Purinergic Signaling in the Cardiovascular System

Geoffrey Burnstock

Abstract: **There is nervous control of the heart by ATP as a cotransmitter in sympathetic, parasympathetic, and sensory-motor nerves, as well as in intracardiac neurons. Centers in the brain control heart activities and vagal cardiovascular reflexes involve purines. Adenine nucleotides and nucleosides act on purinoceptors on cardiomyocytes, AV and SA nodes, cardiac fibroblasts, and coronary blood vessels. Vascular tone is controlled by a dual mechanism. ATP, released from perivascular sympathetic nerves, causes vasoconstriction largely via P2X1 receptors. Endothelial cells release ATP in response to changes in blood flow (via shear stress) or hypoxia, to act on P2 receptors on endothelial cells to produce nitric oxide, endothelium-derived hyperpolarizing factor, or prostaglandins to cause vasodilation. ATP is also released from sensory-motor nerves during antidromic reflex activity, to produce relaxation of some blood vessels. Purinergic signaling is involved in the physiology of erythrocytes, platelets, and leukocytes. ATP is released from erythrocytes and platelets, and purinoceptors and ectonucleotidases are expressed by these cells. P1, $P2Y_1$, $P2Y_{12}$, and P2X1 receptors are expressed on platelets, which mediate platelet aggregation and shape change. Long-term (trophic) actions of purine and pyrimidine nucleosides and nucleotides promote migration and proliferation of vascular smooth muscle and endothelial cells via P1 and P2Y receptors during angiogenesis, vessel remodeling during restenosis after angioplasty and atherosclerosis. The involvement of purinergic signaling in cardiovascular pathophysiology and its therapeutic potential are discussed, including heart failure, infarction, arrhythmias, syncope, cardiomyopathy, angina, heart transplantation and coronary bypass grafts, coronary artery disease, diabetic cardiomyopathy, hypertension, ischemia, thrombosis, diabetes mellitus, and migraine. (*Circ Res*. 2017;120:207-228. DOI: 10.1161/CIRCRESAHA.116.309726.)**

Key Words: blood cells ■ blood vessels ■ heart failure ■ hypertension ■ thrombosis

Purinergic signaling, that is ATP acting as an extracellular signaling molecule, was proposed in 1972[1] and as a cotransmitter in sympathetic nerves.[2] In 1978, separate families of receptors for adenosine (P1) and ATP and ADP (P2) were recognized,[3] and purine and pyrimidine receptors were cloned and characterized in the early 1990s.[4] Four P1 G-protein–coupled receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3), 7 P2X ion channel receptor subtypes (P2X1-7), and 8 P2Y G-protein–coupled receptor subtypes ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$) are recognized.[5] ATP is released by gentle mechanical stimulation from most, if not all, cell types, as well as from dead or dying cells.[6] Much is also known about the ectonucleotidases that break down the released ATP to ADP, AMP, and adenosine.[7] Reviews are available about the physiology and pathophysiology and the therapeutic potential of purinergic signaling.[8,9] There are both short-term purinergic signaling in neurotransmission, neuromodulation, and secretion and long-term (trophic) purinergic signaling in cell proliferation, differentiation, and death in development and regeneration.[10]

Reviews on various aspects of purinergic signaling in cardiac physiology and pathophysiology have been published, including: physiological roles of cardiac P2X and P2Y purinoceptors,[11] roles of adenosine in health and disease,[12] effects of ATP and adenosine on coronary myocytes,[13] purine degradation pathways in the myocardium,[14] myocardial nucleotide transport,[15] nonadrenergic, noncholinergic neural control of the atrial myocardium,[16] vagal cardiovascular reflexes,[17] and genetic modulation of adenosine receptor function.

In the seminal paper by Drury and Szent-Györgyi,[18] it was reported that extracellular purine compounds, in particular AMP, act on the coronary arteries of the guinea pig, cat, rabbit, and dog. It was shown later that adenosine had actions on the human heart and was a powerful dilator of the coronary vessels in the perfused rabbit heart. ATP produces heart block in the guinea pig and is more potent than adenosine.

An influential hypothesis was put forward when Berne,[19] and independently Gerlach et al,[20] suggested that adenosine is the physiological regulator of reactive hyperemia in the heart. However, data obtained in subsequent studies challenged this hypothesis.[21] P1 receptor antagonists block coronary vasodilation by perfused adenosine, but only blocks the later phase of reactive hyperemia. Although reactive hyperemia occurs ≈10 seconds after resumption of blood flow, adenosine does not appear in the perfusate until ≈90 seconds later because ADP, the first breakdown product, inhibits ecto-5′nucleotidase (CD73). Burnstock[22] claimed that ATP, released during hypoxia from endothelial cells promoting the production of nitric oxide

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Nonstandard Abbreviations and Acronyms	
α,β-meATP	α,β-methylene ATP
ADA	adenosine deaminase
AF	atrial fibrillation
K_{ATP}	ATP-sensitive potassium channels
NO	nitric oxide
NTS	nucleus tractus solitarii
PK	protein kinase
SHR	spontaneously hypertensive rats
Up_4A	uridine adenosine tetraphosphate
VEGF	vascular endothelial growth factor

(NO), is the compound initially responsible for reactive hyperemia and that adenosine, after breakdown of ATP, acts later as a dilator via A_1 receptors on smooth muscle.

An important action of adenosine is the inhibition of the myocardial effect of catecholamines, which became known as the indirect anti–β-adrenergic action of adenosine.[23] Purinergic signaling has a pivotal role in the control of vascular tone and remodeling.[24] However, there are differences in the purinergic regulatory mechanisms involving different purinoceptor subtypes in different blood vessels and in different species, related to the specific physiological roles of the particular vessel.

Control of vascular tone was considered for many years to be by antagonistic sympathetic noradrenergic constrictor nerves and parasympathetic cholinergic dilator nerves. However, after the conceptual advances about cotransmission[2] and endothelium-derived relaxing factor(s),[25] it is now recognized that vascular tone is under the dual control of cotransmitters released from perivascular nerves and various substances released from endothelial cells.[26,27] ATP is released together with noradrenaline from sympathetic nerves supplying blood vessels.[28] ATP is also released from endothelial cells in response to changes in blood flow (because of shear stress) and hypoxia[29] (Figure 1). It seems likely that the ATP released from sympathetic nerve varicosities, and endothelial cells in the microvasculature, causes activation of sensory afferent fibers that reflexly increase sympathetic activity cross talk. Another common feature is the long-term (trophic) actions of purines in promoting migration of endothelial cells and proliferation of both smooth muscle and endothelial cells during angiogenesis, restenosis, and atherosclerosis.[30]

The involvement of purinergic signaling in the biology of erythrocytes, platelets, and leukocytes was recognized early and an account is included of later studies leading to our current understanding of the various roles played by purine nucleotides and nucleosides in health and disease.[31] There is also a valuable earlier review about the roles of nucleotide receptors in blood cells.[32] Information is reviewed about purinergic peripheral and central nervous control of the vascular system.[33]

In this review, because of space restrictions, it has been decided to focus on the most recent findings about purinergic signaling in the heart, blood vessels, and blood cells. The reader is recommended to refer to 3 comprehensive recent reviews about these issues for early references to the experimental papers reporting all the findings.[6,24,31]

Heart

Innervation of the Heart

Intrinsic cardiac neurons and sympathetic, parasympathetic, and sensory nerves influence the activities of the heart.

Sympathetic nerves release ATP and noradrenaline as cotransmitters.[34] Stimulation of sympathetic nerves elicits release of ATP and adenosine from the perfused rabbit heart.[35] Prejunctional modulation of noradrenaline release from sympathetic nerves involves P2 receptors and P1 adenosine receptors. Ectonucleoside triphosphate diphosphohydrolase (CD39 or apyrase) and CD73 are present on sympathetic nerve fibers in the heart. Sympathetic nerves regulate heart size and timing of cardiomyocyte cell cycle withdrawal.[36]

Nonadrenergic, noncholinergic parasympathetic neurotransmission was identified in the mammalian heart.[16] Inhibitory adenosine A_1 receptors on cardiac motor nerves are present that have a modulatory action on cardiac nonadrenergic, noncholinergic neurotransmission.

Both A_1 and A_2 receptors are present on ventricular epicardial sensory nerve endings of dorsal root ganglion neurons. It was shown with in situ hybridization that P2X3 receptors are localized on sensory nerves in the heart, whereas in a canine heart model in vivo, P2X2/3 receptors have been identified.[37]

Cardiac intramural nerve cell bodies and nerve fibers in guinea pig atria, many of which, but not all, are parasympathetic, stain positively for quinacrine, which is a marker for high levels of ATP in vesicles containing neuropeptides, suggesting that a subpopulation of intracardiac nerves could release ATP.[38] In ganglia in the atria and in interatrial septum of new born guinea pig heart, 2 types of intracardiac neurons were identified, AH (highly refractory with pronounced after-hyperpolarizations) and M (nonaccommodating tonic firing characteristics) cells.[39] Responses (depolarization that was sometimes followed by hyperpolarization and a slow prolonged depolarization) to ATP are greater than ADP, whereas AMP and adenosine are ineffective. Cardiac neurons activated by ATP greatly enhance cardiac myocyte spontaneous beating frequency. Consequently, it was proposed that intracardiac neurons are not all parasympathetic neurons controlled by nicotinic neurotransmission, but they may also contain intrinsic sensory neurons playing a role in local reflex pathways in the heart.[40] ATP activates $P2Y_2$ receptors on rat intracardiac neurons to transiently raise $[Ca^{2+}]_i$ and activate an outward current. The increase in cytoplasmic Ca^{2+} from a ryanodine-insensitive Ca^{2+} store was suggested to modulate neuronal excitability via the activation of Ca^{2+}-dependent K^+ channels and membrane hyperpolarization.[41] CD39 is localized on intrinsic neurons of human and porcine hearts.[42] Decrease in heart rate and blood pressure by activation of A_1 receptors seems to be mediated in the periphery. The increase in heart rate by activation of A_{2A} receptors seems to be mediated in the central nervous system, whereas the decrease in blood pressure by activation of A_{2A} receptors is most probably mediated in the periphery.[43]

Reflex Control of the Cardiovascular System

There was an early hint that effects of purines in the heart are mediated by a central vagal reflex.[18] The right vagus plays a dominant role in carrying cardiopulmonary vagal afferent traffic, and

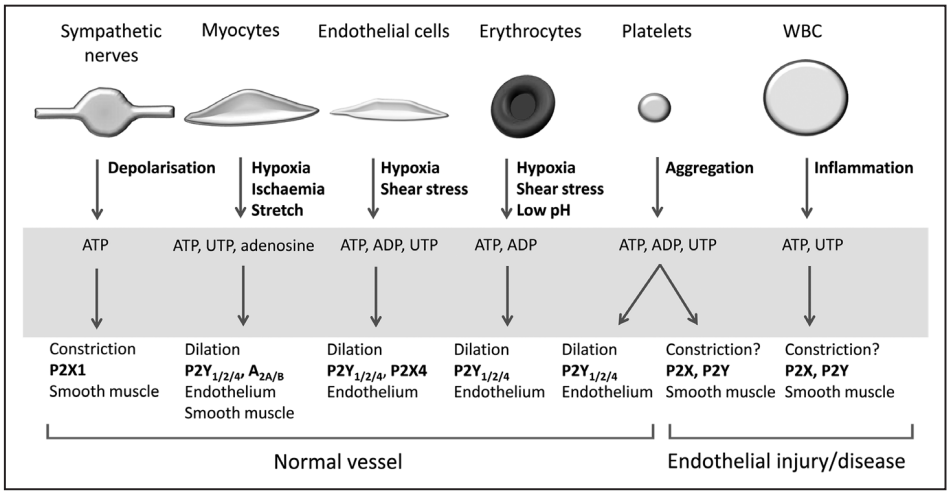

Figure 1. Cellular sources of nucleotides and nucleosides relevant to the control of blood vessel contractility. In healthy blood vessels, vasoconstriction by ATP released from sympathetic nerves and vasodilatation produced by purines and pyrimidines released from endothelial cells, myocytes, and erythrocytes 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). Reprinted from Burnstock and Ralevic[24] with permission of the publisher. Copyright ©2014, The American Society for Pharmacology and Experimental Therapeutics.

there is a dominant role of the right vagus in the ATP-triggered vagal reflex in dogs. This reflex is because of the activation of P2X2/3 receptors localized on vagal sensory nerve terminals in the inferoposterior wall of the left ventricle (Figure 2). ATP attenuates reflex increases in renal sympathetic nerve activity by stimulating left ventricular chemoreceptors with cardiac vagal afferents. A_{2A} receptors in the nucleus tractus solitarii (NTS) mediate the inhibition of the cardiopulmonary chemoreflex control of sympathetic outputs via a GABAergic mechanism.[44]

Reflex regulation of the cardiovascular system seems, in part, dependent on ATP release and activation of P2X receptors; these mechanisms exist both in the periphery and in the NTS. Within the carotid body, the transduction of hypoxia to afferent discharge depends, in part, on ATP release and activation of P2 receptors.[45,46] Purinergic receptor plasticity was revealed in chemoreceptive petrosal afferent neurons in hypertension. Selective blockade of these receptors reduced arterial pressure and may be a novel therapeutic target for hypertension. The NTS is a major integrating station for visceral afferent reflexes and P2X2 and P2X3 receptors in particular have been found in the NTS of rats.[47,48] P2X7 receptors have also been located presynaptically on vagal afferents in the NTS. Microinjection of α,β-methylene ATP (α,β-meATP) into the NTS produced hypotension and bradycardia, which was antagonized by suramin.[49] Blockade of P2 receptors in the NTS abolished both the bradycardia and the pressor/sympathoexcitatory response evoked by carotid body stimulation.[50] During myocardial ischemia, endogenously released ATP activates cardiac spinal afferents mediated by P2X.[51]

Cardiac Actions of Adenine Nucleosides and Nucleotides

References to the experimental papers described in the following sections are available in the review by Burnstock and Pelleg[11]. All 4 subtypes of P1 (adenosine) receptors are expressed on cardiomyocytes and mediate cardioprotection. A_1 receptors mediate the negative chronotropic and dromotropic actions of adenosine and anti–β-adrenergic actions. A_1 receptor transgenic overexpression reverses the inotropic, but not the chronotropic, effects of adenosine in mouse heart. Activation of A_{2A} receptors results in contraction of cardiomyocytes. No evidence has been reported for the presence of A_3 receptors in the atrium, but stimulation of atrial natriuretic peptide secretion

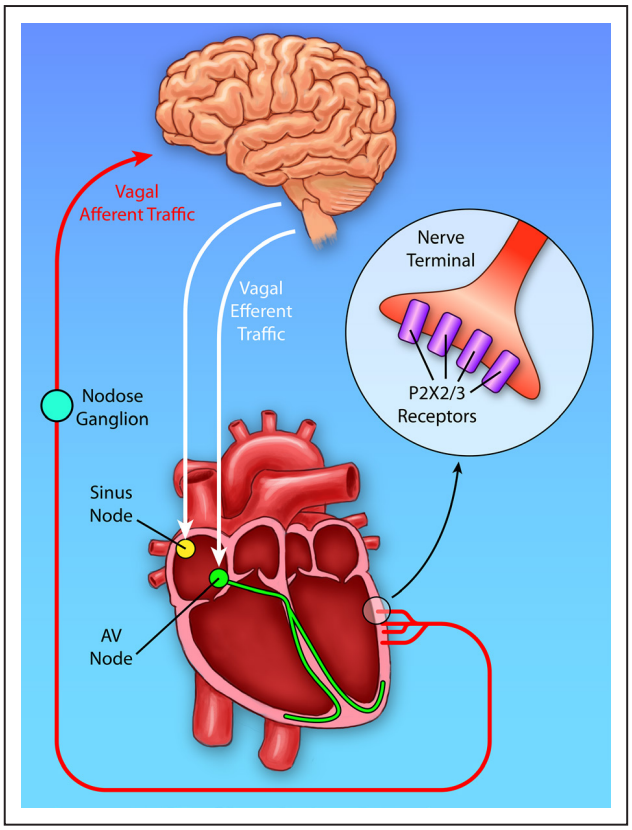

Figure 2. Central vagal cardiocardiac reflex triggered by ATP.[230] Illustration Credit: Ben Smith.

was shown to be mediated by A_3 receptors. Adenosine attenuates cardiomyocyte hypertrophy, with adenosine kinase being an important mediator of this effect. Caffeine disrupts embryonic cardiac function, and its response to hypoxia raised concern about caffeine exposure during embryogenesis in pregnancies with increased risk of embryonic hypoxia. Adenosine deaminase (ADA) is present on midmyocardium of all chambers of the rabbit heart. Heavy exercise training increases the activity of CD73 and ADA in the left ventricle of the rat heart. CD39, ecto-nucleotidase pyrophosphatase, CD73, and alkaline phosphatases are involved in the degradation of ATP to adenosine.

mRNA and protein for all P2X receptor subtypes have been described on cardiac myocytes. mRNA for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors are also expressed.[52] ATP, ADP, and β,γ-methylene ATP have negative chronotropic and inotropic effects on guinea pig atrium, whereas α,β-meATP, acting selectively on P2X1 and P2X3 receptors, produces stimulatory responses. Rat ventricular myocytes release ATP in response to hypoxia in the perfused heart. P2X1 receptors are found in low density on myocytes of the rat heart, but with occasional high-density patches near nerve varicosities. P2X3 and P2X4 receptor mRNA are present in the human heart. P2X1, P2X3, and P2X4 and $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors were cloned and characterized in the human fetal heart.[53] P2X2 and P2X3 receptors are present on afferent nerve fibers. ATP elicits oscillatory contractions and potentiates the amplitude of contractions in rat ventricular myocytes. The diadenosine polyphosphates, adenosine 5′-tetraphosphate and adenosine 5′-pentaphosphate, inhibit contractile and electric activity in the rat heart, mediated by P2 receptors.[54] Stretch of rat atrial myocytes induces release of ATP to act on P2X receptors, including P2X7 receptors mediating cardiomyocyte apoptosis. P2X1 receptors in human myocardium are densely localized in gap junctions at intercalated discs between myocytes. P2X4 receptors mediate increases in myocyte contractility.

ATP increases mechanical activity and inositol trisphosphate production in rat heart, indicating mediation by P2Y receptors. The positive inotropic effects of ATP in mouse cardiomyocytes are mediated by $P2Y_{11}$ receptors. There are interactions between purinergic and adrenergic receptors in the regulation of rat myocardial contractility in postnatal development. ATP via adenosine and P1 receptor activation increases atrial natriuretic peptide secretion, whereas UTP via P2Y receptors decreases atrial natriuretic peptide secretion. P2Y receptor–mediated signaling is involved in the intercellular synchronization of intracellular Ca^{2+} oscillations in cultured cardiac myocytes. Shear stress induces a Ca^{2+} wave via autocrine release of ATP acting on $P2Y_1$ receptors in rat atrial myocytes.[55] The effects of nicotinamide adenine dinucleotide on the rat heart are mediated by P2 receptors.[56] Purinergic signaling seems to be involved in embryonic development of the heart. Activation by ATP and ADP promotes cardiomyogenesis of embryonic stem cells.[57]

AV and SA Nodes

Both adenosine, via A_1 receptors, and ATP inhibit AV nodal conduction. In anesthetized dogs, ATP, but not adenosine, triggers a vagal reflex, which mediates, in part, the transient negative chronotropic and dromotropic effects on the SA and AV nodes, respectively. In human patients intravenously administered ATP produces AV block via P1 receptor mediation. In freely moving mice with overexpression of A_1 receptors, there is AV (and SA) nodal dysfunction and supraventricular arrhythmias.

Adenosine and ATP via P1 receptors suppress pacemaker activity of the SA node. ATP increases SA conduction time in isolated blood-perfused dog atrium and increases sinus cycle length in the canine heart in vivo. mRNAs for P2X1, P2X4, and $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, and $P2Y_{14}$ receptors are expressed in human SA node, with $P2Y_{14}$ receptors showing the highest level.

Papillary Muscle and Endocardium

The mechanism underlying positive inotropy induced by ATP in vitro in rat papillary muscle is mediated, in part, by increased Ca^{2+} inward current. The action potential duration in guinea pig papillary muscle is prolonged by UTP via $P2Y_2$ receptors. Adenosine antagonizes the positive inotropic action mediated by β-, but not by α-adrenoceptors, in rabbit papillary muscle. Stimulation of A_3 receptors reverses myocardial stunning of isolated papillary muscles. ATP, ADP, AMP, and adenosine via P2 and P1 receptors hyperpolarizes guinea pig endocardial endothelium-like cells either in small tissue preparations or in freshly isolated cells.

Cardiac Fibroblasts

Reverse transcription-polymerase chain reaction showed that mRNA for all 4 P1 receptor subtypes, A_1, A_{2A}, A_{2B}, and A_3, are expressed in rat cardiac fibroblasts, with A_2 receptors dominant. Adenosine, acting via A_{2B} receptors, inhibits collagen and protein synthesis in cardiac fibroblasts. Transgenic overexpression of A_{2B} receptors results in a decrease in basal levels of collagen and protein synthesis, whereas underexpression of A_{2B} receptors results in an increase in protein and collagen synthesis. In cultured rat cardiac fibroblasts, P2Y receptors mediate activation of c-fos gene expression and inhibition of DNA synthesis. $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors and $P2Y_{11}$-like receptors are functionally coexpressed through $G_{q/11}$ protein coupling in neonatal rat cardiac myofibroblasts. ATP upregulates proliferation and migration of human cardiac fibroblasts, probably largely via $P2Y_2$ receptors, but P2X4 and P2X7 receptors are also involved. ATP is released from rat and mouse cardiac fibroblasts by hypotonic (mechanical) stimulation via connexin hemichannels. ADP via $P2Y_4$ receptors increases $[Ca^{2+}]_i$ in rat ventricular myofibroblasts and via $P2Y_1$ receptors promotes cell growth and proliferation.[58]

Coronary Circulation

It seems to be appropriate to include coronary blood vessels in this section, rather than in the section on the Vascular System. AMP was identified as a potent dilator of coronary vessels in 1929.[18] Later, adenosine, ATP, and ADP were also reported to dilate coronary vessels.[59] In the rabbit coronary artery, the predominant effect of noradrenaline is vasodilation via β_2-adrenoceptors, whereas the sympathetic cotransmitter ATP causes vasodilation via muscle P2Y purinoceptors. ATP is released from both sympathetic nerves and endothelial cells to control coronary vessel tone. ATP evokes endothelium-dependent vasodilation in isolated human coronary arteries, and there is also smooth muscle relaxation by ATP and UTP of human epicardial coronary arteries.[60] UTP-sensitive P2U receptors ($P2Y_2$ and $P2Y_4$) and $P2Y_1$ receptors mediating vasodilation are present on human cardiac endothelial cells. $P2Y_2$ receptor activation by ATP or UTP induces dramatic upregulation of tissue factor,

an initiator of the coagulation cascade, in human coronary artery endothelial cells.[61] ATP stabilizes, whereas adenosine disrupts barrier function (ie, macromolecule permeability of microvascular endothelial cells and microvessels) of the rat coronary microvasculature. Different roles for $P2Y_2$ and $P2Y_6$ receptors in large and small mouse coronary arteries have been described.[62] Both P2X1 and $P2Y_2$ receptors are expressed on the coronary artery smooth muscle cells of human, porcine, rabbit, and rat hearts, and their activation leads to increases in $[Ca^{2+}]_i$. Reverse transcription-polymerase chain reaction analysis of P2 receptors in human coronary arteries showed dominant expression of P2X1 and $P2Y_2$ receptor mRNA, with weaker expression of $P2Y_1$, $P2Y_4$, and $P2Y_6$ receptor mRNA.[63] ATP constricts human epicardial coronary veins. ATP is a factor controlling coronary blood flow during exercise.

Adenosine is a coronary dilator in all mammalian species studied, including humans. A_{2A} receptors are located on smooth muscle, whereas both A_{2A} and A_{2B} receptor activation of endothelial cells mediates relaxation of human small coronary arteries via NO. In porcine coronary artery, nicotinamide adenine dinucleotide evokes relaxations that are abolished by a selective A_{2A} receptor antagonist.[64] Different adenosine receptor subtypes mediate coronary vasodilation in postnatal and mature rats, and there is a reduction in the response to adenosine with age. Using P1 receptor genetic knockout mice, it was shown that adenosine reduces coronary blood flow, cardiac output, and stroke volume.[65]

ATP in the coronary effluent of saline perfused heart is in the range of 1 nmol/L. This low value reflects the rapid degradation of ATP by CD39 and CD73, which accounts for the high quantities of adenosine detected in the perfusates. ADA is localized on the extracellular surface of endothelial cells of small coronary arteries. The mechanisms of ATP release include vesicular exocytosis from both nerve terminals and vascular endothelial cells, which also release ATP via connexin and pannexin hemichannels.

Proliferation of porcine cultured coronary artery smooth muscle cells is elicited by ATP via P2Y receptors acting synergistically with insulin. There is P2X1 receptor–mediated inhibition of the proliferation of human coronary smooth muscle cells. In both in vitro organ cultures and in vivo stented coronary arteries, there is upregulation of $P2Y_2$ receptors and mitogenic actions of ATP and UTP on coronary artery smooth muscle cells. Second messenger signals of both the extracellular signal–regulated kinase and phosphatidylinositol-3-kinase pathways are involved in ATP-stimulated coronary artery smooth muscle proliferation. Adenosine attenuates human coronary artery smooth muscle proliferation.[66] The mitogenic effect of adenosine on porcine coronary artery smooth muscle cells is mediated by A_1 receptors. Activation of A_3 receptors induces proliferation of primary human coronary smooth muscle cells, involving early growth response genes.

Vascular System

There is dual control of vascular tone by perivascular nerves and endothelial cells (Figure 3).[67]

Perivascular Nerves

There is considerable variation in the proportions of ATP and noradrenaline released as cotransmitters from sympathetic nerves in different vessels. There is a major ATP component of sympathetic neurotransmission in rabbit saphenous artery and mesenteric artery. In the rabbit jejunal artery and guinea pig submucosal arterioles, ATP is the predominant, perhaps the sole, mediator of the contractile response to sympathetic nerve stimulation, whereas coreleased noradrenaline acts as a prejunctional modulator.[68] Evidence for sympathetic cotransmission has also been reported from vascular beds. Fluctuations in mean arterial pressure in conscious rats evoked by noradrenaline and ATP released from sympathetic nerves can be distinguished by their frequency characteristics. A contribution of ATP to sympathetic vasopressor responses has been demonstrated in the pithed rat. Evidence has also been presented showing a significant role of purinergic signaling for sympathetic vascular responses of rat mesenteric artery in vivo.[69] There are separate vesicular stores of noradrenaline and ATP in sympathetic nerve terminals.[70] ATP is released earlier than noradrenaline where ATP induces the initial phase of vasoconstriction, whereas noradrenaline initiates more slowly developing and longer-lasting tonic constrictions. The contractile actions of ATP released from perivascular sympathetic nerves are mediated principally via P2X1 receptors, confirmed by the use of P2X1 genetic knockout mice. However, in some vessels (eg, human omental arteries[71] and rat basilar arteries[72]), smooth muscle cells also express P2X4 receptors. P2X5 and P2X1 receptors are expressed by rat small mesenteric arteries,[73] and P2X1/4 heteromeric receptors mediate constriction of rat cerebral arteries. P2X1 receptor clusters have been described on vascular smooth muscle in regions adjacent to sympathetic nerve varicosities. Raised tone revealed ATP as a sympathetic neurotransmitter in the porcine mesenteric arterial bed, which is relevant to physiological conditions.[74]

P2Y and P2X receptors are expressed by smooth muscle in some blood vessels. For example, $P2Y_1$ receptors in human cystic artery and great saphenous vein[75] and $P2Y_6$ receptors in mouse mesenteric resistance arteries and human subcutaneous arteries mediate contraction.[76] $P2Y_4$ and $P2Y_6$ receptors mediate vasoconstriction in rat cerebral parenchymal arterioles. The involvement of ATP-sensitive potassium channels (K_{ATP}) channels in vascular function has been reviewed.[77] In rat mesenteric arteries, neurally released ATP produces an early junctional calcium transient and this is followed by calcium waves. The L-type calcium channel blocker, nifedipine, inhibits the purinergic component of sympathetic vasoconstriction.

Excitatory junction potentials appear upon stimulation of perivascular sympathetic nerves. Excitatory junction potentials are resistant to prazosin in the rat tail artery but are blocked by the selective P2 receptor desensitizer α,β-meATP (Figure 4A through 4C) and the P2 receptor antagonist suramin (Figure 4D). Intermittent release of single quanta of ATP responsible for excitatory junction potentials was shown in the rat femoral artery. Noradrenaline 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 activity occurs in sympathetic nerves. In rat skeletal muscle, proximal arterioles responded predominantly to α_1- and α_2-adrenoceptor activation, whereas distal arterioles responded most to P2X1 receptor activation.[78]

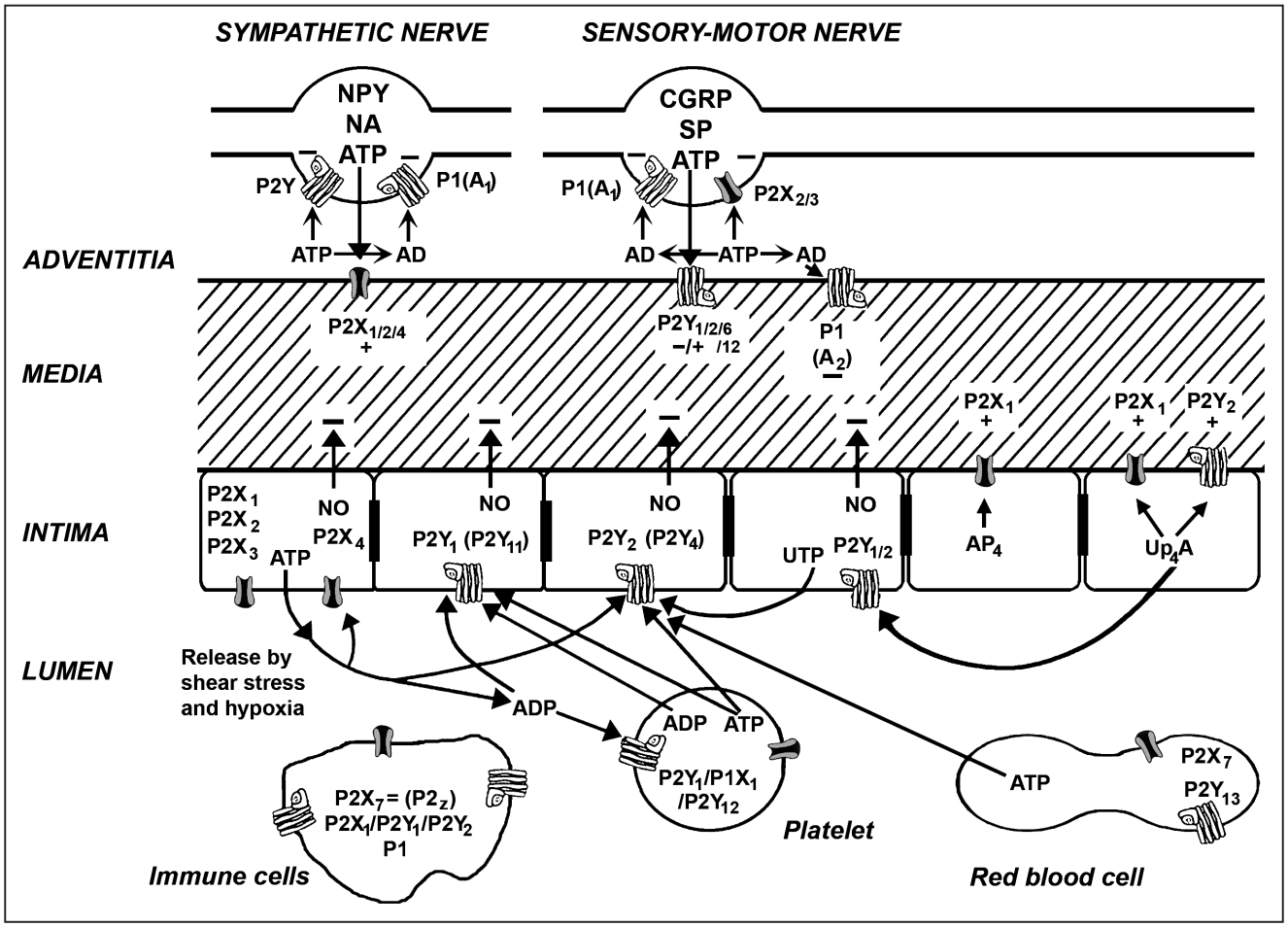

Figure 3. Schematic diagram illustrating the main receptor subtypes for purines and pyrimidines present in blood vessels involved in control of vascular tone. ATP is released as a cotransmitter with noradrenaline (NA) and neuropeptide Y (NPY) from sympathetic nerves in the adventitia to act at smooth muscle P2X1 receptors and, in some vessels, P2X2, P2X4 and $P2Y_1$, $P2Y_2$ and $P2Y_6$ receptors, resulting in vasoconstriction (and rarely vasodilation); 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 (A_1) receptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (arising from ecto-enzymatic breakdown of ATP) modulation of transmitter release. P2X2/3 receptors are present on a subpopulation of sensory nerve terminals. P1 (A_2) receptors on vascular smooth muscle mediate vasodilatation. Endothelial cells release ATP and UTP during shear stress and hypoxia to act on $P2Y_1$, $P2Y_2$ and sometimes $P2Y_4$, $P2Y_{11}$ P2X1, P2X2, P2X3, and P2X4 receptors, leading to the production of nitric oxide (NO) and subsequent vasodilatation. Adenosine tetraphosphate (AP_4) 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 $P2Y_1$ and $P2Y_{12}$ ADP-selective receptors and P2X1 receptors. Immune cells of various kinds possess P2X7 and P1, P2X1, $P2Y_1$, and $P2Y_2$ receptors. ATP released from red blood cells, which express P2X7 and $P2Y_{13}$ receptors, is also involved in some circumstances. The additional involvements of uridine adenosine tetraphosphate (Up_4A) are indicated. Modified from Burnstock[231] with permission of the publisher. Copyright ©1996, Blackwell Science Ltd, UK.

Most blood vessels are not innervated by parasympathetic nerves, with the exception of those supplying salivary glands and some cerebral and coronary blood vessels. Whether ATP is a cotransmitter in these perivascular parasympathetic nerves has not been investigated yet. Reviews that discuss sympathetic and nonsympathetic purinergic neurotransmission are available.[79,80]

Blood vessels are often innervated by sensory-motor nerves, both unmyelinated C fibers and myelinated Aδ fibers. Perivascular sensory innervation of mouse mesenteric arteries is impaired in old age.[81] The main neurotransmitter in perivascular sensory-motor nerves is calcitonin gene-related peptide, which mediates vasorelaxation, but ATP was also shown to be released during antidromic stimulation of sensory nerves in the rabbit ear artery causing vasodilation.[82]

Purinergic Involvement in Cardiac Reflex Activities

Purinergic cotransmission plays a major role in the pressor sinocarotid reflex in urethane-anesthetized rats. Hypothalamic stimulation in anesthetized rabbits evokes skeletal muscle vasodilation, which is mediated by ATP released from sympathetic nerves. Blood flow to the skin after exposure to a cold environment is reduced, preventing heat loss and is achieved by reflex increase in sympathetic tone of cutaneous veins. This is resistant to adrenoceptor antagonism, but is inhibited by desensitization of P2X purinoceptors with α,β-meATP. It may be important for thermoregulation and may explain why purinergic cotransmission is more prominent in cutaneous than in deep blood vessels.

ATP seems to act as a cotransmitter in sensory-motor nerves during vascular axon reflex activity.[83] P2X receptors

play a role in evoking the exercise pressor reflex in rats with peripheral artery insufficiency.[84] There is impaired sympathetic nerve activity to the skin in old age, which is related to impaired reflex vasoconstrictor responses to whole-body cooling in human aging, perhaps mediated by ATP.[85] ATP enhances cholinergic cutaneous vasodilation, but not sweating, in young males and females.[86] Adenosine receptors play a role in evoking the venous distension reflex in humans.[87] The venoarterial reflex, where venous congestion triggers arterial vasoconstriction, is modulated by adenosine.[88]

Endothelial Cells

A major role of endothelial cells is the mediation of vasodilatation to counterbalance the vasocontractile effects or neurally released ATP and noradrenaline. ATP released by shear stress acts on endothelial P2 receptor subtypes to elicit vasodilatation, via release of NO, endothelium-derived hyperpolarizing factor, and prostacyclin. The dominant purine receptors on both animal and human endothelial cells are $P2Y_1$ and $P2Y_2$ nucleotide receptors, as well as A_{2A} and A_{2B} adenosine receptors.[89] However, there are vessel- and species-specific differences in receptor subtype expression[24] (Table 1). With an intact endothelium, released ATP elicits vasodilatation, but when there is endothelial damage ATP may act as a vasoconstrictor via P2 receptors on the vascular smooth muscle, which may lead to local vasospasm.

P2X4 receptor genetic knockout mice show reduced dilation, release smaller amounts of NO, and have higher blood pressure.[90] Expression of P2X4 and P2X7 receptors in human umbilical vein endothelial cells is upregulated under inflammatory conditions. There is selective upregulation of P2X4 receptor gene expression by interferon-γ in endothelial cells of human umbilical vein and aorta and microvascular endothelial cells. P2X7 receptor activation causes release of both pro- and anti-inflammatory interleukin-1 receptor ligands. Oral administration of ATP increases blood flow after exercise in animals and humans. ATP protects endothelial cells against DNA damage caused by irradiation or chemically induced damage.[91] K_{ATP} channels in endothelial cells mediate arteriole relaxation.[92]

There is ATP-stimulated release of ATP by human endothelial cells. ATP released from rat adrenomedullary endothelial cells increases $[Ca^{2+}]_i$, which spreads to neighboring cells forming a Ca^{2+} wave; this is blocked by suramin or apyrase. The mechanism of ATP release from endothelial cells during shear stress is, at least in part, vesicular, but connexin and pannexin 1 channels are also involved.

Adenosine 5′-pentaphosphate is a purinergic endothelium-derived vasoconstrictor in rodent and human microvessels, which acts predominantly through activation of smooth muscle P2X1 receptors. Uridine adenosine tetraphosphate (Up_4A) is also an endothelium-derived vasoconstricting factor.[93]

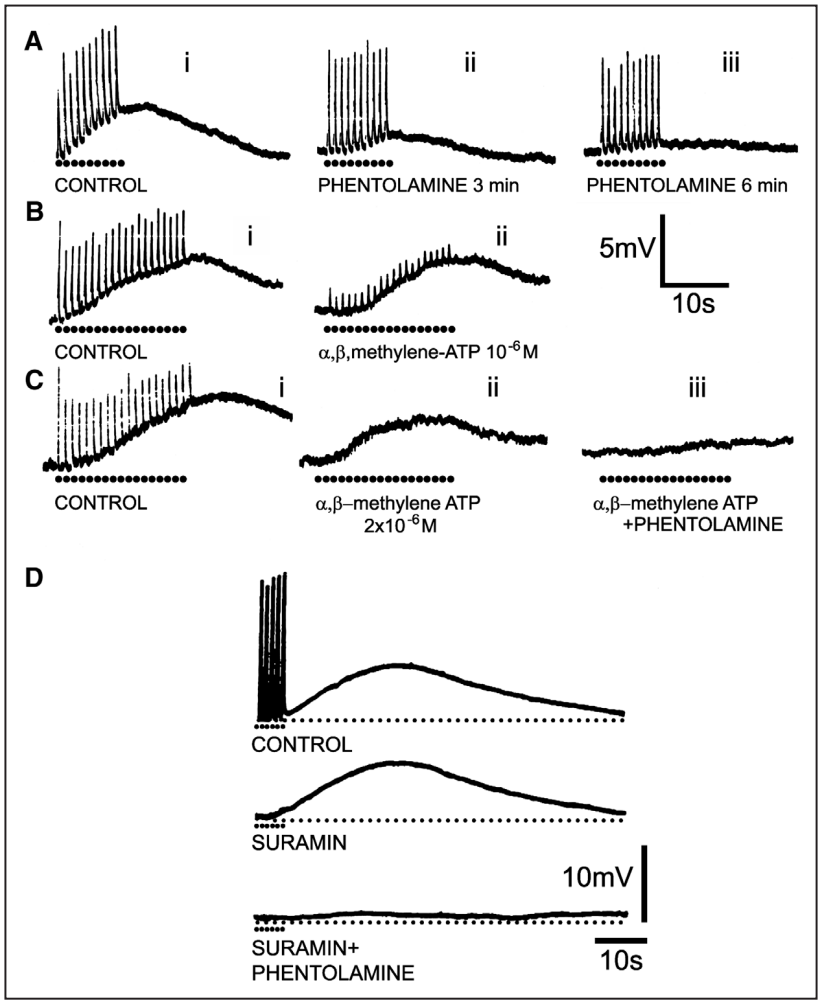

Figure 4. A–C, Intracellular recording of the electric responses of single smooth muscle cells of the rat tail artery to field stimulation of the sympathetic motor nerves (the pulse width was 0.1 ms at 0.5 Hz, indicated by ●). **A**i, Control response of the muscle. Note that to each individual stimulus, there was a rapid depolarization, and as the train of pulses progressed, a slow depolarization developed. Similar responses were obtained in **B**i and **C**i, which are also control responses in Krebs solution. **A**ii and **A**iii, 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 min. **B**ii, The tissues have been in the presence of 10^{-6} M α,β-methylene ATP for >15 min. The fast depolarizations produced by each stimulus were greatly reduced, but the slow depolarization persisted. **C**ii, 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 (**C**iii). Reprinted from Sneddon and Burnstock[232] with permission of the publisher. Copyright ©1984, Elsevier. **D**, Excitatory junction potentials (EJPs) in the main tail artery of the rat. Inhibition of the fast EJPs by suramin (1 mmol/L). Reprinted from Jobling and McLachlan[233] with permission from the authors.

Table 1. Purinoceptor Subtypes on Smooth Muscle and Endothelial Cells in Different Blood Vessels

	P1 Receptors		P2X Receptors		P2Y Receptors	
Blood Vessels	SM	EC	SM	EC	SM	EC
Aorta						
Guinea pig	A_1 A_{2B}					$P2Y_{1,4}$
Rat		A_1 A_{2A} A_{2B}	P2X1			$P2Y_{1,2,4}$
Mouse		A_1 A_{2A} A_{2B} A_3	P2X1		$P2Y_{2,4,6}$	$P2Y_{1,2,6}$
Hamster		A_{2A}				
Rabbit					$P2Y_1$	$P2Y_{1,2,4}$
Pig			P2X5			
Rat tail artery		A_1 A_{2A}	P2X1		$P2Y_{1,2,4}$	
Rabbit ear artery			P2X1		$P2Y_{2,4}$	
Mesenteric vessels						
Artery						
Rat	A_{2A} A_{2B}	A_{2A}	P2X1	P2X1	$P2Y_{2,6}$	$P2Y_{1,2,4}$
Rabbit			P2X1			
Guinea pig			P2X1			
Hamster			P2X1			$P2Y_{2,4}$
Mouse			P2X1	P2X1	$P2Y_6$	
Vein						
Guinea pig			P2X1		$P2Y_{1,2,4}$	
Rat					$P2Y_{2,4}$	
Coronary vessels						
Human	A_{2A} A_{2B}		P2X1		$P2Y_2$	
Guinea pig	A_1	A_1 A_{2A} A_{2B}				$P2Y_{2,4}$
Pig	A_1 A_{2A} A_3	A_{2A}			$P2Y_2$	$P2Y_1$
Dog			P2X1			
Rat						$P2Y_1$
Cerebral vessels						
Rat	A_{2A}	A_{2A} A_{2B}	P2X1		$P2Y_{2,6}$	$P2Y_{1,2}$
Human			P2X1		$P2Y_6$	$P2Y_{2,4,6}$
Rabbit					$P2Y_{2,4}$	$P2Y_1$
Skeletal muscle						
Human						$P2Y_2$
Dog			P2X1			
Rabbit						$P2Y_1$
Femoral artery						
Rabbit			P2X1			
Rat		A_1				
Pulmonary vessels						
Cat	A_1					
Guinea pig	A_1 A_{2B}					

(*Continued*)

Table 1. Continued

	P1 Receptors		P2X Receptors		P2Y Receptors	
Blood Vessels	SM	EC	SM	EC	SM	EC
Rabbit						$P2Y_{2,4}$
Rat			P2X1		$P2Y_{2,4,6}$	$P2Y_1$
Renal vessels						
Artery						
Rat		A_{2A}	P2X1		$P2Y_{2,4,6}$	$P2Y_{1,2}$
Human			P2X1			
Rabbit			P2X1			
Mouse			P2X1		$P2Y_6$	
Afferent arteriole						
Mouse	A_1					
Rat		A_{2A}				
Arcuate artery						
Rabbit	A_1	A_{2A}				
Hepatic vessels						
Artery						
Rabbit			P2X1			
Portal vein						
Rat			P2X1			
Rabbit			P2X1			
Chorionic artery and vein						
Human	A_3	A_{2B}				
Placental vessels						
Human			P2X1		$P2Y_{1,2,4}$	$P2Y_{1,2}$
Skin vessels						
Dog			P2X1			
Carotid artery						
Rat			P2X1			$P2Y_{1,2,4}$

EC indicates endothelial cells; and SM, smooth muscle.

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CD39, which hydrolyzes ATP to AMP, and CD73, which hydrolyzes AMP to adenosine, are expressed by endothelial cells.[94] The balance of ATP-generating (involving ectoadenylate kinase) and ATP-consuming pathways in human cell-free serum controls the duration and magnitude of purinergic signaling in the blood.

The mechanism of ATP-induced NO release from endothelial cells probably involves Ca^{2+}-activated Cl^- channels and AMP-activated protein kinase (PK) may also be involved. In contrast to acetylcholine-induced endothelium-dependent vasodilatation, ATP-mediated vasodilation is not impaired with advancing age in healthy humans. Barrier function of endothelial cells cultured from human pulmonary artery is promoted by β-nicotinamide adenine dinucleotide, involving $P2Y_1$ and $P2Y_{11}$ receptors and PKA- and EPAC1/Rac1-dependent actin cytoskeleton rearrangement.

The blood–brain barrier consists of a single layer of specialized endothelial cells. A_{2A} receptor activation modulates blood–brain barrier permeability[95] (see review by Bynoe et al[96]). Premature newborn infants display elevated levels of adenosine in the blood, and it has been claimed that this is because of blood–brain barrier immaturity.[97] P2X7 receptor suppression preserves blood–brain barrier integrity after intracerebral hemorrhage.[98]

All subtypes of P1 adenosine receptors, A_1, A_{2A}, A_{2B}, and A_3, are expressed by vascular endothelium, A_{2A} and A_{2B} receptors being dominant. Intravenous infusion of adenosine in conscious endothelial NO synthase genetic knockout mice evokes a reduction in mean arterial blood pressure. A_{2A} and A_3 receptors are mediators of human endothelial progenitor cell migration. Table 1 summarizes the distribution of purinoceptor subtypes on both smooth muscle and endothelial cells mediating vasoconstriction and vasodilation in different vessels in the body. Reviews concerned with purinergic signaling in endothelial cells are available.[99–101]

Trophic Vascular Roles of Purinergic Signaling

Reviews describing the effect of purines and pyrimidines on migration, proliferation, and death of different cell types are available[10,30,102,103] (Table 2).

Vascular Smooth Muscle

ATP and ADP stimulate DNA synthesis and proliferation of porcine aortic smooth muscle cells via activation of P2Y receptors. ATP, released from sympathetic nerves and from erythrocytes, platelets, endothelial cells, and damaged smooth muscle, is a mediator of vascular smooth muscle proliferation. ADP released from platelets acting synergistically with peptide growth factors, results in smooth muscle proliferation at sites of vascular injury. $P2Y_2$ and $P2Y_4$ receptors sensitive to ATP and UTP are mediators of proliferation of rat aortic smooth muscle cells. UDP stimulates the growth of smooth muscle cells cultured from rat aorta via activation of $P2Y_6$ receptors. Up_4A stimulates DNA synthesis and proliferation of human aortic vascular smooth muscle cells, mediated by P2Y receptors involving the MAPK and P13K/Akt pathways. Vascular smooth muscle cell migration is induced by UTP and Up_4A acting via $P2Y_2$ receptors and by UDP acting via $P2Y_6$ receptors. Upregulation of $P2Y_2$ receptors on vascular smooth muscle cells is induced by cytokines, resulting in increased mitogenic responses to UTP and ATP. Arrestin-dependent regulation of $P2Y_2$ receptor-stimulated MAPK signaling is essential for the migration of aortic smooth muscle cells, a key event in vascular remodeling.

Low ATP concentrations stimulate expression of genes for the contractile vascular smooth muscle phenotype, whereas high concentrations of ATP produce a phenotypic shift from the contractile to the synthetic phenotype.[104] The differentiated contractile phenotype of vascular smooth muscle expresses predominantly P2X1 receptors. P2X1 receptors are downregulated and the mitogenic $P2Y_1$ and $P2Y_2$ transcripts upregulated in the dedifferentiated smooth muscle synthetic phenotype. ATP promotes vascular smooth muscle cell DNA synthesis and cell proliferation during embryonic and postnatal development via the activation of extracellular signal–regulated kinase 1/2 involving both PKC-δ and Ca^{2+}/calmodulin-dependent PK II-δ_2.

Transgenic overexpression of CD39 decreases vascular smooth muscle cell proliferation and prevents neointima formation after angioplasty. CD39 deletion impairs smooth muscle cell migration in vitro and inhibits neointimal formation in a mouse model of injured carotid arteries. Reviews concerned with the trophic effects of ATP on vascular smooth muscle and endothelial cells are available.[24,105]

A_1 and A_2 receptors mediate stimulation of DNA synthesis in rat cultured arterial smooth muscle cells. A_{2B} receptors, however, inhibit growth of rat and human aortic smooth muscle cells. Adenosine regulates human vascular smooth muscle

Table 2. Purine Receptors Involved in Long-Term Tropic Signaling

	Smooth Muscle			Endothelial Cells		
	P2X	P2Y	P1	P2X	P2Y	P1
Proliferation/ mitogenesis		$P2Y_2$ and/or $P2Y_4$ (+)	A_1 (+)		$P2Y_1$ (+)	A_{2A} (+)
		$P2Y_2$ and/or $P2Y_4$ (–)[a]	A_{2A} (–)[b]		$P2Y_2$ and/or $P2Y_4$ (+)	A_{2B} (+)
		$P2Y_6$ (+)	A_{2B} (–)[c]		$P2Y_{13}$ (+)	
Migration		$P2Y_2$ (+) ($P2Y_4$) (+)			$P2Y_1$ (+)	A_{2A} (+)
		$P2Y_6$ (+)			$P2Y_2$ and/or $P2Y_4$ (+)	A_{2B} (+)
						A_3 (+)[d]
Angiogenesis					$P2Y_1$ (+)	A_{2A} (+)
					$P2Y_2$ and/or $P2Y_4$ (+)	A_{2B} (+)
Apoptosis			A_{2B} (+)	P2X7 (+)		

See[21] for references.

+ indicates stimulation; and –, inhibition.

[a]Human internal mammary artery and saphenous vein.

[b]Pulmonary artery (hypertension and smooth muscle proliferation in A_{2A} receptor knockouts).

[c]Human and rat aortic smooth muscle cells.

[d]Human endothelial progenitor cells.

cell proliferation and migration, in part, by affecting the expression of hyaluronic acid synthase. AMP-activated PK-α2 deficiency promotes smooth muscle cell migration with accelerated neointima formation in vivo.

Vascular Endothelial Cells

ADP promotes human endothelial cell migration by activating $P2Y_1$ receptor-mediated MAPK pathways. Stimulation of human endothelial cells via $P2Y_1$ receptors activates vascular endothelial growth factor (VEGF) leading to angiogenesis. UTP, as well as ATP, has mitogenic and angiogenic actions on vascular endothelial cells, indicating mediation by $P2Y_2$ and $P2Y_4$ receptors. In human umbilical vein endothelial cells, these receptors influence cytoskeletal changes, cellular adhesion, and motility. $P2Y_4$ receptor mRNA is present in primary endothelial cells isolated from mouse heart, and $P2Y_4$ genetic knockout mice show that the $P2Y_4$ receptor is an important regulator of angiogenesis. ATP, acting via $P2Y_{11}$ receptors, impairs endothelial cell proliferation by inducing cell cycle arrest. Stimulation of P2X7 receptors enhances apoptosis of endothelial cells mediated by lipooligosaccharide, which causes release of ATP. ATP increases DNA synthesis, migration, and tube formation in vasa vasorum endothelial cells.

Adenosine increases capillary density and dipyridamole, which inhibits uptake of adenosine, increases capillary proliferation. In pulmonary endothelial cells and human umbilical vein endothelial cells, proliferation involves A_{2A} receptors. Canine retinal microvascular endothelial cell migration and tube formation are stimulated by adenosine as well as proliferation and migration of human retinal, porcine, and rat endothelial cells via A_{2B} receptors. Adenosine increases the migration of human endothelial progenitor cells via the activation of A_{2B} receptors.

Figure 5 illustrates the P1 and P2 receptor subtypes on both smooth muscle and endothelial cells that mediate proliferation.

Blood Cells

Detailed coverage of the literature up to 2015 about purinergic signaling and blood cells has been published.[31,106]

$P2Y_1$ and P2X7 receptors are the dominant nucleotide receptor subtypes expressed by mature erythrocytes, whereas A_{2A} receptors are expressed by embryonic red blood cells. ATP is released from human erythrocytes in response to mechanical deformation or hypoxia.[107] Erythrocytes are not only O_2 carriers but also ATP release via pannexin 1 hemichannels has a direct role in regulation of vascular tone.

Platelets express $P2Y_1$, $P2Y_{12}$, P2X1, and P1 receptor subtypes involved in platelet aggregation and shape change. Platelets were shown early to contain high concentrations of ATP[108] and that extracellular ATP is rapidly broken down to ADP. The ability of ADP to produce platelet aggregation was also recognized early.[109] Platelet shape change is considered to be the main role of $P2Y_1$ receptors although it also contributes to platelet aggregation. A review concerned with the structure and function of platelet $P2Y_{12}$ receptors has been published.[110] The role of P2X1 receptors expressed by platelets has been difficult to assess, because of its rapid desensitization, but the consensus is that it contributes to platelet shape change and adhesion. VNUT, the vesicular nucleotide transporter, is responsible for vesicular storage and release of nucleotides from platelets. There is enhanced CD39 and CD73 activities in platelets in human pregnancy. Red wine inhibits aggregation and increases CD39 activity of platelets in vitro.[111] Adenosine is a competitive inhibitor via A_{2A} receptors of human platelet aggregation by ADP.

Megakaryocytes, which are platelet precursor cells in bone marrow, release ATP. $P2Y_1$, $P2Y_2$ or $P2Y_4$, $P2Y_{12}$, and P2X1 receptors are expressed on megakaryocyte cell lines.

Purinergic signaling in leukocytes, white blood cells that consist largely of immune cells, has been reviewed in detail.[112] ATP release via pannexin 1 channels in endothelium promotes leukocyte adhesion and emigration.[113] ATP releases ATP from human leukocytes via $P2Y_{2,4,6, \text{ and } 11}$ receptors.[114]

Cardiovascular Diseases

ATP injections were used for the treatment of angina pectoris associated with coronary disease in the 1940s, and AMP was used for the treatment of angina. ATP was also used early for the treatment of patients with coronary insufficiency. The emphasis in the recent literature is on the pathophysiology of purinergic signaling in the cardiovascular system and on the therapeutic potential of purinergic drugs (see[11,12,115,116] where references to early papers can be found).

Heart Diseases

These have been discussed in detail in a recent review.[11] So only articles published since this review are reported.

Accumulation of adenosine in chronic heart failure may be because of the reduction of ADA gene expression and increase in CD73. Adenosine therapy is cardioprotective for chronic heart failure mediated by A_1 and A_3 receptors.[117] A_1 receptor activation attenuates cardiac hypertrophy and prevents heart failure in a mouse left-ventricular pressure-overload model and in a rat neonatal cardiac myocyte model.[118] K_{ATP} channels are critical for maintaining myocardial perfusion and high-energy phosphates in the failing heart.[119] It has been suggested that $P2Y_6$ receptors could constitute a therapeutic target to regulate cardiac hypertrophy.[120]A_{2B} receptor activation exerts stronger cardioprotective effects against cardiac ischemia/reperfusion injury compared with A_{2A} receptor activation in rats.[121] CD73 and A_{2B} receptor agonists have been considered as therapeutic agents for myocardial ischemia. There is a protective role of CD39, which leads to increased adenosine, in ischemia-reperfusion injury, while genetic deletion of CD39 leads to enhanced myocardial ischemia-reperfusion injury.[122] Recombinant CD39 may offer a novel therapeutic approach to the damage caused by ischemia by reducing sympathetic activity. Adenosine may regulate inflammatory responses initiated during ischemia-mediated immune injury.[123] Although the early focus was on the role of adenosine in ischemic and reperfusion injuries, there is increasing interest in the role of ATP. Administration of ATP, before or just after cardiac ischemia, is cardioprotective.[124] Reflex responses mediated by cardiac sympathetic afferent nerves during myocardial ischemia, are caused by ATP released from the ischemic myocardium.[125] It was concluded in a recent review that P2Y receptors play an important role as a therapeutic target in myocardial

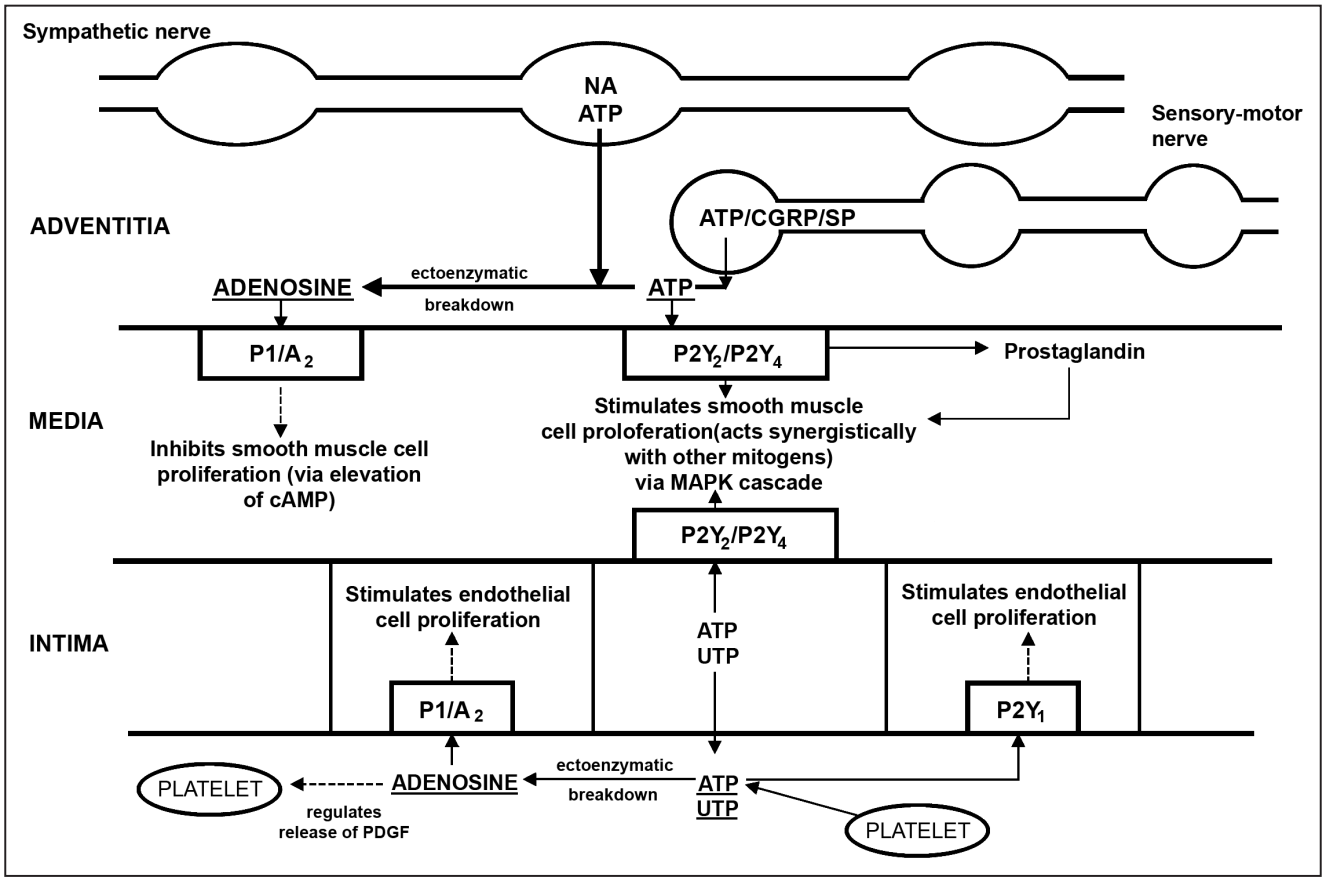

Figure 5. Schematic diagram of long-term (trophic) actions of purines released from nerves, platelets, and endothelial cells (which also release UTP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and sensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via $P2Y_2$ and $P2Y_4$ receptors via a mitogen-activated protein kinase (MAPK) cascade, whereas adenosine resulting from enzymatic breakdown of ATP acts on P1 (A_2) receptors to inhibit cell proliferation (via elevation of cAMP). ATP and UTP released from endothelial cells stimulate endothelial and smooth muscle cell proliferation via $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors. Adenosine resulting from ATP breakdown acts on P1 (A_2) receptors to stimulate endothelial cell proliferation and regulate the release of platelet-derived growth factor (PDGF) from platelets. CGRP indicates calcitonin gene-related peptide; NA, noradrenaline; and SP, substance P. Reprinted from Burnstock[30] with permission of the publisher. Copyright ©2002, Wolters Kluwer Health, Inc.

protection during ischemia/reperfusion.[126] The P2X4 receptor is needed for neuroprotection via ischemic preconditioning.[127] An increase in expression of P2X3 receptors in superior cervical ganglia and dorsal root ganglion neurons was also reported, leading to aggravated sympathoexcitatory reflexes. Mitochondrial K_{ATP} channels are claimed to provide protection against myocardial ischemia/reperfusion injury.[128,129]

AMISTAD clinical trials (Acute Myocardial Infarction Study of Adenosine) showed that the infusion of adenosine for 3 hours resulted in a striking reduction in infarct size.[130,131] Protection against myocardial infarction is mediated by A_1 and probably A_3 receptors in the rabbit heart. Adenosine reduces the incidence of postoperative atrial fibrillation (AF). Upregulation of A_{2A} receptors is linked to abnormal calcium handling in AF. Prevention of A_{2A} receptor activation may be a novel way to maintain uniform beat-to-beat responses at higher beating frequencies in patients with AF.[132] Adenosine-guided pulmonary vein isolation has been recommended, after a randomized clinical trial, for the treatment of paroxysmal AF,[133] but this has been questioned in a more recent clinical trial.[134] The clinical significance of ATP-induced AF has been investigated.[135] The presence of AF does not affect the efficacy of the $P2Y_{12}$ antagonists prasugrel and ticagrelor.[136] Articles discussing the role of adenosine in atrial arrhythmias and fibrillation have been published[137] and also the role of ATP.[138] ATP was used for acute therapy of paroxysmal supraventricular tachycardia in the late 1940s and later utilized by others. Bolus injection of adenosine (Adenocard) is being used clinically to slow conduction time through the AV node, interrupt the reentry pathways through the AV node in patients with paroxysmal supraventricular tachycardia. The treatment of paroxysmal supraventricular tachycardia by ATP and adenosine has been discussed in a recent review.[139] Adenosine and ATP have been used in conjunction with the head-up tilt table test to provoke vasovagal reaction in syncope patients.[140]

Cardiomyopathy may be inherited, but can also be caused by factors such as viral infections, alcoholism, vitamin B deficiency, or amyloidosis. Disruption of ATP synthase has been reported to contribute to diabetic cardiomyopathy.[141] Roles for P2X7 receptors in dilated cardiomyopathy have been reported[142] and also for K_{ATP} channels.[143] $P2Y_{11}$ receptor agonists may be a means to reduce cardiac fibrosis.[144] A recent overview about extracellular nucleotide regulation of signaling in cardiac fibrosis has been published.[145] The P2X7 receptor

antagonist, A740003, attenuates experimental autoimmune myocarditis and is promising for treating clinical myocarditis.[146] Intracoronary administration of adenosine provokes angina pain. Intracoronary administration of a single dose of adenosine in patients with unstable angina undergoing percutaneous coronary intervention produces decreased myonecrosis and improved coronary blood flow.[147] It has been suggested that in vascular pain, including angina, pelvic, and ischemic pain, as well as migraine, ATP released from endothelial cells during reactive hyperemia after vasospasm 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.[148,149] Activation of P2X2/3 receptors expressed on nociceptive airway sensory nerves causes cardiovascular reflexes in conscious rats via reflex modulation of the autonomic nervous system.[150] The responses to adenosine of the transplanted human heart show supersensitivity. Donor pretreatment with AMP-activated PK activator protects cardiac grafts from cold ischemia/reperfusion injury.[151] Short-term treatment with a P2X receptor antagonist prolongs cardiac transplant survival. Atherosclerosis of coronary vessels is known as coronary artery disease or coronary artery syndrome. $P2Y_{12}$ receptor inhibition combined with aspirin is beneficial for patients with acute coronary syndrome undergoing percutaneous coronary intervention (see recent reviews by De Luca et al[152] and Rollini et al[153]). Plasma levels of adenosine are correlated with homocysteine and uric acid concentrations in patients with coronary artery disease.[154]

Vascular Diseases

Hypertension

There seem to be 6 different ways that purinergic signaling contributes to the development of hypertension:

1. There is an increase in sympathetic nerve activity supplying blood vessels in hypertension, associated with hyperplasia and hypertrophy of arterial vessels. ATP, released as a cotransmitter with noradrenaline from sympathetic nerves, acts on P2X1 receptors to constrict vascular smooth muscle.[31] Increased release of ATP as a sympathetic cotransmitter relative to noradrenaline release in spontaneously hypertensive rats (SHR) has been reported.[155,156] Guanethidine was used in early times for the treatment of hypertension, probably by inhibiting the release of both ATP and noradrenaline from sympathetic nerves. Purinergic prejunctional inhibitory modulation of vascular sympathetic neurotransmission in SHR and also in deoxycorticosterone acetate-salt hypertensive rats is diminished via A_1 receptors. In SHR, the vasoconstrictor response of tail arteries to sympathetic nerve stimulation is inhibited by desensitization of P2X1 receptors. When the pressure of rat small mesenteric arteries was raised from 30 to 90 mm Hg, which is similar to the pressure in these arteries in vivo, the contribution of ATP to excitatory sympathetic neurovascular transmission increases. Sympathetic nerve–mediated forearm vasoconstriction persisted after α receptor blockade in hypertensive patients, probably because of ATP release as a cotransmitter. Therefore, P2X1 receptor antagonists are promising for the treatment of hypertension.
2. ATP is released from endothelial cells during shear stress produced in response to changes in blood flow,[157] which then acts on P2 receptors, particularly $P2Y_1$, $P2Y_2$, and P2X4 subtypes on endothelial cells to release NO, resulting in vasodilation resulting in a decrease in blood pressure.[24,158] ATP introduced in vivo into dogs or rats induces hypotension. In the anesthetized mouse, intravenous injection of ATP or UTP causes a decrease in systemic arterial pressure via a cAMP pathway. Hypotension is mediated by A_3 receptors in the pithed rat. Vascular introduction of $P2Y_1$, $P2Y_2$, and P2X4 agonists, which would lead to increased vasodilatation, offers another way to reduce hypertension.[158] The release of ATP from endothelial cells, leading to attenuation of the blood pressure rise seen with advancing age, was increased by all-*cis*-5,8,11,14,17-icosapentaenoate, a major active component of fish oil.
3. Purinoceptors on neurons in the brain stem and hypothalamus mediate sympathetic nerve activity.[159,160] Brain stem hypoxia contributes to the development of hypertension in the SHR, because of release of ATP resulting in central sympathetic drive leading to increase in systemic arterial blood pressure.[161] Antagonists to these purinoceptors would reduce sympathetic nerve activity resulting in vasodilatation and are therefore potential centrally acting drugs for the treatment of hypertension.
4. Recent studies have shown that there is an antihypertensive effect of P2X3 receptor antagonists, because of inhibition of sympathetic nerve activity via increased peripheral chemoreceptor reflex sensitivity involving the carotid body.[46] Microinjections of the selective P2X3 (as well as P2X1) receptor antagonist, α,β-meATP, into the dorsal facial area of the medulla increased common carotid artery blood flow.[162] Therefore, P2X3 receptor antagonists are being explored for the treatment of hypertension.
5. P2X7 receptor antagonists reduce blood pressure in angiotensin-II–treated rats.[163] Glomeruli show an abundance of P2X7 receptor immunostaining (in podocytes, endothelium, and mesangial cells) in the kidney of transgenic hypertensive rats, whereas in kidneys from normal rats, there is only low-level P2X7 receptor immunostaining.[164] Hypertension and renal injury are attenuated in deoxycorticosterone acetate-salt hypertension in P2X7 receptor knockout mice, suggesting that the P2X7 receptor plays a key role in the development of hypertension via increased inflammation. It has been suggested that drugs affecting P2X7 receptor signaling may have promise as clinical antihypertensive agents.
6. In conscious SHR and also in Dahl salt-sensitive rats, plasma adenosine concentrations are elevated. Adenosine activates the vascular renin–angiotensin system in hypertensive subjects. Salt-induced hypertension is reduced by A_1 receptor antagonism. There is an increase in sensory-motor vasodilation of the mesenteric arterial bed in rats with hypertension after treatment with a P1 receptor antagonist, perhaps as a compensatory mechanism. Vasodilation and hypotension in conscious SHR are produced by A_{2A} receptor agonists. A_{2B} receptors are involved in hypertension in Dahl salt-sensitive rats.[165] A_1 receptor knockout mice show a decreased blood pressure response to low-dose angiotensin-II infusion. A_3 receptor agonists have been recommended recently for treatment of essential hypertension.[166]

Polyphenolic compounds contained in red wine cause release of nucleotides from endothelial cells resulting in vasodilation, thereby decreasing blood pressure in SHR.[167] Up_4A, a dinucleotide claimed to be an endothelium-dependent vasoconstrictor, is increased in plasma of juvenile hypertensives and acting via $P2Y_2$ or $P2Y_4$ receptors may contribute to the early development of primary hypertension. Vascular smooth muscle cell proliferation in SHR is stimulated by ATP and UTP via $P2Y_2$ and $P2Y_4$ receptors. ATP released during muscle contraction acts on P2 receptors on skeletal muscle sensory afferents to initiate the metaboreflex in hypertension.[168] $P2Y_6$ receptors form heterodimers with angiotensin AT1 receptors to promote angiotensin-II–induced hypertension.[169] It has been claimed that K_{ATP} channels in vascular smooth muscle plays a major role in blood pressure control.[170]

A review about renal P2 receptors and hypertension is available.[171] A review concerned with blood pressure control by P2 purinoceptors in the kidney[172] and an Editorial on the same topic[173] have been published.

Infusion of ATP-$MgCl_2$ may be clinically useful in the treatment of children with pulmonary hypertension. ATP is a mitogen for pulmonary artery smooth muscle cells, which is relevant for the pathophysiological basis of pulmonary hypertension. In animal models of pulmonary hypertension, there are beneficial effects of ATP infusion. ATP release from erythrocytes may be a novel target for the treatment of pulmonary arterial hypertension.[174] Suppression of endothelial CD39 nucleotidase is associated with vascular remodeling in pulmonary hypertension and may be a novel target for therapy.[175] It has been suggested in a recent article that P2X1 receptor antagonists could serve as a treatment for pulmonary hypertension.[176] A_{2B} receptor antagonists have also been recommended for the treatment of pulmonary hypertension associated with interstitial lung disease. Increased proliferation of endothelial cells and increased smooth muscle hypertrophy occur in pulmonary arteries of A_{2A} receptor genetic knockout mice. This suggests that adenosine, acting at A_{2A} receptors, is a regulatory mechanism to protect against development of pulmonary arterial hypertension.

Elevated maternal blood pressure occurs in preeclampsia, and high adenosine levels are found in the fetal–placental circulation in preeclamptic pregnancies.[177] The Ca^{2+} responses of human hand vein endothelial cells to ATP are larger in pregnant women than in nonpregnant and preeclamptic women. There is increased maternal–fetal plasma ADA and xanthine oxidase activity in preeclampsia. In preeclampsia, fetal endothelial cell proliferation and migration are reduced, which may be related to reduced A_{2A} receptor expression and A_{2A} receptor-mediated responses in placental endothelium. High fetal plasma concentrations of adenosine are found in patients with preeclampsia with chronic uteroplacental ischemia. High levels of ATP have been found in women with preeclampsia and infusion of ATP in pregnant rats induced preeclampsia-like symptoms. Low serum levels of the ATP-binding cassette transporter, ABCA1, are predictive of preeclampsia.[178]

Atherosclerosis

ATP signaling is involved in the development of atherosclerosis.[179,180] Endothelial and smooth muscle cell proliferation and increased expression of VEGF are promoted by adenosine and ATP in atherosclerosis via $P2Y_1$, $P2Y_2$, and P1 receptors.

Adenosine regulates endothelial cell proliferation in angiogenesis. In a human model of hypoxic foam cells, adenosine via A_{2A} receptors modulates hypoxia-inducible factor-1α, VEGF, interleukin-8, and foam cell formation.[181] A_{2B} and A_3 antagonists block steps in atherosclerotic plaque development. CD73-derived adenosine acts as an endogenous modulator protecting against vascular inflammation and monocyte recruitment, thereby limiting the progression of atherosclerosis. Genetic deletion of CD73 in mice promotes atherogenesis, most likely by deinhibition of resident macrophages and T cells.

Elevated levels of circulating ATP and ADP are associated with atherosclerosis and smoking.[182] The mitogenic effects of UTP and ATP via $P2Y_2$ and $P2Y_4$ receptors on vascular smooth muscle are involved in chronic inflammation and atherosclerosis. UTP, via $P2Y_2$ receptors, induces vascular cell adhesion molecule-1 expression in coronary artery endothelial cells leading to the recruitment of monocytes associated with the development of atherosclerosis. Upregulation of $P2Y_2$ receptors mediates intimal hyperplasia in collared rabbit carotid artery and is an indicator of the early stages of atherosclerosis. ATP and UTP, via $P2Y_2$ receptors, are chemotactic for dendritic cells and attract inflammatory cells to atherosclerotic plaques. Proinflammatory cytokines, such as interleukin-1β, accelerate atherosclerosis through the upregulation of $P2Y_2$ receptors.[183] P2X4 and P2X7 receptors modulate high glucose inflammatory responses in endothelial cells.[184]

$P2Y_1$ receptor antagonists serve as a therapeutic target for neointima formation.[185] In diet-induced atherosclerosis, there is increased expression of $P2Y_6$ receptor mRNA in atherosclerotic regions. $P2Y_{12}$ receptors mediate the formation of atherosclerotic lesions in apolipoprotein E–deficient mice. Endothelial P2X4 receptors play a more significant role in intense proliferation in atherosclerosis than $P2Y_2$ receptors, as reflected by the susceptibility of saphenous vein grafts to atherosclerosis compared with internal mammary arteries. ATP contributes to atherogenesis, via $P2Y_2$, $P2Y_6$, P2X4, and P2X7 receptors by inducing leukocyte recruitment in mice.

CD39, expressed on the surface of endothelial cells and leukocytes, is atheroprotective.[186] Stent coating with CD39 mRNA has also been proposed for the treatment of atherosclerotic blood vessels.[187] An increase in CD39 and subsequent ATP and ADP hydrolysis occurs in platelets of hypercholesterolemic patients. Deficiency of ABC transporters A1 and G1 in endothelial cells accelerates atherosclerosis in mice.[188] The trophic roles of purinergic signaling in vascular smooth muscle and endothelial cell proliferation and death are involved in atherosclerosis, and purinergic therapeutic strategies have been proposed.[179,180]

Ischemia

Ischemia results in injury of most organs in the body. Purines and pyrimidine nucleotides, released at the site of cell damage, contribute to injury, but may also have protective effects. Adenosine, after breakdown of released ATP, is also protective of ischemic injury. $P2Y_{12}$ receptor antagonists have been used to prevent ischemic stroke.[189] An account of ischemia in the

heart was included earlier, and detailed coverage of the involvement of purinergic signaling in ischemia in blood vessels in a wide range of organs can be found in a review by Burnstock and Ralevic[24] and in some more recent articles since then.[190,191]

Vascular Injury, Angiogenesis, and Restenosis

Vascular injury is a critical initiating event in the pathogenesis of various vascular diseases. Large amounts of ATP are released from injured cells, and ATP and adenosine have potent actions on smooth muscle and endothelial cell growth, migration, proliferation, and death. ATP and UTP acting via $P2Y_2$ receptors regulate endothelial inflammation and angiogenesis.[192] P2Y receptors may play a role in late postangioplasty restenosis.

A_{2B} receptor activation stimulates angiogenesis in human microvascular endothelial cells.[193] A_{2B} receptor agonists inhibit neointimal lesion development after arterial injury in apolipoprotein E–deficient mice. Adenosine promotes wound healing and mediates angiogenesis in mice in response to tissue injury via A_{2A} receptors. Adenosine stimulation of VEGF via adenosine receptors may present a potential therapeutic target for regulation of angiogenesis. Adenosine, via A_{2A} receptors, downregulates the production by human macrophages of a VEGF receptor (sFlt-1), a potent antiangiogenic factor. Adenosine is an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. Systemic administration of CD39 reduces injury-induced platelet deposition and leukocyte recruitment and stops neointimal hyperplasia. A review focusing on the vascular actions of P2X receptors in renal injury is available.[194]

Thrombosis, Inflammation, and Stroke

Extracellular nucleotides are mediators of vascular inflammation and thrombosis. $P2Y_{12}$ receptor antagonists reduce thromboxane levels. Clopidogrel is a $P2Y_{12}$ antagonist that inhibits platelet aggregation and is used for the treatment of thrombosis and stroke[195] (Figure 6). Other $P2Y_{12}$ antagonists have been developed as antithrombotics, such as ticlopidine, cangrelor, ticagrelor, prasugrel, elinogrel, BX 667, and PSB 0739. There is an association of haplotype H2 gene variants of the $P2Y_{12}$ receptor with lower risk of ischemic stroke and deep venous thromboembolism/pulmonary disease. $P2Y_1$ receptor antagonists are also antithrombotic agents and have been recommended as an alternative or complement to current $P2Y_{12}$ antiplatelet strategies.[196] Synergistic effects of $P2Y_1$ and $P2Y_{12}$ ADP activated receptors have been recommended as a novel approach to rapidly attenuate platelet-mediated thrombosis.[197] Adenosine, acting via A_{2A} and A_3 receptors, has antithrombotic effects, perhaps by blocking induction of circulating tissue factor. CD39 mediates resistance to occlusive arterial thrombus formation after vascular injury in mice. P2X7 receptors are prothrombotic and genetic knockout of the P2X7 receptor gene is protective in a mouse model of coronary artery thrombosis.

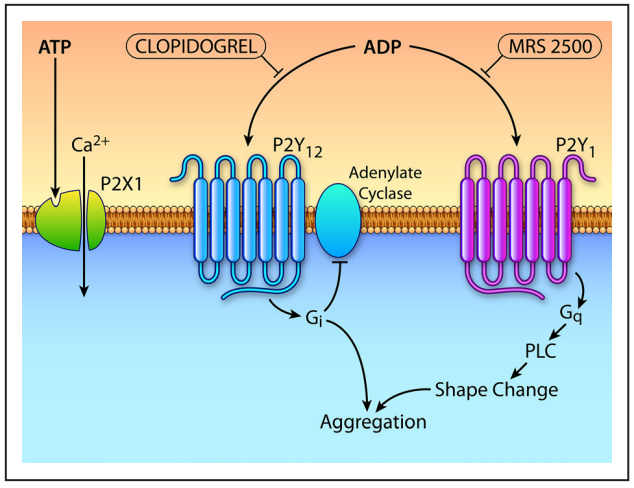

Figure 6. Three P2 receptor subtypes, P2X1, $P2Y_1$, and $P2Y_{12}$, are involved in ADP-induced platelet activation. Clopidogrel is a $P2Y_{12}$ receptor blocker that inhibits platelet aggregation and is in highly successful use for the treatment of thrombosis and stroke. A $P2Y_1$ receptor antagonist, MRS 2500, inhibits shape change.[234] Illustration Credit: Ben Smith.

After exposure to inflammatory stimuli, human microvascular endothelial cells show a selective induction of $P2Y_6$ receptors and inflammatory responses, because of lipopolysaccharide treatment in vivo, are attenuated in $P2Y_6$ knockout mice or after $P2Y_6$ antagonist treatment. This suggests that the $P2Y_6$ receptor may be a therapeutic target for systemic inflammatory responses. Reduction of inflammatory cytokines by glibenclamide is dependent on P2X7 receptor activation of monocytes by release of ATP from erythrocytes during hypoxia. High levels of circulating nucleotides may affect the development of inflammatory diseases by promoting an injury response in vascular tissues. In P2 receptor knockout mice, there is reduction of inflammatory diseases. $P2Y_1$ receptors control leukocyte recruitment in allergic inflammation in mice.[198]

Review articles have been published on various aspects of purinergic signaling in thrombosis and inflammation, including the use of the $P2Y_{12}$ receptor antagonists clopidogrel, prasugrel, cangrelor, and ticagrelor[199,200]; platelets and inflammation[201]; and P2Y receptor polymorphisms and disease.[202]

Diabetic Vascular Disease

In streptozotocin diabetic rats, there is prejunctional A_1 receptor–mediated impairment of sympathetic neurotransmission and impaired ATP-mediated endothelial vasorelaxant function in mesenteric arteries.[203] Vasodilatation to ATP, UTP, and adenosine is attenuated in the skeletal muscle circulation of patients with type 2 diabetes mellitus. In erythrocytes from humans with type 2 diabetes mellitus, ATP release is impaired, consistent with the hypothesis that a defect in erythrocyte physiology could contribute to diabetic vascular disease. There is an increase in vascular smooth muscle cells in diabetes mellitus patients and higher rates of restenosis after coronary angioplasty. P2X7 receptors on monocytes may be involved in the pathological changes of type 2 diabetes mellitus, particularly in patients with high C-reactive protein levels.[204] Up_4A is a vessel constrictor and is associated with diabetes mellitus.[205] Adenosine-insulin signaling in fetoplacental endothelial dysfunction in gestational diabetes mellitus has been reviewed.[206] Enhanced A_{2A} receptor–mediated increase in coronary flow in type I diabetic mice has been reported.[207] The vascular K_{ATP} channel is organ protective in diabetes mellitus.[208] The authors suggest that therapeutic

interventions to maintain functional K_{ATP} channels may help to lower or prevent diabetic organ dysfunction.

Migraine

There are 2 distinct cerebrovascular phases associated with vascular pain: an initial vasoconstriction (not associated with pain), followed by vasodilatation (reactive hyperemia) associated with pain in migraine. A purinergic hypothesis for migraine was put forward in 1981.[209] It was proposed that ATP and its breakdown product adenosine may mediate the vasodilatation during reactive hyperemia associated with pain after the initial vasospasm. It was also suggested that ATP stimulation of P2 receptors on primary afferent nerve terminals located in the adventitia of the cerebral microvasculature was involved in migraine pain. Later studies showed that ATP-induced cerebral vasodilatation was endothelium dependent via activation of P2X and P2Y receptors, resulting in release of endothelium-derived relaxing factors. These findings extended the purinergic hypothesis for migraine in 2 ways. First, they identified the mechanism of purinergic vasodilatation during the headache phase of migraine. Second, they suggested that a purinergic mechanism may also be involved in the initial local vasospasm, via P2X receptors on smooth muscle cells activated by ATP, released either as a cotransmitter from perivascular sympathetic nerves or from damaged cells.[210] Evidence in support of the purinergic hypothesis was presented where it was shown that decreased platelet ATP release was a marker for migraine, reflecting purinergic hypofunction, resulting in a greater tendency for vasoconstriction that predisposes to migraine attacks. Further support came from the identification of P2X3 receptors on primary afferent nerve terminals supplying cerebral vessels arising from trigeminal, nodose, and spinal ganglia.[148,211] Data were also presented recently that was consistent with the purinergic hypothesis of migraine pain.[212] In migraine, peripheral sensitization in the dura-vascular sensory pathway via P2X3 receptors occurs. P2X3 receptor antagonists have been suggested as potential candidates for antimigraine drug development.[213] Calcitonin gene-related peptide, released during migraine attacks from trigeminal neurons, results in sensitization of trigeminal P2X3 nociceptive receptors. The nonsteroidal anti-inflammatory drug, naproxen, widely used for the treatment of migraine pain, was shown to block P2X3 receptor–mediated responses of rat trigeminal neurons. Migraine may also involve a chronic sympathetic nervous system disorder, where there is an increase in release of sympathetic cotransmitter ATP, perhaps contributing to the initial vasospasm. Neutralization of nerve growth factor induces plasticity of ATP-sensitive P2X3 and P2X2/3 receptors of nociceptive trigeminal ganglion neurons involved in migraine. Cultures of the trigeminal ganglia from the knockin mouse genetic model of familial hemiplegic migraine are neuroinflammatory, perhaps facilitating release of ATP to activate P2X3 receptors and amplify nociceptive signals by trigeminal sensory neurons.

Adenosine has also been claimed to be involved in migraine. Infusion of adenosine caused migraine-like symptoms and withdrawal from the use of adenosine receptor antagonists caffeine and theophylline also caused migraine-like symptoms. Clinical trials with dipyridamole, an adenosine uptake inhibitor that increases extracellular adenosine, had to be stopped because of increased migraine attacks in all patients. Overactive glial P2Y receptors may contribute to pain transduction in migraine.

Reviews discuss the role of purinergic signaling in the cause of migraine and the therapeutic potential of purinergic agents.[214,215]

Sepsis and Septic Shock

Degradation of adenine nucleotides is related to irreversibility in hemorrhagic shock.[216] ATP may prevent disruption of glucose homeostasis and development of endotoxin shock by counteracting insulin and blunting hypoglycemia. It was claimed that treatment of shocked animals with ATP-$MgCl_2$ is an effective therapy for experimental hemorrhagic shock although this has been debated. In animal models of sepsis, treatment with ATP-$MgCl_2$ prevents endothelial dysfunction, reduces organ damage, restores immune competence, and increases survival. In the early hemodynamic changes associated with sepsis, a role for adenosine has been claimed. Regional hemodynamic responses to adenosine are altered after lipopolysaccharide treatment in conscious rats.

Calcific Aortic Valve Disease

ATP acts as a survival signal and prevents mineralization of aortic valve that occurs in calcific aortic valve disease.[217] Released ATP causes the survival of valvular interstitial cells in the aortic valve via $P2Y_2$ receptors and a high level of membrane-bound ectonucleotidase ecto-nucleotidase pyrophosphatase 1 is expressed in calcific aortic valve disease. Inhibition of ectonucleotidase with ARL67156 prevented the development of calcific aortic valve disease in warfarin-treated rats. Up_4A activation of P2Y receptors enhanced vascular calcification in vitro.

Blood Cell Diseases

A role of ATP released from erythrocytes in vascular regulation has been suggested to have predictive value in disease processes. Abacavir is linked to cardiovascular disease, and ATP has been shown to play a role in leukocyte accumulation induced by abacavir, via P2X7 receptors.[218] Erythrocyte ATP release is sensitive to increase in temperature. This raises the possibility of treatment of patients with peripheral vascular disease, by using local heating to stimulate erythrocyte ATP release to increase flow and oxygen to limbs. Purinergic signaling inhibits human acute myeloblastic leukemia cell proliferation, migration, and engraftment in immunodeficient mice, via P2X7 and $P2Y_2$ and $P2Y_4$ receptors.[219]

Infection with the malaria protozoan parasite, *Plasmodium falciparum*, induces osmolyte and anion channels in the host erythrocyte membranes involving ATP release. ATP released by the rupture of erythrocytes during the blood-stage of *P. chabaudi* malaria induces an increase in the expression of P2X7 receptors on $CD4^+$ T cells. The ectoenzymes, CD39, CD73, and ADA, on the surface of platelets decreased in rats infected by *Trypanosoma evansi*. A review is available concerned with malaria-infected erythrocytes and purinergic signaling.[220]

Hemolysis mediated by leukotoxin, a virulence factor secreted by some bacteria, is potentiated by ATP release and P2X receptor activation of human erythrocytes. Antagonists

to P2X1 and P2X7 receptors and apyrase inhibit the virulence factor exotoxin α-hemolysin–induced lysis of erythrocytes. Therefore, selective P2X receptor antagonists may ameliorate symptoms during sepsis with hemolytic bacteria. *Escherichia coli* α-hemolysin causes ATP release and P2 receptor–mediated Ca^{2+} influx in human erythrocytes through the toxin pore.

In type 2 diabetes mellitus, erythrocytes are less deformable leading to lowered levels of deformation-induced ATP release. A combination of C-peptide–mediated rescue of low O_2-induced ATP release from erythrocytes and insulin may help in the prevention and treatment of peripheral vascular disease associated with diabetes mellitus.[221]

Adenosine is a potentially important therapeutic target for the treatment and prevention of sickle cell disease.[222] However, a complication is that adenosine signaling also induces hemoglobin S polymerization, promoting sickling, vasoocclusion, hemolysis, and organ damage. Circulating adenosine levels are elevated in pregnant women with sickle cell disease.[223] Amyloid β peptide inhibits ATP release from deoxygenated erythrocytes by activating red cell caspase 3, suggesting that there may be a pathophysiologic role for vascular amyloid peptide in Alzheimer disease.

Perspectives and Future Directions

It is clear from this review that purinergic signaling is involved in different ways in both the physiology and pathophysiology of the cardiovascular system. ATP is released as a cotransmitter from nerves and as an autocrine or paracrine messenger from non-neuronal cells in the heart. Both P1 and P2 receptors play multiple roles in cardiac physiology and pathophysiology. Many cardiovascular diseases involve inflammation, which involves purinergic signaling, especially release of inflammatory cytokines via P2X7 receptor activation.[224,225] Micro-RNAs, which modulate purinergic signaling, are gaining interest as putative novel disease biomarkers and therapeutic targets.[226]

The physiological and pathophysiological roles of purinergic signaling in blood vessels are clearer, and several important conclusions can be drawn from this review about purinergic signaling in the vasculature. Purinergic signaling plays a major role in control of both vascular tone and remodeling. Vascular tone is regulated by a dual control balance between ATP release as a cotransmitter during sympathetic nerve vasoconstrictor activity and ATP release from endothelial cells mediating vasorelaxation via endothelium-derived relaxing factor, mostly NO. The actions of other locally released purine and pyrimidine nucleotides (ADP, UTP, and UDP) and by adenosine also influence vascular tone. Adenosine acting via P1 receptors is predominantly a vasodilator, acting predominantly on smooth muscle via A_2 receptors but with some actions via A_2 receptors on endothelial cells. Contractile P2X1 receptors are dominantly expressed on the smooth muscle of all blood vessels. Adenosine and purine and pyrimidine nucleotides elicit long-term (trophic) signaling, producing cell proliferation, differentiation, and death in angiogenesis and regeneration of damaged vessels. A_2 and $P2Y_1$ receptors mediate proliferation of endothelial cells, whereas stimulation of A_2 and $P2Y_2$/$P2Y_4$ receptors results in proliferation of smooth muscle cells.

Most of the therapeutic strategies for the variety of heart disorders based on the manipulation of purinergic signaling are not yet well defined for most cardiac diseases, and the side effects of treatments need to be considered and strategies to overcome them defined. Newly developed stable, small molecules that act as selective agonists and antagonists at purinergic receptors, which are orally bioavailable and are stable in vivo, as well as novel nucleotidase inhibitors and ATP transport blockers, are likely to be a major step forward toward resolving these problems. Inhibitors of ATP release may also enhance our understanding of the relevant mechanisms and the genetic variations in response to purinergic compounds. Immunologic factors are attracting increasing attention and should also be taken into account.[227] Human embryonic stem cells are pluripotent cells with the properties of self-renewal and differentiation potential into various cell types, including cardiovascular progenitor cells. This in vitro differentiation system is being explored for cardiac regenerative therapy.[228]

All cells in the vascular system express one or more types of purine or pyrimidine receptors, so this raises the possibility that purine receptors may be potential targets in vascular disease.[24,229] ATP release mechanisms, receptors, and ectonucleotidases are all potential targets for drug development for treatment of vascular diseases such as hypertension, atherosclerosis, and thrombosis. Clopidogrel and other $P2Y_{12}$ receptor antagonists are widely used antithrombotic drugs. Of promise seem to be the development of A_3 agonists to protect against ischemia-reperfusion injury. The importance of ATP as a sympathetic cotransmitter in arteries of spontaneously hypertensive and obese rats is becoming recognized. Therefore, antagonists at smooth muscle P2X1 receptors could be beneficial in these diseases. The drawback is that P2X1 receptors are widely distributed. For example, P2X1 genetic knockout mice show male infertility and an increase in blood pressure. Characterization of vascular smooth muscle P2X and P2Y receptor subtypes and endothelial $P2Y_2$, $P2Y_4$, $P2Y_6$, and P2X4 receptors is needed. There are differences in purinoceptor subtype expression in different blood vessels, related to their physiological roles. The heroic efforts of the medicinal chemists working in this field are leading to a promising emergence of subtype-specific P2 ligands that can be used orally, which will open up new avenues for research into their therapeutic potential for the treatment of cardiovascular disorders. Therapeutic strategies involving purinergic signaling are being developed for the treatment of heart failure, hypertension, atherosclerosis, and cardiovascular cancers.

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None.

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