Roles of P₂-Purinoceptors in the Cardiovascular System

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P urines exert potent and diverse effects on the cardiovascular system; the physiological significance of these actions is receiving increasing attention. Much progress has been made since the initial observations of Drury and Szent-Györgyi of the effects of adenosine and AMP on the mammalian heart and circulation; they showed that intravenous injection of these purines into whole animals induced a decrease in heart rate and arterial blood pressure, dilation of coronary blood vessels, and inhibition of intestinal movements. Subsequently, observations of the potent vasodilator actions of extracellular ATP led to the rapid expansion of the field of cardiovascular pharmacology of purines.

Two of the first studies to suggest physiological roles for adenosine and ATP in local regulation of blood flow resulted in 1) the observation that stimulation of the rabbit great auricular nerve caused antidromic dilatation of the ear vessels and concomitant release of ATP, suggesting a role for ATP in this neurally mediated response, and later, 2) the detection of breakdown products of adenosine in the cardiac perfusate after a period of hypoxia, which formed the basis for the hypothesis that adenosine is a physiological regulator of coronary blood flow. It is now clear that these early studies predicted examples of only two of the many different roles played by adenosine and ATP in the physiological regulation of the cardiovascular system.

The heterogeneity of the purine receptor population was first formally recognized in 1978, when Burnstock named two classes, P₁ and P₂, and proposed criteria for their identification. An agonist potency order of adenosine > AMP > ADP > ATP; selective antagonism by methylxanthines; and receptor-activated changes in intracellular cyclic AMP (cAMP) levels identified the P₁-purinoceptor. At the P₂-purinoceptor, the agonist potency order was proposed to be ATP > ADP > AMP > adenosine; there was no antagonism by methylxanthines and no change in intracellular cAMP levels after receptor occupancy. However, in some cases, there was stimulation of prostaglandin biosynthesis. P₁-Purinoceptors have been further subdivided into A₁ and A₂ (Reference 7) or R₁ and R₂ (Reference 8) (according to alternative systems of nomenclature), which inhibit (A₁/R₁) or activate (A₂/R₂) adenylate cyclase.

Subdivision of P₂-purinoceptors has also recently been suggested (see “Subclassification of P₂-Purinoceptors”).

The direct effects of purine nucleotides can be manifested as vasoconstrictor or vasodilator responses, which have been described in a large number of vessels and vascular beds (see “Actions of P₂ Agonists in Animals and in Humans” and References 9 and 10). Evidence has been presented for a number of different local sources of purine nucleotides: ATP has been shown to be released as a cotransmitter with noradrenaline from perivascular sympathetic nerves and as a transmitter from purinergic and sensory nerves, and ATP and ADP are components of blood-borne elements, such as platelets and erythrocytes, and can also be released from endothelial and smooth muscle cells. The relation between the release of purines from these sources and stimuli such as ischemia, hypoxia, and sheer stress is the subject of much investigation. Several factors are important in determining the direction of the vascular response elicited by purine nucleotides, including the nature of the purinoceptor subtype involved and its location in relation to the structural components of the vascular wall, although there is usually a close correlation between the two. Activation of specific P₂-purinoceptors located on the smooth muscle can produce direct vasoconstrictor or vasodilator effects; activation of purinoceptors located on endothelial cells usually produces relaxation via production of endothelium-derived relaxing factor (EDRF), although endothelium-mediated contractions can also occur. In light of evidence for the crucial role played by the vascular endothelium in the relaxant responses elicited by many agents, including ATP and ADP, there is particular interest in mechanisms that perturb endothelial function or integrity because these have the potential to abolish a response in one direction (endothelially mediated vasodilatation) to allow an opposite effect (vasoconstriction at the underlying smooth muscle) to take place.

Prejunctional and postjunctional neuromodulatory effects of purines have been described. Prejunctional

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inhibition of noradrenaline release by ATP and adenosine has been demonstrated in many blood vessels.\textsuperscript{16,17} Most often, the prejunctional inhibitory effects of ATP are mediated through the P\textsubscript{2y}-purinoceptor after its ectoenzymatic breakdown to adenosine because the prejunctional inhibition is blocked by methylxanthines but not mimicked by the stable analogue of ATP, \(\alpha,\beta\text{-methylene ATP.}\) In some tissues, including the rat caudal artery and rat vas deferens, a prejunctional P\textsubscript{2} receptor has been proposed that mediates the suppression of noradrenaline release.\textsuperscript{18,19} It has been suggested that this purinoceptor recognizes the structure of both nucleosides and nucleotides because it is antagonized by 8-phenyltheophylline and other P\textsubscript{1} antagonists, yet it is activated by \(\beta,\gamma\text{-methylene ATP.}\) However, because \(\beta,\gamma\text{-methylene ATP is degraded to its adenosine analogue within 1 minute in most tissues, this cannot be regarded as decisive evidence; nevertheless,} \(\alpha,\beta\text{-methylene ATP has been shown to attenuate the release of acetylcholine from guinea pig longitudinal muscle.}\textsuperscript{20}\) It has recently been suggested that ATP may act directly to facilitate noradrenergic transmission in the rabbit ear artery.\textsuperscript{21} This demonstrates the variability of purine-mediated effects at the prejunctional level; it also suggests that a possible further diversity of purinoceptor subtypes may exist. ATP, ADP, AMP, and adenosine are inextricably linked by purine metabolic pathways compounding the complexity of physiological purine mechanisms. This review, however, will concentrate on the involvement of P\textsubscript{2}-purinoceptors (principal effectors are ATP and ADP) in the local regulation of vascular tone.

**Subclassification of P\textsubscript{2}-Purinoceptors**

P\textsubscript{2}-Purinoceptors have been subdivided into two major classes: P\textsubscript{2x} and P\textsubscript{2y}, which elicit contraction and relaxation, respectively, and are characterized primarily by the order of potency of ATP and its analogues\textsuperscript{22} (Table 1). Excitatory effects at the P\textsubscript{2x}-purinoceptor, located on vascular smooth muscle, are mediated by agonists in the following order: \(\alpha,\beta\text{-methylene ATP} \gg \beta,\gamma\text{-methylene ATP} > \text{ATP} = 2\text{-methylthio ATP, whereas at the} \ P_2\gamma\text{-purinoceptor agonist, potency is} \ 2\text{-methylthio ATP} > \text{ATP} > \alpha,\beta\text{-methylene ATP,} \ \beta,\gamma\text{-methylene ATP. In general, the} \ P_2\gamma\text{-purinoceptor is located on endothelial cells, although in some vessels it is present on the smooth muscle.}\textsuperscript{23–25} \ \alpha,\beta\text{-Methylene ATP has found widespread use in characterization of P\textsubscript{2}-purinoceptor subclasses because of its selective desensitization of the} \ P_2\text{x receptor.}\textsuperscript{22,26} A number of structural analogues of ATP have been synthesized with different degrees of selectivity and potency at P\textsubscript{2x} and P\textsubscript{2y} receptors, some of which have the added advantage of being relatively resistant to dephosphorylation (see Reference 27). 1-Adenosine 5’-(\(\beta,\gamma\text{-methylene})\) triphosphate and its analogues are selective agonists of the P\textsubscript{2x}-purinoceptor,\textsuperscript{28} whereas adenosine 5’-(2-fluorodiphosphate) is a specific agonist for the P\textsubscript{2y}-purinoceptor.\textsuperscript{29}

Although potentially more useful in the characterization of P\textsubscript{2}-purinoceptor subtypes, the development of selective antagonists has proved more difficult. Arylazidoaminopropionyl ATP (ANAPP\textsubscript{3})\textsuperscript{30} has been used successfully in the rat aorta\textsuperscript{31} and in some nonvascular tissues to antagonize responses to ATP or its analogues mediated via P\textsubscript{2x}-purinoceptors. The anthraquinone sulfonic acid derivative, Reactive blue 2, competitively inhibits P\textsubscript{2y}-purinoceptor-mediated responses in some preparations, although its use has been limited by a narrow effective concentration range.\textsuperscript{32–34} The trypanocidal drug suramin has been reported to have P\textsubscript{2}-purinoceptor antagonistic activity in the rabbit ear artery\textsuperscript{35} and in some nonvascular preparations, but it does not appear to display selectivity between P\textsubscript{2x}- and P\textsubscript{2y}-purinoceptors.\textsuperscript{36} For a more detailed report on these antagonists, see References 10 and 37.

In the rabbit basilar artery, the virtual lack of a vasoconstrictor response to \(\alpha,\beta\text{-methylene ATP and its inability to cause desensitization of ATP-mediated contractions has led to the proposal that a vasoconstriction-mediating P\textsubscript{2}-purinoceptor, distinct from the P\textsubscript{2x}-purinoceptor, exists in this vessel.}\textsuperscript{38} This receptor has tentatively been called the P\textsubscript{2x}-receptor. Other, nonvascular P\textsubscript{2}-purinoceptor subtypes, P\textsubscript{2T} and P\textsubscript{2Z},\textsuperscript{12} which are indirectly associated with actions on vascular tone by virtue of their effects on platelets and mast cells, respectively (Table 1), and P\textsubscript{2S}\textsuperscript{39} have also been described.

**P\textsubscript{2x}-Purinoceptor–Mediated Vasoconstriction of Vascular Smooth Muscle**

**P\textsubscript{2x}-Purinoceptors and Sympathetic Cotransmission**

The P\textsubscript{2x}-purinoceptor is responsible for the vasoconstrictor responses produced as a consequence of the release of ATP from sympathetic perivascular nerves, which is appropriate considering the close spatial relation between its situation on vascular smooth muscle and the source of its effector.\textsuperscript{40} Relatively little is known about the mechanisms involved in P\textsubscript{2x}-mediated vasoconstriction, although production of phosphatidylinositol and mobilization of intracellular and extracellular Ca\textsuperscript{2+} is likely to be involved.\textsuperscript{41–43}

Cotransmission, the concept that nerves can store and release more than one transmitter, is now widely accepted.\textsuperscript{17,44–46} Several lines of study provide evidence supporting cotransmission of noradrenaline and ATP in sympathetic nerves. Biochemical analyses indicate that noradrenaline and ATP may be co-stored in the same vesicles in sympathetic nerves in various ratios.\textsuperscript{47} Other studies have taken advantage of purine catabolic pathways to prime tissues with tritiated (\(^3\text{H}\))-adenosine and then use tritium efflux as a measure of ATP release, having established that this largely represents \(^3\text{H}\text{-ATP.}\) Su\textsuperscript{48} was the first to use this method to demonstrate corelease of ATP.
and noradrenaline from blood vessels by showing that electrical stimulation of the rabbit aorta and portal vein evoked the release of $^3$H-purine together with noradrenaline and that this release was tetrodotoxin and guanethidine sensitive, indicating an origin from sympathetic nerves. The same technique has been used to show cotransmission of noradrenaline and ATP in the dog basilar artery, rabbit pulmonary artery, and rabbit mesenteric artery. The use of a bioluminescence assay for direct measurement of endogenous ATP has proved invaluable in demonstrating corelease of ATP and noradrenaline from the vas deferens, a tissue with a high density of sympathetic nerves. Its use with vascular preparations, however, has been severely restricted by the fact that release is below the borderline of detection.

The sympathetic nerve-mediated vasoconstrictor response may be pharmacologically dissected into its two distinct components: the adrenergic component, which is sensitive to block by $\alpha$-adrenoceptor antagonists such as prazosin, and the purinergic component, which is susceptible to desensitization by $\alpha,\beta$-methylene ATP. The rabbit saphenous artery provides a classic example of a vessel in which pharmacological manipulations have been used to identify the contribution of ATP to sympathetic transmission. In this vessel, sympathetic nerve stimulation produces a contractile response of which less than 30% is blocked by prazosin, whereas the remainder is abolished by $\alpha,\beta$-methylene ATP. Reserpine treatment, which depletes sympathetic nerves of their catecholamine content, failed to abolish nerve-mediated contractions despite a more than 95% reduction in tissue noradrenaline content, whereas after exposure to 6-hydroxydopamine, which destroys sympathetic nerves, no nerve-mediated responses were observed. A pharmacological approach has also been used to identify ATP as a sympathetic cotransmitter in many other vessels, including dog cerebral and mesenteric arteries and rabbit hepatic artery (Figure 1).

Electrophysiological studies have also been used to examine the components of the sympathetic nerve-mediated response. In a number of vessels, electrical stimulation of perivascular nerves induces excitatory junction potentials (EJPs) in the vascular smooth muscle that are resistant to $\alpha$-adrenoceptor blockade with prazosin or phentolamine but are blocked by $\alpha,\beta$-methylene ATP and abolished by guanethidine or tetrodotoxin. Iontophoretic application of ATP induces a depolarization with a rapid decay, which mimics nerve-induced EJPs, whereas noradrenaline evokes a depolarization that decays with a slow time course. These results are consistent with the concept that ATP acts as a cotransmitter with noradrenaline from perivascular nerves.

There is considerable variation in the proportions of ATP and noradrenaline used as cotransmitters in the many vessels in which a sympathetic purinergic component has been demonstrated. In rabbit small
mesenteric arteries, for example, the purinergic component has been reported to account for 80% of the response to sympathetic nerve stimulation, whereas in the rabbit ear artery, the purinergic component is relatively small. Species differences also occur, for example, in mesenteric arteries of various species; the contribution of the purinergic component to the sympathetic nerve-mediated response ranges from predominant in the rabbit to medium in the dog and small in the rat.

In the rat mesenteric arterial bed preparation, perivascular sympathetic nerve stimulation produces a contractile response that is entirely or almost entirely blocked by prazosin. However, in myograph-mounted small mesenteric arteries (150–250-μm diameter) from the same species, both the EJPs and a significant proportion of the contractile response are blocked by α,β-methylene ATP. This apparent anomaly raises some important points about sympathetic cotransmission. First, this may be a consequence of different proportions of ATP used as a cotransmitter in mesenteric resistance vessels compared with larger mesenteric arteries (which contribute to the vascular resistance of the whole bed preparation). Second, this difference may be related to the different parameters of stimulation that are used to elicit contraction in the two preparations. In the rabbit ear artery, it has been shown that the purinergic component is optimal with short bursts of low frequency stimulation, whereas longer durations of higher frequency favor adrenergic transmission. The parameters of stimulation required to elicit contraction of the mesenteric arterial bed are of relatively high frequency and long duration, whereas the small isolated vessels will respond to stimulation at lower frequencies and of shorter duration. This point is supported by the observation that in small mesenteric arteries of the rat, 80% of the contraction to a single stimulation at high frequency is resistant to α-adrenoceptor blockade by prazosin, whereas with increasing duration of stimulation, noradrenaline becomes the dominant transmitter.

Several possibilities for the functional significance of cotransmission have been discussed by Burnstock and Kennedy. First, different proportions of cotransmitters can be released according to the number, frequency, and pattern of impulses (i.e., the relative rates of release of cotransmitters may depend on the functional activity of the nerve terminal). The results of the experiments with the rabbit ear artery and isolated rat mesenteric vessels discussed above are consistent with this idea. Second, there may be differences in the time course of release and in events occurring after discharge of transmitter from the nerve terminal (i.e., in diffusion, postjunctional action, or inactivation of cotransmitters). The relatively short duration for which transmitters are in transit in the extracellular space makes it difficult to discriminate among these events; however, there are many examples of the delayed action of a peptide acting as a cotransmitter with a classic transmitter. Third, corelease of two transmitters may be linked to a postjunctional synergistic interaction between two substances because of events occurring at or beyond the cell membrane. A synergistic interaction between ATP and noradrenaline has been described in the rat femoral artery, guinea pig and rat portal veins, and rat mesenteric arterial bed.

Adrenergic and purinergic components of sympathetic transmission may be differentially affected by physiological and pathophysiological conditions. It has been suggested that the relative importance of cotransmitters may vary with age. In the rat tail artery, younger rats apparently produce entirely ATP-mediated contractions, whereas mesenteric vessels from older rats do not always produce a purinergic response, which is consistent with the notion of a higher ATP-to-noradrenaline ratio in sympathetic nerves of younger animals. In spontaneously hypertensive rats, it has been suggested that the ATP component in sympathetic nerves of rat tail arteries is more prominent. The radioligand 3H-α,β-methylene ATP, which has been used to preferentially localize P2X-purinoceptors in vascular and nonvascular tissues, may prove valuable in quantifying changes in this receptor population with age and in disease.

P2X-Purinoceptors and Nonsympathetic Transmission

Nonsympathetic purinergic excitatory transmission has recently been described in rat intrapulmonary arteries. This provides a physiological role for the vasoconstrictor P2X-purinoceptors shown to be present in the isolated rat lung preparation.

P2Y-Purinoceptor–Mediated Vasodilatation of Vascular Smooth Muscle

P2Y-Purinoceptors and Sympathetic Cotransmission

P2Y-Purinoceptors have been identified on smooth muscle cells of rabbit coronary arteries, and it has been suggested that these may be implicated in mediating the relaxant response to ATP after its release as a cotransmitter with noradrenaline from sympathetic nerves. The vasodilatation that occurs in rabbit skeletal muscle in response to hypothalamic stimulation has also been suggested to be mediated by ATP released from sympathetic nerves.

P2Y-Purinoceptors and Nonsympathetic Transmission

In the rabbit portal vein, the P2Y-purinoceptor, which is usually located on endothelial cells, is located on the vascular smooth muscle. There is substantial evidence to suggest that this smooth muscle P2Y-purinoceptor is associated with ATP released from a population of nonsympathetic nerves. Stimulation of nonadrenergic, noncholinergic nerves in the rabbit portal vein produces vasodilatation that is mimicked by ATP. The rabbit portal vein was the vessel originally used by to provide evidence for the release of ATP from perivascular nerves; he showed that although this release was abolished by tetrodotoxin, a significant proportion was resistant to block by guanethidine, suggest-
Figure 2. Representative tracings showing responses of raised-tone rat femoral artery and rabbit hepatic artery to ATP in presence and absence of endothelium. Vasodilator responses of rat femoral artery are entirely dependent on P2Y-purinoceptors present on intact endothelium; in its absence, only vasoconstriction is produced. In contrast, ATP elicits vasodilatation of rabbit hepatic artery even after removal of endothelium, via P2Y-purinoceptors located on vascular smooth muscle. NA, noradrenaline. Modified with permission; courtesy of A. Brizzolara.

Vessels that also have their P2Y-purinoceptors on the smooth muscle. Unlike the portal vein, these do not appear to be associated with a discrete population of purinergic nerves. Although these P2Y-purinoceptors may be associated with ATP released as a cotransmitter from parasympathetic nerves, more evidence exists for their physiological function as mediators of the response to ATP coreleased with substance P and/or calcitonin gene–related peptide from sensory nerves during “axon reflex” vasodilatation. ATP released from sensory nerve collaterals in the skin could participate in the mediation of neurogenic vasodilatation and plasma extravasation by its action on vascular P2Y-purinoceptors and by causing the

ing that this component arose from ATP released from a nonsympathetic source. Complementary results were obtained with fluorescence histochemical localization of quinacrine, which binds to high levels of ATP, demonstrating a dense plexus of perivascular ATP-containing nerves in the rabbit portal vein that were resistant to chemical sympathectomy. More recently, it has been shown that the nonadrenergic, noncholinergic (nonsympathetic) vasodilatation of the rabbit portal vein is inhibited by the P2Y-antagonist, Reactive blue 2, at a concentration that selectively antagonizes vasodilator responses to ATP.

The rabbit mesenteric artery, human pulmonary arteries, and rabbit hepatic artery (Figure 2) are
release of histamine from mast cells, presumably via ATP$^+$ acting on P$_{2Y}$-purinoceptors. It is appropriate that the P$_{2Y}$-purinoceptors involved in antidromic vasodilatation should be located on vascular smooth muscle cells in close juxtaposition to sensory nerves.

Evidence for an involvement of ATP as a transmitter in sensory nerves comes from several sources. Holton and Holton originally proposed that ATP released from sensory nerve endings is responsible for the antidromic vasodilatation that occurs as a result of stimulation of the rabbit great auricular nerve. They also pointed out that this made it likely that ATP was involved in central synaptic transmission from sensory afferent neurons. This was later substantiated by the localization of fluoride-resistant acid phosphatase, which acts selectively on $5'$-nucleotidase substrates, in a discrete population of dorsal root ganglion neurons. Furthermore, ATP excites populations of neurons in ganglia and areas of the spinal cord associated with termination of primary afferent fibers and hence with pain mechanisms. ATP has recently been shown to activate cation channels in rat sensory neurons.

**P$_{2Y}$-Purinoceptor Vasodilatation via Endothelium**

In the majority of blood vessels, P$_{2Y}$-purinoceptors are located on endothelial cells where their activation by ATP, ADP, and some structural analogues produces vasodilatation. In some vessels, the P$_{2Y}$-purinoceptor is located on the vascular smooth muscle (see "P$_{2Y}$-Purinoceptor–Mediated Vasodilatation of Vascular Smooth Muscle"). The importance of the endothelium in vascular relaxation was first recognized in 1980 by Furchgott and Zawadzki, who described an endothelial mediator of the relaxant response, EDRF. This has since been identified as nitric oxide synthesized from l-arginine and is produced in response to stimulation of endothelial receptors, including the endothelial P$_{2Y}$-purinoceptor. The functional significance of the location of P$_{2Y}$-purinoceptors on the intimal surface of blood vessels is related to the sources of the effector purines, including platelets, erythrocytes, and endothelial cells, and to the physiological and pathophysiological conditions affecting their release from these sources (see References 11, 12, 98). Under normal conditions, purines released from these sources will produce an endothelium-mediated relaxation via the P$_{2Y}$-purinoceptor. In situations where the intimal smooth muscle is exposed to the blood, as in cases of endothelial cell injury, the purines may act at P$_{2X}$-purinoceptors to cause vasospasm.

The definitive experiments showing an involvement of the endothelium in the relaxant response to ATP involve removal of the endothelium by a variety of mechanical and chemical means. De Mey and Vanhoutte were the first to show that rubbing the canine femoral artery to remove the endothelium abolished ATP-induced relaxation. Similarly, in the rat femoral artery, destruction of the endothelium abolished relaxation to ATP and also unmasked vasoconstrictor responses resulting from a direct action of ATP on the smooth muscle (Figure 2). In the rat mesenteric arterial bed preparation, removal of the endothelium with the detergent sodium deoxycholate abolishes vasodilator responses to ATP and 2-methylthio ATP, confirming an involvement of the P$_{2Y}$-purinoceptor in this response. Inhibitors of the effects of EDRF such as hemoglobin, methylene blue, and hydroquinone attenuate responses to endothelium-dependent relaxing agents, including ATP and its analogues. It has recently been shown that derivatives of l-arginine, such as N-monomethyl l-arginine (LNMMA) and N-nitro l-arginine (L-NOARG), act as potent inhibitors of the formation of nitric oxide from its precursor l-arginine. L-NOARG methyl ester has been shown to block vasodilator responses to ATP but not those to the endothelium-independent vasodilator sodium nitroprusside in the isolated, perfused rabbit liver and rat mesenteric arterial bed. Studies on aortic endothelial cells in culture have shown directly that ATP releases nitric oxide from these cells.

The P$_{2Y}$-purinoceptor is also linked to the production of prostacyclin (PGI$_2$), which may contribute to vasodilatation in some vessels. Endothelial cells are prolific producers of prostaglandins, and both ATP and ADP stimulate the release of prostaglandin from vascular beds and PGI$_2$ from endothelial cells in culture. 2-Methylthio ATP mimics this response, whereas $\alpha,\beta$-methylene ATP is inactive, confirming the involvement of the P$_{2Y}$-purinoceptor. The mobilization of intracellular Ca$^{2+}$ as a consequence of generation of inositol 1,4,5-triphosphate from the hydrolysis of membrane-bound phosphatidylinositol 4,5-biphosphate is important in the mechanism of production of PGI$_2$, whereas extracellular Ca$^{2+}$ appears to be more important in the generation of nitric oxide. Although PGI$_2$ is a potent relaxing agent in some systems, it is more likely to be involved in homeostatic mechanisms, whereas EDRF is relatively more important in causing vascular relaxation.

**Actions of P$_2$ Agonists in Animals and in Humans Isolated Vessels**

The response of a vessel to exogenous ATP and its analogues will be a balance between opposing effects mediated by P$_{2X}$- and P$_{2Y}$-purinoceptors. A contribution of P$_1$-purinoceptors, largely following ectoenzymatic breakdown of ATP to adenosine, also has to be considered. Manipulations of the tone of isolated vessels can be used in pharmacological studies to favor either of these two P$_2$-purinoceptor subtypes (Figure 3). Hence, at low tone, the vascular response to ATP is generally a contraction exerted via P$_{2X}$-purinoceptors on the smooth muscle, whereas when the tone is raised, relaxant effects via P$_{2Y}$-purinoceptors on endothelial cells are favored. This is useful in the identification and characterization of receptor subtypes, although closer approximations of "real"...
effects are obtained from studies using vascular beds and whole animals at physiological tone. Vasoconstrictor and vasodilator effects mediated by P₂-purinoceptors have been described in large arteries, including the pig aorta,¹¹² rat femoral artery,⁷¹ rabbit portal vein,²³ dog coronary artery,¹¹³ and rabbit mesenteric artery.³² Biphasic responses to ATP, where contraction is preceded by a relaxation, have been described in the canine basilar artery¹⁴ and to adventitial application of ATP in the rabbit mesenteric artery.¹¹⁴ In the isolated human umbilical artery, ATP causes a contraction that is depressed by indomethacin, suggesting that prostaglandins may be involved.¹¹⁵ Small arteries, including peripheral branches of the rabbit mesenteric artery⁶² and human pulmonary arteries,²⁵ have also been shown to be constricted by ATP. Other examples of the effects of P₂-purinoceptor activation on isolated vessels have been mentioned in this review (see Reference 9 for additional information).

The structural and functional integrity of the endothelium is of crucial importance to vascular responsiveness; anything affecting it is likely to reduce the contribution of endothelial P₂Y-purinoceptors to regulation of vascular tone and may even expose vasoconstrictor P₂X-purinoceptors on the underlying smooth muscle. Pathological models for atherosclerosis have been used to investigate the mechanisms of altered vascular responsiveness associated with this disease. In rabbits made atherosclerotic by severe hypercholesterolemia, an impairment of endothelial-dependent relaxations of the aorta to acetylcholine and ATP has been reported.¹¹⁶ Similarly, endothelium-dependent relaxations to ADP and aggregating platelets are impaired in hypercholesterolemic pig basilar arteries¹¹⁷ and in coronary resistance arteries of cholesterol-fed rabbits.¹¹⁸ There is also evidence that old age can affect endothelial-mediated and, hence, P₂Y-mediated vasodilatation. Relaxation of the rabbit aorta to ATP is impaired with age,¹¹⁹ and in pial arterioles of old rats, the endothelium-dependent relaxation to ADP was reduced by more than 50%.¹²⁰

**Vascular Beds**

Vascular beds comprise small resistance arteries and arterioles, which are physiologically more relevant to the control of peripheral vascular resistance. ATP and ADP are potent vasodilators and, in some

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**Figure 3.** Isolated rat mesenteric arterial bed preparation. Panels a and b: Representative tracings showing responses of a single preparation to doses of ATP (expressed as -log moles): (a) vasoconstrictor responses elicited by ATP in preparation at low tone—each dose is labeled; and (b) vasodilator responses elicited by ATP when tone of preparation is raised (by addition of 30 μM noradrenaline) — alternate doses only are labeled. Doses not labeled are, from the left, 9.3, 8.3, and 7.3. Panels c and d: Dose/response curves showing (c) vasoconstriction to ATP (n=20) and (d) vasodilatation to ATP (n=11).
cases, constrictors of a large number of vascular beds, including the cerebral, coronary, renal, hindlimb, and forelimb. The mechanisms underlying the vascular dilatation that occurs in response to increased metabolic demands of the tissue have been the subject of much investigation. The vasodilator properties of adenosine have been known for many years, and the adenosine hypothesis proposing adenosine as a local regulator of vascular tone has received much support. The potent vasodilator actions of ATP and ADP in most vascular beds have also implicated these nucleotides as local regulators of blood flow.

ATP affects both heart function and tone. In general, the effects of ATP on cardiac function are depressant (i.e., decreased force of heart rate and contraction), although these are largely mediated at P1-purinoceptors after breakdown to adenosine. The negative inotropic effects of adenine compounds are particularly marked in the atrium of mammals. Positive inotropic effects of ATP have also been shown in some mammalian ventricular preparations and in the frog heart. This effect of ATP appears to be a result of a direct action at specific P2-purinoceptors because the effects are mimicked by α,β-methylene ATP but not by adenosine and can be antagonized by α,β-methylene ATP. In rat ventricular preparations, P2-purinoceptor activation was shown to produce both a positive inotropy and an increase in inositol phosphate formation, whereas in single frog ventricular cells, P2-purinoceptor stimulation was shown to increase the Ca2+ current by a pathway that might also involve phosphoinositide turnover. An ATP receptor–induced influx of Ca2+ has been implicated in the enhancement of cytosolic calcium in isolated rat ventricular myocytes; furthermore, the rank order of potency of ATP and its analogues in increasing intracellular calcium was consistent with the activation of purinoceptors of the P2Y-subtype.

The potent cardiodyetic effects of ATP have also been long known. In 1934, Gillespie showed that injection of ATP into the isolated rabbit heart caused an increase in coronary blood flow. The rat coronary vascular bed responds to ATP by producing both vasoconstriction and vasodilatation, manifest as a biphasic response, which is mediated through activation of P2X- and P2Y-purinoceptors, respectively. A study by Paddle and Burnstock showed that ATP is released during hypoxic stimulation of the heart and provided early, although indirect, evidence for a role for ATP as a physiological modulator (along with adenosine) of coronary flow. In a more recent study,
Hopwood et al.\textsuperscript{102} showed that hydroquinone, an antagonist of EDRF-mediated effects, blocked both the dilatation to 2-methylthio ATP at the \(P_{2\gamma}-\text{purinoceptor}\) and the hypoxic relaxation, implicating both ATP and EDRF in the cardiac dilatation to hypoxia. The source of this ATP is not entirely clear. ATP has been shown to be released into the perfusate of the working rat heart in response to hypoxia\textsuperscript{134} and from hypoxic cardiomyocytes\textsuperscript{135} in concentrations high enough to account for the dilator response. However, purinergic nerves\textsuperscript{136} and endothelial cells\textsuperscript{98,137} represent alternative sources of releasable ATP. Isolated cardiac endothelial cells in culture have recently been shown to contain and release large amounts of adenine nucleotides,\textsuperscript{138} which is consistent with the hypothesis that endothelial cells represent a physiological source of the ATP that is released during hypoxia.\textsuperscript{139}

ATP and ADP cause vasodilatation of the hindlimb of the cat.\textsuperscript{140} ATP and ADP were shown to be more potent than adenosine in this preparation, and the order of vasodilator potency of 2-methylthio ATP > ADP > ATP > \(\beta3\gamma\)-methylene ATP, \(\alpha\beta\)-methylene ATP is consistent with an action at \(P_{2\gamma}\)-purinoceptors.\textsuperscript{34} In the rat hindlimb, a biphasic response to ATP is produced, with vasoconstriction being preceded by transient vasodilatation, although 5-hydroxytryptamine is likely to be the mediator of the pressor response.\textsuperscript{141}

In the perfused rabbit hindlimb in vivo, the vasodilatation resulting from ATP was severely reduced (by 88%) by gossypol, a potent and irreversible inhibitor of EDRF; in some cases, vasoconstrictor responses were then observed.\textsuperscript{142,143} It is possible that ATP contributes to the local regulation of blood flow in skeletal muscle during exercise because it has been shown to be released from active frog skeletal muscle in vitro\textsuperscript{122} and is present in the venous effluent of contracting human forearm muscles.\textsuperscript{144}

The potenti effects of purine nucleotides on cerebral blood flow (they can cause either vasodilatation or vasoconstriction) has led to the hypothesis that they are involved in cerebral vasospasm and migraine.\textsuperscript{145–147} It has also been suggested that they may be released from the brain, possibly from endothelial cells, as part of a protective mechanism of vasodilatation in response to hypoxia.\textsuperscript{147}

In the isolated, perfused rabbit liver at low tone, ATP, 2-methylthio ATP, and \(\alpha\beta\)-methylene ATP produce vasoconstriction, with agonist potency order indicating an effect at \(P_{2\gamma}\)-purinoceptors. In the raised-tone preparation, ATP and 2-methylthio ATP but not \(\alpha\beta\)-methylene ATP elicit vasodilatation consistent with an action at \(P_{2\gamma}\)-purinoceptors; furthermore, vasodilator responses were not affected by the \(P_{1}\)-antagonist 8-phenyltheophylline, confirming that relaxation to ATP did not occur at \(P_{1}\)-purinoceptors after breakdown to adenosine.\textsuperscript{148} The attenuation of relaxations to ATP but not those to adenosine by the nitric oxide to L-arginine antagonist LNOARG methyl ester implicates nitric oxide produced by endothelial cells as a mediator of the vasodilator response to ATP.\textsuperscript{105} Vasoconstrictor responses to ATP and ADP have also been described in the isolated, perfused rat liver preparation.\textsuperscript{149} The functional significance of these receptors in the hepatic vasculature may be related to the participation of \(P_{2}\)-purinoceptor mechanisms in the compensatory dilatation of the hepatic arterial vasculature in response to reductions in portal blood flow, the "buffer response,"\textsuperscript{150} which has until recently\textsuperscript{151} been largely attributed to \(P_{1}\)-purinoceptor mechanisms.

\(P_{2}\)-Purinoceptor subtypes have been characterized in the isolated rat mesenteric arterial bed\textsuperscript{100} (Figure 3). ATP and its analogues produced vasoconstriction of the preparation at low tone with a potency order of \(\alpha\beta\)-methylene ATP > 2-methylthio ATP > ATP, which is characteristic of an action at \(P_{2\gamma}\)-purinoceptors. Vasodilatation of the raised-tone preparation was elicited by 2-methylthio ATP > ATP, whereas \(\alpha\beta\)-methylene ATP was without effect, which is characteristic of effects mediated at \(P_{2\gamma}\)-purinoceptors. Removal of the endothelium did not reduce \(P_{2\gamma}\)-mediated contraction but abolished \(P_{2\gamma}\)-mediated relaxation, indicating that these receptors are present on the smooth muscle and on endothelial cells, respectively, where they are likely to contribute to local regulation of mesenteric blood flow.

\textbf{Whole Animals}

When ATP is infused or injected into animals and humans, it produces a profound decrease in blood pressure, which is often associated with bradycardia. Early studies include those of Gillespie,\textsuperscript{2} who showed that ATP injected into the jugular vein of the cat caused a decrease in systemic blood pressure. Wayne et al.\textsuperscript{152} showed that ATP injected into the jugular vein of the guinea pig had marked effects on the heart, causing a depression of normal function with sinus slowing and the appearance of heart block as well as a decrease in blood pressure. Similarly, Emmelin and Feldberg\textsuperscript{153} found that bradycardia and a decrease in systemic blood pressure accompanied intravenous or intra-arterial injection of ATP in cats, rabbits, and dogs.\textsuperscript{153} The suggestion of an involvement of the vagal reflex in the electrophysiological actions of ATP but not of adenosine was later confirmed in the canine heart where it was shown that the negative chronotropic and dromotropic actions on the sinoatrial and atrioventricular nodes, respectively, were attenuated by either atropine or vagotomy plus autonomic blockade with propranolol.\textsuperscript{154,155} Forrester et al.\textsuperscript{145} reported that ATP injected into the carotid artery of cats and baboons caused a "profound increase in cerebral blood flow"; ATP had a greater effect than adenosine. However, Newberg et al.\textsuperscript{156} who looked at the cerebral and systemic effects of adenosine and ATP in dogs, found that although the systemic vascular resistance decreased significantly by 40–62%, there was a large decrease in cerebral perfusion pressure and flow and
no difference between the effectiveness of ATP and that of adenosine. These observed differences may be the result of different routes of administration (carotid artery versus femoral vein) and modes of administration (dose versus continuous infusion) used by the different investigators.

In these studies, it appears that a large part of the effects of ATP are mediated after its breakdown to adenosine. Sollevi et al.\(^{157}\) showed that inferior vena cava infusion of adenosine and ATP in dogs produced equal decreases in systemic vascular resistance and demonstrated that ATP was largely degraded in the lung before it reached the vascular arterial bed. More recently, it has been suggested that the depressor effects on systemic arterial pressure of ATP injected intra-arterially in the anesthetized rat are effected at P\(_1\)-purinoceptors after breakdown to adenosine, although the potent vasoconstrictor actions of \(\alpha,\beta\)-methylene ATP imply a direct action at P\(_2\)-purinoceptors.\(^{158}\) It is not yet clear what the implications are from the recent discovery that in vivo administration of AMP, ADP, or ATP, but not adenosine, results in an expansion of red blood cell ATP pools and blood plasma ATP levels,\(^{159}\) although it has been suggested to explain the differences in cardiovascular effects of ATP administration compared with adenosine administration in humans.

Because of its potent vasodilator properties in most vascular beds, ATP has been investigated with respect to pharmacologically induced hypotension, which is frequently used during surgery to optimize operating conditions and decrease chances of hemorrhage. For example, ATP infusion has recently been used by Aso et al.\(^{160}\) to control blood pressure in the treatment of pheochromocytoma cases.\(^{160}\) The advantages of ATP-induced hypotension are the absence of tachyphylaxis, the stability of blood pressure with well-preserved hemodynamics, fast onset, and rapid reversibility with no rebound hypotension; however, many authors agree that metabolic acidosis and cardiac arrhythmias impose constraints on its clinical use.\(^ {161,162}\) Furthermore, the negative inotropic and chronotropic effects that are responsible for the lack of a tachycardiac response during hypotension can, with excessively large doses of adenyl compounds, be responsible for complete atrioventricular block and asystole.\(^ {152,163}\)

ATP has been used successfully, given intravenously or intra-arterially, to treat various pathologic conditions of the coronary and peripheral circulations. There are several examples in which ATP has been used in the treatment of cardiovascular disease, including arthritis (see Reference 164). Recently, intravenous infusion of ATP in humans was investigated with regard to its therapeutic use in patients with chronic obstructive pulmonary disease\(^ {165}\); ATP produced a significant pulmonary vasodilatation and at the low infusion rates used was well tolerated, short acting, and selective, although there was an undesirable rebound vasoconstriction on cessation of infusion and a worsened hypoxemia. The potent depressant effects of ATP on the sinoatrial and ventricular nodes of the human heart have resulted in the recommendation of ATP for the treatment of various types of tachycardia.\(^ {163,166,167}\)

**Summary**

Characterization of P\(_2\)-purinoceptor subtypes has facilitated understanding of the many diverse effects produced by purine nucleotides (see Figure 4). P\(_{2X}\)-Purinoceptors are located on vascular smooth muscle where they mediate vasoconstriction resulting from ATP released as a cotransmitter with noradrenaline from sympathetic nerves. P\(_{2Y}\)-Purinoceptors are usually located on the vascular endothelium where they have a role as mediators of vascular relaxation by locally produced ATP. In some vessels, P\(_{2Y}\)-purinoceptors are also located on the smooth muscle, perhaps in association with purinergic or sensory nerves, where they can elicit direct relaxation to neurally released ATP. The net effect of ATP and its analogues on isolated vessels or on vascular beds will be the result of actions mediated by P\(_{2X}\)- and P\(_{2Y}\)-purinoceptor subtypes, although changes in vascular tone and in integrity of nerves and endothelial cells may alter the balance of the response. Such changes have been observed in diseased states (e.g., atherosclerosis) and may have important implications for the involvement of P\(_2\)-purinoceptors in, for example, vasospasm. The development of selective and potent antagonists to P\(_{2X}\) and P\(_{2Y}\)-purinoceptors has so far remained elusive, and their therapeutic potential can only be guessed.

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