Chen and Lin, 1999).

Porcine pulmonary vessels also express P2 receptors, eliciting PGI2 release (Hellewell and Pearson, 1984). Intercellular communication via Ca^{2+} waves upon mechanical stimulation of calf pulmonary artery endothelial cells is mediated by nucleotides (Moerenhout et al., 2001).

Shear stress evokes release of ATP from endothelial cells, which causes NO release and rat pulmonary artery vasodilation (Hassésson et al., 1993). ATP release induced by NA from endothelial cells was greater in the distal (smaller) compared with proximal pulmonary arteries of the rabbit (Takeuchi et al., 1995). Shear stress stimulates human pulmonary artery endothelial cells to release ATP, which then activates Ca^{2+} influx in endothelial cells via P2X4 receptors (Yamamoto et al., 2003). P2X4 receptor knockout mice do not exhibit normal endothelial cell responses to shear stress, showing reduced vasodilation, impaired [Ca^{2+}]_{i} influx and subsequent NO production, and impaired vascular remodeling (Ando and Yamamoto, 2009). In addition to ATP released from endothelial cells, ATP release from erythrocytes contributes to release of NO and subsequent vasodilation (Sprague et al., 1996; Kotsis and Spence, 2003). Distension of the main pulmonary artery in anesthetized dogs stimulates vagal afferent activity (Moore et al., 2004), perhaps via purinergic mechanosensory transduction, where ATP released in response to stretch acts on P2X3 receptors expressed on sensory nerve terminals in the vessel wall (see Burnstock, 2007). A previously published article (Dieterle et al., 1978) described hydrolysis of ATP to adenosine by cultures of porcine pulmonary endothelial cells and uptake of adenosine, which was rapidly phosphorylated by adenosine kinase. Inhibition of ecto-ATPase by ATPγS, α,β-meATP, and 5′-adenylylimidodiphosphate in endothelial cells of bovine pulmonary artery has been reported (Chen and Lin, 1997). Adenosine mediates vasodilation via P1 receptors but does not mediate the vasodilator response to ATP in the feline pulmonary vascular bed (Neely et al., 1989). P1 receptors also mediate relaxation of bovine bronchial arteries (Alexander and Eyre, 1985). In human pulmonary arteries, the vasodilator effect of adenosine is mediated by A2 receptors (McCormack et al., 1989b). Adenosine markedly increases fetal ovine...
pulmonary blood flow (Smolich et al., 2012). Adenosine, acting via A₂ receptors, dilates juvenile rabbit pulmonary arteries and veins (Steinhorn et al., 1994) and adult rabbit perfused lung (Pearl, 1994); it is likely that the A₂ receptors are located on the smooth muscle because in rabbit isolated pulmonary artery the vasodilator effect of adenosine is endothelium independent (El-Kashef et al., 1999). Adenosine also constricts the pulmonary vasculature of a number of species (Neely et al., 1991; Wiklund et al., 1987; Biaggioni et al., 1989). In the feline pulmonary vascular bed this involved A₁ receptors (Neely et al., 1991; Cheng et al., 1996); adenosine caused vasoconstriction via A₁ receptors and vasodilation via A₂ receptors but not by releasing NO (Cheng et al., 1996). Similarly, adenosine elicits both contractile and relaxant effects on the pulmonary artery of guinea pigs via A₁ and A₂B receptors, respectively (Szentmiklősi et al., 1995); the contractile effect was abolished by cyclooxygenase inhibition with indomethacin (Biaggioni et al., 1989).

Compared with ovine pulmonary artery rings from postnatal animals, ovine fetal pulmonary artery rings have diminished endothelium-mediated relaxation to ADP (Abman et al., 1991). ATP and adenosine increase pulmonary blood flow in perinatal lamb via P₁ and P₂ receptors that are independent of PGI₂ synthesis (Konduri et al., 1992); these receptors were later identified as A₂A and P₂Y₁ receptors, respectively (Konduri et al., 2000), and shown to act via NO (Konduri and Mital, 2000). An increase in ATP release during oxygen exposure may contribute to birth-related pulmonary vasodilation in fetal lambs (Konduri and Mattei, 2002). ATP causes NO-mediated pulmonary vasodilation predominantly via endothelial P₂Y₂ receptors in newborn rabbits (Konduri et al., 2004).

It has long been known that, unlike in most other vascular beds, hypoxia induces pulmonary vasoconstriction (von Euler and Liljestrand, 1946), which may lead to pulmonary hypertension. Hypoxia can also result in pulmonary vasodilation, and this was shown not to be mediated by adenosine (Gottlieb et al., 1984). The pulmonary hypertensive response to endotoxin was first reported by Hinshaw and colleagues (1957), and damage to the endothelium may play a role in the development of pulmonary hypertension in humans (Greenberg et al., 1987). ATP-MgCl₂ has been shown to reduce the vasoconstriction associated with hypoxic pulmonary hypertension (Paidas et al., 1988). It was subsequently proposed that ATP and adenosine may have a beneficial role in the management of pulmonary hypertension in children (Konduri, 1994). Adenosine can decrease pulmonary artery pressure and vascular resistance in patients with primary pulmonary hypertension who respond to calcium channel blockers (Inbar et al., 1993). ATP-induced pulmonary vasodilation has been described in patients with chronic obstructive pulmonary disease (Gaba and Préfaut, 1990).

Extracellular nucleotides enhance leukocyte adherence to pulmonary artery endothelial cells via P₂Y receptors, and because adherence of leukocytes is an important early step in acute vascular injuries, it was speculated that nucleotide-induced leukocyte adherence could be important in mediating vascular injuries in such conditions as respiratory distress syndrome and septic shock (Dawicki et al., 1995). Permeability edema is a life-threatening complication accompanying acute lung injury. Extracellular ATP, produced during inflammation, induces a rapid and dose-dependent increase in transendothelial electrical resistance, and restoration of endothelial barrier integrity is achieved via ATP and adenosine acting on endothelial P₂Y and A₂ receptors (Lucas et al., 2009). LPS binds to and activates A₁ receptors on human pulmonary artery endothelial cells and thus may be involved in acute lung injury because of Gram-negative septicemia and endotoxemia (Wilson and Batra, 2002).

Extracellular ATP is a proangiogenic factor for pulmonary artery vasa vasorum endothelial cells, potentiating the effect of both vascular endothelial growth factor and basic fibroblast growth factor (Gerasimovskaya et al., 2008). Hypoxia, through the hypoxia-inducible transcription factors (HIF)-1α and HIF-2α, induces angiogenesis by upregulating cytokines. The adenosine A₂A receptor is an angiogenic target of HIF-2α in human pulmonary endothelial cells and mediates increases in cell proliferation, cell migration, and tube formation (Ahmad et al., 2009). The authors further show that there is increased expression of A₂A receptors in human lung cancer.

Dormant pulmonary vein conduction can be transiently restored by ATP after extensive encircling pulmonary vein isolation, and it has been claimed that radiofrequency application for provoked ATP-reconnection may reduce atrial fibrillation recurrence (Hachiya et al., 2007). Adenosine restores atriovenous conduction after ostial isolation of pulmonary veins (Tritto et al., 2004). Spontaneous ectopy arising from pulmonary veins is a trigger for atrial fibrillation, and adenosine can induce pulmonary vein ectopy (Cheung et al., 2012).

Reviews, which include discussion of the roles of purines in control of pulmonary vascular tone, have been published (Liu and Barnes, 1994).

K. Carotid Artery

ATP produced vasoconstriction that was more potent than that produced by ADP in dog internal and external carotid arteries (Chiba et al., 1980). ATP vasoconstriction was via an increase in Ca²⁺ influx into the smooth muscle cells of canine internal carotid artery (Kawai et al., 1984). Endothelium-dependent hyperpolarizations in intimal smooth muscle cells were elicited by ATP and ADP in rabbit carotid arteries, whereas in adventitial smooth muscle cells the nucleotides produced depolarizations (Chen and Suzuki, 1991).
Abluminal application of ATP and ADP produced vasoconstriction of rabbit carotid arteries via P2X receptors, whereas intraluminal application of ADP produced endothelium-mediated vasodilatation via P2Y receptors (Kaul et al., 1992). The endothelium-dependent dilator effect of ATP via P2Y1 receptors was via production of NO in rat carotid arteries, whereas the endothelium-dependent dilations produced by UTP mediated by P2U (P2Y2 or P2Y4) receptors did not involve NO but probably EDHF (Malmsjö et al., 1998).

Adenosine produced vasodilation in dog internal and external carotid arteries (Chiba et al., 1980). Adenosine is also a vasodilator in internal carotid arteries of adult pigs but is less effective at eliciting vasodilatation in newborn pigs (Laudignon et al., 1990). The ability of adenosine to release NO and cause vasodilation in the carotid artery and its circulation was shown to be greater in mature than in juvenile or middle-aged rats (Omar and Marshall, 2010). Adenosine acts as a prejunctional inhibitory modulator of sympathetic neurotransmission to the canine cavernous carotid artery (Fujiwara et al., 1986). In A2A Receptor knockout mice, there was enhanced homing ability of leukocytes and increased formation of the neointima of injured carotid arteries (Wang et al., 2010a).

L. Splenic Vessels

Adenosine was shown early to evoke mouse splenic arteriolar dilatation (Reilly and McCuskey, 1977). Later ATP was shown to constrict canine perfused splenic arteries (Ren et al., 1994). EJPs were recorded in arteriolar smooth muscle of splenic vessels of guinea pigs and rats in response to sympathetic nerve stimulation (Jobling, 1994). Later it was shown that ATP and NA were cotransmitters in sympathetic nerves supplying canine and rabbit splenic arteries, the purinergic component being predominant at low stimulation frequencies (Ren et al., 1996; Ren and Burnstock, 1997). αβ-MeATP abolished the purinergic component of sympathetic nerve-stimulated responses of canine splenic arteries (Yang and Chiba, 1999b), consistent with activation of P2X1 receptors. Yang and Chiba (2001) later showed that activation of prejunctional NPY1 receptors enhanced the prolonged adrenergic vasoconstriction but not the transient purinergic vasoconstriction. Prejunctional P1 receptors, α2 adrenoceptors, and Ang-II receptors can modulate the release of NA and ATP (Yang and Chiba, 1999a, 2003). Precontraction with the thromboxane-mimetic U46619 potentiated P2X receptor-mediated contractions in porcine isolated splenic arteries (Roberts, 2012).

M. Uterine Artery

EJPs were recorded in guinea pig uterine artery smooth muscle cells in response to sympathetic nerve stimulation, and there was a decrease in amplitude of EJPs during pregnancy (Tare et al., 1998). ATP released by sympathetic nerve stimulation to the human uterine artery produced contractions via P2 receptors and inhibition of NA release via prejunctional A1 receptors (Neta et al., 1999). A study of purine and pyrimidine receptors in human uterine artery led to the conclusion that there are P2X2 (or P2X3) and P2Y2 receptors on smooth muscle mediating contraction, whereas stimulation of P2Y2 receptors on endothelium produces NO, inducing relaxation (Fontes Ribeiro et al., 1999).

Endothelial cells from ovine uterine arteries respond to extracellular ATP via P2Y receptors, occupation of which leads to Ca2+ release from the endoplasmic reticulum; a pregnancy-specific modulator role of mitochondria in this signaling mechanism has been described (Yi and Bird, 2005). Pregnancy-enhanced gap junction intercellular communication supports sustained phase ATP-induced [Ca2+]i bursts in sheep uterine artery endothelial cells (Yi et al., 2005). Studies of purinergic signaling to uterine artery endothelial cells from pregnant and non-pregnant ewes identified pregnancy-enhanced Ca2+ responses to ATP; P2Y2 receptors were identified as the principal receptor subtype mediating these effects in both pregnant and nonpregnant conditions, although expression of P2X1, P2X2, P2X7, P2Y1, P2Y6, and P2Y11 receptors was also found on uterine endothelial cells (Gifford et al., 2006).

N. Human Umbilical and Placental Vessels

1. Human Umbilical Vessels. Pharmacological and histochemical evidence for P2X receptors on the smooth muscle of both human umbilical artery and vein was presented (Bo et al., 1998). On the basis of pharmacological experiments, P2X1, P2X4, and P2X7 receptors were identified on the smooth muscle of human umbilical cord and chorionic blood vessels (Valdecantos et al., 2003). Calcium oscillations were induced by ATP acting on P2Y receptors on human umbilical arterial muscle cells (Meng et al., 2007). ATP, via P2X1 receptors, produced fast, transient cationic currents and increased [Ca2+]i in human umbilical artery smooth muscle cells (Enrique et al., 2012). The expression of P2X receptors on human umbilical cord lymphocyte CD34+ cells was significantly higher than that of adult human blood cells, and it was suggested that this may reflect early involvement of P2X receptors in differentiation of human hematopoietic cells (Kazakova et al., 2011).

Because there are no nerves in umbilical vessels, this implies an important role of locally released nucleotides in purinergic regulation of umbilical vascular function. Thrombin, at physiologic concentrations, is a potent stimulus of HUVEC ATP release involving activation of the thrombin-specific, protease-activated receptor 1, and intracellular calcium mobilization (Gödecke et al., 2012). Reducing pannexin 1 expression reduced ATP release, whereas downregulation of connexin 43 was ineffective (Gödecke et al., 2012). Hypoxia caused
ATP to be released from HUVECs (To et al., 2011). The G protein agonist compound 48/80 also stimulated release of ATP from HUVECs (Gruenhausen and Yeung, 2004). Because pannexin 1 is activated by anoxia and ischemia (MacVicar and Thompson, 2010), it would be interesting to investigate the possible involvement of pannexins in ATP release from HUVECs during hypoxia and other stimuli.

ATP evokes hyperpolarization and NO synthesis in endothelial cells cultured from human umbilical vein; the pathway for NO synthesis was distinct from that used by estrogen to produce NO (Sheng et al., 2008). A more recent study showed that P2Y2 receptors are the principal nucleotide receptors in HUVECs mediating rapid Ca\(^{2+}\) mobilization, membrane hyperpolarization, and NO production, events that underlie vasorelaxation to ATP and UTP via P2Y2 receptors (Raqueeb et al., 2011). P2 receptors were characterized on the HUVEC cell lines ECV304 (Conant et al., 1998) and EAhy 926 (Graham et al., 1996) and both P2Y1 receptors, sensitive to Ap3A, and P2Y2 receptors, sensitive to UTP but insensitive to diadenosine polyphosphates, were identified. ATP acting on P2Y receptors on HUVECs regulates PGI2 release via transient elevation of [Ca\(^{2+}\)]\(_i\) from intracellular bound stores (Carter et al., 1988). Desensitization of PGI2 synthesis by ATP is likely to be due to uncoupling of the P2Y receptor from phosphoinositidase C but does not involve PKC activation (Carter et al., 1990). Cultured HUVECs were shown to synthesize platelet-activating factor as well as PGI2 in response to ATP (McIntyre et al., 1985). P2Y receptors activate MAPK/extracellular signal-regulated kinase (ERK) through a pathway involving P13K/PDK1/PKC-ζ in HUVECs (Montiel et al., 2006). In another HUVEC cell line (EAhy 926 cells), P2Y receptor-mediated inhibition of TNFα was demonstrated (Paul et al., 2000).

HUVECs have high ATP synthesizing activity on their cell surface, and this appears to be involved in the proliferation of these cells in cancer (Arakaki et al., 2003). ADP breakdown to adenosine by cultured HUVECs was shown early (Dosne et al., 1979). Plasma membrane ATPases were first identified on the HUVEC cell line ECV 304 (Mehgji and Burnstock, 1995). Later ENTPDase 1/CD39 was shown to be present on HUVECs (Koziak et al., 1999). Extracellular ATP formation in cultured HUVECs is mediated by ectonucleotide kinase activities via phosphotransfer reactions (Yegutkin et al., 2001). Perindopril, an angiotensin-converting enzyme inhibitor, augments ecto-ATP diphosphohydrolase activity and platelet aggregation in HUVECs (Kishi et al., 2003). LPS upregulates ecto-5′-nucleotidase activity on HUVECs, and it was suggested that because adenosine is an anti-inflammatory molecule, 5′-nucleotidase upregulation may protect endothelial cells against inflammatory damage (Li et al., 2008b). Adenosine concentration in umbilical venous blood increased significantly in anemic fetuses, suggesting that the fetus responds to hypoxia by increasing blood adenosine (Ross Russell et al., 1993). Functional A1 and A2B receptors were shown to be expressed on the HUVEC-derived ECV304 cell line (Kobayashi et al., 2005). A2B receptors have also been claimed to be present on HUVECs (Fang and Olah, 2007).

2. Placental Vessels. Adenosine causes a biphasic response: vasoconstriction followed by vasodilation, in ovine fetal placental vasculature (Reid et al., 1990). In human chorionic arteries A2B receptors, which are normally vasorelaxant (see Table 2), caused contraction mediated by actions at both the endothelium and smooth muscle and induction of the arachidonic acid cascade (Donoso et al., 2005). It was suggested that A3 receptors mediate smooth muscle relaxation in human chorionic arteries (Donoso et al., 2005).

ATP contracts human placental vascular smooth muscle via P2X receptors; UTP mediates weak vasoconstriction via P2Y2 (or P2Y4) receptors on smooth muscle; ADP, ATP, and UTP dilate placental vessels, likely via endothelial P2Y1 and P2Y2 receptors, and involve release of NO (see Read et al., 1993; Ralevic et al., 1997; Buvinic et al., 2006). Interestingly, contractile responses to ATP and α,β-meATP were sustained in human perfused placental cotyledons and human placental chorionic surface arteries (Dobronyi et al., 1997; Ralevic et al., 1997) in contrast with the rapidly desensitizing responses observed to α,β-meATP via P2X1 receptors in most other blood vessels. Furthermore, responses to ATP and α,β-meATP were blocked by suramin and insensitive to PPADS (a P2 receptor antagonist that blocks P2X1 responses in other vessels), whereas UTP elicited little or no contraction, which might indicate the involvement of heteromeric P2X receptors (Dobronyi et al., 1997; Ralevic et al., 1997). An elegant study by Buvinic et al. (2006) showed that P2Y1 and P2Y2 (or P2Y4) receptors were coupled to both contraction and relaxation in human perfused placental cotyledons and that these receptors were unevenly distributed along the placental vascular tree; in the chord and chorionic vessels P2Y1 and P2Y2 receptor distribution was mainly in the smooth muscle, whereas in the cotyledon vessel these receptors were equally distributed between the endothelium and smooth muscle cells (Buvinic et al., 2006).

O. Saphenous Vessels

1. Saphenous Artery. The rabbit saphenous artery exhibits a large purinergic component of the contractile response to sympathetic nerve stimulation (Burnstock and Warland, 1987a; Warland and Burnstock, 1987). At a stimulation frequency of 2 Hz, the principal transmitter is ATP, while at higher frequencies both NA and ATP are involved (Zhang and Ren, 2001). EJPs recorded in smooth muscle of the guinea pig saphenous artery were inhibited by arylazido amino propyl ATP,
a P2 receptor antagonist (Cheung and Fujioka, 1986) and also by suramin (Nally and Muir, 1992). The purinergic contractile component of rabbit saphenous (and ileocolic) arteries was antagonized by nifedipine, a calcium channel blocker (Bulloch et al., 1991). Prejunctional α2-adrenoceptors and P2 receptors were suggested to play a role in the autoregulation of neuromuscular transmission in the guinea pig saphenous artery (Fujioke and Cheung, 1987). NPY potentiates the purinergic component of the neural response in guinea pig saphenous artery (Cheung, 1991). ET-1 modulates cotransmission in the rabbit saphenous artery by potentiating postjunctionally the purinergic component of the contractile response to nerve stimulation and to exogenous ATP (Mutafova-Yambolieva and Radomirov, 1994). NF023, a selective P2X1 receptor antagonist (Lambrecht, 1996), blocked the constrictor response of rabbit saphenous artery to α,β-methylATP but not the endothelium-dependent and -independent responses mediated by P2Y receptors (Ziyal et al., 1997). Substantial release of ATP from endothelial cells from rat saphenous artery in response to α1-adrenoceptor activation has been reported (Shinozuka et al., 1997).

2. Saphenous Vein. Sympathetic cotransmission involving NA, ATP, and NPY has been described for the human saphenous vein (Rump and von Kugelgen, 1994; Racchi et al., 1999; Loesch and Dashwood, 2009) and dog saphenous vein, where an involvement of α1- and α2-adrenoceptors and P2X receptors was shown (Hiraoka et al., 2000). ATP elicited a transient inward current and increased [Ca2+]i in freshly isolated smooth muscle cells of human saphenous vein via P2X receptors (Loirand and Pacaud, 1995). NPY, acting postjunctionally, potentiated the vasoconstriction elicited by ATP as well as NA (Donoso et al., 2004). Diadenosine polyphosphates are coreleased with ATP into the blood stream from the dense granules of platelets, and Ap3A was an effective agonist at eliciting calcium responses at P2Y1 and P2Y2 receptors expressed on human cultured saphenous vein endothelial cells; other diadenosine polyphosphates were largely ineffective agonists (Conant et al., 2000). Downregulation of P2X1 receptors mediating contraction and upregulation of P2Y1 and P2Y2 receptors has been demonstrated in human long saphenous vein during varicose disease (Metcalfe et al., 2007).

P. Renal Vessels

1. Microcirculation. Adenosine was shown early to increase vascular resistance in the kidneys of pigs, dogs, rabbits, and rats, whereas it vasodilates most other nonrenal blood vessels (Thurau, 1964; Scott et al., 1965; Osswald, 1975; Sakai et al., 1981). Sympathetic nerve-mediated vasoconstriction results in release of adenosine into the perfusate of the kidney vascular bed (Fredholm and Hedqvist, 1978), in retrospect probably released from endothelial cells in response to shear stress induced by changes in blood flow. Ecto-5’-nucleotidase controls the renal production of adenosine (Ramos-Salazar and Baines, 1986). It was suggested that the intrarenal vasoconstrictor effects of adenosine were mediated by an angiotensin mechanism (Spielman and Osswald, 1979; Dietrich et al., 1991), and inhibition of adenosine A1 receptor-mediated glomerular vasoconstriction in angiotensin AT1A receptor-deficient mice has been reported (Traynor et al., 1998). Conversely, attenuated renovascular constrictor responses to Ang-II have been reported in A1 receptor knockout mice (Hansen et al., 2003). However, one article (Barrett and Droppleman, 1993) presented evidence against an interaction between renovascular A1 and angiotensin AT1 receptors. The vasoconstriction elicited by intravenous infusion of adenosine acting via A1 receptors is short lasting, being replaced within 1–2 minutes by vasodilation, probably mediated by A2 receptors on endothelial cells generating NO (Hansen and Schnermann, 2003; Hansen et al., 2005).

ATP has a vasodilatory effect in the renal circulation, but only when given directly into the renal artery; ATP is used clinically to induce hypotension, but is not effective if administered intravenously (Hashimoto et al., 1988). ATP dilates the rat perfused kidney via endothelial release of NO (Radermacher et al., 1990). In rat isolated perfused kidneys, it was shown that P2X receptors, and perhaps also P2U (P2Y2 and/or P2Y4) receptors mediating vasoconstriction, are located on smooth muscle and that endothelial P2Y receptors mediate vasodilation involving NO but not prostanoids (Churchill and Ellis, 1993a; Eltze and Ullrich, 1996). P2X1 receptors have been localized using autoradiography and immunochemistry on vascular smooth muscle cells of rat intrarenal arcuate and interlobular arteries and afferent (but not efferent) glomerular arterioles (Chan et al., 1998). Renal periarterial (sympathetic) nerve stimulation induced vasoconstriction at low frequencies and was primarily due to ATP release in the rat perfused kidney (Schwartz and Malik, 1989). Neuronal- and paracrine-released nucleotides evoke renal vasoconstriction of mouse perfused kidney via activation of P2X1, P2X3, and P2Y6 receptors (Vonend et al., 2005b). α2A-Adrenoceptor activation inhibits both NA and ATP release from mouse renal sympathetic nerves (Vonend et al., 2007). P2 receptor antagonists enhanced noradrenergic pressor responses of rat perfused kidney to sympathetic nerve stimulation by blocking prejunctional receptors (Bohmann et al., 1997).

Diadenosine polyphosphates evoke transient constrictions of the rat renal microcirculation, which are mediated by A1 and P2 receptors (Gabriels et al., 2000). Ap3A-induced vasoconstriction of human renal resistance vessels appeared to be mediated by P2X receptors, whereas vasodilation due to Ap4A appeared to be mediated by P2Y receptors (Steinmetz et al., 2003).
Uridine, UMP, and UDP had vasoconstrictor actions in the rat isolated perfused kidney (Macdonald et al., 1984). UpD₄A was claimed to act as a potent vasoconstrictor of the rat isolated perfused kidney via P₂X₁ and P₂Y₂ receptors, whereas endothelium-dependent vasodilation was mediated by P₂Y₁ and P₂Y₂ receptors (Tölle et al., 2010).

2. Renal Arteries. Adenosine dilates rabbit renal arteries, and it was suggested that this was via P₁ receptors on the smooth muscle (Gagnon et al., 1980). However, subsequent studies showed that adenosine-mediated vasodilation of rabbit renal arteries is endothelium dependent, but independent of NO (Rump et al., 1998, 1999). A₂A receptors mediate vasodilation in rabbit renal arcuate artery, and this has been claimed to involve K⁺ channel activation (Prior et al., 1999). In rat renal artery A₂A receptors have been suggested to mediate endothelium-dependent relaxation, which may involve EDHF (Grbović et al., 2000).

β,γ-Methylene ATP, a P₂X receptor agonist, produced weak vasoconstrictor responses of human renal arteries (von Kügelgen et al., 1995). Extracellular ATP increased cytosolic calcium in rat cultured renal artery smooth muscle cells (Inscho et al., 1996). Freshly isolated renal vascular smooth muscle cells from interlobular and arcuate arteries of rat kidney express genes encoding P₂X₁ and P₂X₄ receptors (Harhun et al., 2009). Sympathetic nerve stimulation releases both ATP and NA in human renal arteries; ATP elicits vasoconstrictor responses via P₂X receptors, whereas vasodilator responses are mediated via P₂Y receptors (Rump et al., 1996). In the rat renal artery, P₂X₁, P₂Y₁, and P₂Y₂ receptors on the smooth muscle mediate vasoconstriction in response to ATP released as a cotransmitter from sympathetic nerves (Knight et al., 2003). An unusual absence of endothelium-dependent or -independent vasodilation to purines and pyrimidines (but not to ACh) was reported in the rat renal artery (Knight et al., 2003). The authors speculated that the absence of endothelium-mediated vasodilation may reflect species variation, because in rabbits P₂X receptor endothelium-mediated vasodilation via NO is present (Rump et al., 1998).

3. Juxtamedullary Afferent Arterioles. Afferent arteriolar A₁ receptors mediate constriction, whereas A₂ receptors on afferent and efferent arterioles mediate dilation (Murray and Churchill, 1985; Holz and Steinhausen, 1987; Givertz, 2009). Afferent arterioles perfused with adenosine responded with persistent vasoconstriction via A₁ receptors on the smooth muscle (Hansen and Schnermann, 2003; Hansen et al., 2007). The preglomerular vasorelaxant adenosine receptors were identified as A₂ₐ receptors (Carroll et al., 2006), whereas dilation of efferent arterioles may be mediated by A₂b receptors (Al-Mashhadi et al., 2009). Vasodilation via low-affinity A₂ receptors and vasoconstriction via high-affinity A₁ receptors and by α,β-meATP-sensitive P₂ receptors (likely P₂X₁) has been demonstrated in rat juxtamedullary afferent arterioles using a blood-perfused juxtamedullary nephron preparation combined with videomicroscopy to measure arteriolar diameter (Inscho et al., 1991); the P₂X receptors were later shown to be present on the afferent, but not efferent, arterioles (Inscho et al., 1992). Similarly, Weihprecht et al. (1992) identified the P₂ receptor causing vasoconstriction of rabbit afferent arteries as a P₂X receptor and also showed a vasoconstrictor effect of adenosine via A₁ receptors in the afferent arteriole.

ATP evokes a biphasic vasoconstriction of the preglomerular vasculature, and it was suggested that the smooth muscle of juxtamedullary afferent arterioles expresses both P₂X₁ and P₂Y₂ receptors (Inscho et al., 1998, 1999). Both the initial and sustained phases of ATP-mediated juxtamedullary afferent arteriolar vasconstriction are dependent on the influx of extracellular Ca²⁺, and the sustained constriction is dependent on Ca²⁺ entry via voltage-gated L-type calcium channels (Inscho et al., 1995). P₂X₁ receptor activation of pregglomerular microvascular smooth muscle cells increases [Ca²⁺], via L-type Ca²⁺ channels (White et al., 2001), but P₂Y₂ receptors mediating vasoconstriction are also present (Inscho and Cook, 2002). P₂X receptors on smooth muscle cells showed no difference in distribution along the length of rabbit afferent arterioles (Gutiérrez et al., 1999). 20-Hydroxyeicosatetraenoic acid, a metabolite of the arachidonic pathway, plays an important role in the regulation of renal vascular and tubular function, and 20-hydroxyeicosatetraenoic acid has been shown to play a significant role in the renal microvascular smooth muscle cell [Ca²⁺] response to P₂X receptor activation (Zhao et al., 2004).

In keeping with sympathetic cotransmitter control of afferent arterioles, ATP increases their reactivity to low concentrations of NA (Hultström et al., 2007). UpD₄A, a novel EDCF that acts via P₂X receptors, is a potent vasoconstrictor of rat juxtamedullary afferent arterioles (Inscho et al., 2008). Intraglomerular ectonucleotidases regulate purine activities (Bakker et al., 1993).

In the kidney, there is some independence of glomerular filtration rate and renal blood flow from arterial pressure between approximately 90 and 180 mm Hg. This uncoupling occurs because of autoregulation and ensures that there is a relative maintenance of fluid and solute excretion in the face of fluctuations of blood pressure. Autoregulation is brought about by adjustments in the diameter of the afferent arteriole. It involves a myogenic mechanism, common to many arteries, in which the afferent arteriole contracts in response to pressure and stretch and involves calcium entry through voltage-dependent calcium channels. It also involves the tubuloglomerular feedback mechanism in which the macula densa senses the sodium chloride concentration of the filtrate and produces
a signal that causes afferent arteriolar constriction. Tubuloglomerular feedback signals are coupled to autoregulatory preglomerular vasoconstriction through ATP-mediated activation of P2X1 receptors (Inscho et al., 2004). Evidence has been presented that autoregulation is impaired in P2X1 receptor knockout mice, suggesting that ATP released from the macula densa directly stimulates afferent arteriolar constriction via P2X1 receptors (Inscho et al., 2004). Autoregulation and P2X1 receptor-mediated afferent arteriolar responses were impaired in a rat model of hypertension (Inscho et al., 2011). However, seemingly conflicting evidence has shown that the tubuloglomerular feedback response is also abolished in A1 adenosine receptor knockout mice (Brown et al., 2001; Sun et al., 2001).

ATP can stimulate secretion of renin from juxtaglomerular cells (Churchill and Ellis, 1993b), whereas adenosine depresses renin secretion in sodium-restricted rats (Osswald et al., 1978). A1 and A2 receptors mediate inhibition and stimulation of renin secretion, respectively (Murray and Churchill, 1985). Mechanical stimulation of a single juxtaglomerular cell initiated propagation of an intracellular Ca2+ wave to up to about 12 surrounding cells; this was prevented by apyrase, which catalyzes the hydrolysis of ATP, implicating ATP as the mediator responsible for the propagation of Ca2+ signaling (Yao et al., 2003). These authors also claimed that administration of ATP into perfused rat kidney induced a rapid, potent, and persistent inhibition of renin secretion with a transient elevation of renal vascular resistance, perhaps after breakdown to adenosine. An ATP-mediated intracellular Ca2+ wave has been demonstrated in renal juxtaglomerular endothelial cells, which may serve an important feedback role during vasoconstriction (Bansal et al., 2007). Bovine glomerular endothelial cells are equally sensitive to mobilization of calcium by UTP and ATP (Briner and Kern, 1994), suggesting that P2Y2 and/or P2Y4 receptors may be involved.

There have been reviews about purinergic regulation of renal blood flow (Inscho et al., 1994; Navar et al., 1996; Inscho, 2001, 2009; Nishiyama et al., 2004; Jankowski, 2008; Schnermann and Briggs, 2008). It has been proposed that adenosine mediates hemodynamic changes in adult renal failure (Churchill and Bidani, 1982; Smith et al., 2000). Cyclosporine has become a standard component of the immunosuppressive regimen in both solid organ and bone marrow transplantation as well as for the treatment of autoimmune diseases. However, a limiting factor in its use has been the development of nephrotoxicity and hypertension in many patients. Hypertension is a feature of chronic renal disease, and it has been claimed that this is largely due to sympathetic overactivity triggered by afferent signals emanating from the kidney and resetting sympathetic tone by stimulation of hypothalamic centers (Orth et al., 2001). Both essential hypertensive patients and patients with renal artery stenosis show a dose-dependent vasodilation after adenosine infusion (Wierema et al., 1998, 2005). In raised-tone perfused kidneys from normal rats ATP elicited constriction at low doses and vasodilatation at higher doses; ATP-mediated vasoconstriction was increased in hyperthyroid kidneys and severely attenuated in kidneys from hypothyroid rats, whereas the vasodilator response in kidneys from hypothyroid rats was abolished (Vargas et al., 1996). A detailed review of purinergic signaling in renal ischemia can be found later in this review.

Q. Hepatic Artery and Portal Vein

1. Hepatic Artery. Portally infused ATP leads to transhepatic vasodilatation via P2Y receptors on the endothelium, whereas ATP released from sympathetic nerves leads to vasoconstriction of hepatic arteries via P2X receptors on the muscle (Brizzolara and Burnstock, 1990, 1991; Phillips et al., 1998). Both endothelium-dependent and endothelium-independent vasodilation of rabbit hepatic artery mediated by purines has been described (Brizzolara and Burnstock, 1991). In the rabbit perfused liver, both P2X vasoconstrictor and P2Y vasodilator receptors (leading to release of NO) were identified (Mathie et al., 1991b; Ralevic et al., 1991). In the rat sinusoidal vascular bed, ATP impairs the flow-limited distribution of [3H]water (Fernandes et al., 2003). ATP-MgCl2 restores depressed hepatocellular function and hepatic blood flow after hemorrhage and resuscitation (Wang et al., 1991). Adenosine-induced dilation of the rabbit hepatic arterial bed is mediated by A2 receptors (Mathie et al., 1991a).

2. Portal Vein. The first suggestion that ATP might be a neurotransmitter in nonadrenergic vasodilator nerves supplying the rabbit portal vein was published by Che Su (Su and Sum, 1974; Su, 1975), and later ATP was shown to be a cotransmitter with NA in sympathetic vasoconstrictor nerves supplying this vein (Su, 1978a). This was supported by subsequent experiments in the rabbit, but not the guinea pig, portal vein (Burnstock et al., 1979). Brizzolara et al. (1993) showed that both ATP and NO are inhibitory cotransmitters in NANC nerves supplying the rabbit portal vein. Most of the ATP released during transmural electrical stimulation of the rabbit portal vein is from nerves (Levitt and Westfall, 1982). Nerve stimulation and ATP contracted the rat portal vein at basal tone via P2X receptors, but caused relaxation in veins with tone raised with ergotamine, probably via P2Y receptors (Reilly et al., 1987). A nonadrenergic contractile response of the guinea pig portal vein to electrical field stimulation has been described, which is mimicked by the response to UTP, but not ATP (Ishizaki et al., 1997), perhaps indicating an involvement of P2Y4 receptors on smooth muscle. A1 receptors were identified as mediating inhibition by adenosine of nerve stimulation-induced contractions.
of the rabbit portal vein (Brown and Collis, 1983). Inhibition of adrenergic neurotransmission by adenosine in the rat portal vein was also reported (Enero, 1981). However, evidence was presented by Kennedy and Burnstock (1984) that the inhibitory prejunctional actions of adenosine on the rat portal vein are mediated by A2 rather than A1 receptors. Experiments carried out on electrical stimulation of the portal vein in freely moving rats showed that overflow of NA is under facilitatory control of its cotransmitter ATP through prejunctional P2Y receptors (Smit et al., 1998). ATP causes postjunctional potentiation of noradrenergic contractions in the portal vein of guinea pig and rat (Kennedy and Burnstock, 1986).

There is evidence for two types of P2 receptor on the longitudinal muscle of the rabbit portal vein: a P2 receptor mediating contractile responses to α,β-meATP (P2X) and an endothelium-independent vasorelaxant P2 receptor activated by ATP and 2-MeSATP (P2Y) (Hughes and Vane, 1967; Burnstock et al., 1979; Kennedy and Burnstock, 1985b). ATP activates cation currents in the rabbit portal vein (Xiong et al., 1991; Pacaud et al., 1994), consistent with P2X receptor activation (Orre et al., 1996). The P2 receptor mediating contraction of the longitudinal muscle of the rat portal vein is also the P2X receptor subtype (Reilly and Burnstock, 1987), and P2X receptors have been identified on rat portal vein myocytes (Orre et al., 1996; Mironneau et al., 2001). ATP, ADP, and UTP, but not adenosine, introduced into the portal circulation of the rat isolated perfused liver increased perfusion pressure (probably via P2X, P2Y2, and/or P2Y4 receptors), whereas infusion of ATP, ADP, and adenosine, but not UTP, transiently increased generation of NO (probably via endothelial purine receptors) (Takemura et al., 1998). Endothelium-independent relaxation of rat portal vein smooth muscle by ATP is mediated, after breakdown by ectonucleotidase, by adenosine activating via A2A receptors (Guibert et al., 1998). The restoration of hepatic blood flow with ATP-MgCl2 treatment after hemorrhage in rats is due to increased portal vein blood flow (Wang et al., 1992b).

R. Other Blood Vessels

From a study of the responses of canine lingual artery to perivascular nerve stimulation, it was concluded that neurogenic contractions were induced largely by ATP acting via P2X receptors (Okamura et al., 1998).

P2Y1, P2Y2 (and/or P2Y4), and P2Y9 receptors were claimed to mediate endothelium-dependent vasodilatation of human left internal mammary artery; no evidence for the involvement of endothelial P2X4 receptors in vasodilation was found (Wihlborg et al., 2003). P2Y12 receptor mRNA was shown to have a high expression among P2 receptors in human internal mammary vascular smooth muscle, and P2Y12 receptors were claimed to mediate contraction of human internal mammary artery and small veins (Wihlborg et al., 2004).

Articular vessels supplying the rabbit knee joint express smooth muscle P1 receptors, which mediate vasodilatation, and P2 receptors, which mediate vasoconstriction; an endothelium-dependent vasodilator effect of ATP and ADP is also evident (Ferrell and Khosbaten, 1990; Matsuda et al., 2009).

Purinoceptors have been shown to be involved in the regulation of local blood flow in the eye (Ziganshina et al., 2012). ATP induced contraction, probably via P2X1 receptors, in bovine ophthalmic and posterior ciliary arteries and relaxation, probably via P1 receptors. Inhibition of bovine retinal microvascular pericyte proliferation was mediated by adenosine (Jackson and Carlson, 1992).

Two subtypes of P2 receptors exist on the pancreatic vascular bed: in general P2X receptors mediate vasoconstriction and P2Y receptors mediate vasodilatation (Chapal and Loubatieres-Mariani, 1983; Hillaire-Buys et al., 1991). Adenosine-5′-(β-thio)diphosphate, a P2Y-selective agonist, caused endothelium-dependent vasodilatation of the rat isolated perfused pancreas, which was mediated partly by NO and PGI2 but also through another mechanism that did not involve KATP, SKCa, and BKCa channels (Saäg et al., 1996; Hillaire-Buys et al., 1998). P2X1, P2X2, P2Y1, and P2Y2 receptor expression was shown using immunohistochemistry in small blood vessels of the rat pancreas (Coutinho-Silva et al., 2003). An endothelial vasocontractile P2Y14 receptor, sensitive to UDP-glucose and UDP, was described in porcine pancreatic arteries (Alsaqati et al., 2013). The role of purine receptors in regulating endocrine and exocrine functions of the pancreas has been reviewed (Novak, 2008).

S. Lymphatic Vessels

Transmural stimulation of bovine mesenteric lymphatics elicited a contraction followed by short- or long-lasting relaxation; it was proposed that nonadrenergic inhibitory nerves may contribute to the long-lasting relaxation (Ohhashi and Roddie, 1981). Studies of neuromuscular transmission in bovine mesenteric lymphatics using the sucrose-gap technique reported EJPs in response to nerve stimulation that, on repetitive stimulation, summed and facilitated to reach threshold potential for firing of action potentials and contraction (Allen and McHale, 1986), reminiscent of EJPs mediated by ATP in response to sympathetic nerve stimulation in the vas deferens and blood vessels (see Burnstock and Holman, 1961; Burnstock, 1990b). α,β-MeATP caused an intense, transient excitatory effect on sheep isolated mesenteric lymphatics (Harty et al., 1993). Hollywood and McHale (1994) presented evidence that stimulation of nerves supplying the sheep mesenteric artery produced excitation mediated by release of ATP and NA. ATP increases lymphatic
smooth muscle [Ca$^{2+}$]$_i$ and vasomotion via activation of both P2X$_1$ and P2Y$_2$ receptors (Zhao and Van Helden, 2002).

In addition to direct excitation of lymphatic smooth muscle, P2Y receptors on endothelial cells mediate ATP-induced constriction via an endothelium-derived excitatory factor, probably thromboxane A$_2$ (Gao et al., 1999). ATP-induced dilation and inhibition of pump activity of lymph vessels via A$_2$ receptors on smooth muscle and on endothelium has been reported, probably acting via NO (Kousai et al., 2004). Shear stress produces release of ATP from lymphatic endothelial cells, which activates P2 receptors, thereby facilitating production of NO and protein expression (Kawai et al., 2010).

The endolymphatic sac epithelium of the mammalian inner ear absorbs endolymphatic fluid generated from the stria vascularis in the cochlea to regulate endolymph volume, and ATP has been shown to increase [Ca$^{2+}$]$_i$ in these epithelial cells via P2Y receptors (Wu and Mori, 1999).

### IV. Long-Term Trophic Roles of Purines and Pyrimidines

There are reviews describing the effect of purines and pyrimidines on proliferation, differentiation, and death of different cell types (Abracchio, 1996; Abracchio and Burnstock, 1998; Neary and Abracchio, 2001; Burnstock, 2002; Adair, 2004; Burnstock and Verkhratsky, 2010).

An important early article showed that intact innervation of the rabbit ear artery was necessary for normal development and maintenance of the artery wall, and trophic factors were implicated (Bevan and Tsuru, 1981). Comparable findings were later obtained with denervated pulmonary and mesenteric arteries (Bevan, 1989). Impaired endothelial-mediated relaxation was observed after long-term denervation of the auricular and central ear arteries (Mangiarua and Bevan, 1986). Both endothelial and smooth muscle cell migration was shown to take place in the repair of injured vessel walls (Gotlieb, 1983). ATP and ADP were shown to be mitogenic (Van Coevorden et al., 1989) and ATP release from endothelial cells was recognized (LeRoy et al., 1984; see Burnstock, 1999). P1 and P2Y receptors mediate the trophic effects of adenosine, ATP, ADP, UTP, and UDP on smooth muscle and endothelial cells (Table 4).

#### A. Vascular Smooth Muscle

Vascular smooth muscle cell migration and proliferation are critical stages in the pathogenesis of atherosclerosis, postangioplasty restenosis/neointimal hyperplasia and chronic allograft rejection.

Extracellular ATP and ADP stimulate proliferation of porcine aortic smooth muscle cells (Wang et al., 1992a). ATP, released as a cotransmitter from sympathetic nerves as well as from platelets, endothelial cells, and damaged smooth muscle was proposed as a mediator of vascular smooth muscle proliferation, which may play a role in atherosclerosis and hypertension (Erlinge et al., 1995). In addition, ADP released from platelets, in synergy with peptide growth factors, leads to smooth muscle proliferation at sites of vascular injury (Crowley et al., 1994). ATP-induced modulation of cell cycle-dependent gene mRNA levels, leading to rat aortic smooth muscle cell proliferation, appeared to be mediated by P2Y rather than P2X receptors, and it was suggested that they may play a role in late postangioplasty restenosis (Malam-Souley et al., 1993), probably involving P2Y$_2$ and/or P2Y$_4$ receptors because the effects were mimicked by UTP (Malam-Souley et al., 1996). In a separate study, P2Y$_2$ and/or P2Y$_4$ receptors sensitive to ATP and UTP, but not P2X$_1$ receptors, were similarly identified as mediators of proliferation of rat aortic smooth muscle cells (Erlinge et al., 1995). UDP acts as a growth factor for smooth muscle cells cultured from rat aorta via activation of P2Y$_6$ receptors (Hou et al., 2002). Up4A stimulates DNA synthesis and proliferation of human vascular smooth muscle cells derived from the aorta; this is mediated by P2Y receptors involving the MAPK and PI3K/Akt pathways (Gui et al., 2011). Up4A is also a strong inducer of vascular smooth muscle cell migration via P2Y$_2$ receptors (Wiedon et al., 2012). UTP and UDP have been claimed to induce rat aortic smooth muscle cell migration via P2Y$_2$ and P2Y$_6$ receptors, respectively (Pilois et al., 2002). Antiproliferative effects of UTP on cultured vascular smooth muscle cells derived from human internal mammary artery and saphenous vein have been claimed (White et al., 2000; Boarder et al., 2001). More recently, P2X$_1$ receptors were shown to mediate inhibition of proliferation of human coronary artery smooth muscle cells via induction of the transcription factor NR4A1 (Hinze et al., 2013).

Proliferation of vascular smooth muscle has consistently been shown to be accompanied by an increased expression of P2Y receptors. The differentiated contractile phenotype of vascular smooth muscle expresses predominantly P2X$_1$ receptors, whereas in the dedifferentiated synthetic phenotype, P2X$_1$ receptors are downregulated and the mitogenic P2Y$_1$ and P2Y$_2$ transcripts upregulated (Erlinge et al., 1998). In a study of human umbilical vein, it was claimed that shear stress led to decreased expression of P2X$_1$ receptors on smooth muscle (leading to reduced tone) and increased expression of P2Y$_2$ and P2Y$_6$ receptors (which stimulate migration and growth of muscle cells), a similar phenotypic shift as seen from cultured contractile to synthetic smooth muscle cells (Wang et al., 2003a). Upregulation of P2Y$_2$ receptors and the mitogenic actions of ATP and UTP on coronary artery smooth muscle cells have been demonstrated in both in vitro organ cultures and in vivo stented coronary arteries (Shen et al., 2004). Cytokines...
induce upregulation of P2Y2 receptors on vascular smooth muscle cells, resulting in increased mitogenic responses to UTP and ATP (Hou et al., 2000). Arrestin-dependent regulation of P2Y2 receptor-stimulated MAPK signaling is essential for migration of aortic smooth muscle cells, a key event in vascular remodeling (Morris et al., 2012).

The mechanisms by which nucleotides promote mitogenesis have been investigated. Low ATP concentrations stimulate expression of genes for the contractile vascular smooth muscle phenotype, whereas high concentrations of ATP cause a phenotypic shift from the contractile to the synthetic phenotype; this shift is dependent on a transient activation of PKA, which inhibits activation of a serum response factor (Hogarth et al., 2004). ATP promotes vascular smooth muscle cell DNA synthesis and cell proliferation during embryonic and postnatal development after injury and in atherosclerosis through the activation of ERK1/2 involving both PKC- and Ca2+-calmodulin-dependent protein kinase II-b2 (Ginnan et al., 2004). ATP-stimulated coronary artery smooth muscle proliferation requires second messenger signals of both the ERK and phosphatidylinositol-3-kinase pathways (Wilden et al., 1998). Phosphorylation of z-catenin, a protein that controls cell-cell adhesion and cell proliferation by PKA, promotes ATP-induced proliferation of vascular smooth muscle cells (Taurin et al., 2008). ATP stimulates prostaglandin E2/cyclin D1-dependent proliferation of vascular smooth muscle via STAT3 activation and involves PKC-dependent NADPH oxidase/ROS generation (Lee et al., 2013). Tyrophostin, a specific inhibitor of tyrosine kinases, inhibited ATP-induced DNA synthesis, cell proliferation, and fos-protein expression, but not ATP-induced Ca2+ influx or vasoconstriction, in rat aortic smooth muscle cells (Erlinge et al., 1996). The expression of osteopontin, a chemoattractive agent for several cell types, is involved in vessel remodeling and is stimulated by UTP (Renault et al., 2005). Overexpression of E-NTPDase 1/CD39 decreases vascular smooth muscle cell proliferation and prevents neointima formation after angioplasty, consistent with an involvement of nucleotides in these processes (Koziak et al., 2008). E-NTPDase 1/CD39 is expressed on mouse carotid artery smooth muscle cells and is required for neointimal formation (Behdad et al., 2009). These authors showed further that E-NTPDase 1/CD39 deletion impairs smooth muscle cell migration in vitro and inhibits neointimal formation in a murine model of injured carotid arteries.

Vascular calcification is associated with atherosclerosis, type 2 diabetes, end-stage renal disease, and aging. Extracellular PPi is a critical inhibitor of calcification. The PPi-generating proteins include ectophosphodiesterase/pyrophosphatase, which catalyzes the hydrolysis of released ATP to produce PPi. Addition of ATP rescued cAMP- and phosphate-treated vascular smooth muscle cell cultures from progression to the calcified state (Prosdocimo et al., 2010).

Reviews concerned with the trophic effects of ATP on vascular smooth muscle and endothelial cells are available (Erlinge, 1998; Burnstock, 2002). A1 receptors mediate the mitogenic effect of adenosine on porcine coronary artery smooth muscle cells; 8-cyclopentyl-1,3-dipropyl-xanthine, a selective antagonist of the A1 receptor, prevented the proliferation of smooth muscle (Kang et al., 2009). Evidence for adenosine stimulation of pig coronary artery smooth muscle cell proliferation via A1 receptors has similarly been presented (Shen et al., 2005). Adenosine, acting via both A1 and A2 receptors, stimulates DNA synthesis in rat cultured arterial smooth muscle cells (Jonzon et al., 1985). However, adenosine, via A2B receptors, has been reported to inhibit growth of rat and human aortic smooth muscle cells (Dubey et al., 1996, 1998; Jackson et al., 2011). Strong effects of adenosine on gene induction in human coronary artery vascular smooth muscle cells via A2B receptors have been described associated with the antiproliferative effects of adenosine.

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on vascular smooth muscle cells (Mayer et al., 2011). A2B receptor agonists have been shown to inhibit neointimal lesion development after arterial injury in apolipoprotein E-deficient mice, and it was suggested that A2B agonism may be a therapeutic approach in the prevention of restenosis (Bot et al., 2012). Adenosine-induced apoptosis of human arterial smooth muscle cells has also been shown to involve A2B receptors (Peyot et al., 2000). A3 receptor activation induced proliferation of primary human coronary smooth muscle cells, involving the induction of early growth response genes (Hinze et al., 2012). The activity and expression of ecto 5′-nucleotidase/CD73 are increased by thyroid hormones, which would be expected to result in higher extracellular levels of adenosine and consequently influence vascular smooth muscle cell proliferation (Tamajusuku et al., 2006). Adenosine has been claimed to regulate human vascular smooth muscle cell proliferation and migration, in part by affecting the expression of hyaluronic acid synthase (Grandoch et al., 2011).

B. Vascular Endothelial Cells

It was recognized early that nucleotides can induce endothelial cell proliferation and migration (McAuslan et al., 1983; Van Coevorden et al., 1989). ADP promotes human endothelial cell migration by activating P2Y1 receptor-mediated MAPK pathways, perhaps contributing to re-endothelialization and angiogenesis after vascular injury (Shen and DiCorleto, 2008). Evidence has been presented that stimulation of human endothelial cells via P2Y1 receptors activates VEGF-2 to stimulate angiogenesis (Rumjahn et al., 2009). It has been claimed that proliferation of endothelial cells is induced by a VEGF-A165-ATP complex (Gast et al., 2011). ATP causes proliferation of bovine cultured corneal endothelial cells via P2 receptors (Cha et al., 2000). P2Y1 and P2Y13 receptors mediate Ca2+ signaling and proliferation of pulmonary artery vasa vasorum endothelial cells and may represent targets for treatment of pathologic vascular remodeling involving vasa vasorum expansion (Lyuibchenko et al., 2011). Small conductance Ca2+-activated K+ channels function to enhance cell proliferation by ATP on brain endothelial cells (Yamazaki et al., 2006). UTP, as well as ATP, has mitogenic and angiogenic actions on vascular endothelial cells, implicating mediation by P2Y2 and possibly P2Y4 receptors (Satterwhite et al., 1999). P2Y2 and possibly P2Y4 receptors mediate the trophic actions of UTP and ATP in HUVECs, influencing cytoskeletal changes, cellular adhesion, and motility (Kaczmarek et al., 2005). P2Y4 mRNA was detected in primary endothelial cells isolated from mouse heart (but not lung), and studies of P2Y4 knockout mice led to the conclusion that the P2Y4 receptor is an important regulator of angiogenesis and inflammation (Horckmans et al., 2008). It has been suggested that ATP, via P2Y11 receptors, impairs endothelial cells proliferation by inducing cell cycle arrest and sensitizing the cells to cisplatin-induced death (Xiao et al., 2011). There is evidence that stimulation of P2X7 receptors enhances apoptosis of endothelial cells mediated by lipopolysaccharide, which causes release of ATP (Sylte et al., 2005).

Angiogenic expansion of the vasa vasorum contributes to the progression of systemic and pulmonary vascular diseases. Vasa vasorum endothelial cells were isolated from the pulmonary artery adventitia, and ATP release during hypoxia was shown to have mitogenic actions (Gerasimovskaya et al., 2005). ATP was shown to dramatically increase DNA synthesis, migration, and tube formation in vasa vasorum endothelial cells (Gerasimovskaya et al., 2008). Vasa vasorum endothelial cells are a potent source of extracellular ATP in the pulmonary artery wall, and P13K and Rho/ROCK pathways in response to ATP are the critical signaling components of the autocrine/paracrine signaling loop linking ATP release and ATP-induced angiogenic responses (Woodward et al., 2009). P2Y receptor-mediated stimulation of brain capillary endothelial cells enhanced cell proliferation by apamin-sensitive small-conductance Ca2+-activated K+ channels in the majority of cells but also triggered cell death in a subpopulation (Yamazaki et al., 2011). Thymosin β-4 (Tβ4) is a peptide released by platelets to improve wound healing. It has been shown recently that Tβ4 increases cell surface ATP levels via ATP synthase and that P2X4 receptors are required for Tβ4-induced HUVEC migration (Freeman et al., 2011).

Enzymatic regulation of extracellular levels of nucleotides influences endothelial cell proliferation and differentiation and angiogenesis. Pigment epithelium-derived factor (PEDF) is a potent blocker of angiogenesis in vivo and of endothelial cell migration. Notari et al. (2010) suggested that PEDF-mediated inhibition of ATP synthase may form part of the biochemical mechanisms by which PEDF exerts its antiangiogenic activity. Ecto-F1F0-ATP synthase/F1-ATPase, an enzymatic complex responsible for ATP synthesis in mitochondria, is also expressed on endothelial cells, where it binds angiotatin, regulates surface ATP levels, and inhibits endothelial cell proliferation and differentiation (Burwick et al., 2005; Champagne et al., 2006). Stimulation of cell surface F1-ATPase activity by apolipoprotein A-1 inhibits endothelial cell apoptosis and promotes proliferation (Radojkovic et al., 2009). Deletion of E-NTPDase 1/CD39, the principal ectonucleotidase expressed by endothelial cells, resulted in inhibition of angiogenesis, causing decreased growth of implanted tumors and inhibiting development of pulmonary metastases; abnormalities in endothelial cell adhesion and integrin dysfunction were observed, involving decreased activation of focal adhesion kinase and extracellular signaling-regulated kinase-1 and -2.
in endothelial cells (Jackson et al., 2007). It was suggested that the adhesion defect occurred through P2 receptor desensitization because it was corrected by apyrase treatment of the E-NTPDase/CD39 knockout mice (Jackson et al., 2007). More recently, it was shown that vascular E-NTPDase/CD39 expressed by liver sinusoidal endothelial cells promotes tumor cell growth by scavenging extracellular ATP (Feng et al., 2011).

It was suggested early that adenosine increased capillary density; dipyridamole, which inhibits uptake of adenosine, making more extracellular adenosine available, increases capillary proliferation (Wright et al., 1981). Long-term vasodilation, perhaps via the action of adenosine (after breakdown of released ATP) stimulated capillary growth in rabbit heart and skeletal muscle (Ziada et al., 1984). Hypoxia-induced angiogenesis in chick chorioallantoic membranes is mediated by adenosine (Dusseau and Hutchins, 1988). Adenosine and hypoxia stimulated proliferation and migration of bovine aortic and coronary venular endothelial cells (Meininger et al., 1988; Ziche et al., 1992) and HUVECs (Ethier et al., 1993) via A2 receptors (Sexl et al., 1995; Ethier and Dobson, 1997). In HUVECs and pulmonary endothelial cells, proliferation involved A2A receptors (Sexl et al., 1995; Ahmad et al., 2013). Adenosine stimulates canine retinal microvascular endothelial cell migration and tube formation (Lutty et al., 1998) and proliferation and migration of human retinal endothelial cells (Grant et al., 1999) via A2B receptors (Grant et al., 2001). Adenosine increases the migration of human endothelial progenitor cells via A2B receptor activation (Rolland-Turner et al., 2013). A2B receptors mediate proliferation of porcine and rat arterial endothelial cells (Dubey et al., 2002). A2B receptors in human skin microvascular endothelial cells modulate expression of angiogenic factors (Feoktistov et al., 2002). Adenosine, via A2A receptors, promotes wound healing and mediates angiogenesis in mice in response to tissue injury (Montesinos et al., 2002).

Adenosine cooperates with hypoxia to stimulate VEGF, and it was suggested that adenosine receptors may present a potential therapeutic target for regulation of angiogenesis (Ryzhov et al., 2007). Macrophages play a key role in induction of angiogenesis, and there is synergistic upregulation of VEGF expression in macrophages by A2A receptor agonists and endotoxin (Ramanathan et al., 2007). Adenosine, acting via A2A receptors, downregulates the production by human macrophages of a soluble form of the receptor for VEGF (sFlt-1), a potent antiangiogenic factor that acts by trapping circulating VEGF (thereby preventing its binding to VEGF receptors) and upregulates the membrane form (mFlt-1), identifying a mechanism whereby adenosine may cause angiogenesis (Léonard et al., 2011). Adenosine A2A receptors were shown to be a target of HIF-2α in pulmonary endothelial cells and were implicated in angiogenesis, as assessed by increases in cell proliferation, cell migration, and tube formation (Ahmad et al., 2009). It has been claimed that adenosine is an endogenous inhibitor of neutrophil-mediated injury to endothelial cells (Cronstein et al., 1986). A1 receptor blockade abolishes ischemic preconditioning observed in humans during coronary angioplasty produced by repeated coronary balloon inflation (Tomai et al., 1996).

Figure 8 summarizes the P1 and P2 receptor subtypes on both smooth muscle and endothelial cells that mediate proliferation.

V. Vascular Diseases

Vascular remodeling plays a pivotal role in the progression of conditions associated with ischemic hypoxia and inflammation in diseases such as hypertension, atherosclerosis, restenosis, vascular insufficiency, neoplasia, and transplant rejection. Reviews about the involvement of purinergic signaling in cardiovascular diseases are available (Ralevic and Burnstock, 2003; Erlinge and Burnstock, 2008; Ralevic, 2009; Burnstock and Verkhratsky, 2012; Schuchardt et al., 2012; Headrick et al., 2013). The relevance of ectonucleotidases for the treatment of cardiovascular disorders has also been reviewed (Mathieu, 2012). A number of cardiovascular purine receptors are now targeted for clinical use. P2Y12 receptors mediate platelet aggregation, and clopidogrel and prasugrel, P2Y12 antagonists, have been very successful clinically against thrombosis and stroke (Hollopeter et al., 2001; Baker and White, 2009). Bolus injection of adenosine (Adenocard; Astellas Pharma US, Inc., Northbrook, IL) is used clinically to slow conduction time through the A-V node, interrupt the reentry pathways through the A-V node, and restore normal sinus rhythm in patients with paroxysmal supraventricular tachycardia. A1 receptor agonists were clinical candidates to treat paroxysmal supraventricular tachycardia (Rankin et al., 1992) and afford protection in ischemia-reperfusion injury (Mizumura et al., 1996). P2 receptors have been implicated in migraine and vascular pain (Burnstock, 1989b), diabetic microvascular disease (Nilsson et al., 2006), angiogenesis, and vascular remodeling (see Erlinge and Burnstock, 2008). Detailed analysis of the roles of purinergic signaling in hypertension, atherosclerosis, and diabetes follows and is summarized in Table 5.

A. Hypertension

Increased sympathetic nerve activity occurs in hypertension, and there is associated hyperplasia and hypertrophy of arterial walls, probably involving brain stem hypoperfusion in driving increased sympathetic activity (see Cates et al., 2012). Guanethidine was used in early times for the treatment of hypertension (Bauer et al., 1961), in retrospect inhibiting the release of both NA and ATP cotransmitters from sympathetic nerves.
Hypertrophy of cerebral vessels occurs in spontaneously hypertensive rats (SHR), and it was suggested that sympathetic nerves may be involved in this trophic effect (Hart et al., 1980). Inhibitory prejunctional purinergic modulation of vascular sympathetic neurotransmission in SHR is diminished via both adrenoceptors and A1 receptors (Kamikawa et al., 1980, 1983; Jackson, 1987; Illes et al., 1989; Yu et al., 1997; Park et al., 2010a). There is also impaired function of prejunctional A1 receptors on perivascular sympathetic nerves in deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Sangsiri et al., 2013).

The vasoconstrictor response of tail arteries of SHR to sympathetic nerve stimulation was inhibited by desensitization of P2X receptors with α,β-meATP, although it was little affected in Wistar-Kyoto (WKY) normotensive rats (Vidal et al., 1986; Bulloch and McGrath, 1992; Brock and Van Helden, 1995). It was concluded that ATP plays a more important role in sympathetic vasoconstriction in SHR compared with normotensive rats (see also Goonetilleke et al., 2013), although this has been contested (Dalziel et al., 1989).

Pressor responses to renal nerve stimulation at 1 Hz in SHR kidneys was claimed to be due entirely to the release of ATP from sympathetic nerves (Rump et al., 1990). EJPs are enhanced in the smooth muscle of mesenteric arteries in SHR (Brock and Van Helden, 1995). In contrast, sympathetic nerve-stimulated release of ATP appeared to be unaltered or impaired in rat mesenteric arteries in DOCA-salt hypertension, although release of NA was increased (Luo et al., 2004; Demel and Galligan, 2006, 2008). However, P2X3 receptor agonist-induced afferent arteriolar vasoconstriction is impaired in DOCA-salt hypertensive rats (Guan et al., 2012). The contribution of ATP to excitatory sympathetic neurovascular transmission increased when the pressure of rat small mesenteric arteries was raised from 30 to 90 mmHg, which is similar to the pressure in these arteries in vivo (Rummery et al., 2007). Sympathetic nerve-mediated vasoconstriction was augmented during diet-induced obesity because of an increase in sympathetic nerve density and release of ATP (Haddock and Hill, 2011). NPY postjunctional augmentation of ATP vasoconstrictions after release from sympathetic nerves in the tail artery of SHR was also greater than in WKY rats (Brock and Van Helden, 1995).

Fig. 8. Schematic diagram of long-term (trophic) actions of purines released from nerves, platelets, and endothelial cells (which also release UTP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and sensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via P2Y2 and/or P2Y4 receptors via a mitogen-activated protein kinase (MAPK) cascade, whereas adenosine resulting from enzymatic breakdown of ATP acts on P1 (A2) receptors to inhibit cell proliferation (via elevation of cAMP). ATP and UTP released from endothelial cells stimulate endothelial and smooth muscle cell proliferation via P2Y1, P2Y2, and P2Y4 receptors. Adenosine resulting from ATP breakdown acts on P1 (A2) receptors to stimulate endothelial cell proliferation and regulate the release of platelet-derived growth factor (PDGF) from platelets. (Reproduced from Burnstock, 2002, with permission from Lippincott, Williams and Wilkins).
perivascular nerves is lost in kidneys of adult SHR, possibly as an adaptive mechanism (Vonend et al., 2005a).

Sympathetic vasoconstriction was not mediated by $\alpha$-adrenoceptors in forearm arteries of patients with essential hypertension (Taddei et al., 1989). Autonomic dysreflexia, a potentially life-threatening episodic hypertension that often develops after spinal cord lesion, is induced by increased sympathetic activity, and it seems likely that ATP released as a cotransmitter from sympathetic nerves is involved as well as NA (Groothuis et al., 1998). Increased responsiveness of SHR shows potentiated responses to exogenous ATP (Schluter et al., 2000), and ATP-induced vasoconstriction is significantly potentiated in SHR aorta (Fernandez et al., 2004). In contrast, P2X$_{1}$ receptor-mediated vasoconstriction of afferent arterioles in the rat kidney is attenuated in Ang-II-dependent hypertension (Inscho, 2009). Immunosuppression preserves renal autoregulatory function and microvascular P2X$_1$ receptor reactivity in Ang-II-hypertensive rats (Guan et al., 2013). Treatment of cultured vascular smooth muscle cells with $\alpha$-meATP in SHR has also been reported (Fernandez et al., 2000), and ATP-induced vasoconstriction is significantly potentiated in SHR aorta (Yang et al., 2004). The concentration of Up$_4$A, a dinucleotide claimed to be an endothelium-dependent vasoconstrictor, is increased in plasma of juvenile hypertensives and may contribute to the early development of primary hypertension (Jankowski et al., 2007). Up$_4$A-induced contractions, mediated by P2X$_1$, P2Y$_2$, or P2Y$_4$ receptors, were increased in renal, basilar, and femoral, but not in pulmonary arteries from DOCA-salt hypertensive rats (Matsumoto et al., 2011, 2012). The authors attributed this to enhanced P2Y signaling in the renal artery, because contractions mediated by P2Y$_2$, P2Y$_{2/4}$, and P2Y$_6$ receptor agonists were also enhanced; there was no change in renal artery protein expression of P2Y$_2$, P2Y$_4$, and P2Y$_6$ receptors in DOCA-salt hypertension, but ERK activation stimulated by

### Table 5

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<th>Pathology</th>
<th>Smooth Muscle</th>
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<td>P1</td>
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<td>Hypertension</td>
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<td>P2X$_2$↑protein</td>
<td>P2Y$_1$↑knockout protective$^e$</td>
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<td>Atherosclerosis</td>
<td>P2X$_3$↑protein</td>
<td>P2Y$_4$ Overexpression in heart beneficial$^f$</td>
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<td>Heart failure</td>
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<td>Inflammation</td>
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$^a$ Afferent arterioles of rat kidney (Inscho, 2009) and human subcutaneous veins (Lind et al., 1997).

$^b$ A$_{2A}$ receptor knockout led to pulmonary artery hypertension and smooth muscle proliferation in mice (Xu et al., 2011).

$^c$ P2Y$_{1}$/apolipoprotein E double-knockout mice had reduced atherosclerotic lesions (Hechler et al., 2008).

$^d$ Diet-induced (apolipoprotein E-deficient mice) atherosclerosis (Guns et al., 2010).

$^e$ Transgenic A$_1$ receptor overexpression increased myocardial resistance to ischemia (Matherne et al., 1997) and genetic deletion of A$_1$ receptors limits myocardial ischemic tolerance (Reichelt et al., 2005).

$^f$ Human myocardium in chronic heart failure (Bann et al., 2005).

$^g$ Reduced adenosine-mediated vasodilatation involving smooth muscle and/or endothelium.

CNS, central nervous system.
Purinergic Signaling and Blood Vessels

UP4A was enhanced (Matsumoto et al., 2011). P2Y2 receptor gene haplotypes are associated with essential hypertension in Japanese men (Wang et al., 2010b).

ADP produces endothelium-mediated vasodilation (probably via P2Y1 receptors) and decreases the increased vasoconstrictor nerve-mediated stimuli in renovascular hypertension in rats; it was suggested that this may be significant when ADP (probably after degradation of ATP) is released from platelets and erythrocytes (Almeida et al., 1992). Red blood cell concentrations of ATP and GTP are higher in SHR than WKY rats (Yeung et al., 2008). However, there is impaired release of ATP from red blood cells of humans with primary pulmonary hypertension (Sprague et al., 2001, 2003). Endothelium-dependent relaxation to ACh, but not to ATP, was augmented in estrogen-treated SHRs (Williams et al., 1988). The Ca2+ responses of human hand vein endothelial cells to ATP were significantly larger in pregnant women than in those from nonpregnant and preeclamptic women (Mahdy et al., 1998). All-cis-5,8,11,14,17-eicosapentaenoate, a major active component of fish oil, increases the release of ATP from endothelial cells, leading to attenuation of the blood pressure rise seen with advancing age (Hashimoto et al., 1994a). In the aorta of SHR, endothelium-dependent contractions elicited by ATP involve the release of thromboxane A2 and PGI2 and possibly also prostaglandin H2 (Gluaís et al., 2007). There is enhanced production of EDCF in SHR and old WKY rats, which would counteract the action of EDRF, thus reducing the vasodilator action of adenine nucleotides (Mombouli and Vanhoutte, 1993).

It was proposed that infusion of ATP-MgCl2 may be clinically useful in the treatment of children with pulmonary hypertension (Fineman et al., 1990; Brook et al., 1994; Nalos et al., 2003). Endothelium-dependent relaxation to ATP has been demonstrated in human pulmonary arteries (Greenberg et al., 1987). On the other hand, ATP is a mitogen for pulmonary artery smooth muscle cells, which may be relevant for the pathophysiological basis of pulmonary hypertension (Zhang et al., 2004). ATP and UTP stimulate vascular smooth muscle cell proliferation in SHR via P2Y2 and/or P2Y6 receptors (Chang et al., 1995; Harper et al., 1998). Moreover, intrapulmonary arteries respond by vasoconstricting to ATP in broiler chickens susceptible to idiopathic pulmonary arterial hypertension (Kluess et al., 2012). Again, the balance between endothelial versus smooth muscle stimulation by ATP may regulate blood pressure in different directions, and we are far from a full understanding of the role of the purinergic system in pulmonary hypertension.

Data have been presented that indicate the potential beneficial effects of clopidogrel by improving hypertension-related vascular functional changes (Giacchini et al., 2010). It was suggested that the effects were not due to the direct actions of clopidogrel on the vasculature, but rather that activated platelets contribute to endothelial dysfunction, perhaps via impaired NO bioavailability. Endothelial dysfunction can potentially affect purinergic signaling involving both P2 and P1 receptors (see below) as well as having broader prohypertensive effects.

In the kidney of transgenic hypertensive rats, the glomeruli showed an abundance of P2X7 receptor immunostaining (in podocytes, endothelium, and mesangial cells), whereas in kidneys from normal rats there was only low level P2X7 receptor immunostaining (Vonend et al., 2004). In P2X7 receptor knockout mice, hypertension and renal injury were attenuated in DOCA-salt hypertension, suggesting that the P2X7 receptor plays a key role in the development of hypertension via increased inflammation (Ji et al., 2012). Common genetic variations in a region of the P2X7 and P2X4 receptor genes had a small, but significant, effect on blood pressure in humans, and it was suggested that drugs affecting P2X receptor signaling may have promise as clinical antihypertensive agents (Palomino-Doza et al., 2008).

Plasma adenosine concentrations are elevated in conscious SHR (Yamada et al., 1992) and in Dahl salt-sensitive rats (Yamada et al., 1995). Adenosine has been claimed to activate the vascular renin-angiotensin system in hypertensive subjects (Taddei et al., 1992). Adenosine, after breakdown of ATP released from sympathetic nerves, was claimed to have trophic effects on both vascular smooth muscle and endothelial cells (Osswald and Azevedo, 1991). A1 receptor antagonism was shown to reduce salt-induced hypertension and was associated with an attenuation of renal injury and a reduction of plasma renin activity and aldosterone concentration (Uehara et al., 1995). In rats with hypertension due to 7 days of treatment with the P1 receptor antagonist 1-3-dipropyl-8-sulfophenylxanthine, there was an increase in sensory-motor vasodilation of the mesenteric arterial bed (Ralevic et al., 1996), perhaps as a compensatory mechanism. A2A receptor knockout mice had increased proliferation of endothelial cells and increased smooth muscle hypertrophy and collagen deposition in the pulmonary arteries and increased wall area thickness and smooth muscle actin immuno-reactivity in the pulmonary resistance vessels, suggesting that adenosine, acting at A2A receptors, is an important regulatory mechanism to protect against development of pulmonary arterial hypertension (Xu et al., 2011). Increased ATP hydrolysis by ectonucleotidases resulting in an accumulation of adenosine in the kidney of hypertensive animals has been reported (Fürstenau et al., 2010).

A2 receptor agonists produce vasodilation and hypertension in conscious SHR (Webb et al., 1990; Yagil and Miyamoto, 1995), identified later as A2A receptors (Leal et al., 2012). A2 receptors mediate vasorelaxation in part via the endothelium and release of NO, and
endothelial dysfunction may cause the attenuation of adenosine receptor-mediated responses seen in aortae of SHR (Fahim et al., 2001; Nejime et al., 2009). However, NO plays, at most, a minor part in adenosine-induced responses (Jonzon et al., 1985). The authors speculated that adenosine could be one of several regulatory factors in the development of atherosclerosis and might also regulate the release of a smooth muscle mitogen, platelet-derived growth factor. It was later shown that A2B receptors may mediate inhibition of the growth of human aortic smooth muscle cells (Dubey et al., 1999). There is now evidence that adenosine also regulates endothelial cell proliferation in angiogenesis (Adair, 2005). Endothelial cell proliferation is mediated by adenosine A2A and A2B receptors, and some of the mitogenic effects are mediated via modulation of VEGF signaling. Inactivation of A2A receptors protects apolipoprotein E-deficient mice from atherosclerosis (Wang et al., 2009a). Adenosine modulates HIF-1α, VEGF, IL-8, and foam cell formation in a human model of hypoxic foam cells (Gessi et al., 2010). The authors claim that A2B and A3 antagonists may be able to block steps in the atherosclerotic plaque development. However, A3 receptor deficiency does not influence atherosclerosis (Jones et al., 2004). A recent review focuses on the influence of adenosine and A2A receptors in particular as a regulatory mechanism to control the formation of foam cells under conditions of lipid loading (Reiss and Cronstein, 2012). It has been shown that ecto-5'-nucleotidase/CD73-derived adenosine acts as an endogenous modulator protecting against vascular inflammation and monocyte recruitment, thus limiting the progression of atherosclerosis. Deletion of ecto-5'-nucleotidase/CD73 in mice, which leads to a reduction of adenosine, promotes atherogenesis, most likely by de-inhibition of resident macrophages and T cells (Buchheiser et al., 2011), but did not alter angiogenesis (Böring et al., 2013). ET4 receptor antagonism restores the myocardial perfusion response to adenosine in experimental hypercholesterolemia (Bonetti et al., 2003). Hypercholesterolemia abolishes voltage-dependent K+ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles (Heaps et al., 2005). Recent articles discuss the role of adenosine, acting via A2B receptors, in the development of atherosclerosis and the risk factors associated with this pathology (Koupenova et al., 2012a, 2012b).

B. Atherosclerosis and Coronary Artery Disease

ATP signaling is involved in atherosclerosis (Burnstock, 2002, 2008b; Di Virgilio and Solini, 2002; Plank et al., 2006, 2007; Seye et al., 2006; Guns et al., 2010; Zerr et al., 2011). Adenosine and ATP promote endothelial and smooth muscle cell proliferation and increase expression of VEGF and growth of new vessels (Burnstock, 2002). Hypoxia is an important stimulus to vascular growth, and it is believed that ATP, which is released from endothelial cells during hypoxia, and its breakdown product adenosine have important roles as mediators of blood vessel growth. An early study reported that adenosine produces an increase in cAMP and decrease in DNA synthesis in cultured arterial smooth muscle cells and suggested that this might result in the regulation of cell proliferation through actions at adenosine A1 and A2 receptors (Jonzon et al., 1985). The authors speculated that adenosine could be one of several regulatory factors in the development of atherosclerosis and might also regulate the release of a smooth muscle mitogen, platelet-derived growth factor. It was later shown that A2B receptors may mediate inhibition of the growth of human aortic smooth muscle cells (Dubey et al., 1999). There is now evidence that adenosine also regulates endothelial cell proliferation in angiogenesis (Adair, 2005). Endothelial cell proliferation is mediated by adenosine A2A and A2B receptors, and some of the mitogenic effects are mediated via modulation of VEGF signaling. Inactivation of A2A receptors protects apolipoprotein E-deficient mice from atherosclerosis (Wang et al., 2009a). Adenosine modulates HIF-1α, VEGF, IL-8, and foam cell formation in a human model of hypoxic foam cells (Gessi et al., 2010). The authors claim that A2B and A3 antagonists may be able to block steps in the atherosclerotic plaque development. However, A3 receptor deficiency does not influence atherosclerosis (Jones et al., 2004). A recent review focuses on the influence of adenosine and A2A receptors in particular as a regulatory mechanism to control the formation of foam cells under conditions of lipid loading (Reiss and Cronstein, 2012). It has been shown that ecto-5'-nucleotidase/CD73-derived adenosine acts as an endogenous modulator protecting against vascular inflammation and monocyte recruitment, thus limiting the progression of atherosclerosis. Deletion of ecto-5'-nucleotidase/CD73 in mice, which leads to a reduction of adenosine, promotes atherogenesis, most likely by de-inhibition of resident macrophages and T cells (Buchheiser et al., 2011), but did not alter angiogenesis (Böring et al., 2013). ET4 receptor antagonism restores the myocardial perfusion response to adenosine in experimental hypercholesterolemia (Bonetti et al., 2003). Hypercholesterolemia abolishes voltage-dependent K+ channel contribution to adenosine-mediated relaxation in porcine coronary arterioles (Heaps et al., 2005). Recent articles discuss the role of adenosine, acting via A2B receptors, in the development of atherosclerosis and the risk factors associated with this pathology (Koupenova et al., 2012a, 2012b).
It was claimed recently that P2Y12 receptors in the development of atherosclerosis has been suggested, whereby UTP induces vascular cell adhesion molecule-1 expression in coronary artery endothelial cells, leading to the recruitment of monocytes associated with the development of atherosclerosis (Kim et al., 2002, 2003; see also Otte et al., 2009). There was upregulation of vascular smooth muscle P2Y2 receptor mRNA by MAPK-dependent growth factor, which may be important in atherosclerosis and neointima formation after balloon angioplasty (Hou et al., 1999). Intimal hyperplasia in collared rabbit carotid artery is mediated by upregulation of P2Y2 receptors (Seye et al., 2002) and may be a useful indicator of the early stages of atherosclerosis (Elmaleh et al., 1998). An important role of P2 receptors in homocysteine-induced atherosclerosis has been shown (Sharma et al., 2007). ATP and UTP are chemotactic for dendritic cells via the P2Y2 receptor and can attract inflammatory cells to the atherosclerotic plaque (Idzko et al., 2002). UTPA has been reported to be a potent mediator of pro-inflammatory responses in the atherosclerotic vascular wall involving Nox1-dependent ROS activation via P2Y2 receptors (Schuchardt et al., 2011). A major contribution of endothelial P2Y1 receptors in the regulation of TNFα-induced vascular inflammation has been claimed (Zerr et al., 2011). Reduced atherosclerotic lesions were found in P2Y1/apolipoprotein E double-knockout mice, and it was concluded that P2Y1 receptors contribute to atherosclerosis, possibly through their role in nonhematopoietic-derived cells (Hechler et al., 2008). Cytokines and chemokines play roles in the initiation and progression of atherosclerosis, where they promote recruitment and migration of inflammatory cells into the atherosclerotic lesion. Among the chemokines is the C-C motif ligand 2 (CCL2), and it has been shown that extracellular nucleotides can induce CCL2 expression in human aortic smooth muscle cells, probably via P2Y1 and/or P2Y11 receptors (Kim et al., 2011). In diet-induced (apolipoprotein E-deficient mice) atherosclerosis, there was increased expression of P2Y6 receptor mRNA in the atherosclerotic regions, and the P2 receptor antagonists suramin and PPADS both reduced plaque size (Guns et al., 2010). Apolipoprotein A-I is the main protein constituent of high-density lipoprotein. It was claimed recently that P2Y12 receptors mediate the formation of atherosclerotic lesions in apolipoprotein E-deficient mice (Cavelier et al., 2012; Li et al., 2012a). The vasodilatory responses to ATP, but not adenosine, are impaired in young apolipoprotein E-deficient mice that might represent the critical initiating event in pathologic vascular remodeling and atherogenesis (Mercier et al., 2012). Atherosclerotic damage results in the disappearance of endothelium-dependent responses to ATP (Burnstock, 2006). Impairment of ATP endothelium-mediated relaxations occurred in the aorta of atherosclerotic rabbits (Ragazzi et al., 1989). This was also the case in Watanabe heritable hyperlipidemic rabbits, except in 12- to 14-month-old rabbits, when ATP-mediated relaxations were increased, suggesting that compensatory responses evolve during atherogenesis to maintain vasodilator functions (Chinellato et al., 1991). Nucleotide receptor activity (via unknown receptors) is preserved for longer than that of other endothelial receptors, including P2Y receptors in Watanabe heritable hyperlipidemic rabbits (Ragazzi et al., 1993; Chinellato and Ragazzi, 1995). Low-density lipoprotein causes attenuation of NO-mediated endothelial responses in coronary arteries induced by adenosine receptor activation (Abebe and Mustafa, 1997).

Vascular injury represents a critical initiating event in the pathogenesis of various vascular diseases. Large amounts of ATP are released from injured cells and, as described above, ATP and adenosine have potent actions on smooth muscle and endothelial cell growth, migration, proliferation, and death. 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 leads to a substantial release of ATP and UTP from endothelial cells (Burnstock, 1999), and these purines might mediate alterations in the balance between proliferation and apoptosis. Apoptotic cell death is recognized to occur in a number of vascular diseases, including atherosclerosis, restenosis, and hypertension (Thomas et al., 1976; Mallat and Tedgui, 2000). ATP release from endothelium and smooth muscle may occur across connexin hemichannels, and connexin hemichannel formation has been shown to be regulated by ATP (Jiang et al., 2005; Lurtz and Louis, 2007). The pattern of vascular connexins is altered during atherosclerotic plaque formation and in restenosis, affecting gap-junctional communication between smooth muscle cells, and genetically modified connexin expression alters the course of atherosclerosis and restenosis (Chadjichristos et al., 2006). Connexins have been claimed to participate in the initiation and progression of atherosclerosis (Morel et al., 2009).

ATP release from endothelial cells in the rat caudal artery is impaired in atherosclerosis (Shinozuka et al., 1994). A high-cholesterol diet decreased the spontaneous and NA-evoked release of ATP from caudal artery of aged rats, leading to an increase in blood pressure, and it was suggested that the main source of ATP was the endothelial cells, with a lesser contribution of smooth muscle (Hashimoto et al., 1998b). On the other hand, ATP release from endothelial cells by shear stress is enhanced in inflammatory states (Bodin and
Burnstock, 1998), and this might represent a protective mechanism by inhibiting natural killer (NK) cell recruitment and consequently NK cell-mediated plaque formation, a mechanism suggested to involve P2Y11 receptors (Gorini et al., 2010). NK cells represent the main source of interferon-γ that contributes to atherosclerotic plaque progression; fractalkine expressed by endothelial cells mediates NK recruitment, and its killing of endothelial cells and high fractalkine mRNA is found in advanced atherosclerotic lesions in human arteries. ATP releases histamine from mast cells and releases inflammatory cytokines such as IL-1 from immune cells via P2X7 receptors.

Saphenous vein, internal mammary, and radial arteries have been used as grafts for coronary bypass surgery; the level of endothelial P2Y2 receptors is comparable in all three vessels, but endothelial P2X4 receptors vary from high in saphenous vein to significantly lower in the other two vessels. It has been suggested that P2X4 receptors play a more significant role in intense proliferation in atherosclerosis and restenosis than P2Y2 receptors, as reflected by the susceptibility of saphenous vein grafts to atherosclerosis compared with internal mammary arteries (Ray et al., 2002). In another study, P2X1 and P2Y4 receptors mediated more prominent contractions in the saphenous vein compared with the internal mammary artery; it has been suggested that selective antagonists to these receptors may prevent vasospasm and restenosis in the saphenous vein during and after revascularization surgery (Borna et al., 2003).

Atherosclerosis is an inflammatory disease induced by hypercholesteremia, and an increase in E-NTPDase 1/CD39 (apyrase) and subsequent ATP and ADP hydrolysis has been shown in platelets of hypercholesteremic patients (Duarte et al., 2007). The authors suggest that this might be beneficial in reducing thrombus formation, but may also contribute to future fatal events such as unstable angina and myocardial infarction. Newly developing vascular endothelia express very high levels of the ectonucleotidase E-NTPDase1/CD39 also seen under hypoxic conditions (Eltzschig et al., 2003). Angiogenesis requires the dynamic interaction of endothelial cell proliferation and differentiation with orchestrated interactions between extracellular matrix and surrounding cells (such as vascular smooth muscle and/or pericytes). Such interactions could be coordinated by interplay between nucleotide release, P2 receptor modulation, and altered NTPDase expression (Goepfert et al., 2001). Although the relationship between atherosclerosis and aneurysm growth and rupture is still not clear, atherosclerotic plaques have been found in the aneurysmal wall; using a numerical model of ATP transport in aneurysms found at arterial bends, a low concentration of ATP was indicated at the proximal side of the aneurysmal site, which may be significant in pathologic changes in the growth and rupture of aneurysms (Imai et al., 2010). Reduction of ectoNTPDase1 and 2 (but not ecto-5′-nucleotidase) was shown in atherosclerotic blood vessels of patients with abdominal aortic aneurysm, and it was suggested that this may be responsible for the development of atherosclerotic-like diseases (Lecka et al., 2010). A recent review considers the therapeutic potential of NTPDases to prevent intimal hyperplasia (Kaczmarek and Koziai, 2011).

Atherosclerosis of coronary vessels is often known as coronary artery disease. Administration of ATP into the left coronary artery has been used to treat patients with coronary artery disease in preference to papaverine (Sonoda et al., 1998). ATP acts on endothelial P2Y receptors to cause release of NO and subsequent vasodilation. Intravenous infusion of ATP has also been used for detecting coronary artery disease in patients unable to perform conventional exercise stress testing adequately (He et al., 2002). Platelet adhesion and aggregation is a key step in the development of inflammation, thrombosis, and atherosclerosis. Thus platelet aggregation inhibitors, such as clopidogrel, an antagonist of ADP-sensitive P2Y12 receptors, have been used to prevent and treat coronary artery disease (Zeidner et al., 2008). It has been suggested that ADP-mediated migration of host smooth muscle-like cells and CD45+ leukocytes, via P2Y12 receptors, may play a role in the development of transplant arteriosclerosis, partly by monocyte chemoattractant protein-1 (Harada et al., 2011). Prasugrel, a P2Y12 receptor antagonist, has been used for the treatment of acute coronary syndrome (Jauregui et al., 2009). Clinical trials with clopidogrel and ticlopidine (P2Y12 receptor antagonists) in patients with atherosclerotic disease have shown significant benefit compared with aspirin. Intravenous adenosine in cardiovascular magnetic resonance imaging is safe and well tolerated even in patients with severe coronary artery disease (Karamitsos et al., 2009).

In conclusion, adenosine, ATP, ADP, and UTP stimulate several inflammatory responses known to be important for atherosclerosis development (Wang et al., 2009a; Gessi et al., 2010; Erlinge, 2011). The long-term (trophic) roles of purinergic signaling in vascular smooth muscle and endothelial cell proliferation and death, which have been implicated in atherosclerosis and restenosis, suggest that therapeutic strategies in relation to these events should be explored (Di Virgilio and Solini, 2002; Ralevic and Burnstock, 2003; Seye et al., 2006; Burnstock, 2008b; Erlinge and Burnstock, 2008; Ralevic, 2009).

C. Ischemia

Ischemia leads to injury of most organs in the body (see Linden, 2006). Purines and pyrimidine nucleotides, released at the site of cell damage, generally contribute to injury, but protective effects have also
been described. Adenosine, released during ischemia, is generally protective (see Grenz et al., 2012b).

1. Heart. An influential article by Berne (1963) suggested that adenosine was the physiologic regulator of blood flow during reactive hyperemia after hypoxia, and early articles supported this hypothesis (Scott et al., 1979; Belardinelli et al., 1981). However, evidence was later presented that questioned the hypothesis. For example, although theophylline, an adenosine receptor antagonist, blocked coronary vasodilation by perfused adenosine, it did not block reactive hyperemia (Rehncrena et al., 1978; Heistad et al., 1981; Pinard et al., 1989). It was also noted that although reactive hyperemia occurred after about 10 seconds, adenosine did not appear in the perfusate until about 90 seconds. In a counterhypothesis, Burnstock (1993b) proposed that the initial phase of vasodilation after hypoxia was due to ATP released from endothelial cells to cause vasodilation via NO, whereas adenosine (after breakdown of ATP) contributed only to the later stages of reactive hyperemia by acting on P1 receptors on the smooth muscle. The delay in appearance of adenosine in the perfusate was explained by the fact that ADP (after rapid breakdown of ATP) inhibits 5'-nucleotidase, the enzyme that mediates breakdown of AMP to adenosine.

Endogenous adenosine is an important mediator of ischemic preconditioning and postconditioning (see Riksen et al., 2009). The cardioprotective effect of ischemic preconditioning is dependent on activation of adenosine A1 receptors in the first few minutes of reperfusion (Solenkova et al., 2006). In contrast, the infarct size-limiting effect of myocardial ischemic postconditioning is mediated by the activation of adenosine A2A receptors at the time of reperfusion (Morrison et al., 2007). Transgenic A1 receptor overexpression increased myocardial resistance to ischemia (Matherne et al., 1997). Conversely, genetic deletion of A1 receptors limits myocardial ischemic tolerance (Reichelt et al., 2005). In male patients with stable angina, capadenoson, an oral A1 receptor agonist, lowers exercise heart rate, which is associated with prolongation of time to ischemia (Tendera et al., 2012). In situ ischemic preconditioning conferred cardioprotection in A1, A2A, and A3, but not A2B knockout mice or in wild-type mice after inhibition of the A2B receptors, and it was suggested that 5'-nucleotidase and A2B agonists might be considered as therapy for myocardial ischemia (Eckle et al., 2007; see also Aherne et al., 2011). Activation of A3 receptors protects against myocardial ischemia-reperfusion injury in mice, an effect that disappears in A3 knockout mice (Harrison et al., 2002; Ge et al., 2006; Wan et al., 2011). CD73-derived adenosine promoted cardiac remodeling and recovery of ventricular performance after ischemia-reperfusion, most likely by acting on T cells (Bönner et al., 2012).

Activation of adenosine receptors with exogenous ligands mimics the cardioprotective effect of preconditioning. Intracoronary adenosine protects against reperfusion injury after coronary occlusion (Olafsson et al., 1987; Babbitt et al., 1989; Ledingham et al., 1990; Thornton et al., 1992), an action mediated by A1 receptors (Liu et al., 1991). Activation of A1 and A2A receptors has been shown to reduce ischemia-reperfusion injury in the heart (Schlack et al., 1993; Lozza et al., 1997). Brief intravenous infusion of ATL-146e, a selective adenosine A2A receptor agonist (added 30 minutes before reperfusion), reduced myocardial infarct size at 48 hours after reperfusion after a period of ischemia (Patel et al., 2009). Selective A3 receptor activation is cardioprotective in wild-type hearts and hearts overexpressing A1 receptors. A more recent article (Xi et al., 2009) claimed that A2A and A2B receptors work in concert to induce strong protection against reperfusion injury in rat hearts, and cooperative activation of A1 and A2A receptors to produce cardioprotection in ischemia-reperfused mouse heart has also been reported (Urmaliya et al., 2010). Evidence that activation of both A1 and A2 receptors during hypoxia can attenuate myocardial injury was claimed (Stambaugh et al., 1997; Tracey et al., 1997; Liang and Jacobson, 1998). Endogenous adenosine makes a significant contribution to A1 agonist-mediated prevention of necrosis in a cardiac cell model of ischemia by cooperative interactions with both A2A and A2B receptors, but does not play a role in A3 agonist-mediated protection (Urmaliya et al., 2009; Methner et al., 2010). It was reported that A1 and A3 receptor agonists reduce hypoxic injury through the involvement of p38 MAPK (Leshem-Lev et al., 2010). Adenosine stimulates the recruitment of endothelial progenitor cells to the ischemic heart to enhance revascularization (Goretti et al., 2012).

A reduced sensitivity to adenosine has been found in the ischemic or hypoxic heart (Zucchi et al., 1989; Vanhaecke et al., 1990). A2 receptor binding in the heart is modified by ischemia (Zucchi et al., 1992), and ischemia-reperfusion selectively attenuated coronary vasodilatation mediated by A2, but not A1 receptor agonists (Cox et al., 1994). Attenuation of responsiveness would tend to counteract the cardioprotective effects of adenosine, but the importance of this is not yet clear.

The mechanism underlying cardioprotection by adenosine is not fully understood. Adenosine activation of the reperfusion injury salvage kinase pathway, involving phosphorylation of Akt and/or ERK1/2, which leads to inhibition of mitochondrial permeability transition pore formation (Hausenloy et al., 2005; Hausenloy and Yellon, 2006; Murphy and Steenbergen, 2008) may be involved. One study concluded that cardioprotection by adenosine is dependent on NO and is mediated principally by activation of a neurogenic pathway (Manintveld et al., 2005). It was suggested that ischemic stress-induced preconditioning is dependent on the concomitant
stimulation of both adenosine and NA receptors, and it was claimed that P1 receptor-mediated cardioprotection occurs only if $\alpha_1$-adrenoceptor activation is intact (Winter et al., 1997). The infarct-sparing effect of A$_{2A}$ receptor activation has been suggested to be primarily due to inhibition of CD$^+$ T-cell accumulation and activation in the reperfused heart (Yang et al., 2006). Another report claims that adenosine triggers the nuclear translocation of PKC$\varepsilon$ in cardiomyoblasts (Xu et al., 2009).

It was claimed early that reflex responses mediated by cardiac sympathetic afferent nerves during myocardial ischemia were caused by adenosine released from the ischemic myocardium mediated by A$_1$ receptors, but more recently it was suggested that ATP activates sympathetic afferents (Fu and Longhurst, 2010). There is decreased adenosine protection in reperfusion injury in aging rats (Gao et al., 2000). Reduced A$_3$ receptor transcription may contribute to improved ischemia tolerance in aged hearts (Ashton et al., 2003). Low-dose adenosine infusion reduced the ischemic burden and improved left ventricular regional systolic function in the ischemic walls of patients with exercise-induced myocardial ischemia (Sadigh et al., 2009). Reviews concerned with the role of adenosine in preconditioning and ischemia-reperfusion injury are available (Sollevi, 1986; de Jong et al., 2000; Ganote and Armstrong, 2000; Sommerschild and Kirkeboen, 2000; Lasley et al., 2001; Przyklenk and Whittaker, 2005; Cohen and Downey, 2008; Drew and Kingwell, 2008; Laubach et al., 2011).

Although the early focus was on the role of adenosine in ischemic and reperfusion injury, there is increasing interest in the role of ATP in this condition. Indeed, extracellular ATP and adenosine appear to play complementary, protective roles in ischemic preconditioning through P2Y and P1 receptors, respectively (Ninomiya et al., 2002). A possible role for nucleotides in cardiac ischemia was first raised in 1948, with the emphasis on degradation of nucleotide, which appears to take place within the muscle cells during ischemia (Stoner et al., 1948). Following this way of thinking, local infusion of ATP was used to delay successfully the onset of irreversible ischemic injury and the role of high energy phosphate in preservation of ischemic myocardium recommended (Kaul et al., 1977). Delayed resynthesis of ATP after its depletion during myocardial ischemia was proposed (Reimer et al., 1981), and ATP-MgCl$_2$ was used for the treatment of ischemia (see Chaudry, 1983; McDonagh et al., 1984; Kopf et al., 1987). ATP-loaded liposomes have been recommended for the treatment of myocardial ischemia (Hartner et al., 2009). Others have shown that ischemia is accompanied by an increased release of ATP from cardiac myocytes and sympathetic nerves (Lai and Nishi, 2000; Sesti et al., 2003; Clarke et al., 2009). It has been suggested that release of ATP from cardiomyocytes is strictly regulated during ischemia by a negative feedback mechanism consisting of maxi-anion channel-derived ATP-induced suppression of ATP release via hemichannels in cardiomyocytes (Kunugi et al., 2011). In a recent article (Cosentino et al., 2012), it was shown that ischemic/hypoxic stress induced rapid ATP release from cultured cardiomyocytes and that distinct P2 receptors regulated cardiomyocyte death, perhaps via P2X$_7$ and P2Y$_2$ receptors. It has been claimed that P2X$_7$ receptor-mediated inhibition in stellate ganglia prevents increased sympathoexcitatory reflex via sensory-sympathetic coupling induced by myocardial ischemic injury (Tu et al., 2013). Ischemia-induced accumulation of ATP in the extracellular space was suggested to trigger enhancement of [Cl$^-$] in ventricular muscle during ischemic conditions (Lai and Nishi, 2000). Release of ATP from cardiac myocytes in response to ischemia is likely to be via connexin hemichannels (Clarke et al., 2009). In another study, it was suggested that ATP release from cardiac myocytes via pannexin-1 channels during early ischemia may be an early paracrine event leading to profibrotic responses to ischemic cardiac injury (Dolmatova et al., 2012). Tissue transglutaminase 2 protects cardiomyocytes against ischemia-reperfusion injury by regulating ATP synthesis (Szondy et al., 2006). Pannexin 1/P2X$_7$ receptor channels mediate release of cardioprotectants induced by ischemic pre- and postconditioning (Vessey et al., 2010). There is upregulation of P2X$_7$ receptors in rat superior cervical ganglia after myocardial ischemic injury (Kong et al., 2013). It has been claimed that ATP postconditioning provides a powerful protective effect in a rat model of ischemia-reperfusion due partly to antioxidation and partly to inhibition of inflammation (Yao and Liu, 2010).

Extracellular ATP protects against reperfusion-induced failure of the endothelial barrier in rat hearts (Gündüz et al., 2006). Extracellular ATP also protects human cultured myocardial endothelial cells from apoptotic cell death during hypoxia by activating P2Y$_2$ receptor-mediated MEK/ERK- and P13K/Akt-signaling (Urban et al., 2009). UTP, released during cardiac ischemia, was claimed to act via P2Y$_2$ (and/or P2Y$_4$) receptors to play a substantial role in mediating cardioprotection from hypoxic damage (Yitzhaki et al., 2005; Erlinge et al., 2005; Wei et al., 2007). It has been suggested that the P2Y$_4$ receptor could be a therapeutic target to regulate cardiac remodeling and posts ischemic revascularization (Horckmans et al., 2012). It has been suggested that P2Y$_9$ and P2Y$_{11}$ receptors are involved in pyridoxal-5'-phosphate-induced cardiac preconditioning in rat hearts (Millart et al., 2009). ADP acting on endothelial P2Y$_1$ receptors plays a major role in coronary flow during posts ischemic hyperemia (Olivecrona et al., 2004). ADP, acting via P2Y$_1$ receptors, mediates release of tissue plasminogen activator during ischemia and posts ischemic hyperemia; tissue plasminogen activator is involved in maintaining the endothelial
Extracellular ATP augments cardiac contractility by elevating intracellular calcium in cardiac myocytes. Impairment of extracellular ATP-induced Ca$^{2+}$ mobilization in rat hearts after ischemia and reperfusion has been described (Saini et al., 2005). In a rat model of ischemic congestive heart failure, there was selective downregulation of the P2X receptor-mediated pressor effects, whereas the hypotensive effects mediated by endothelial P2Y receptors were unaffected (Zhao et al., 2000). Cardiac-specific overexpression of human P2X$_4$ receptors confers a beneficial effect in the left anterior descending artery ligation model of ischemic cardiomyopathy (Sonin et al., 2007). Transgenic overexpression of P2X$_4$ receptors in mouse heart led to an enhanced cardiac contractile performance after ischemic infarction and increased survival at 1 and 2 months after infarction; the actions suggest that enhanced contractile function via P2X$_4$ receptors of the non-infarcted areas was likely to be a rescuing mechanism (Sonin et al., 2008). A recent review discusses P2X$_4$ receptors as targets for cardiac ischemia (Yang and Liang, 2012). There was selective upregulation of P2X$_6$ receptors in the myocardium of patients undergoing heart transplantation because of chronic heart failure (Banfi et al., 2005).

P2X$_3$ receptors have been identified on afferent terminals in the heart activated by ATP, perhaps associated with angina pectoris (Zhang et al., 2008). P2X$_3$ receptors were upregulated in rat stellate ganglia after myocardial ischemic injury and may underlie ischemic pain (Shao et al., 2007; Wang et al., 2008, 2009d). Myocardial ischemic injury induced an increase in expression of P2X$_3$ receptors in superior cervical and dorsal root ganglion neurons, which led to aggravated sympathoexcitatory reflexes (Li et al., 2011a). The authors also claimed that oxymatrine, a Chinese herbal remedy for ulcers and tumors, may decrease the expression of P2X$_3$ receptors and depress the aggravated sympathoexcitatory reflex induced by ischemic injury. Mesenchymal stem cell-derived exosomes increase ATP levels and decrease oxidative stress to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia-reperfusion injury (Arslan et al., 2013).

A protective role of E-NTPDase 1/CD39 in ischemia-reperfusion injury has been shown. Targeted deletion of E-NTPDase 1/CD39 led to heightened levels of myocardial ischemia-reperfusion injury, which was corrected by infusion of AMP or apyrase (which degrades nucleotides) (Köhler et al., 2007). More recently, transgenic expression of human E-NTPDase 1/CD39 in pigs was shown to protect against myocardial ischemia-reperfusion injury (Wheeler et al., 2012). Interestingly, robust and selective induction of E-NTPDase 1/CD39 was shown after preconditioning in mouse heart (Köhler et al., 2007). Similarly, ischemic preconditioning increases 5’-nucleotidase activity and adenosine release during myocardial ischemia and reperfusion in dogs (Kitakaze et al., 1993). Ectonucleotidase on sympathetic nerve endings attenuates ATP and NA exocytosis in myocardial ischemia (by reducing ATP levels and consequently the prejunctional facilitatory effects of ATP) (Sesti et al., 2003). Conversely, overexpression of E-NTPDase 1/CD39 resulted in enhanced removal of exogenous ATP (Corti et al., 2011) and protection against murine myocardial ischemic injury (Cai et al., 2011). Because ATP availability greatly increases in myocardial ischemia, it has been suggested that recombinant E-NTPDase 1/CD39 may offer a novel therapeutic approach to the damage caused by ischemia by reducing sympathetic activity (Corti et al., 2011). E-NTPDase 1/CD39 deletion led to an attenuation of the activity of sympathetic pre- and postsynaptic P2X receptors (attributed to P2X receptor desensitization) perhaps because of prolonged exposure to ATP that accompanies E-NTPDase 1/CD39 deletion, and it was suggested that E-NTPDase 1/CD39 can potentially prevent the transition from myocardial ischemia to infarction (Schaefer et al., 2007).

2. Brain and Spinal Cord. The hypothesis of Berne (1963) that adenosine is the physiologic regulator of reactive hyperemia was supported for the cerebral circulation by some authors (e.g., Winn et al., 1980; Emerson and Raymond, 1981; Kung et al., 2007) but not by others when the increase in blood flow that occurred in hypoxia was shown not to be clearly related to changes in adenosine concentration (Rehncrona et al., 1978; Heistad et al., 1981; Pinard et al., 1989). ATP, as well as adenosine, was measured in the perfusate of rat cerebral cortex during hypoxia (Phillis et al., 1993). Real-time measurements showed release of purines during ischemia in hippocampal slices and suggested that ATP and adenosine release during ischemia are, to a large extent, independent processes (Frenguelli et al., 2007). The roles of adenosine and
adenine nucleotides as regulators of cerebral blood flow in hypertension, hypoxia/ischemia, and hypercapnia/acidosis have been reviewed (Burnstock, 1982; Phillis, 2004; Dale and Frenguelli, 2009; Thauerer et al., 2012). Extracellular nucleotides modulate BBB responses to ischemic events, and NTPDase activity was shown to significantly increase after ischemia/hypoxia in vitro (Ceruti et al., 2011a).

The GPR17 (P2X-like) receptor appears on microglia/macrophages after ischemia; it may play a role in both inducing neuronal death in ischemia and in orchestrating the local repair responses (Lecca et al., 2008; Zhao et al., 2012). Cortical spreading depression releases ATP into the extracellular space, and the subsequent activation of P2Y receptors makes a major contribution to the induction of ischemic tolerance in the brain (Schock et al., 2007). Endothelial P2Y2 receptor-mediated dilations of rat middle cerebral artery to UTP were potentiated after ischemia-reperfusion, whereas P2Y1 receptor-mediated dilation was attenuated after ischemia-reperfusion (Marrelli et al., 1999). Astrocyte P2Y1 receptors were involved in the regulation of cytokine/chemokine transcription and brain damage in a rat model of cerebral ischemia (Kuboyama et al., 2011). A role for P2Y12 receptors in ischemia in terms of microglial mediated neurotoxicity has been proposed, suggesting an additional benefit of clopidogrel (Webster et al., 2010).

Increased extracellular glutamate levels and subsequent excitotoxicity can lead to neuronal death. Activation of P2X receptors and consequent Ca2+ influx might contribute to the ischemia-induced facilitation of glutamate release (Zhang et al., 2006). Purinergic facilitation, via P2X and A2A receptors, of glutamate release in ischemic hippocampus was shown (Sperlágh et al., 2007). Delayed P2X1 receptor expression in microglia after hypoxia-ischemia in postnatal (P3) rat brain has been reported, and it was suggested that this may play a role in neuroinflammation and injury progression (Wixey et al., 2009). Young age and low temperature, but not female sex, delay ATP loss and glutamate release and protect Purkinje cells during simulated ischemia in cerebellar slices (Mohr et al., 2010). Mild hypothermia has been considered as a neuroprotective strategy for the management of ischemic brain injury. However, it was reported recently that ATP induction of mild hypertension in rats has a strikingly detrimental impact on focal cerebral ischemia (Zhang et al., 2013a). P2 receptor stimulation plays a deleterious role during severe ischemic conditions in rat hippocampal slices (Coppi et al., 2007).

ATP and a,b,meATP, likely via P2X receptors, have been shown to accelerate recovery from hypoxic/hyperglycemic perturbations of guinea pig hippocampal neurotransmission (Aihara et al., 2002). Neuroprotective effects of the P2 receptor antagonist PPADS on focal cerebral ischemia-induced injury in rats have been reported (Lämmer et al., 2006). Reactive blue 2, a P2 receptor antagonist, partially protected against neurologic impairment after cerebral focal ischemia in rats (Melani et al., 2006). P2X1 and P2X2 receptor knockout mice were significantly protected in a model of ischemic stroke (Bargiotas et al., 2011).

Recent experiments showed that P2X7 receptors mediate ischemic damage in oligodendrocytes (Domercq et al., 2010) and blockade of P2X7 receptors attenuates postischemic injury (Matute, 2011; Arbela et al., 2012). P2X7 receptors may be involved in hypoxic-ischemic injury of radial glia clone L2.3 cells (Zeng et al., 2012). There is upregulation of P2X7 receptors after ischemia in the cerebral cortex of rats (Franke et al., 2004). Supersensitivity of P2X7 receptors in cerebrocortical cell cultures after in vitro ischemia has also been reported (Wirkner et al., 2005). P2X7 receptor expression on microglia was increased by both ischemia and reactive blue 2 in rats; hence, the protection against ischemic neurologic impairment by reactive blue 2 was suggested to be mediated through increased expression of P2X7 receptors (Melani et al., 2006). Downregulation of P2X7 receptor expression in rat oligodendrocyte precursor cells occurs after hypoxic ischemia (Wang et al., 2009b). There is a recent review that discusses the role of P2X7 receptors in cerebral ischemia (Bai and Li, 2013). Endothelial cells at the BBB were shown to be extremely susceptible to cell death in ischemic conditions, whereas the other BBB cells, astrocytes, and pericytes, were more resistant (Kittel et al., 2010).

Early articles showed release of adenosine from ischemic brain (Berne et al., 1974; Winn et al., 1979; Phillis et al., 1987; see Chu et al., 2013), and adenosine was shown to have a protective effect against ischemic injury (Hagberg et al., 1987; Dux et al., 1990; Boissard et al., 1992; Miller and Hsu, 1992; Rudolphi et al., 1992; Schubert and Kreutzberg, 1993). An involvement of adenosine in cerebral blood flow regulation in hypercapnia has also been reported (Phillis and Delong, 1987; Phillis et al., 2004). During the postischemic period, adenosine is elevated in the cerebrospinal fluid and could affect reperfusion (Meno et al., 1991). Prevention of spinal cord injury was achieved with regular infusion with adenosine (Herold et al., 1994; Reece et al., 2008). In the early stages of hypoxia, adenosine is likely to be released per se from rat striatum rather than being derived from released ATP (Melani et al., 2012); furthermore, repeated hypoxia results in reduced production of extracellular adenosine, and this may underlie the increased vulnerability of the mammalian brain to repetitive or secondary hypoxia/ischemia (Pearson et al., 2003). Endogenous adenosine, acting via A1 receptors, inhibits striatal GABAergic transmission during in vitro ischemia (Centonze et al., 2001). Inosine, as well as adenosine, reduced astroglial injury in ischemia, and it was suggested that adenosine
was acting after breakdown to inosine (Haun et al., 1996). The diadenosine polyphosphate Ap4A protects against injury induced by ischemia in rat brain through actions at P1 adenosine receptors (Wang et al., 2003b).

Hypoxia can induce upregulation of CD73 expression in brain microvessel endothelial cells (Li et al., 2006), which would be expected to lead to increased formation of adenosine extracellularly. Transgenic overexpression of adenosine kinase, which removes adenosine through phosphorylation to AMP, renders the brain more susceptible to injury from ischemia, perhaps by reducing the extracellular levels of adenosine (Pignataro et al., 2007). Downregulation of hippocampal adenosine kinase after focal ischemia appears to be an endogenous neuroprotective mechanism (Pignataro et al., 2008).

There is a general consensus that agonist stimulation of A1 receptors reduces brain damage after experimentally induced ischemia. Hence, it has been suggested that A1 agonists may be used clinically in the treatment of ischemic brain disorders (Bischofberger et al., 1997). A1 receptor agonists have been claimed to play a pivotal role in protection from hypoxic insults (Sebastião et al., 2001). A1 receptor activation decreases cytotoxic amino acid release from both neurons and glial cells (Schubert et al., 1994; Von Lubitz et al., 1996; Pearson et al., 2006). A1 receptor agonists were particularly effective against cerebral ischemia (Von Lubitz et al., 1994, 1999; Chen et al., 2006) and cerebral ischemia preconditioning (Heurteaux et al., 1995). However, deletion of A1 receptors was reported not to alter neuronal damage after ischemia in vivo or in vitro (Olsson et al., 2004). Short cerebral ischemic preconditioning upregulates A1 and A2B receptors in the hippocampal CA1 region of rats (Zhou et al., 2004). An increase in A1 receptor gene expression in cerebral ischemia in rats has been reported (Lai et al., 2005). The A1 receptor enhancer PD 81,723 failed to protect against ischemia-reperfusion evoked cerebral injury (Cao and Phillis, 1995) but provided significant neuroprotection against hypoxic-ischemic injury in newborn rats (Halle et al., 1997). A1 receptors might contribute to the enhancement of inhibiting synaptic transmission in CA1 pyramidal neurons after forebrain ischemia, leading to delay in the process of neuronal cell death (Liang et al., 2009). In vitro ischemic preconditioning allows CA1 hippocampal neurons to become resistant to prolonged exposure to ischemia; adenosine, by stimulating A1 receptors, plays a crucial role. A2A receptors are not involved, whereas A3 receptor activation is harmful to ischemic preconditioning (Pugliese et al., 2003), although it was claimed in a recent article that A3 receptor agonists reduced brain ischemic injury and inhibited inflammatory cell migration in rats (Choi et al., 2011). Activation of A1 receptors has been shown to promote postischemic electrocortical burst suppression (Ilie et al., 2009).

A2 receptor antagonists reduced brain ischemic injury (Gao and Phillis, 1994), and A2A receptor antagonists reduced brain injury in neonatal and adult cerebral hypoxia-ischemia (Bona et al., 1997; Monopoli et al., 1998; Pedata et al., 2005; Stone and Behan, 2007; Mohamed et al., 2012). The protective effects of A2A antagonists in brain ischemia may be largely due to reduced A2A receptor-mediated glutamate outflow from neurons and glial cells (Pedata et al., 2007). Moreover, adenosine, acting via A2A receptors, enhances glutamate release during ischemia (Marroli et al., 2004). A2A receptor deficiency reduces striatal glutamate outflow and attenuates brain injury induced by transient focal ischemia in mice (Chen et al., 1999; Gui et al., 2009), but another study claimed that there was aggravated brain damage after hypoxic ischemia in immature A2A knockout mice (Adén et al., 2003). It has been claimed that A2A receptor-stimulated cascade in bone marrow-derived cells is an important modulator of ischemic brain injury (Yu et al., 2004). A2A receptor antagonism reduced JNK MAPK activation in oligodendrocytes after cerebral ischemia (Melani et al., 2010). In A2A receptor knockout mice there was a decrease in early ischemic vascular injury after subarachnoid hemorrhage (Sehba et al., 2010). Nearly 50% of cerebral hypoxic hyperemia was attenuated in A2A knockout mice (Miekišiak et al., 2008). In the CA1 area of the hippocampus, stimulation of A2A receptors attenuates A1 receptor-mediated depression of synaptic transmission induced by in vitro ischemia (Latini et al., 1999). Early (onset within hours), but not late (duration of days) neuroprotection due to preconditioning is associated with upregulation of A2B receptor mRNA (von Arnim et al., 2000). Thus, A1 receptor agonists and A2A and P2 receptor antagonists have protective effects against cerebral ischemic injury.

3. Lung. Ischemia-reperfusion lung injury was attenuated by ATP-MgCl2 in rats, although its effect was in part mediated by its breakdown product, adenosine, via A2 receptors, probably on leukocytes, acting to prevent the production of damaging O2- derived free radicals (Hsu et al., 1994; Hirata et al., 2001; Chen et al., 2003). A2A receptor antagonists attenuated cardiac dysfunction arising from pulmonary ischemia-reperfusion injury (Reece et al., 2005; Ellman et al., 2008; Gazoni et al., 2008; Sharma et al., 2009). It has been claimed that A2B Receptors play a role in mediating lung inflammation after ischemia-reperfusion by stimulating cytokine production and neutrophil chemotaxis (Anvari et al., 2010). Activation of A2B receptors has also been claimed to attenuate lung injury after in vivo reperfusion (Rivo et al., 2004; Mulloy et al., 2013). A1 receptor antagonists have been reported to block ischemia-reperfusion injury of feline lung (Neely and Keith, 1995). In summary, A1, A2A, A2B, and A3 receptor activation all ameliorate lung ischemia-reperfusion injury (Gazoni et al., 2007, 2010;
Fernandez et al., 2013). CD39 and CD73 ectonucleotidase activities were severely impaired in the vasa vasorum endothelial cells from pulmonary arteries of chronically hypoxic neonatal calves, with increases in extracellular ATP and ADP levels associated with higher proliferative responses (Yegutkin et al., 2011).

4. Kidney. Adenosine levels are raised in ischemic kidney after 10 minutes of ischemia (Miller et al., 1978) but decreased after 30 minutes of ischemia (López-Martí et al., 2003). It was suggested that tissue levels of adenosine can modulate renal ischemia-reperfusion injury via effects on NO and superoxide (López-Martí et al., 2003). In contrast to most other vessels, extracellular adenosine produces vasoconstriction of the renal vasculature via A1 receptors and hence is a candidate for the decrease in renal blood flow and gomeral filtration rate that occurs in ischemic kidneys. Theophylline, a P1 adenosine receptor antagonist, reduced the vasoconstriction in response to arterial occlusion and that to adenosine (and ATP) (Sakai et al., 1979b). It was suggested that renal ischemic preconditioning can protect against ischemia-reperfusion injury by decreasing the renal interstitial concentrations of adenosine and ATP, and it was claimed that A1 receptor activation during ischemia-reperfusion injury is detrimental to renal function (Li et al., 2005). However, later articles claim short-term and delayed protection against renal ischemia and reperfusion injury with A1 receptor activation (Joo et al., 2007; Moosavi et al., 2009). More recently, an A1 receptor enhancer, PD-81723, was shown to be protective against renal ischemia-reperfusion injury (Park et al., 2012). A1 receptor activation has also been shown to inhibit inflammation, necrosis, and apoptosis after renal ischemia-reperfusion injury in mice (Lee et al., 2004a). A1 receptor knockout mice exhibit increased renal injury after ischemia-reperfusion injury (Lee et al., 2004b), but Kim et al. (2009) shows the reverse effect.

Other studies showed that A2A receptor agonists reduce renal injury after ischemia-reperfusion (Okusa et al., 1999, 2001; Day et al., 2003). The A2A agonist protective effect on ischemic-reperfusion injury was blocked in macrophage-depleted A2A knockout mice (Day et al., 2005a). ATL146 [4-[3-[6-amino-9-(5-ethylcarbamoyl-3,4-dihydroxy-tetrahydro-furan-2-yl)-9H-purin-2-yl]-prop-2-ynyl]-cyclohexanecarboxylic acid methyl ester], an A2A agonist receptor antagonist, has been tested in phase 3 clinical trials to treat acute kidney injury leads to cell death (Lee, 2004). Adenosine uptake mediates recovery of ATP content in postischemic cultured renal tubular cells (Cadnapaphornchai et al., 1991). Enhanced recovery from acute renal failure in rats by postsischemic infusion of ATP-MgCl2 was described earlier (Osias et al., 1977; Glazier et al., 1978; Siegel et al., 1980; Gaudio et al., 1982; Hirasawa et al., 1983; Sumpio et al., 1984; Wang et al., 1992b; 1992d; Martin et al., 1992). Renal injury can arise as a consequence of ischemia and reperfusion during renal transplant surgery. Kidneys subjected to warm ischemia before transplantation are salvaged by addition of ATP-MgCl2 to the perfusate (Lytton et al., 1981). The mechanism underlying the beneficial effects of ATP is still not clear, although several mechanisms have been proposed. The protective effect of ATP-MgCl2 after warm ischemia in the kidney is not mediated by adenosine (Sumpio et al., 1987). ATP given after ischemia augmented the expression of genes critical to cell proliferation that may underlie its beneficial mechanism of action (Paller et al., 1998). ATP-MgCl2 has been shown to be effective in preventing lipid peroxidation if given before reperfusion, but not before ischemia, in rabbit kidneys (Mocan et al., 1999).

Trimetazidine is an antioxidant agent used to protect kidney grafts from ischemia-reperfusion injury; tissue concentrations of ATP and ADP were significantly increased in kidneys from rats treated with trimetazidine, and it was suggested that this drug protects against dephosphorylation of nucleotides and ischemic damage (Domanski et al., 2006). In an in vitro model of ischemic injury involving severe depletion of intracellular ATP in rabbit cultured proximal tubule cells, P2Y receptor agonists were shown to be protective, and the mechanism involved reduced activation of NF-κB, an inducible transcription factor associated with inhibitory proteins (Lee and Han, 2005). Suramin, a nonselective P2 receptor antagonist, promotes recovery from renal ischemia-reperfusion injury in mice; suramin-treated animals had a significant reduction in apoptosis and increase in proliferation of tubular cells and infiltrating leukocytes (Zhuang et al., 2009).
There was a robust and selective induction of E-NTPDase 1/CD39 protein in mouse renal tissues after renal ischemic preconditioning (Grenz et al., 2007b), as observed after preconditioning in mouse heart (Köhler et al., 2007). Transient overexpression of E-NTPDase 1/CD39 was shown to protect against renal ischemia-reperfusion and transplant vascular injury in mice (Crikis et al., 2010; see Roberts et al., 2013). Conversely, renal protection by ischemic preconditioning was abolished in E-NTPDase 1/CD39 knockout mice, whereas apyrase treatment (to hydrolyze nucleotides) restored renal protection by ischemic preconditioning in the knockouts; it was proposed that apyrase treatment may be a therapeutic strategy in treating acute ischemic injury of the kidneys (Grenz et al., 2007b). Ischemia, followed by reperfusion for durations of over 30 minutes, was associated with loss of glomerular ATP diphosphohydrolase activity and potentiated the deleterious effects of reperfusion (Candinias et al., 1996). E-NTPD3 activity, located on the surface of LLC-PK1 cells, was significantly inhibited by ischemia (Ribeiro et al., 2012). Ecto-5'-nucleotidase/CD73 is the rate-limiting enzyme for extracellular adenosine formation, and increases in renal adenosine concentration with ischemic preconditioning were attenuated in ecto-5'-nucleotidase/CD73 knockouts (Grenz et al., 2007a). Moreover, renal ischemic preconditioning was shown to induce ecto-5'-nucleotidase/CD73 (Grenz et al., 2007a). However, there is a lack of consensus about the role of ecto-5'-nucleotidase/CD73 in tissue protection during renal ischemia. In kidneys of ecto-5'-nucleotidase/CD73 knockout mice renal protection by ischemic preconditioning was abolished and was completely restored after intraperitoneal injection of the knockout mice with soluble 5'-nucleotidase (Grenz et al., 2007a). Moreover, renal injury after ischemia in the knockouts was also attenuated by treatment with soluble 5'-nucleotidase (Grenz et al., 2007a). Others have similarly shown that in kidneys of ecto-5'-nucleotidase/CD73 knockout mice there was greater injury and lipid peroxidation, and free radical concentrations were higher than in wild-type kidneys, supporting the concept that ecto-5'-nucleotidase/CD73 may protect the kidney from ischemia-reperfusion injury through adenosine production (Jian et al., 2012). However, there are other reports that ecto-5'-nucleotidase/CD73 knockout in mice has protective effects in renal ischemia-reperfusion injury (Lu et al., 2008; Rajakumar et al., 2010a). It has been claimed that CD39 is protective in kidney ischemia-reperfusion injury via adenosine A2B receptor signaling (Rajakumar et al., 2010b).

5. Gut. Superoxide dismutase is beneficial in restoring the ATP and GTP concentrations of rat small intestine during posts ischemic reperfusion (Siems et al., 1991). ATP-MgCl2 reduces intestinal permeability during mesenteric ischemia (Kreienberg et al., 1996). Both adenosine and ATP were reported to attenuate intestinal dysfunction produced by ischemia but not that caused by reperfusion (Taha et al., 2010a, 2010b). A more recent study showed that treatment with adenosine attenuated small bowel motor and neural malfunctions produced by ischemia and reperfusion in rats (Haddad et al., 2012). Extracellular adenosine restores cellular ATP after intestinal epithelial ischemia (Mammen et al., 2004). External application of ATP protects mucin in the mucosal barrier during early periods of gut ischemia (Chang et al., 2012). A2B receptor signaling is claimed to provide potent protection during intestinal ischemia-reperfusion injury (Eltzschig et al., 2009; Hart et al., 2009). Pretreatment with 5'-N-ethylcarboxamidoadenosine, a nonselective A1/A2 agonist, provides partial improvement in intestinal ischemia-reperfusion injury in rats (Özacmak et al., 2009). Ischemia-reperfusion of the rat intestine causes a decrease in the expression of P2X2 receptors on neurons of the myenteric and submucosal plexuses, as well as a decrease in density and of neurons immunoreactive for the P2X3 receptor (Paulino et al., 2011). After intestinal ischemia-reperfusion injury, P2Y2 receptor mRNA expression was increased in murine lung and kidney and A3 receptor mRNA was increased in lung but decreased in kidney, but there was no change in mRNA expression of these receptors in the intestine (Milano et al., 2008). Protection from intestinal ischemia-reperfusion injury by hypoxia-inducible factor-1α involves CD73 and A2B receptors (Hart et al., 2011). The roles of ATP and adenosine in hypoxia during intestinal ischemia and inflammation are discussed in a recent review (Grenz et al., 2012b).

6. Liver. It was claimed many years ago that infusion of ATP-MgCl2 improved hepatic function and survival after hepatic ischemia (Hirasawa et al., 1980; Ohkawa et al., 1983; Frederiks and Fronik, 1986; Jeong and Lee, 2000) and after reperfusion (Clemens et al., 1985b). It has been claimed that the beneficial effect of ATP-MgCl2 treatment after trauma-hemorrhage is associated with a downregulation of circulating levels of the inflammatory cytokines TNF and IL-6 (Wang et al., 1992c). Another suggestion was that the improvement in ischemic damage by ATP-MgCl2 infusion may be mediated through improvement in mitochondrial energy metabolism (Jeong and Lee, 2000). There is a 90% ATP loss from hepatocytes during 60 minutes of ischemia (Gruene et al., 1997). Ischemic preconditioning protects the liver against necrosis, perhaps through protecting against tissue ATP loss (Selzner et al., 2003). ATP treatment was particularly effective for treatment of ischemia in old mice (Selzner et al., 2007). Aging of the liver is associated with mitochondrial dysfunction, resulting in poorer tolerance against ischemic injury; improvement in intrahepatic ATP levels in old livers by glucose injection protects the old liver against ischemic injury.
and restores the protective effect of preconditioning (Selzner et al., 2007). Provision of adenosine during reperfusion enhanced restoration of ATP concentrations in rat liver (Palombo et al., 1991).

P2Y2 receptors promote hepatocyte resistance to hypoxia by downmodulating ERK1/2-mediated signals that promote Na+ influx through the Na+/H+ exchanger (Carini et al., 2001). A study demonstrated that administration of UTP before induction of ischemia can attenuate, via P2Y2 and/or P2Y4 receptors, posts ischemic hepatocyte apoptosis and thereby reverse liver damage (Ben-Ari et al., 2009). The authors suggest that the UTP-mediated protective effect may be regulated through NF-κB inactivation. P2Y receptor antagonism prevents cold preservation-induced cell death independent of cellular ATP levels in a human hepatocyte cell line (Anderson et al., 2007). After hepatic ischemia and reperfusion injury, vascular NTPDase activity is lost and deletion of E-NTPDase 1/CD39 in mice leads to significantly increased injury and decreased survival, consistent with a protective role of endogenous nucleotides in ischemia-reperfusion injury (Imai et al., 2000).

There is compelling evidence that adenosine can play a protective role against ischemia-reperfusion injury (Dunne et al., 1998; Nilsson et al., 2000). This probably occurs through activation of A2 receptors (Peralta et al., 1999) located on sinusoidal endothelial cells (Arai et al., 2000) and in particular involving A2A receptors (Carini et al., 2001; Lappas et al., 2006). Protection is lost in A2A receptor knockout mice (Day et al., 2004, 2005b). However, administration of an adenosine A1 receptor antagonist before ischemia attenuates ischemia-reperfusion injury (Magata et al., 2007; Kim et al., 2008). Ozone oxidative preconditioning is mediated by A1 receptors in a rat model of liver ischemia-reperfusion (León Fernández et al., 2008). Selective intrarenal human A1 receptor overexpression reduced acute liver (and kidney) injury after hepatic ischemia-reperfusion in mice (Park et al., 2010b). It is claimed that ischemic preconditioning promotes liver regeneration via A2 receptors on Kupffer cells (Arai et al., 2007). Hepatic ischemia-reperfusion injury induces expression of A2B receptors early after partial portal occlusion and A2B receptor activation has a protective effect (Zimmerman et al., 2011). Hypoxic preconditioning in vivo was reported to protect against liver ischemia-reperfusion injury via A2B receptors (Choukér et al., 2012). Ischemia-driven expression of 5′-nucleotidase/CD73 leads to increased adenosine production conferring tissue protection during liver ischemia-reperfusion (Hart et al., 2008b).

7. Bladder. Ischemia-reperfusion of the urinary bladder may result in dysfunction of the contractile response to nerve stimulation and NO may act as a tissue damaging agent in ischemia-reperfusion injury (Saito et al., 1998). Contractile responses to ATP were similarly sensitive to ischemia (Bratlavsky et al., 1999).


9. Testis and Prostate. ATP-MgCl2 prevented reperfusion injury in testicular torsion (Abes et al., 2001). It has been suggested that adenosine-induced vasodilatation may be useful to prevent prostatic ischemia (Ribeiro et al., 2011).


11. Skeletal Muscle. A very early article implicated ATP in the responses to experimental limb ischemia (Stoner and Green, 1945). Hagberg et al. (1985) concluded that reduction in the glycogen available for ATP resynthesis during rabbit skeletal muscle ischemia drastically reduces the ability of skeletal muscle to withstand prolonged ischemia. Vascular injury in dog gracilis nuclei during ischemia-reperfusion was attenuated by pretreatment with ATP-MgCl2 (Korthuis et al., 1988; Hayes et al., 1990). Attenuation of apoptosis in ischemia-reperfusion injury in vivo in mouse skeletal muscle by P2Y6 receptor activation has been claimed (Mamedova et al., 2008). Release of nucleotides in ischemic tissue may induce a pronounced activation of the endogenous fibrinolytic system (Hrafnskoldtir et al., 2001). ATP released from ischemic muscle works together with acid by increasing the sensitivity of sensory fibers when P2X and acid sensing ion channel receptors form a molecular complex resulting in ischemic pain (Birdsong et al., 2010). In young healthy humans, oxygen delivery is matched to oxygen demand during hypoxia and exercise by increases in skeletal muscle blood flow. However, in aging humans there is impaired vasodilation and functional sympatholysis in response to hypoxia (Kirby et al., 2012).

Adenosine pretreatment applied prior to ischemia protects against ischemia-reperfusion injury (Lee and Lineaweaver, 1996). Intra-arterial adenosine administration during reperfusion preserves endothelium-dependent relaxation in the rabbit hindlimb (Farooq et al., 1997). A2A receptors were not found to contribute to the hyperemia after ischemia of skeletal muscle in the hindlimb of anesthetized cats (Poucher, 1997). Adenosine appears to act via A1 receptors during acute ischemic preconditioning of pig skeletal muscle, protecting against infarction (Pang et al., 1997). A more recent paper describes a protective role for A1, A2A, and in particular A3 receptors in skeletal muscle ischemia and reperfusion injury (Zheng et al., 2007a, 2007b). Remote postconditioning may attenuate ischemia-reperfusion injury in murine hindlimb via activation of adenosine receptors (Tsubota et al., 2009). Mg-ATP
elicits a protective effect on ischemic skeletal muscle by acting primarily through adenosine receptors (Maldonado et al., 2013).

12. Eye. The concentration of adenosine in rat retina is elevated after retinal ischemia and during reperfusion (Roth et al., 1997). It was proposed that blockade of A<sub>2A</sub> receptors combined with stimulation of A<sub>1</sub> receptors may be potential strategies for the prevention of ischemic damage of the retina (Li et al., 1999). However, a more recent study suggested that A<sub>2A</sub> receptors mediate the protective effect of adenosine on retinal ischemia-reperfusion injury (Konno et al., 2006). Evidence was presented that glutamate-sine on retinal ischemia-reperfusion (Roth et al., 1997). It was proposed that blockade of A<sub>2A</sub> receptors combined with stimulation of A<sub>1</sub> receptors may be potential strategies for the prevention of ischemic damage of the retina. However, a recent study suggested that A<sub>2A</sub> receptors mediate the protective effect of adenosine on retinal ischemia-reperfusion injury (Konno et al., 2006). Evidence was presented that glutamate evoked purinergic P2Y<sub>1</sub> receptor signaling of glial cells is involved in cell volume homeostasis of the retina, and it was suggested that this mechanism may contribute to the protective effect of purines in ischemic tissue (Uckermann et al., 2006). Atrial natriuretic peptide (ANP) inhibits osmotic glial cell swelling in the ischemic rat retina; because ANP evokes release of ATP from Müller glial cells, it was suggested that P2Y<sub>1</sub> receptors and also A<sub>1</sub> receptors (after ATP breakdown to adenosine) on glial cells are involved in the protective actions of ANP (Kalisch et al., 2006). It has been suggested that suppressing neuronal necrosis using ATP-liposomes in ischemia-reperfusion-challenged neuronal tissues could promote a neuroprotective environment and reduce tissue damage (Dvorianchikova et al., 2010). P2X<sub>7</sub> receptor activation causes retinal ganglion cell death in a human retina model of ischemic neurodegeneration (Niyadurupola et al., 2013).

D. Thrombosis and Stroke

Extracellular nucleotides are recognized as mediators of vascular inflammation and thrombosis (Robson et al., 2001). ATP released from endothelial cells during changes in blood flow (producing shear stress) is broken down by ectonucleotidases to ADP, which acts on P2Y<sub>1</sub> and P2Y<sub>12</sub> receptors on platelets to cause aggregation. Clopidogrel is a P2Y<sub>12</sub> antagonist that inhibits platelet aggregation and has been used for the treatment of thrombosis and stroke (Herbert et al., 1993; see Saraff et al., 2012). Other P2Y<sub>12</sub> antagonists have been developed to follow clopidogrel as antithrombotics, such as ticlopidine, cangrelor, ticagrelor, prasugrel, elinogrel, BX 667, AZD6140, and PSB 0739 (see Cattaneo, 2009; Khoynezhad et al., 2009; Wallentin, 2009; Angiolillo and Ferreiro, 2010; Nawarskas and Snowden, 2011; Ahmad and Storey, 2012). Evidence has been presented for an association of haplotype H2 gene variants of the P2Y<sub>12</sub> receptor with lower risk of ischemic stroke and deep venous thromboembolism/pulmonary disease (Zee et al., 2008). P2Y<sub>1</sub> receptor antagonists have also been proposed as antithrombotic agents (see Gachet, 2008). Adenosine has antithrombotic effects by blocking induction of circulating tissue factor (Deguchi et al., 1998) via A<sub>2A</sub> and A<sub>3</sub> receptors (Sitkovsky et al., 2004). Ectonucleotidases are regulators of purinergic signaling in thrombosis (Deaglio and Robson, 2011). The ectonucleotidase CD39 mediated resistance to occlusive arterial thrombus formation after vascular injury in mice (Huttinger et al., 2012).

E. Diabetic Vascular Disease

In rats with streptozotocin diabetes there was prejunctional impairment of sympathetic neurotransmission and impaired ATP-mediated endothelial vasorelaxant function in mesenteric arteries (Ralevic et al., 1995a). Attenuated vasodilatation to ATP, UTP, and adenosine in the skeletal muscle circulation of patients with type 2 diabetes was demonstrated (Thaning et al., 2010).

Red blood cells may stimulate vasodilatation via release of ATP, which may be important in maintaining perfusion in the microcirculation. Interestingly, in erythrocytes from humans with type 2 diabetes ATP release is impaired, consistent with the hypothesis that a defect in erythrocyte physiology could contribute to the vascular disease associated with this clinical condition (Sprague et al., 2006). This group later showed that the phosphodiesterase 3 inhibitor cilostazol rescues low PO2-induced ATP release from erythrocytes of humans with type 2 diabetes (Sprague et al., 2011).

High extracellular glucose releases ATP and/or UTP in endothelial cells and pancreatic β-cells (Parodi et al., 2002; Hellman et al., 2004). An increase in glucose from 5 to 15 mM (equivalent to normal and hyperglycemic levels, respectively) results in a marked increase in the pro-atherogenic nuclear factor of activated T cells signaling pathway in vascular smooth muscle cells (Nilsson et al., 2006). The effect is mediated via glucose-induced release of ATP and UTP that subsequently activate P2Y<sub>2</sub> but also P2Y<sub>6</sub> receptors (after degradation to UDP). Thus, nucleotide release is a potential metabolic sensor for the arterial smooth muscle response to high glucose. Diabetic patients experience microvascular disease characterized by increased wall-lumen ratio, mainly because of an increase in vascular smooth muscle cells, and have higher rates of restenosis after coronary angioplasty. High-glucose-induced release of extracellular nucleotides acting on P2Y<sub>2</sub> receptors to stimulate vascular smooth muscle cell growth via nuclear factors of activated T-cells may provide a link between diabetes and diabetic vascular disease (Nilsson et al., 2006). There is a recent review about adenosine-insulin signaling in fetoplacental endothelial dysfunction in gestational diabetes (Guzmán-Gutiérrez et al., 2013).

F. Migraine and Vascular Pain

There are two distinct cerebrovascular phases: an initial vasoconstriction (not associated with pain)
followed by vasodilatation (reactive hyperemia) associated with pain in migraine, with early hints of the involvement of ATP. A “purinergic” hypothesis for migraine was put forward in 1981 as a basis for the reactive hyperemia and pain during the headache phase (Burnstock, 1981). It was proposed that ATP and its breakdown product adenosine were contenders for mediating the vasodilatation after the initial vasoconstriction and subsequent hypoxia. It was also suggested that ATP was implicated in migraine pain via stimulation of primary afferent nerve terminals located in the adventitia of the cerebral microvasculature. Later studies have shown that ATP-induced cerebral vasodilatation is endothelium dependent via activation of P2X and P2Y receptors on endothelial cells, with subsequent release of EDRFs, and that the endothelial cells are the main local source of ATP involved, although ADP and ATP released from aggregating platelets may also contribute to this vasodilatation. These findings have extended the purinergic hypothesis for migraine in two ways. First, they clarify the mechanism of purinergic vasodilatation during the headache phase of migraine. Second, they suggest that a purinergic mechanism may also be involved in the initial local vasoconspasm, via P2X receptors on smooth muscle cells activated by ATP, released either as a cotransmitter with NA from perivascular sympathetic nerves or from damaged endothelial cells (Burnstock, 1989b).

Evidence in support of the purinergic hypothesis was reported where it was suggested that decreased platelet ATP release was a marker for migraine, reflecting hypofunction of the purinergic system, resulting in a greater tendency for vasoconstriction, which predisposes to migraine attacks (Joseph et al., 1986). Further support was the identification of P2X3 receptors on primary afferent nerve terminals supplying cerebral vessels arising from trigeminal, nodose, and spinal ganglia (Chen et al., 1995, Burnstock, 1996a, 2001b). Peripheral sensitization in the dural vessel sensory pathway via P2X3 receptors in migraine was also reported (Jennings and Cho, 2007). P2X3 receptor antagonists were suggested as possible candidates for antimigraine drug development (Waebcr and Moskowitz, 2003). CGRP, released during migraine attacks from trigeminal neurons, leads to sensitization of trigeminal P2X3 nociceptive receptors; thus it was suggested that trigeminal P2X3 receptors may be a potential target for the early phase of migraine attacks (Fabbretti et al., 2006). The nonsteroidal anti-inflammatory drug naproxen, which is widely used for the treatment of migraine pain, has been shown to block P2X3 receptor-mediated responses of rat trigeminal neurons (Hautaniemi et al., 2012). Migraine may also involve a chronic sympathetic nervous system disorder, with an increase in release of sympathetic cotransmitters, including ATP (Peroutka, 2004), perhaps contributing to the initial vasoconspasm. It has been shown that neutralization of NGF induces plasticity of ATP-sensitive P2X3 receptors of nociceptive trigeminal ganglion neurons involved in migraine (D’Arco et al., 2007). Familial hemiplegic migraine calcium channel mutation R192Q enhances ATP-gated P2X3 receptor activity of mouse sensory neurons mediating trigeminal pain, suggesting a role for P2X3 receptors (Wirkner et al., 2007; Fabbretti, 2010; Nair et al., 2010). According to Gnanasekaran et al. (2011), it was shown that P2X3 receptors of trigeminal nerves were preferentially localized to lipid rafts, which is associated with a molecular phenotype characterized by stronger responses to P2X3 receptor activation. Cultures of the trigeminal ganglia from the knockin mouse genetic model of familial hemiplegic migraine had a neuroinflammatory profile that may facilitate release of ATP to activate P2X3 receptors and amplify nociceptive signals by trigeminal sensory neurons (Franceschini et al., 2012, 2013). Vascular endothelial cells mediate endothelium-induced hyperalgesia via release of ATP to activate P2X2/3 nociceptive receptors (Joseph et al., 2013).

Adenosine has also been implicated in migraine. For example, during migraine, biosynthesis of ATP is elevated and, after release, degrades to adenosine and the blood level of adenosine increased by 47% (Guieu et al., 1994). Infusion of adenosine causes migraine-like symptoms; and withdrawal from caffeine and theophylline (adenosine receptor antagonists) addiction also causes migraine-like symptoms (Spencer 1996; Shapiro, 2007). Early clinical trials with dipyridamole, an adenosine uptake inhibitor that increases extracellular adenosine, had to be stopped because of increased migraine attacks in all patients (Hawkes, 1978). The efficacy of caffeine against migraine is poor (Tokola et al., 1984), but it is effective when used in combination with analgesics (Diener et al., 2005). An A1 receptor agonist, GR79236 [(2R,3R,4S,5R)-2-(6-[(1S,2S)-2-hydroxycyclopentyl]amino)-9H-purin-9-yl]-5-(hydroxymethyl)oxolane-3,4-diol], acting on the trigeminovascular system, was claimed to be effective against migraine, perhaps by reducing CGRP release (Goadesby et al., 2001; Mehrtra et al., 2008). A2A receptors facilitate the effects of CGRP and vasoactive intestinal peptide, two neuropeptides claimed to be involved in migraine pathophysiology and are a target of caffeine, used in migraine treatment in combination with analgesics; A2A receptor gene variation may contribute to the pathogenesis of migraine with aura (Hohoff et al., 2007).

It has been suggested that in vascular pain, including angina, pelvic, and ischemic pain, as well as migraine, ATP released from endothelial cells during the reactive hyperemia after vasoconspasm (not associated with pain) diffuses through the wall of microvessels to reach P2X3 receptors on sensory perivascular nerves to
initiate impulses that travel via the spinal cord to pain centers in the brain (Burnstock, 1996a). Data have been presented to suggest that overactive glial P2Y receptors may contribute to pain transduction in migraine (Ceruti et al., 2011b; see Magni and Ceruti, 2013).

G. Sepsis and Septic Shock

An early theory was that degradation of adenine nucleotides is related to irreversibility in hemorrhagic shock, and a marked reduction in ATP contents of the pancreas, liver, and duodenum was demonstrated (Cunningham and Keaveny, 1977). It was suggested that ATP prevented disruption of glucose homeostasis and development of endotoxin shock by counteracting insulin and blunting hypoglycemia (Chaudry et al., 1976; Filkins and Buchanan, 1977). Treatment of shocked animals with ATP-MgCl$_2$ was reported to be an effective therapy for experimental hemorrhagic shock (Kraven et al., 1980; Machiedo et al., 1981; Engelbrecht and Mattheyse, 1986; Zambon et al., 1994), although this was debated (Schloerb et al., 1981). In animal models of sepsis, treatment with ATP-MgCl$_2$ has been shown to prevent endothelial dysfunction, reduce organ damage, restore immune competence, and increase survival (Wang et al., 1999; Nalos et al., 2003).

Human microvascular endothelial cells showed a selective induction of the P2Y$_6$ receptor after exposure to inflammatory stimuli, and in vivo inflammatory responses due to LPS treatment were attenuated in P2Y$_6$ knockout mice or after P2Y$_6$ antagonist treatment, implicating the P2Y$_6$ receptor as a therapeutic target for systemic inflammatory responses (Riegel et al., 2011). Glibenclamide reduced proinflammatory cytokines in an ex vivo model of human endotoxinemia under hypoxic conditions (Schmid et al., 2011). It was suggested that glibenclamide inhibition was dependent on P2X$_7$ receptor activation of monocytes by ATP-releasing erythrocytes during hypoxia. A role for adenosine in the early hemodynamic changes associated with sepsis has been claimed (Conlon et al., 2005). Regional hemodynamic responses to adenosine were shown to be altered after LPS treatment in conscious rats, such that renal and hindquarters vasodilator responses were abolished (Jolly et al., 2008).

H. Hyperhomocysteinemia

Hyperhomocysteinemia is a risk factor for cardiovascular disease; the mechanisms causing the cardiovascular complications are not fully understood, although endothelial dysfunction appears to be involved. In this condition, there is evidence that constitutive and evoked adenosine release is decreased, and it has been suggested that as a consequence the cardio- and vasoprotective actions of adenosine are attenuated, identifying a key role for adenosine in the pathogenesis of hyperhomocysteinemia (Riksen et al., 2003).

I. Calcific Aortic Valve Disease

ATP acts as a survival signal and prevents mineralization of aortic valve that occurs in calcific aortic valve disease (Osman et al., 2006; Côté et al., 2012b). Released ATP promoted the survival of valvular interstitial cells in the aortic valve via P2Y$_2$ receptors, and it was also shown that a high level of membrane-bound ectonucleotidase ENPP1 was expressed in calcific aortic valve disease. Inhibition of ectonucleotidase with ARL67156 prevented the development of calcific aortic valve disease in warfarin-treated rats (Côté et al., 2012a).

J. Metabolic Syndrome

Metabolic syndrome is an obesity disorder in which abnormal metabolism of glucose and lipid is associated with the development of chronic inflammatory diseases. Metabolic syndrome has pronounced effects on small blood vessels, and these result in many chronic complications in other organ systems (Sparks and Chatterjee, 2012).

VI. Conclusions and Future Directions

Several important conclusions can be drawn from this review:

1. Purinergic signaling plays a major role in control of both vascular tone and remodeling. Vascular tone is regulated by moment-to-moment changes in sympathetic nerve activity that coordinates with endothelium-mediated vasorelaxation (or less commonly contraction). ATP has a pivotal role as it is released as a cotransmitter with NA from sympathetic nerves to contract vascular smooth muscle and is also released from endothelial cells in response to changes in blood flow (producing shear stress) and hypoxia to act on adjacent endothelial cells with subsequent vasodilation. Vascular tone is also regulated by the actions of other locally released purine and pyrimidine nucleotides (ADP, UTP, and UDP) and by adenosine.

2. $P1$ adenosine receptors: Adenosine is predominantly a vasodilator, acting via $A_{2A}$ and/or $A_{2B}$ receptors on the endothelium and smooth muscle, but mediates vasoconstriction, mainly via $A_1$ receptors, in pulmonary arteries, renal afferent arterioles and some aortae.

3. $P2$ receptors for purine and pyrimidine nucleotides: Contractile $P2X_1$ receptors appear to be expressed on the smooth muscle of all blood vessels. Vascular smooth muscles also express contractile $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors activated by ATP, UTP, and UDP (Table 3). Some blood vessels express vasorelaxant $P2Y_2$ receptors on their smooth muscle (hepatic veins and aortae and coronary arteries from some species), but in
most blood vessels vasorelaxant P2Y receptors are expressed on the endothelium.

4. Adenosine and purine and pyrimidine nucleotides elicit long-term (trophic) signaling, modulating cell proliferation, differentiation, and death in angiogenesis and regeneration of damaged vessels (see Table 4). In summary, A2 and P2Y receptors mediate proliferation of endothelial cells, whereas A2 and P2Y2/P2Y4 receptors stimulate proliferation of smooth muscle cells.

5. Significant changes in purinergic signaling pathways occur in pathologic conditions (see Table 5).

Thus, the field of purinergic signaling in the vasculature is complex, but major patterns are emerging, which we have summarized in the figures. Within blood vessels, the effects of purines are influenced by the sources of purine release and endothelial integrity.

In general, purines released at the adventitia (e.g., ATP from sympathetic nerves) are vasocontractile, whereas those released at the intima (e.g., from erythrocytes, endothelial cells) mediate endothelium-dependent vasodilatation or contraction where there is endothelial damage. A balance between purine release and the activities of ectonucleotidases will determine the extracellular levels of ATP, ADP, UTP, and adenosine and consequently the prevailing target effects.

Clearly, purines contribute to a number of processes involved in normal vascular function, and disturbances in purinergic signaling are involved in some vascular diseases. As all cells in the vascular system express one or more types of purine receptor, this raises the possibility that purine receptors may be potential targets in vascular disease (see Schuchardt et al., 2012). Purine release mechanisms, receptors, and ectonucleotidases are all potential targets for drug development. Drugs against purinergic receptors already exist, giving reason for optimism. Clodigrel is a widely used antithrombotic drug; the active metabolites of this prodrug bind to platelet P2Y12 receptors to inhibit aggregation. The success of this compound owes much to the fact that P2Y12 receptors are found mainly on platelets. However, purine receptors within the vasculature are also expressed elsewhere in the body, and this is a major drawback to the development of drugs targeting these receptors; as with all established/potential drugs, target selectivity is not guaranteed. Of some promise may be the development of A3 agonists to protect against ischemia-reperfusion injury. The A3 receptor has a relatively limited expression, and it has been shown to be cardioprotective and adenosine can safely be administered to humans. An increased importance of ATP as a sympathetic cotransmitter in arteries of spontaneously hypertensive and obese rats (Haddock and Hill, 2011; Goonetilleke et al., 2013 and references therein) suggests that antagonists at smooth muscle P2X1 receptors could be beneficial in these diseases. The drawbacks are that P2X1 receptors are widely distributed, and P2X1 knockout mice showed male infertility and an increase in blood pressure (Mulryan et al., 2000). E-NTPDase1 knockout augmented purinergic vasorelaxation in vitro and the hypotensive effects of purines in vivo (Kauffenstein et al., 2010b), and thus enzyme inhibition might be useful in conjunction with purinergic vasodilator drugs, especially those targeting the endothelial P2Y1 receptor; potential pitfalls are an increased desensitization of the P2Y1 receptor (Kauffenstein et al., 2010b) and prothrombotic effects. An area with future potential involves the characterization of vascular smooth muscle and endothelial P2Y2, P2Y4, and P2Y6 receptors because there is evidence of profound species differences and some restriction in their expression within blood vessels. However, the development and use of ligands with good subtype specificities that are orally bioavailable and do not degrade in vivo is needed to exploit this. The considerable efforts of the clinical biochemists working in this field are leading to a promising emergence of subtype-specific P2 ligands. This will open up new avenues for research into the physiologic roles of purine receptors and their therapeutic potential for the treatment of vascular disorders.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Burnstock, Ralevic.

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