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Editorial Review

Cardiovascular pharmacology of purines

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1. This review focuses on the extracellular actions of ATP and adenosine, and in particular their role in cardiovascular regulation.
2. ATP serves as a co-transmitter within the sympathetic nervous system, and is also released from endothelium and aggregating thrombocytes. ATP acts on P2x purinoceptors on vascular smooth muscle cells to induce vasoconstriction. Stimulation of P2y purinoceptors on endothelial cells releases endothelium-derived relaxing factors and causes vasodilatation. This dual action of ATP may have pathophysiological importance by inducing vasospasm at sites of impaired endothelial function and thrombus formation.
3. Adenosine is generated by enzymic degradation of ATP. Its formation is enhanced during ischaemia. Adenosine inhibits noradrenaline release from sympathetic nerve endings, causes vasodilatation via endothelium-dependent and endothelium-independent actions, has important anti-arrhythmic properties and prevents deleterious sequelle of ischaemia. In humans, adenosine evokes a sympahto-excitatory reflex mediated by chemically sensitive receptors and afferent nerves in the kidney, heart and forearm. This reflex may be active during exercise and ischaemia and, because of its potential adverse consequences, it should be considered when developing new therapies to potentiate the anti-ischaemic actions of endogenous adenosine in humans. Adenosine appears to mediate ischaemia-induced pain; a reduced sensitivity to adenosine may underlie silent ischaemia.
4. New drugs that interact with adenosine formation or degradation or with adenosine receptors are under development. These have potential therapeutic application in the treatment of ischaemia and other circulatory disorders.

INTRODUCTION

The cardiovascular actions of extracellular nucleosides and nucleotides have been recognized since the beginning of this century [1], but a comprehensive understanding of their interstitial formation, degradation and mode of action and their (patho-)physiological significance has only recently been accomplished [2]. They appear to be involved in modulation of central nervous system actions, immune function, thrombocyte aggregation and regulation of renal and gastrointestinal homeostasis, and may influence virtually every body function through their interaction with the autonomic nervous system [3]. This review will focus on the extracellular actions of two purines: adenosine and ATP, and in particular their role in the modulation of the cardiovascular system. Although purine receptors in the brain may contribute to resuscitation, we consider these effects beyond the scope of this review.

FORMATION, RELEASE AND DEGRADATION OF ADENOSINE AND ATP

Within the cell, adenosine is primarily formed by two metabolic routes [4]. One is from ATP, by degradation, via ADP and AMP to adenosine. This pathway is accelerated when there is a mismatch between energy supply and demand and probably represents the main route of adenosine formation during ischaemia. The hydrolysis of AMP is catalysed by 5′-nucleotidase, which is present both in the intracellular (cytosolic form) as well as the extracellular space (membrane-bound form). In the second pathway, adenosine is formed by hydrolysis of ATP via S-adenosylmethionine to S-adenosyl-
homocysteine which in turn is hydrolysed to adenosine. This reaction is catalysed by S-adenosylhomocysteine hydroxylase. This route is oxygen insensitive and is probably of minor importance during ischaemic conditions. Adenosine diffuses through the cellular membrane between the extracellular and intracellular space. This bidirectional process [5] is gradient dependent and is facilitated by a nucleoside transporter that is located in the plasma membrane of many cells such as the endothelial cells, erythrocytes, cardiomyocytes and vascular smooth muscle cells.

Apart from a cellular source of adenosine, adenosine formation may occur from extracellular ATP that can be degraded rapidly to adenosine by ectonucleotidases which are widespread and present on the extracellular surface of vascular smooth muscle cells and endothelium [6]. The endothelium appears to play a key role in uptake and metabolism of luminal applied adenosine [7], but it is also a major source of endogenous adenosine nucleosides and nucleotides. The endothelial release of adenosine nucleosides and nucleotides is increased by β-adrenoceptor stimulation [6]. Noradrenaline-induced activation of 5'-nucleotidase may play a role in this interaction between endothelial adenosine formation and the sympathetic nervous system [8].

ATP is released by exocytosis from aggregating thrombocytes and from sympathetic nerve endings where it is co-released with noradrenaline [3, 9]. In most cells, cytoplasmic ATP concentrations are high and simple diffusion of ATP through the cell membrane may partly account for extracellular ATP. During increased blood flow [10], sympathetic nerve stimulation [11], or application of well-known vasodilators like bradykinin and acetylcholine [12], endothelial cells appear to release ATP. Cardiac ischaemia evokes an increase in plasma nucleotide concentration in the venous effluent which is underestimated by vascular ectonucleotidase activity [6].

During normal oxygenation, intracellular adenosine and AMP concentrations are low. In this situation, extracellular adenosine will be transported rapidly to the intracellular compartment where it is further inactivated by phosphorylation to AMP or deamination to inosine. An extracellular form of adenosine deaminase exists. Intracellular deamination after uptake is probably of greater importance in humans [13].

**PURINERGIC RECEPTORS**

Cardiovascular responses to purines are mediated by membrane-bound receptors that are subdivided into P1- and P2-purinergic receptors (Table 1) [14]. This subdivision is based on the potency order of agonists (ATP > ADP > AMP > adenosine and adenosine > AMP > ADP > ATP for the P2- and P1-purinergic receptor respectively), the P1- but not P2-purinergic receptor antagonistic properties of xanthine derivatives, and differences in intracellular second messengers [15].

P1-purinergic receptors were originally subdivided into A1 and A2 receptors. This differentiation has been verified by molecular techniques [16]. Both receptors are linked to G-proteins. A1-receptor stimulation evokes a Gi-mediated decrease in adenylyl cyclase activity with subsequent reduction in intracellular cAMP levels. A1 receptors are linked to other effector systems (phospholipase C, potassium and calcium channels [17, 18]). A1-receptor-mediated opening of KATP-channels [19] may play a role in the phenomenon of ischaemic preconditioning (see below). The A2 receptor is associated with a Gs binding protein and stimulation of this receptor increases adenylyl cyclase activity with a subsequent increase in intracellular cAMP levels. Recently, the existence of a distinct A3 adenosine receptor has been demonstrated [20]. This receptor is less sensitive to antagonism by methylxanthines and can be stimulated specifically by a synthetic adenosine derivative, N6-2-(4-aminophenyl)ethyladenosine (APNEA). Like the other adenosine receptors, the A3 receptor is coupled to a G-protein. Stimulation of the A3 receptor reduces the intracellular cAMP concentration. In pithed rats, intravenous infusion of APNEA evokes a hypotensive response which is mediated by mast cell degranulation [21]. In the rabbit heart this receptor has been implicated in ischaemic preconditioning (see below) [22]. Advances in molecular biology will probably reveal many other subclasses of the adenosine

<table>
<thead>
<tr>
<th>Main class</th>
<th>Most potent endogenous ligand</th>
<th>Subclass</th>
<th>Structure</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Adenosine</td>
<td>A1</td>
<td>Cloned</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A2</td>
<td>Cloned</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A3</td>
<td>Cloned</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>P2x</td>
<td>Cloned</td>
<td>Ligand-operated cation channel</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>P2y</td>
<td>Cloned</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>P2m</td>
<td>Cloned</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td>ADP</td>
<td>P1</td>
<td>Not known</td>
<td>G-protein coupled</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>P2</td>
<td>Not known</td>
<td>Non-selective pore</td>
</tr>
<tr>
<td></td>
<td>A1, A2</td>
<td>P3</td>
<td>Not known</td>
<td>Not known</td>
</tr>
<tr>
<td></td>
<td>A3, P1</td>
<td>P4</td>
<td>Not known</td>
<td>Not known</td>
</tr>
</tbody>
</table>

Table 1. Classification of purine receptors
receptor whose specific pharmacological or physiological effects, distinct from A1- or A2-receptor stimulation, will need to be elucidated.

The vascular P2-purinergic receptor has been subdivided into P2x- and P2y-purinergic receptors based on rank-order differences in agonist potencies and different vascular effects (Table 1) [23]. The existence of a separate vascular 'pyrimidine' or 'nucleotide' receptor with affinity for both ATP and UTP has also been suggested [24, 25]. Whether this represents a distinct, so-called P2x-purinergic receptor, or is a member of the 'P2x purinoceptor family' [26] depends on whether UTP acts as its endogenous ligand [27]. Based on pharmacological evidence, a further subdivision of P2x- and P2y-purinoceptors has been proposed [26, 28].

A more definite characterization of cardiovascular P2-receptors is hindered by the lack of selective, competitive receptor antagonists [27]. The P2x-purinergic receptor agonist, α,β-methylene ATP, has been successfully applied to perform radioligand studies on P2 purinoceptors and to desensitize P2x-purinergic receptors [29, 30]. Although desensitization with α,β-methylene ATP may selectively antagonize P2x-purinoreceptor-mediated responses in vitro, repeated application of α,β-methylene ATP in vivo not only inhibits the vasoconstrictor effect of ATP, but may also attenuate the vasoconstrictor effect of noradrenaline, angiotensin II and vasopressin [31]. In addition, the ability of α,β-methylene ATP to inhibit ATP-induced effects varies enormously [32]. Suramin has been reported to inhibit P2x purinoceptors. However, this drug also inhibits the P2y purinoceptor and several enzymes including possibly ectophosphatases [33, 34]. A classification of P2 purinoceptors, based on potency differences between various ATP derivatives, can be confounded by differences in degradation of ligands by ectophosphatases [35].

A P2x-like receptor with seven transmembrane domains has been isolated from turkey and chicken brain but is not expressed in the heart [36, 37]. A P2x receptor has been cloned from human airway and colonic epithelium [38]. This receptor also contains seven membrane-spanning domains, characteristic of G-protein-coupled receptors, but is expressed in the heart. Two pharmacologically and structurally different P2x-purinergic receptors have been cloned from rat vas deferens and phaeochromoctoma PC12 cells [39, 40]. As anticipated from voltage-clamp studies, both receptors are ligand-gated ion channels. Thus, molecular techniques confirm a heterogeneity of P2x purinoceptors as anticipated by pharmacological observations.

Apart from vascular P2x-purinergic receptors, specific P2y-purinergic receptors have been demonstrated on thrombocytes [41]. ADP is the most potent endogenous agonist for this receptor and ATP is an endogenous antagonist [42]. Receptor stimulation results in thrombocyte activation and subsequent thrombus formation. A selective and possibly competitive antagonist of this receptor has been synthesized [43]. In the future, this may have clinical importance as an anti-thrombotic agent. Stimulation by ATP4- of a P2y purinoceptor in non-excitable cell types such as leucocytes, parotid acinar cells and 3T6-transformed fibroblasts induces non-selective pores and may be involved in cell fusion [44, 45]. Finally, a P2y purinoceptor has been recognized with diadenosine polyphosphates (ApnA; n = 3–6) as endogenous ligands. ApnA is stored in synaptic vesicles and chromaffin granules and may play a role as a co-transmitter in the nervous system [46]. This receptor (class?) has not been fully characterized. Some of the described effects of diadenosine polyphosphates may be mediated by other P2 receptors [46, 47]. When more structural data, as obtained from molecular techniques, and truly selective antagonists become available, major changes in this classification of P2 purinoceptors will probably occur. As yet, no generally accepted classification system for P2 purinoceptors exists [27].

**CARDIOVASCULAR EFFECTS OF PURINERGIC RECEPTOR STIMULATION**

**Vascular wall**

ATP exerts two vascular actions that are opposite and mediated by two distinct P2-purinergic receptors located on endothelium and vascular smooth muscle cells (Table 2). The endothelial receptor evokes an endothelium-mediated vasodilator response (Fig. 1) [49]. On vascular smooth muscle cells, P2x-purinergic receptor stimulation results in a vasoconstrictor response [50] that has also been observed in human vessels in vitro [51], but since the endothelium plays an important role in degradation and uptake of purines, only a minor part of luminally applied ATP will probably reach the vascular smooth muscle cells [52]. Indeed, if endothelial function is intact, ATP as released during thrombocyte aggregation causes vasodilatation [53]. The receptor on vascular smooth muscle cells will preferentially be stimulated by intermittently released ATP as occurs from sympathetic nerve endings (Fig. 1). Biochemical, histochernical, electrophysiological and pharmacological evidence supports the hypothesis that ATP is released from sympathetic nerve endings, together with noradrenaline [3, 54]. In the mesenteric vascular bed of the rat, subthreshold doses of ATP potentiate the vasoconstrictor response to noradrenaline but not to 5'-hydroxytryptamine or potassium chloride, indicating a specific facilitating postsynaptic interaction between ATP and noradrenaline [55]. A direct, endothelium-independent, relaxing effect of ATP on vascular smooth muscle cells has also been observed, suggesting the presence of P2y purinoceptors [56, 57].

When infused into the brachial artery of healthy humans, ATP induces a dose-dependent vasodilator response, with high concentrations inducing maximal
vasodilatation, indicating that the vasodilator action of luminally applied ATP is not limited by stimulation of P2x purinoceptors or the release of endothelium-derived contracting factors [58]. These responses to ATP could not be inhibited by theophylline and the vasodilator response to ATP was approximately 10 times more potent than the vasodilator response to equimolar doses of adenosine, indicating that P2x purinoceptor activation is not responsible for the vasodilator response to ATP [58].

Adenosine induces a vasodilator response via A2-purinergic receptor activation in most vessels (see Fig. 1) [59]. Although the A1-purinergic receptor may mediate vascular relaxation via opening of ATP-dependent potassium channels [18], this receptor subtype is generally believed to be involved in a vasoconstrictor action of adenosine in glomerular afferent arterioles (see below) and possibly also in the pulmonary vascular bed [60]. In humans however, only a vasodilator response to adenosine in the pulmonary vascular bed has been described [61].

An intact endothelium is not obligatory for the vasodilator action of adenosine [62], but because some studies show a reduced vasodilator response to adenosine when the endothelium is removed, adenosine receptors may be present on these cells [49]. Inhibition of nitric oxide synthase has been shown to inhibit adenosine-mediated vasodilatation [63].

Recently, A2-adenosine receptors have been demonstrated on human endothelial cells [64], but there is some debate with regard to their functional significance. The site of adenosine application or formation may determine whether the vasodilator response is mediated by a direct effect on the vascular smooth muscle cells (interstitial adenosine) or whether the endothelium is involved (luminal adenosine) [65]. In humans, infusion of adenosine into the brachial or coronary arteries results in vasodilatation [66, 67]. The latter serves as the basis for the adenosine stress test for inducible ischaemia in patients undergoing this test for the evaluation of coronary artery disease [68]. This vasodilatation can be antagonized by caffeine and theophylline, indicating a P1-purinoceptor-mediated effect. Nitric oxide participates in this vasodilator response [69], supporting a role for the endothelium in the vascular action of intraluminally applied adenosine in vivo.

A1 purinoceptors are also reported to mediate a vasodilator effect via opening of ATP-dependent potassium channels [18]. This potassium channel can also be activated by stimulation of A2 purinoceptors [70]. We have not been able to demonstrate a role for ATP-dependent potassium channels in the forearm vasodilator response to adenosine in humans [69].

Apart from their direct influence on vascular tone, purines inhibit noradrenaline release from sympathetic nerve endings by presynaptic modulation (see Fig. 1). Most studies on this subject point towards an adenosine-induced A1-purinergic receptor-mediated mechanism. ATP, via yet unclas-

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**Table 2. Cardiovascular effects of purine receptor stimulation**

<table>
<thead>
<tr>
<th>Receptor subtype</th>
<th>Tissue distribution</th>
<th>Effect of receptor stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Juxtaglomerular cells</td>
<td>Vasodilation</td>
</tr>
<tr>
<td></td>
<td>Sympathetic efferent nerves</td>
<td>Autonomic reflex with sympathetic excitation</td>
</tr>
<tr>
<td></td>
<td>Smooth muscle cells in glomerular afferent arterioles and possibly in pulmonary vascular bed</td>
<td>Vasodilatation</td>
</tr>
<tr>
<td></td>
<td>Cardiac conduction system</td>
<td>Depression of sinus node activity, prolongation of atrioventricular conduction time, attenuation of ischaemia- and catecholamine-induced arrhythmias</td>
</tr>
<tr>
<td>A2k</td>
<td>Cardiomyocytes</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>A2b</td>
<td>Vascular smooth muscle cells</td>
<td>Increase in contractility</td>
</tr>
<tr>
<td>A2b (P) [48]</td>
<td>Thrombocytes</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>A3</td>
<td>Endothelium</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>P1x</td>
<td>Not expressed in cardiovascular system</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>P2x</td>
<td>Vascular smooth muscle cells</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>P2y</td>
<td>Cardiomyocytes</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>P2y</td>
<td>Endothelial cells</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>P2x</td>
<td>Vascular smooth muscle cells</td>
<td>Thrombocyte aggregation</td>
</tr>
<tr>
<td>P2x</td>
<td>Sympathetic efferent nerves (?)</td>
<td>May be involved in macrophage fusion</td>
</tr>
<tr>
<td>P2x</td>
<td>Endothelial cells</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>P2x</td>
<td>Vascular smooth muscle cells</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>P2x</td>
<td>Thrombocytes</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>P2x</td>
<td>No known distribution in cardiovascular system</td>
<td>Vasoconstriction</td>
</tr>
<tr>
<td>P2x</td>
<td>Endothelial cells (A2x-A)</td>
<td>Release of EDRFs</td>
</tr>
<tr>
<td>P2x</td>
<td>Vascular smooth muscle cells (A2x-A)</td>
<td>Vasoconstriction</td>
</tr>
</tbody>
</table>
sified purinergic receptor subtypes (P₂ and a possible P₃ purinoceptor), may be involved as well [71, 72]. Using nucleoside transport inhibition to increase adenosine concentrations at sites of formation, we recently demonstrated presynaptic modulation of noradrenaline release from sympathetic nerve endings in humans in vivo [73].

Based on the experimental data above, Burnstock and Sneddon [3] hypothesized that ATP is co-released with noradrenaline by sympathetic nerve endings, induces a vasoconstrictor response in concert with noradrenaline and is rapidly degraded to adenosine which presynaptically inhibits sympathetic neurotransmitter release thus providing a negative-feedback loop. The activity of this feedback loop may be controlled by ectonucleotidase activity [74].

Heart

In addition to causing coronary vasodilatation and presynaptic inhibition of noradrenaline release, purines have negative chronotropic, dromotropic and inotropic effects in isolated heart preparations. ATP is thought to induce its negative chronotropic and dromotropic effects after degradation to adenosine [75]. However, under certain experimental conditions, atropine or vagotomy can attenuate these actions, indicating that this effect is at least partially vagally mediated [76].

**Negative chronotropic actions.** Adenosine depresses sino-atrial node activity and shifts the earliest site of atrial activation from the sino-atrial node towards the crista terminalis region. At high concentrations, adenosine may even cause sino-atrial exit block. Adenosine may also depress atrioventricular and ventricular escape rhythms. In patients with a ventricular escape rhythm, heart rate is reduced in response to adenosine, especially when the escape rhythm is accelerated by isoproterenol. These effects are probably mediated by A₁-adenosine receptor stimulation [77]. In the atrium, this receptor is coupled via a G-protein to an acetylcholine-dependent potassium channel. Activation of this channel results in hyperpolarization of pacemaker cells and subsequent reduction in the frequency of depolarizations [78]. In the intact organism, the
Adenosine-induced stimulation of reflex mechanisms may result in an increase in heart rate, which strongly contrasts with its effect on the sino-atrial node (see below).

**Negative dromotropic actions.** Adenosine prolongs atrioventricular conduction time in a dosedependent manner. Its site of action is probably the proximal portion of the atrioventricular junction. Based on the rank order of potency of different adenosine analogues and the use of specific antagonists, A₁-adenosine receptors are thought to be involved [79, 80]. The ability of adenosine to induce atrioventricular block has led to its widespread clinical use in the diagnosis and treatment of broad complex tachycardias [81, 82]. Bolus infusions are used for this purpose to prevent the reflexly induced sympathetic excitation that occurs when adenosine is infused more slowly [83].

**Anti-arrhythmic properties.** Adenosine can attenuate ischaemia- and catecholamine-induced arrhythmias in experimental preparations [84, 85] via A₁-adenosine receptors, probably by preventing catecholamine-induced calcium influx and subsequent afterdepolarizations [77]. ATP also inhibits isoproterenol-induced calcium influx, probably by stimulating A₁-adenosine receptors after its degradation to adenosine [77]. When ATP alone (i.e., without isoproterenol pretreatment) is applied to isolated myocardial cells, calcium influx is increased. This effect is thought to be related to P₂-purinergic receptor stimulation [86] and may contribute to ATP-induced automaticity. Alternatively, in isolated myocardial cells, ATP hydrolysis may activate the chloride/bicarbonate exchanger. The subsequent intracellular acidification may induce arrhythmic activity, providing a P₂-purinergic receptor-independent mechanism [87].

**Negative inotropic actions.** Adenosine has a negative inotropic effect on both atrial and ventricular myocardium when contractility is increased with isoproterenol, probably by antagonizing the isoproterenol-induced increase in intracellular cAMP [77]. In the atrium, adenosine also has a direct, i.e. without isoproterenol pretreatment, negative inotropic action, probably by increasing potassium efflux and subsequent hyperpolarization. These effects are mediated by A₁-adenosine receptor stimulation [77]. In contrast to adenosine, ATP exerts a positive inotropic action on both atrial and ventricular myocardial cells [88] by increasing the intracellular calcium concentration.

**Kidney**

In contrast to most other vascular beds, adenosine induces A₁-receptor-mediated vasoconstriction in the outer renal cortex that appears to be confined to the glomerular afferent arterioles. Simultaneously, adenosine dilates the efferent glomerular arterioles via A₂-adenosine receptor stimulation [89]. Acting in concert, these effects of adenosine lower glomerular filtration rate. The vasoconstrictor effect occurs immediately upon adenosine infusion, while the vasodilator response develops more slowly. These opposing effects of adenosine may explain the biphasic response of renal blood flow but a constant depression of glomerular filtration rate during infusion of adenosine into the renal artery [89]. In the rat kidney, adenosine and angiotensin II act synergistically on afferent glomerular arterioles [90]. Adenosine-induced release of endothelium-derived relaxing factor (EDRF) probably contributes to the efferent arteriolar vasodilatation [91]. Inhibition of tubuloglomerular feedback by selective adenosine receptor antagonists and adenosine deaminase, as well as its stimulation by adenosine deaminase inhibition and nucleoside transport inhibition, is important evidence in favour of adenosine as a modulator of these phenomena [2].

Adenosine inhibits renal renin release via A₁-adenosine receptor stimulation [92, 93], resulting in reduced angiotensin II production and subsequent aldosterone release. Renal erythropoietin production is increased by A₂-adenosine receptor stimulation [94].

The renal effects of ATP are less well assessed. In the rat, ATP induces a vasoconstrictor response that appears to be most pronounced in afferent (preglomerular) arterioles. The efferent arterioles are not affected by ATP [95]. ATP may also be involved in tubuloglomerular feedback [96]. Mesangial cells contain P₂y purinoceptors [97], but their physiological role is, to our knowledge, not clear.

The direct tubular effects of purine receptors are beyond the scope of this review.

**EFFECTS OF ADENOSINE ON AFFERENT NERVE ENDINGS**

In conscious humans and some animals, adenosine stimulates carotid and aortic chemoreceptors [83] and afferent nerves in the kidney [98], heart [99, 100] and forearm muscle [101]. Stimulation of these afferent nerves activates the sympathetic nervous and respiratory systems [102, 103], causing a subsequent increase in systolic blood pressure, plasma renin activity and ventilation [83, 104, 105]. The increase in heart rate is probably mediated by concomitant deactivation of the parasympathetic nervous system, since it can be antagonized by atropine but not by propranolol [106]. These excitatory effects of intravenous adenosine infusion are dependent on an intact autonomic reflex arc [83], which probably explains why these effects are blunted in anaesthetized humans [107]. An increase in systolic blood pressure is not always observed during intravenous infusion of adenosine into healthy volunteers [108, 109], probably due to differences in the degree of their caffeine abstinence. In the studies in which no increase in systolic blood
pressure is observed, caffeine abstinence is invariably brief or totally absent. After ingestion of two cups of regular coffee, plasma caffeine concentrations are in the range of 4–5 mg/l [110], which is sufficiently high to antagonize the haemodynamic effects of intravenous adenosine infusion [104]. The plasma half-life time of caffeine after ingestion of two cups of coffee ranges from 2 to 8.5 h [110]. Therefore, a 24 h period of caffeine abstinence is important to prevent underestimation of adenosine-induced haemodynamic and neurohumoral effects. An increase in systolic blood pressure, heart rate and plasma catecholamines has also been observed in resting man after administration of the adenosine transport inhibitor dipyridamole [111]. These changes could be inhibited by previous administration of caffeine or theophylline [111, 112], suggesting that adenosine is formed under baseline conditions and has functionally important actions. These effects of endogenous adenosine have recently been replicated by the more selective nucleoside transport inhibitor drafazoline [113]. The excitatory effects of dipyridamole might explain its lack of benefit on cardiovascular mortality in large trials [114, 115].

In the heart and skeletal muscle, stimulation of adenosine-sensitive afferent nerves induces ischaemia-like pain [116]. Whether anginal pain and sympathetic activation are elicited by stimulation of the same afferent nerves is unknown. In healthy volunteers, sympathetic-excitation is a more sensitive marker of adenosine-sensitive afferent nerve stimulation than the induction of chest discomfort [113]. In patients with atherosclerotic heart disease, intracoronary infusion of adenosine can mimic both the nature and location of ischaemic pain [117], suggesting that adenosine may be the chemical mediator of angina in this condition. Chest pain is not provoked when adenosine is infused into patients with total cardiac afferent denervation, i.e. orthotopic cardiac transplant recipients [118]. In contrast to subjects with intact cardiac innervation, systolic blood pressure falls in these transplant patients, suggesting that a significant contributor to the sympathetic-excitation observed when adenosine is infused in normal subjects is a reflex response to stimulation of adenosine-sensitive cardiac afferent nerve endings [99, 119, 120].

Patients with coronary artery disease manifest a spectrum of responses to ischaemia, from chest discomfort to a total lack of awareness. The syndrome of silent ischaemia, in which no chest pain is experienced, has attracted considerable attention, but the mechanism responsible for this variation in the expression of ischaemia between subjects, and within the same subject on different occasions, remains uncertain. The severity of chest pain in response to intravenous adenosine infusion is somewhat less in those with predominantly silent ischaemia than in subjects who experience angina [121], but the role of endogenously formed adenosine in this variable perception of ischaemia is as yet unexplored. As the extent of myocardium exposed to ischaemia may have a bearing on whether or not this stimulus is perceived as pain [122], there may be a quantitative relationship between the amount of adenosine generated and the development of angina. Alternatively, silent ischaemia may be caused by selective loss of adenosine-sensitive cardiac afferent nerves (certainly silent ischaemia is associated with autonomic neuropathy in patients with diabetes mellitus [123]), or by decreased responsiveness of receptors on these afferent nerve endings to adenosine. We are actively pursuing these latter hypotheses.

Apart from sympathetic afferent nerves, the heart contains adenosine-sensitive vagal afferent nerves that are located in the inferoposterior wall of the left ventricle [124]. Stimulation of these afferent nerves may explain a recent observation that the effect of dipyridamole infusion on heart rate is related to the site of the perfusion defect on thallium scanning – with attenuation of the heart rate response in patients with inferoposterior, as opposed to anterior left ventricular hypoperfusion (A. Zahedi, J. S. Floras and R. J. Burns, unpublished work).

**ADENOSINE: A NATURAL DEFENCE AGAINST THE CONSEQUENCES OF ISCHAEMIA**

Extracellular formation of adenosine is increased during hypoxia and ischaemia [125]. Several effects of locally formed adenosine are assumed to be beneficial in situations of reduced oxygen supply [126]. In the heart, its negative inotropic and chronotropic effects will reduce oxygen demand, and adenosine-induced vasodilatation will increase oxygen supply. Its electrophysiological actions should reduce the risk of ischaemia-induced arrhythmias. Endogenous adenosine inhibits noradrenaline release and sympathetic neurotransmission during ischaemia [127, 128]. Presynaptic inhibition of noradrenaline release should attenuate catecholamine-induced arrhythmogenesis, vasoconstriction, thrombocyte aggregation and cytotoxicity [129]. In addition, adenosine inhibits thrombocyte aggregation [130] and may reduce the formation of leukocyte-derived free radicals during ischaemia and reperfusion [131]. In several animal models, adenosine has been shown to reduce myocardial infarct size, prevent ischaemia-induced cardiac arrhythmias and reduce myocardial stunning [132–134].

Adenosine appears to be involved in preconditioning, defined as the increased tolerance of myocardium to a prolonged ischaemic insult, achieved by an initial brief exposure to ischaemia and reperfusion [135]. Preconditioning probably occurs in humans [136]. In animals, adenosine-induced preconditioning is mediated by A<sub>1</sub> receptors [137]. An A<sub>1</sub>-adenosine receptor may also be
involved [22]. Protein kinase C appears to play a key role in the post-receptor mechanism of preconditioning [138], although interspecies differences exist in the importance of protein kinase C in ischemic preconditioning [139]. Activation of protein kinase C facilitates opening of ATP-dependent potassium channels, which are important in mediating ischemic preconditioning [132, 140]. Alternative proteins that play a role in ischemic preconditioning and whose function could be modulated by protein kinase C activation are ecto-5'-nucleotidase and the nucleoside transporter [141-144]. The role of nitric oxide in ischemic preconditioning is not completely understood [144, 145]. Recently, ischemic preconditioning has also been demonstrated in skeletal muscle of the pig [146] and in the brain [147], indicating that rhythmic contractions are not obligatory for this phenomenon and suggesting that this protective mechanism against the sequelae of ischemia might also be operative in patients with intermittent claudication or with cerebral ischemia. Adenosine receptor stimulation and elevation of endogenous adenosine levels appear to reduce cerebral infarct size [148] and mediate cerebral ischemic preconditioning [147] in animals. Adenosine attenuates the synaptic release of the cytotoxic amino acids glutamate and aspartate by stimulation of presynaptic A1-adenosine receptors. Postsynaptically, adenosine depresses membrane calcium permeability and increases potassium and chloride permeability, resulting in hyperpolarization and a reduced oxygen demand [149]. Endogenous adenosine prevents the occurrence of convulsions [150]. Dilation of cerebral arteries will increase oxygen supply.

The role of the sympathetic nervous system in ischemic preconditioning is controversial. In rabbits, pretreatment with reserpine prevents ischemic preconditioning from reducing infarct size [151]. Noradrenaline is able to augment ischemia-induced adenosine formation by activation of 5'-nucleotidase [8]. Furthermore, noradrenaline (like adenosine) has been shown to induce ischemic preconditioning [8]. In contrast, ischemic preconditioning occurs in cultured human myocardial cells, a model that lacks sympathetic innervation [152]. In the isolated perfused rat heart, ischemic preconditioning reduced noradrenaline release during sustained ischemia ('neural preconditioning') [153]. Unlike the beneficial effects of ischemic preconditioning on infarct size, this reduction in noradrenaline release could not be attributed to endogenous adenosine release and could only partially be prevented by inhibitors of protein kinase C. These authors suggested a role for neural preconditioning in mediating the anti-arrhythmic effect of ischemic preconditioning.

Despite the increasing amount of evidence that adenosine is beneficial in cardiac ischemia, some conflicting results should also be mentioned. While in animals infusion of the adenosine receptor antagonist 8-phenyltheophylline in combination with adenosine deaminase exacerbates myocardial ischemia during exercise [154], in humans administration of theophylline, aminophylline or bamilphyl- line may reduce exercise-induced ischemia as assessed by electrocardiographic parameters [155, 156]. Recently, this apparent discrepancy has been clarified. Aminophylline has a-adrenoceptor agonistic properties which redistribute myocardial flow during mild ischemia in favour of the subendocardial myocardium [157, 158], reducing ischemia in this area. However, during severe ischemia, aminophylline exacerbates myocardial injury, probably by antagonizing the cardioprotective effects of endogenous adenosine [157]. In the rat heart, ischemic preconditioning may not be mediated by a-adrenoceptors or adenosine receptors [159]. In the kidney, adenosine only marginally affects renal blood flow (due to a combination of afferent arteriolar constriction and efferent vasodilation) but reduces tubular oxygen demand by attenuating glomerular filtration rate and tubular function [160]. Notwithstanding, the adenosine receptor antagonist theophylline prevented renal ischemic damage in rabbits [161]. In human studies, theophylline attenuated nephrotoxicity from contrast media while the nucleoside transport inhibitor dipyridamole augmented the contrast media-induced reduction in glomerular filtration rate [162].

PHARMACOLOGICAL INTERVENTIONS

To date, pharmacological interventions are primarily directed towards adenosine-mediated effects. P1- and P2-purinergic receptor agonists and antagonists with varying degrees of selectivity have been described. For a detailed description, there are some excellent reviews on this topic [27].

Apart from compounds that directly interact with the adenosine receptor, some drugs with an indirect action have been developed. These can enhance adenosine release (AICA riboside [163]) or reduce adenosine degradation by inhibiting cellular uptake of adenosine (e.g. nucleoside transport inhibitors such as nitrobenzylthionoisine, dipyridamole, dilazep and mioflazine derivatives like draflavine [164]) or inhibiting adenosine deaminase activity (deoxycolor- mycin). In addition, some compounds facilitate the binding of adenosine to its receptor through an allosteric mechanism. These allosteric enhancers may have the theoretical advantage of a possible adenosine receptor subtype specificity [165]. What these indirectly acting drugs have in common is that they affect adenosine-mediated actions only at sites of endogenous adenosine release, thereby preventing those systemic side-effects that are associated with intravenous infusion of adenosine or its analogues. For example, low-grade nucleoside transport inhibition is able to potentiate the forearm
vasodilator response to adenosine infusion into the brachial artery without inducing unwanted side-effects [113].

CONCLUSIONS

The naturally occurring purines adenosine and ATP can induce a variety of effects that are mediated by receptors located on the plasma membrane. Studies in vitro and animal studies in vivo indicate that ATP is released from sympathetic nerve endings and aggregating thrombocytes, and can induce vasoconstriction via stimulation of P2x purinoceptors located on vascular smooth muscle cells and vasodilatation via P2Y receptors that are located on the endothelium and to a small extent also on smooth muscle cells. Adenosine, the major pharmacologically active breakdown product of ATP, can induce vasodilatation via endothelium-dependent and endothelium-independent mechanisms. Furthermore, adenosine is able to reduce noradrenaline release from sympathetic nerve endings by a presynaptic mechanism. Adenosine can protect the heart against the sequelae of ischaemia and can induce ischaemic preconditioning. Many of these effects have now also been observed in humans in vivo. Exploitation of the cardioprotective effects of endogenous adenosine is a major challenge for cardiovascular pharmacology. In this respect, specific low-grade nucleoside transport inhibition is very promising.

Important progress has been made in the molecular characterization of P2 purinoceptors. New insights into the structure of these receptors should facilitate the development of specific P2 purinoceptor antagonists and agonists that could further characterize the physiological importance of purines in the regulation of various body functions and direct the design of pharmacological interventions of clinical benefit.

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