All cells in the cardiovascular system express one or more subtypes of purine receptors making them possible targets for therapeutic strategies in cardiovascular diseases.

Involvement of Purinergic Signaling in Cardiovascular Diseases

by Vera Ralevic and Geoffrey Burnstock

Summary
Purine receptor subtypes are expressed with some selectivity on different types of cells in the cardiovascular system and are possible targets for therapeutic strategies in cardiovascular diseases. The considerable efforts of the clinical biochemists working in this field is leading to a promising emergence of subtype-specific P2 ligands. This will open up new avenues for research into the physiological roles of purine receptors and their therapeutic potential. © 2003 Prous Science. All rights reserved.

All cells in the heart and blood vessels express one or more subtypes of purine receptors for extracellular adenosine, ATP, ADP, UTP and UDP. P1 receptors, of which there are four subtypes (A_1, A_{2A}, A_{2B}, A_3), are activated by adenosine.[1] There are two distinct families of receptors for purine and pyrimidine nucleotides: P2X ligand-gated ionotropic channels and P2Y metabotropic G-protein-coupled receptors.[2] There are currently eight members of the P2X receptor family ($P2X_{1-8}$) and seven members of the P2Y receptor family ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$ and $P2Y_{13}$)[1,3,4]. These subtypes are expressed with some selectivity on different types of cells in the cardiovascular system, and are possible targets for therapeutic strategies in cardiovascular diseases. Figure 1 illustrates the distribution of P1 and P2 receptors in blood vessels.

Roles of purines and pyrimidines in the cardiovascular system

The extracellular effects of purines in the cardiovascular system have been known for many years.[5-7] The first suggestion that purines acted in a cardioprotective role came with the demonstration that adenosine mediated vasodilatation during hypoxia to increase blood flow and thus maintain oxygen delivery to the heart.[8,9] It is now clear that adenine and pyrimidine nucleotides are released from the heart during hypoxia,[10-12] particularly from endothelial cells,[13,14] and act on P2Y receptors on endothelial cells to produce nitric oxide (NO) and subsequent vasodilatation, before breaking down to adenosine.[15-17] Furthermore, the cardioprotective effects of purine nucleosides and nucleotides involve not only their actions as potent vasodilators, but also effects on platelet aggregation and trophic actions on smooth muscle and endothelial cells.[18,19] It follows that perturbation of these functions may be involved in some cardiovascular diseases, and indeed there is some evidence for altered levels of expression of P2 receptors and purine receptor signaling in some conditions.

In most blood vessels, $P2X_1$ receptors are the principal subtype of P2X receptor expressed on the smooth muscle and mediate contraction; they are involved in fast excitatory neurotransmission, being activated by ATP released as a cotransmitter with noradrenaline from perivascular sympathetic nerves.[20] Contractile $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors and vasorelaxant A_2 receptors are also expressed on vascular smooth muscle.[1,21] Some vascu-

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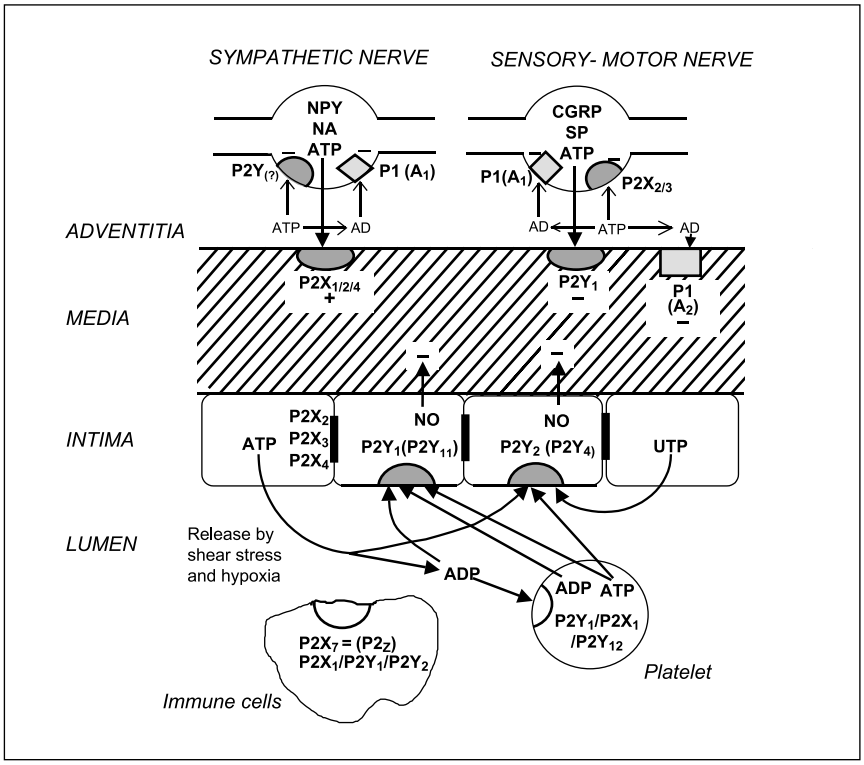

Fig. 1. Short-term (acute) purinergic signaling controlling vascular tone. Schematic illustrating the main receptor subtypes for purine and pyrimidines present in most blood vessels. Perivascular nerves in the adventitia release ATP as cotransmitter: ATP is released with noradrenaline (NA) and neuropeptide Y (NPY) from sympathetic nerves to act on smooth muscle $P2X_1$- and in some vessels $P2X_2$ and $P2X_4$ purinoceptors, resulting in vasoconstriction; it is released with calcitonin gene-related peptide (CGRP) and substance P (SP) from sensory nerves during 'axon reflex' activity to act on smooth muscle P2Y-purinoceptors resulting in vasodilatation. P1 (A1)-purinoceptors on nerve terminals of sympathetic and sensory nerves mediate adenosine (arising from enzymatic breakdown of ATP) modulation of transmitter release. $P2X_3$ purinoceptors are present on a subpopulation of sensory nerve terminals. P1 (A_2)-purinoceptors 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 $P2Y_4$ purinoceptors leading to the production of NO and subsequent vasodilatation. ATP, following its release from aggregating platelets, also acts on these endothelial receptors. Blood-borne platelets possess $P2Y_1$ and $P2Y_{12}$ ADP-selective purinoceptors as well as $P2X_1$ receptors, while immune cells of various kinds possess $P2X_7$, as well as $P2X_1$, $P2Y_1$ and $P2X_2$ purinoceptors. $P2X_2$, $P2X_3$ and $P2X_4$ receptors have also recently been identified on endothelial cell membranes. (Reproduced with permission from Burnstock, G. *Development and perspectives of the purinoceptor concept* . J Auton Pharmacol 1996, 16: 295-302.)

lar smooth muscle cells, notably those in coronary arteries of a variety of species, also express vasorelaxant P2Y receptors ($P2Y_1$-like).[22-26] P2Y and A_2 receptors expressed on smooth muscle cells additionally have roles in control of cell proliferation (Fig. 2).[19]

The vascular endothelium expresses principally $P2Y_1$, $P2Y_2$ and A_2 receptors, which mediate vasorelaxation to ADP, UTP/ATP and adenosine, respectively, following the release of the purines and pyrimidines from the endothelium, platelets and erythrocytes.[1] P2Y and A_2 receptors expressed on endothelial cells are also involved in control of cell proliferation (Fig. 2).[19] Recent evidence suggests that P2X receptors are also expressed on the endothelium.[27-30] There is evidence that shear stress causes Ca^{2+} influx via activation of $P2X_4$ receptors on human endothelial cells.[31-33] The function of these endothelial P2X receptors is uncertain, but roles in cell permeability and adhesion[34] have been proposed.

The nerves in the adventitia of blood vessels express purine receptors. Inhibitory A_1 receptors are expressed on sympathetic and sensory nerves, inhibitory P2Y receptors are expressed on some sympathetic nerves, and heteromeric $P2X_{2/3}$ receptors, which may be involved in nociception, have been described on sensory nerves.[1,35]

In the heart, adenosine is a potent coronary vasodilator and causes tachycardia and slows atrioventricular node conductance.[36] It inhibits neutrophil activation, reduces infarct size and preserves endothelial function during inflammation and is also involved in preconditioning via activation of A_1 and A_3 receptors. All four subtypes of adenosine receptor have been localized in the heart;[1,37] the A_1 receptor is associated with the negative cardiac inotropic, chronotropic and dromotropic effects of adenosine, while A_2 receptors mediate coronary vasodilatation.[36,38] Mice lacking the adenosine A_{2A} receptor were found to have an increase in heart rate and high blood pressure (as well as being more aggressive and hypoalgesic), identifying a key role of the A_{2A} receptor in controlling cardiovascular function.[39]

ATP generally elicits positive inotropic effects, and upon rapid application to cells induces various forms of arrhythmia.[40] It elicits a variety of effects on single cardiomyocytes including activation of nonspecific depolarizing cationic and Cl^- currents, increases and decreases in Ca^{2+} currents, activation of inwardly rectifying currents and outward K^+ currents, an increase in cAMP, and activation of tyrosine kinases to produce Cl^-/HCO_3^- exchange and large transient acidosis.[40] Other possible roles of ATP in the heart include hypertrophy, preconditioning and apoptosis. Which of the several subtypes of P2X and P2Y receptors expressed in the heart[41-47] is associated with each of these effects is currently unclear.

The $P2Y_{12}$ receptor is found almost exclusively on platelets and is responsible (together with the $P2Y_1$ and $P2X_1$

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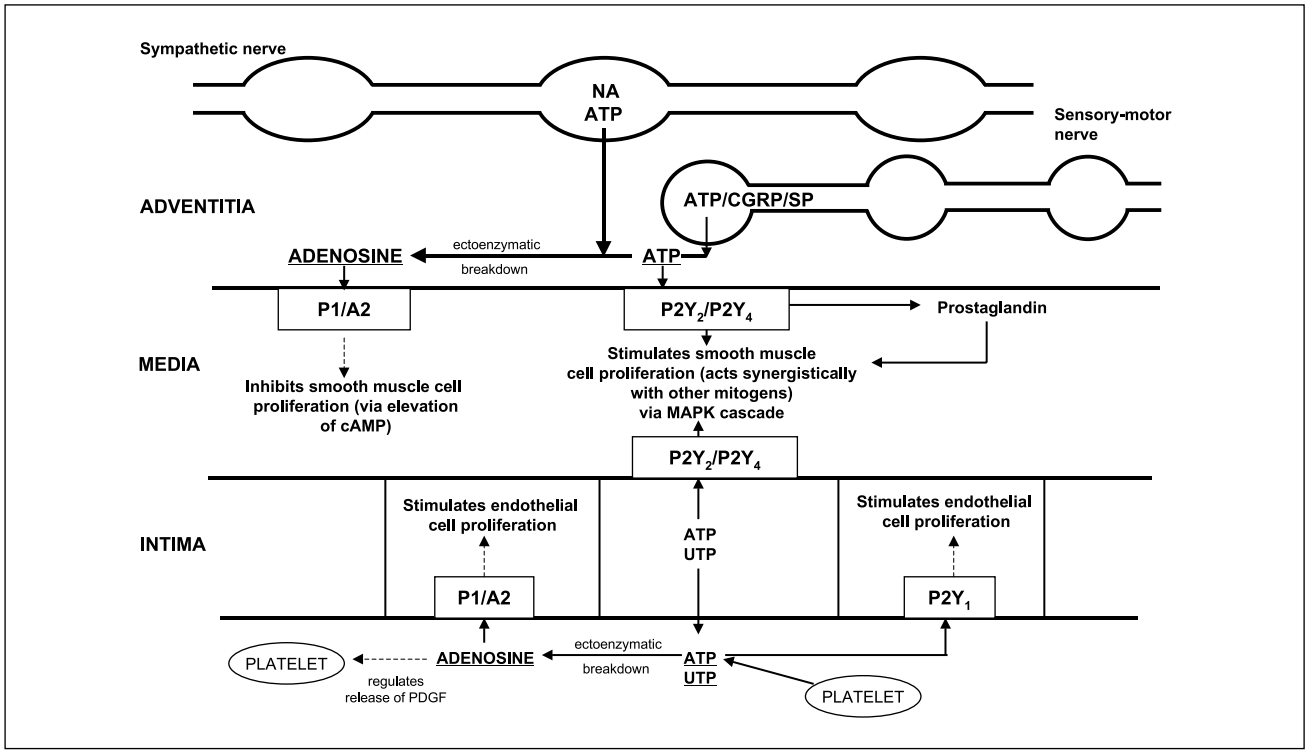

Fig. 2. Schematic of long-term (trophic) actions of purines released from nerves, platelets and endothelial cells (which also release U TP) acting on P2 receptors to stimulate or inhibit cell proliferation. ATP released as a cotransmitter from sympathetic nerves and s ensory-motor nerves (during axon reflex activity) stimulates smooth muscle cell proliferation via $P2Y_2$ and/or $P2Y_4$ receptors via a MAPK cascade, while adenosine resulting from enzymatic breakdown of ATP acts on P1 (A2) receptors to inhibit cell proliferation (via elevation of cA MP). ATP and UTP released from endothelial cells stimulate both endothelial and smooth muscle cell proliferation via $P2Y_1$, $P2Y_2$ and $P2Y_4$ receptors. Adenosine resulting from ATP breakdown acts on P1 (A2) receptors to stimulate endothelial cell proliferation and regulate releas e of PDGF from platelets. (Reproduced with permission from Burnstock, G. *Purinergic signalling and vascular cell proliferation and death.* Arterioscler Thromb Vasc Biol 2002, 22: 364-73.)

receptors) for mediating the aggregatory properties of adenine nucleotides.[3,18,48]

Ectonucleotidases

Once released, ATP is rapidly degraded to ADP, AMP and adenosine by the actions of a family of ectonucleotidases[49-51] and adenosine is further rapidly metabolized via a series of breakdown products to, ultimately, uric acid. The degradation of ATP and adenosine is extremely rapid.[52,53] Ectonucleotidases are situated at the surface of cells and may also be co-released with ATP, for example from sympathetic nerves[54] and endothelial cells.[55] Not only does this limit the actions of ATP at P2 receptors by removing it through enzymatic breakdown, but it can evoke opposite effects via the actions of the breakdown products ADP and adenosine at P2Y and P1 receptors, respectively. When ATP is released from cells there is likely a cascade of events involving its metabolic breakdown products and activation of multiple subtypes of purine receptors.

Purinergic signaling in cardiovascular diseases: Therapeutic approaches

Until recently, exploiting cardiovascular purine P2 receptors as therapeutic targets has been limited by the lack of subtype-specific ligands for these receptors. There is evidence, however, that this is changing. For example, $P2Y_{12}$ receptor antagonists are an evolving therapeutic strategy as antithrombotics in cardiovascular disease,[56,57] while a highly specific $P2Y_1$ receptor antagonist, MRS-2279,[58] has recently been developed, holding promise that this or future compounds may find application as antithrombotics, as well as providing new insights into the physiological role of the $P2Y_1$ receptor. In addition, TNP-ATP is a powerful antagonist for the $P2X_1$ receptor that mediates vasoconstriction.[59]

Antithrombotics

This is a success story in the use of purine receptors as therapeutic targets. ATP and ADP are involved in many of the hemostatic mechanisms that become prominent at sites of vascular injury. When collagen becomes exposed due to endothelial cell damage, platelets begin to adhere to it, aggregate and release AT; this is broken down to ADP, which promotes further aggregation via activation of $P2Y_{12}$ receptors.[3,18,60] ADP can also act on $P2Y_1$ receptors, either on endothelial cells or on exposed vascular smooth muscle cells, and thereby evoke dilatation. Platelet aggregation is inhibited by ATP and certain

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adenine dinucleotides, acting as competitive antagonists on the platelet $P2Y_{12}$ receptor, so high local concentrations of these compounds arising as a result of degranulation could serve to control the extent of aggregation. The $P2Y_{12}$ receptor is the molecular target of the antithrombotic drugs clopidogrel and ticlopidine[3,56,61-63] and $P2Y_1$ receptor antagonists are currently being explored as antithrombotic agents by several pharmaceutical companies. A $P2X_1$ receptor is also present on platelets that modulates calcium influx, but its functional significance is unclear.

A recent study has shown that ATP causes platelet aggregation in whole blood, but not in platelet-rich plasma, implying that ectonucleotidases normally present in whole blood have a significant role in converting the ATP to ADP, which is active at platelet $P2Y_1$ and $P2Y_{12}$ receptors; it is likely that the levels of these ectonucleotidases vary between individuals, with the possibility that levels are low in individuals with impaired blood clotting (S. Heptinstall, personal communication). Increasing or decreasing ATP breakdown could be exploited to alter the balance of purine effects in the cardiovascular system, and in this respect ectonucleotidases are a novel drug target in cardiovascular disease.

Supraventricular tachycardia

Slowing of the heart rate occurs via A_1 receptors on sinoatrial and atrioventricular nodes, causing bradycardia and heart block, respectively, while the inotropic effects include a decrease in atrial contraction and action potential duration.[36] This aspect of A_1 receptor activation has found application in the clinical use of adenosine to treat supraventricular tachycardia,[64] and in the use of adenosine receptor antagonists in the treatment of bradyarrhythmias. ATP has a similar efficacy to adenosine, likely acting via adenosine following metabolic breakdown.[65]

Adenosine has been used as a diagnostic tool of wide QRS complex tachycardias where the mechanism is uncertain.[66-69] Wide complex ventricular tachycardia is often misdiagnosed as wide complex supraventricular tachycardia with aberrancy, and the use of verapamil in misdiagnosed patients can lead to severe hypotension and cardiac arrest.[67-69] Adenosine can help to differentiate between these conditions because of its specific action on AV nodal conductance.

A recent report has described that the gap junctions in the intercalated disks between human cardiac muscle show some colocalization and some alternate immunostaining for connexin43 and $P2X_1$ (L. Jiang, personal communication).

Ischemia and reperfusion

During ischemia there is an accumulation of adenosine in the ischemic tissue which may exert protective effects during the reperfusion period via cardiovascular, metabolic and anti-inflammatory effects. Adenosine has, therefore, been used as a cytoprotective agent during myocardial ischemia and reperfusion, although the exact mechanism by which it exerts its beneficial effects is still unclear.[70-72] There is evidence that A_1 receptors are involved, at least in the heart.[73-80] The mechanism may involve A_1 receptor activation of K_{ATP} channels, as infarct size reduction after activation of A_1 receptors is abolished by the blockade of K_{ATP} channels.[73,81,82]

There is a recent report that in congestive heart failure in rats there is an increase by 2.7-fold and 4.7-fold of $P2X_1$ and $P2Y_2$ mRNA levels, respectively.[83]

Hypertension, diabetes and blood pressure regulation

There is evidence that ATP plays a significantly greater role as a sympathetic cotransmitter in spontaneously hypertensive rats.[84-86] Defective prejunctional P1 receptor-mediated modulation has been claimed to contribute to enhanced sympathetic neurotransmission in hypertension.[87-89] Constriction of the isolated kidney to renal nerve stimulation was increased in spontaneously hypertensive rats compared with controls and appeared to be entirely due to ATP released from sympathetic nerves.[90] In the kidney of a transgenic, hypertensive model, the glomeruli show an abundance of $P2X_7$ immunostaining and a similar expression of $P2X_7$ receptors has been found in the glomeruli of diabetic rats with kidney damage.[91]

Intravenous infusion of ATP in humans has been investigated with regard to its therapeutic use in patients with chronic obstructive pulmonary disease.[92] Pulmonary hypertension can be a major problem after thoracic surgery in patients with chronic obstructive pulmonary disease. Low-dose intravenous ATP or adenosine have been used as vasodilators under these conditions as they have been shown to have predominantly pulmonary vasodilating effects,[93,94] an advantage over vasodilators with predominant systemic effects. Pulmonary hypertension can also be a serious problem in children after surgical repair of congenital heart defects, and in this respect intravenous ATP produced a reduction or disappearance of the pulmonary hypertensive crisis without a change in mean systemic arterial pressure and systemic vascular resistance.[95]

Because it is a potent vasodilator 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.[69,96] 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.

Atherosclerosis and restenosis

Adenosine and ATP have a number of cardiovascular protective effects in addition to vasodilatation, including the promotion of endothelial and smooth muscle cell proliferation and an increase in the expression of vascu-

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lar endothelial growth factor (VEGF) mRNA (Fig. 2), which play an important role in the development of intimal thickening during arterial diseases, such as atherosclerosis, and in restenosis after angioplasty, and in the growth of new vessels that takes place during wound healing and in tumors.[19] Hypoxia is an important stimulus to vascular growth and it is believed that adenosine and ATP, both of which are released from cells during hypoxia, have important roles as mediators of blood vessel growth.

Endothelial cell proliferation is mediated by A_{2A} and A_{2B} receptors, and some of the mitogenic effects are mediated via the modulation of VEGF signalling. There is evidence that A_{2B} receptors inhibit the growth of human aortic smooth muscle cells.[97] ATP and ADP stimulate DNA synthesis and cell proliferation of porcine aortic smooth muscle cells[98] via activation of P2Y receptors. UTP also has powerful mitogenic actions on vascular smooth muscle, and since the mitogenic effects of UTP and ATP were approximately equipotent,[99,100] this would suggest that the receptor involved is either a $P2Y_2$ or $P2Y_4$ subtype. There was upregulation of vascular smooth muscle $P2Y_2$ receptors by MAPK kinase-dependent growth factor, which the authors suggested may be important in atherosclerosis and neointimal formation after balloon angioplasty.[47] ATP and ADP have also been shown to stimulate endothelial cell migration and proliferation,[101,102] probably via P2Y receptors.

Apoptotic cell death is recognized to occur in a number of vascular diseases, including atherosclerosis, restenosis and hypertension.[103,104] ATP releases histamine from mast cells and releases inflammatory cytokines such as IL-1 from immune cells.[105] In addition, occupation of P2 receptors leads to prostaglandin and COX-2 synthesis,[106] both involved in inflammatory processes. Vascular endothelial cells are continuously exposed to variations in blood flow, which plays an important role in vessel growth or regression and in the local development of atherosclerosis. The shear stress leads to a substantial release of ATP and UTP from endothelial cells[16] and these purines might mediate alterations in the balance between proliferation and apoptosis.[107]

In restenosis following balloon angioplasty, there is a peak in the proliferation and apoptosis of vascular smooth muscle at about 14 days.[108] The first balloon inflation during coronary angioplasty is a preconditioning stimulus leading to a decrease in ischemia in later inflations; intracoronary adenosine administration before coronary angioplasty modifies the preconditioning effect of the first inflation.[109]

Vascular inflammation

Adenosine is released at sites of inflammation and has antiinflammatory effects via multiple mechanisms. It inhibits neutrophil rolling and adhesion to vascular endothelium, decreases oxygen-free radical production by neutrophils via activation of A_{2A} receptors and also has effects on endothelial cell permeability via A_1 and A_{2A} receptors.[110] Adenosine also inhibits macrophage production of the proinflammatory cytokine tumor necrosis factor-α (TNFα) and suppresses TNFα mRNA expression and plasma levels.[110] A recent study in knockout A_{2A} mice showed an important role of A_{2A} receptors in preventing liver damage in inflammation and maintaining lower levels of serum TNFα.[111] P2 receptors can also be involved in inflammation, as ATP induces a localized inflammatory response in rat paw.[112]

Vascular pain

It has been suggested that in vascular pain, including migraine, angina, pelvic pain and ischemic pain, that ATP released from endothelial cells during the reactive hyperemia following vasospasm (not associated with pain) diffuses through the wall of microvessels to reach $P2X_3$ receptors on sensory perivascular nerves to initiate impulses that travel via the spinal cord to pain centers in the brain.[35] Furthermore, ATP activation of $P2Y_1$ receptors potentiates vanilloid VR1 currents evoked by capsaicin or protons and reduces the temperature threshold for activation of VR1.[113,114]

Adenosine alleviates neuropathic pain, hyperalgesia and allodynia,[115,116] an action which may be mediated by inhibitory A_1 receptors expressed on sensory nerves. Intravenous ATP in mice was found to have analgesic activity on hot plate and phenyl quinone-induced stretching assays.[117-119]

Conclusions

There is evidence that purines contribute to a number of processes involved in normal cardiovascular function, and that disturbances in purinergic signaling are involved in some cardiovascular diseases. As all cells in the cardiovascular system express one or more types of purine receptor, this raises the possibility that purine receptors may be potential targets in cardiovascular disease. Indeed, antagonists of $P2Y_{12}$ receptors are now used as antithrombotics, and P1 agonists for supraventricular tachycardia in humans. It is an advantage that there is some selectivity in the subtypes of P1 and P2 receptors that are expressed on different structures in the heart and blood vessels. However, the development and use of ligands with good subtype specificities that do not degrade *in vivo* is needed to exploit this. The considerable efforts of the clinical biochemists working in this field is leading to a promising emergence of subtype-specific P2 ligands. This will open up new avenues for research into the physiological roles of purine receptors and their therapeutic potential.

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Dr. Vera Ralevic is a Lecturer in Cardiovascular Sciences and Royal Society Research Fellow at the School of Biomedical Sciences, University of Nottingham Medical School, Queen's Medical Centre, United Kingdom. Prof. Geoffrey Burnstock, FRS, is Professor of Anatomy, Director of Autonomic Neuroscience Institute, Department of Anatomy and Developmental Biology, Royal Free and University College Medical School, London, United Kingdom;*
E-mail: g.burnstock@ucl.ac.uk;
Tel: +44(0)20-7830-2948;
Fax: +4 4(0)20-7830-2949.
**Correspondence.*

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