Role of adenosine in the heart and circulation

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1. Introduction

Adenosine (Ado), a metabolite of adenine nucleotides, is a ubiquitous biological compound found in every cell of the human body [1]. Since the classic work by Drury and Szent-Györgyi in 1929 [2], in which the coronary vasodilator and antiarrhythmic properties of this nucleoside were first described, and in which its role as a regulator of coronary blood flow was first proposed, it has become increasingly clear that Ado plays not only a biological role in cellular metabolism, but also an important physiological role in the cardiovascular system [1-4]. Interest in the subject was rekindled by Berne in 1963, who presented experimental evidence for the ‘Ado hypothesis’, which suggests that Ado is an endogenous dilator of coronary vessels and is released during reduced myocardial O₂ supply or increased myocardial workload [5]. Many of the cardiovascular actions of Ado are homeostatic and protective in nature, and the nucleoside has therefore been termed a ‘retaliatory metabolite’, equalizing local energy requirements with energy supply [6,7]. It is only in the last decade that the clinical relevance of these actions has started to be recognized, greatly increasing interest in the therapeutic and diagnostic potential of Ado [4,8].

The aim of this review is to put recent research and major advances in our understanding of the role of Ado in the heart and circulation into perspective, and to demonstrate the importance of Ado in cardiology today. Emphasis is placed on the therapeutic and diagnostic implications of the regulatory functions of Ado, and on exciting opportunities with novel agents that modulate the actions of this nucleoside.

2. The adenosine hypothesis

The development of the Ado hypothesis was intimately associated with an increased understanding of coronary hemodynamics. Early studies showed that arterial inflow to the heart is precisely regulated by the coronary vasculature over a wide range of cardiac activity, maintaining a nearly constant and high level of O₂ extraction by the myocardium, and resulting in low coronary venous O₂ saturation levels (20–30%) [9-11]. Since this high level of O₂ extraction is maintained even during basal, or resting, conditions, little reserve capacity exists for increasing O₂ uptake when O₂ demand is increased. Furthermore, the myocardium has a very limited capacity for anaerobic metabolism, and is largely dependent on oxidative metabolism. Substantial increases in cardiac activity cannot therefore be met by increased O₂ extraction or anaerobic metabolism, hence prolonged alterations in O₂ demand must be met by proportional changes in coronary blood flow [11]. In the myocardium, blood flow appears to be under the control of the nervous system (neurogenic control), of a myogenic mechanism, and of chemical substances originating from the myocytes (metabolic autoregulation). The relative importance of each mechanism varies in different tissues.

Some evidence suggests that the decreased O₂ tension that results from an inadequate coronary inflow has a direct effect on coronary vascular smooth muscle relaxation [12]. However, a much larger body of evidence suggests that an indirect regulatory process is involved, in which hypoxia leads to metabolic alterations that modify the flux of vasoactive substances, which in turn act on...
coronary smooth muscle to cause vasodilatation [11,13]. Vasoactive substances therefore act as error signals, increasing in concentration when myocardial perfusion is inadequate, and decreasing as the balance between myocardial energy needs and coronary artery flow is restored [11,14]. Although a number of vasoactive substances have been proposed to contribute to this homeostatic regulation of coronary flow, the relative importance of each has yet to be established. The theory favoring Ado as a primary mediator, although far from complete, has received extensive experimental support and has led to the development of the Ado hypothesis of coronary blood flow regulation (Fig. 1) [11,14]. However, it should be borne in mind that the Ado hypothesis does not necessarily exclude a role for other substances, and it is unlikely that any one single factor can fully explain the regulation of coronary blood flow [11]. Indeed, there is evidence that, in addition to Ado, ATP (released from sympathetic nerves, from sensory-motor nerves, or from endothelial cells) is also involved in the control of blood flow, including in the coronary bed [15]. In addition, peptides such as the calcitonin gene-related peptide, substance P and neurokinin A, all released by capsaicin-sensitive sensory-motor nerves, may also be involved [16].

### 3. Metabolism of adenosine

#### 3.1. Formation of adenosine

Ado may be formed in tissues by two major pathways: the dephosphorylation of AMP to Ado and phosphate, and the hydrolysis of S-adenosylhomocysteine (SAH) to Ado and homocysteine (Fig. 2) [8]. The relative importance of the two pathways varies under different circumstances and possibly also in different tissues. Nonetheless, it appears that an imbalance between O$_2$ supply and O$_2$ demand is the most important stimulus for the formation of Ado [17]. In the heart, the main sites of Ado formation are the cardiac vascular endothelium and the cardiomyocytes [17].

##### 3.1.1. Dephosphorylation of adenosine monophosphate

The major source of Ado is probably via dephosphorylation of intracellular AMP, the concentration of which is a function of intracellular ATP concentration and energy charge. Thus, an increase in the hydrolysis of ATP, or a decrease in the rate of ATP synthesis, leads to a drop in energy charge and to an increase in AMP concentration (see Fig. 1) [14]. AMP is then dephosphorylated by the enzyme 5'-nucleotidase to Ado. It has been clearly demonstrated that stimuli that lower energy levels (including hypoxia, ischemia, and exercise) greatly increase the activity of the enzyme and hence the conversion of AMP to Ado [5,18].

The enzyme 5'-nucleotidase is localized in the cytosol (cytosolic 5'-nucleotidase) and at the surface membrane (ecto-5'-nucleotidase) of cardiomyocytes. Ado can therefore be formed either intracellularly or extracellularly by the degradation of AMP [17–19]. Although it was originally thought that the ecto-enzyme was primarily responsible for the increased release of Ado during hypoxia, studies by Meghi et al. [20] have shown that a monoclonal antibody against this enzyme has no effect on Ado release from polymorphonuclear leukocytes and neonatal heart cells under conditions where Ado release is stimulated. Furthermore, other studies have found that the ecto-enzyme inhibitor α,β-methylene-ADP has no effect on hypoxia-induced Ado release [21]. These findings, together with the fact that Ado release during hypoxia in the isolated guinea pig heart and rat cardiocytes is prevented by Ado transport inhibitors, suggest that Ado is formed in the cardiomyocyte by the action of cytosolic 5'-nucleotidase and is then released by a nucleoside carrier into the extracellular space, where it performs its actions [20,22]. In the rat heart, the cytosolic enzyme is activated by ATP, which makes its role in hypoxia-induced Ado release questionable. The presence of a lysosomal 5'-nucleotidase has also been demonstrated. Since this enzyme is activated by AMP but inhibited by ATP, it is a likely candidate for the key enzyme in Ado release during hypoxia [23]. However, in the dog heart an AMP-specific cytosolic 5'-nucleotidase has been purified, that is also inhibited by ATP and activated by Mg$^{2+}$ [24].

Nucleotidase activity is regulated by Ado [25] and by α$_1$-adrenergic receptor stimulation [26]. Both the endo-
and ecto-enzymes are partially inactivated after 30 min exposure to Ado [25]. This down-regulation may serve to protect the heart from excessive Ado accumulation. The effect of α,β-adrenergic stimulation may be partly mediated by the inotropic effect on the myocardium [27].

Adenine nucleotides released from platelets and endothelial cells are also potential extracellular sources of Ado [28]. It should also be noted that AMP may alternatively undergo conversion to inosine monophosphate (IMP), a reaction catalyzed by adenylic deaminase. However, in cardiac tissues this conversion is inhibited by increased intracellular concentrations of inorganic phosphate and decreased levels of ATP, conditions typical of hypoxia, thus facilitating the formation of Ado via 5'-nucleotidase [29].

3.1.2. Hydrolysis of S-adenosylhomocysteine: transmethylation pathway

Ado may also be formed intracellularly via the transmethylation pathway, in which SAH is hydrolyzed to Ado and homocysteine (Fig. 2). This reaction is catalysed by the enzyme SAH-hydrolase [30]. The rate of Ado release via this pathway is approximately equal to the rate of total Ado release from the heart during normoxia, and does not appear to increase significantly during hypoxia [30]. This finding, together with direct evidence from studies using intracellular nucleotide-labelling techniques, indicates that dephosphorylation of AMP by the nucleotidases is the main mechanism responsible for the increased formation of Ado during hypoxia [31]. It has therefore been suggested that the transmethylation pathway may be of more regulatory significance under conditions other than hypoxia [8].

3.2. Inactivation of adenosine

An autacoid such as Ado requires a local re-uptake and/or inactivation mechanism to limit its activity. Ado inactivation and salvage are also critical for the regeneration of cardiomyocyte adenine nucleotide pools, depleted after an ischemic or hypoxic episode, since the pathway for de novo nucleotide synthesis accounts for only 0.4% of the total nucleotide pool per hour [31]. Ado inactivation occurs via three mechanisms: phosphorylation to AMP by Ado kinase (re-incorporation into the ATP pool [17]), which represents the preferential pathway of Ado inactivation; degradation to inosine by Ado deaminase; or washout in the circulation (Fig. 2) [6,32]. At physiological concentrations, Ado is predominantly metabolized to AMP, while at higher concentrations, e.g. following exogenous administration, it is deaminated to inosine [33].

The inactivation of Ado can take place in cardiomyocytes as well as in other cells (endothelial cells, erythrocytes). Since both Ado kinase and Ado deaminase are
cytosolic enzymes, Ado must first be taken up by these cells before being inactivated. This occurs via a nucleoside transport system that is present in endothelial cells, erythrocytes, and cardiomyocytes, and which can be inhibited by nucleoside transport inhibitors [34].

Following the degradation of Ado to inosine, inosine is further degraded to hypoxanthine, which can subsequently be phosphorylated to IMP before being converted to AMP [32]. Ado can also be converted to adenosine, which is then ribosylated to AMP. These salvage pathways represent mechanisms whereby Ado and its metabolites contribute to the preservation of the adenine nucleotide pool during periods of oxygen deprivation and reperfusion [6]. In addition, adenine and hypoxanthine have both been found effective in preserving postischemic ATP and myocardial function [35].

3.3. Activation of the adenosine pathway

Ado is continuously released into the interstitial fluid from normal and ischemic myocytes [10] and has been shown to accumulate even during brief periods of anoxia in dog hearts [29,36]. Anoxia and graded hypoxia induce an increase in myocardial Ado production and content, as well as in release of Ado into the coronary sinus effluent, probably as a result of an increased breakdown of ATP to AMP and the subsequent conversion of AMP to Ado [37,38]. In addition, constriction of the thoracic aorta of the rat has been shown to increase the formation of Ado and its degradation products [39]. In studies on cardiac performance in the dog during treadmill exercise, endocardial Ado levels were shown to increase in response to exercise, supporting the hypothesis that Ado also regulates coronary blood flow during increased metabolic activity [40].

In the original formulation of the Ado hypothesis, Berne suggested that an inadequate O₂ supply for the prevailing metabolic rate would increase the Ado level, resulting in vasodilatation, and thereby increase O₂ delivery [5]. This would then feed back through the system, increasing the tissue O₂ tension and subsequently lowering the Ado concentration. Ado concentration would therefore inversely reflect tissue O₂ tension [5]. Rubio and Berne [29] proposed that Ado production was coupled to the rate of myocardial metabolism, implying the existence of a relationship between the consumption of high-energy phosphate bonds and the release of Ado from the myocardial cell. It has been suggested that Ado production may be regulated by myocardial phosphocreatine, and that the increase in free Mg²⁺ observed when ATP is broken down during contraction may reverse the phosphocreatine-mediated inhibition of S'-nucleotidase activity [41]. The reasoning behind this is that phosphocreatine is known to inhibit the action of S'-nucleotidase. With increased myocardial metabolism, the phosphocreatine level falls, resulting in de-inhibition of the enzyme and a subsequent increase in Ado production.

4. Adenosine receptors

4.1. Adenosine receptor classification and regulation

Ado causes its various effects by interacting with specific cell surface receptors [42,43]. Although in the past the existence of three types of Ado receptors - A₁ (R₁), and two subtypes (A₂a and A₂b) of A₂ (R₂) - has been documented [44,45], which are classified according to their relative affinity for various Ado receptor agonists, subtypes of the A₁ receptor have also been described [46,47], in addition to the novel A₃ [45,48] and A₄ receptors [49]. The four Ado receptor subtypes that have been cloned to date, namely A₁, A₂a, A₂b, and A₄, all couple to guanine nucleotide-binding (G) proteins [45].

Sensitivity to Ado may be altered by up- or down-regulation of the receptor number. This phenomenon has been demonstrated in laboratory animals and cultured cardiac cells in which long-term exposure to Ado receptor agonists reduced the A₁ receptor number and the cellular responsiveness to Ado (down-regulation) [50,51]. In contrast, chronic administration of an Ado receptor antagonist such as theophylline results in an increase in A₁ receptor number and a parallel increase in the response to Ado (up-regulation) [52]. More recently, preliminary studies have shown that chronic intake of caffeine by humans sensitizes platelets to the anti-aggregator actions of Ado, thus indicating up-regulation of Ado receptors [17].

4.2. A₁- Receptor

The A₁-receptor is located on cardiomyocytes and is coupled to its effectors (ionic channels and adenylyl cyclase) via a pertussis toxin-sensitive G protein, presumably Gₓ [53-55]. Evidence for a coupling of the A₁-receptor to protein kinase C (PKC) has been recently obtained [56], but the nature of the G protein involved has not been elucidated.

Various subtypes of the A₁-receptor have been proposed [46-48] - A₁a, A₁b, and A₁ (which is different to the recently described novel A₃ receptor [57]) - although none of these putative subtypes have yet been identified by molecular cloning [43]. Results of studies by Gurden et al. did not support the existence of these A₁ receptor subtypes [44].

Interaction of Ado with the cardiomyocyte A₁ receptor results in the activation of Gₓ [53-55]. Activated Gₓ then causes the opening of specific K⁺ channels (called Kₐd or Kₐcha channels) [53,55,58] and the inhibition of cAMP production by adenylyl cyclase [59]. This last effect is only present under conditions where cAMP synthesis has been stimulated (e.g. by catecholamines, histamine). In this way, Ado antagonizes the cAMP-dependent stimulation of L-type Ca²⁺ channels, delayed rectifier K⁺ channels, Cl⁻ channels, and pacemaker ion channels (Fig. 3) [53,55,58]. Coupling of Ado receptors to ATP-sensitive K⁺ (or Kₐtp)}
channels has received increased attention: Ado-induced activation of these channels [60,61] may play a role, at least in part, in the direct effect of Ado in ventricular tissues and has been implicated in the myocardial protection afforded by Ado or by ischemic preconditioning [62,63].

Ado activation of the A1 receptor also reverses the increase in protein phosphorylation induced by β-adrenoceptor stimulation [64], probably as a result of the inhibitory effect on cAMP accumulation and on the resulting activation of protein kinase A (cAMP-dependent kinase).

Overall, activation of the A1-receptor gives rise to effects (negative chronotropic and dromotropic effects, anti-adrenergic effects) which cause a reduction in cardiac work and myocardial O2 consumption. In addition, activation of A1-receptors delays ischemic contracture [65] and improves glucose utilization by stimulation of anaerobic glycolysis [6,38,66].

Although very little is currently known about the role of Ado in the regulation of blood pressure and renal sodium balance, it has been shown that A1-receptor activity may be involved and that antagonism of this receptor may be used for the treatment of essential hypertension [67]. Furthermore, one study has shown that exogenous Ado inhibits the release of atrial natriuretic peptide (ANP), a hormone that regulates salt and water balance, and blood pressure [68]. This effect of Ado may be achieved in part by its negative chronotropic effect, and also possibly by its direct inhibitory effect on ANP release mechanisms in atrial myocardium [68], and both effects may be mediated by the A1-receptor [69]. The physiological role of Ado in regulating ANP, however, remains to be determined [68].

4.3. A2-Receptor

The A2-receptor is present on coronary vascular smooth muscle and endothelial cells [1,17,42,43]. To date, the existence of two A2-receptor subtypes has been proposed: the receptor found in dog coronary artery, human platelets, and neutrophils, which may correspond to the A2a subtype; and the receptor found in the guinea pig aorta, which may be of the A2b subtype [44]. Interaction of Ado with the A2-receptor leads to a stimulation of adenylate cyclase activity and a subsequent increase in intracellular cAMP levels [45]. Activation of the A2-receptor elicits vasodilatation, believed to be cAMP-dependent [70]. In addition, activation of the A2-receptor is thought to be involved in the stimulation of gluconeogenesis [8], and in the release of endothelium-derived relaxing factor [54]. It has been reported that activation of the receptor is associated with a reduction in local damage through inhibition of neutrophil endothelial adherence and plugging [71], reduction of toxic free radical formation by neutrophils or ischemic cells [6,72,73], and inhibition of platelet aggregation [8] and plugging [6,74,75].

In addition to the receptor present in vascular muscle, a functional A2-receptor capable of mediating an increase of intracellular cAMP and of contractility is expressed in chick embryonic myocytes [76]. The A2-receptor is also expressed in adult cardiac tissue, is coupled to phosphoinositide metabolism and mediates positive inotropic action [77,78].

4.4. A3-Receptor

Zhou and colleagues [48] recently described the existence of a novel A3-receptor, different from the A1-receptor subtype that has also been designated A3 [57]. Furthermore, despite its designation, this new receptor does not correspond to the pharmacologically proposed A3-receptor [45]. Activation of the A3-receptor has been reported to mediate hypotension in the isolated rat whose blood pressure has been raised to normal levels by angiotensin II [57]. In this same study, classical Ado receptor agonists were found to be active at this A3-receptor.

5. Effects of adenosine

5.1. Mechanisms of action of adenosine

Once Ado interacts with its specific cell receptors, a series of events are triggered off which lead to various effects, depending on the specific tissue involved.

5.1.1. Electrophysiological and contractile effects

Based on the pharmacological response and mechanism of action of Ado, the cardiac actions of the nucleoside have been tentatively classified as direct (cAMP-indepen-
dent) and indirect (cAMP-dependent) [4,38,42,53,55,79]. The former effects are characteristic of supraventricular tissues and occur in the absence of catecholamines or other cardioactive drugs; such effects include the negative inotropic effect of Ado in atrial tissue, the negative chronotropic effect on the sinoatrial (SA) node, the negative dromotropic effect on the atrioventricular (AV) node, and coronary vasodilatation. The indirect, or anti-adrenergic, effects are observed in all cardiac cells, in the presence of β-adrenergic agonists or of other substances that increase cellular cAMP levels; these effects are associated with the attenuation of an agonist-stimulated rise in cellular cAMP.

5.1.1.1. Supraventricular tissue. In supraventricular tissues (SA node, AV node, atria) the principal effect of Ado is to activate the K\textsubscript{Ado} channels, thus inducing an outward K\textsuperscript{+} current [17,53,55,58]. Activation of this specific current elicits a shortening of the action potential duration in atrial myocytes, a hyperpolarization and a decrease in the rate of pacemaker depolarization in SA nodal cells, and a depression of the action potential in AV nodal cells.

Ado also affects L-type Ca\textsuperscript{2+} channels. Whereas there is still controversy as to whether Ado causes an attenuation (12–18%) of basal, non-β\textsubscript{1}-adrenergically stimulated Ca\textsuperscript{2+} current, there is no doubt that Ado produces a marked reduction in the stimulation of the Ca\textsuperscript{2+} current by β-adrenergic agonists [17,55,58]. As mentioned above, this is a result of a G\textsubscript{i}-mediated inhibition of adenylate cyclase, with an attendant reduction in intracellular cAMP content.

In SA nodal cells, Ado potently attenuates the catecholamine-enhanced pacemaker current [17]. An analysis of the ionic basis of the Ado depressant effect on AV nodal conduction indicates that activation of the K\textsubscript{Ado} current is the principal mechanism responsible for the AV block caused by Ado [80]. It is not clear to which extent an Ado-induced decrease in Ca\textsuperscript{2+} inward current could also be involved [17,55].

Ado can be expected to reverse the cAMP-dependent effects of catecholamines on delayed rectifier K\textsuperscript{+} channels and on Cl\textsuperscript{−} channels. Since the deactivation of the delayed K\textsuperscript{+} current may play a role in pacemaking, this reversal of catecholamine effects may also contribute to the antiadrenergic action of Ado. The physiological significance of the effect on Cl\textsuperscript{−} channels needs to be assessed.

5.1.1.2. Ventricular tissue. It is still widely believed that no direct effects of Ado are present in mammalian ventricular tissue and that ventricular myocytes do not possess the distinct subset of K\textsuperscript{+} (K\textsubscript{Ado} or K\textsubscript{ACR}) channels that are activated by Ado in atrial cells. However, the distribution of such channels may be heterogeneous among ventricular tissues of different species, or within the myocardium of a given species. Recent studies have identified K\textsubscript{Ado} channels in ventricular myocytes of various species, including of human ventricle [81,82]. In addition, it has been shown that K\textsubscript{ATP} channels, which are present in ventricular as well as in atrial myocytes of probably all species, can be activated by Ado under some experimental conditions [60,61]. The functional importance of this coupling of Ado receptors to K\textsubscript{ATP} channels remains unclear [62,63,83].

Ado antagonizes the positive inotropic and arrhythmogenic effects of β-adrenergic agonists on ventricular myocardium, a response similar to that seen in supraventricular tissues [17,84,85]. Again, this anti-adrenergic effect of Ado is attributable to its antagonism of the increase in L-type Ca\textsuperscript{2+} [17] and transient inward currents [84] caused by catecholamines. Furthermore, it accounts for the observation that Ado decreases the amplitude of delayed after-depolarizations and suppresses triggered activity induced by agents known to increase cellular cAMP [84].

It has been suggested that Ado regulation of L-type Ca\textsuperscript{2+} current in cardiomyocytes may be different from the regulation by muscarinic agonists [86]. Ado was not (while carbachol was) efficacious in decreasing the stimulation of the Ca\textsuperscript{2+} current or the cAMP accumulation induced by isoproterenol in myocytes from adult rabbits or by forskolin in myocytes from newborn rabbit. This difference was explained by assuming that Ado only acts at the level of adenylate cyclase, while muscarinic inhibition involves additional steps distal to the enzyme [86].

5.1.1.3. Vascular tissue. Ado also produces potent vasodilatation in the majority of vascular beds through activation of A\textsubscript{2}-receptors, and several mechanisms for this effect have been proposed [17]. There appears to be evidence for a coupling of Ado receptors on vascular smooth muscle to adenylate cyclase and guanylate cyclase [17]. The coupling to guanylate cyclase may involve nitric oxide release by Ado, since inhibitors of NO formation markedly reduce the Ado-mediated vasodilation [87,88]. Furthermore, Ado has been demonstrated to inhibit the accumulation of inositol phosphates caused by noradrenaline, to depress Ca\textsuperscript{2+}-dependent action potentials in coronary arteries, and to inhibit the uptake of \textsuperscript{45}Ca in cultured vascular smooth muscle cells [17].

Recently it has been suggested that the Ado-induced vasodilatation is associated with the opening of K\textsubscript{ATP} channels in vascular smooth muscle [89,90], again possibly through mediation via A\textsubscript{2}-receptors [91]. However, contrasting results have been reported in this area. First, Nakhostine and Lamontagne showed that although Ado contributed to hypoxia-induced vasodilatation through activation of K\textsubscript{ATP} channels, this was achieved by mediation primarily via the A\textsubscript{1}-type receptor [92]. It has been proposed that at least two receptors are involved in the vasodilatation [93]. Secondly, despite the indication that K\textsubscript{ATP} channels are coupled to A\textsubscript{1}-receptors by G\textsubscript{i}, in ventricular myocytes [60,61], Furukawa et al. have suggested that a G-protein was not involved in the opening of these channels for the A\textsubscript{2}-receptor-mediated vasodepression in resistance vessels of rats [91]. Finally, contrary to
the prevailing literature, which has implicated $K_{\text{ATP}}$ channels in the vasodilatation and bradycardia induced by Ado [89,90]. Fozard and Carruthers found no evidence to support the role for such channels in the $A_1$- or $A_2$-receptor mediated cardiovascular effects in the pithed rat [94]. Others have found that glibenclamide causes a parallel rightward shift of Ado agonist dose–response curves, indicating that $K_{\text{ATP}}$ channels are not the sole effectors of Ado-mediated vasodilatation [93]. With the emergence of such contrasting results, it is clear that this is an area that warrants further investigation.

5.1.2. Metabolic effects

The initial metabolic response of the myocardium to hypoxia or ischemia is an increase in glucose uptake and utilization to preserve myocardial energy supply by glycolysis [95]. In this context, a number of studies appear to indicate that Ado plays a role in glucose metabolism [66] by enhancing glucose uptake during periods of reduced $O_2$ supply [66].

Ado has been reported to delay the onset of ischemic contracture during global ischemia in isolated rat hearts via interaction with $A_1$-subtype receptors [65,96]. This effect was associated with an increase in lactate release, thus confirming the involvement of Ado in glucose metabolism [95]. Although the enhancement of glucose uptake by Ado provides yet another possible mechanism whereby an $O_2$ supply–demand imbalance may be corrected, it remains a controversial issue [97]. Moreover, whether enhanced glucose uptake has any beneficial effects on cell viability also remains to be determined [6]. More recent studies suggest that Ado slows ischemic metabolism [98] and decreases the intracellular acidosis and the $Ca^{2+}$ accumulation associated with ischemia [99].

5.1.3. Presynaptic inhibition of neurotransmitter release

The effect of Ado also involves a presynaptic attenuation of the release of noradrenaline from sympathetic nerve endings in addition to its postsynaptic effects [100]. Even short periods of myocardial ischemia have been shown to result in the release of sufficient amounts of Ado to reduce significantly the amount of noradrenaline released from nerve fibres in the heart [100]. In addition, Ado exerts a prejunctional inhibitory action on the release of peptides from the capsaicin-sensitive sensory-motor nerves [16].

5.2. Role of adenosine in the regulation of cardiac function

The multiple effects of Ado are relevant to the modulation of heart function and the regulation of myocardial $O_2$ supply–demand balance (Fig. 4) [17]. Ado achieves an increase in myocardial $O_2$ supply by causing coronary vasodilatation. It also minimizes the workload of the heart and lowers $O_2$ demand by decreasing heart rate through depression of SA node activity, and by depressing AV nodal conduction. The magnitude of these negative chronotropic and dromotropic effects of Ado, however, appears to be species dependent, and caution should therefore be exercised when extrapolating results from Ado studies in one species to another, including man [101]. A reduction in $O_2$ requirement is also achieved by Ado antagonizing the increase in cardiac contractility that results from stimulation by $\beta$-adrenergic agonists.

The earliest response to an inadequate $O_2$ supply for myocardial needs (due to decreased supply caused by hypoxia or ischemia, or to increased demand caused by an increase in cardiac work or metabolism) is dilatation of the coronary resistance vessels [6,97]. As mentioned earlier, a large body of evidence supports the concept that ischemia–hypoxia-induced vasodilatation is mediated by the release of Ado from the myocardial cells, and that the degree of vasodilatation and Ado release are directly proportional to the degree of $O_2$ deprivation [16,37,41,101]. A number of studies with the Ado receptor antagonist theophylline support this concept since the antagonist has been shown to decrease coronary flow during hypoperfusion in isolated, perfused, and in situ hearts [1]. Further support has been gained from studies involving the intracoronary administration of Ado deaminase in which a significant attenuation of the increase in coronary flow during systemic hypoxia has been observed [38].

When the coronary resistance vessels have reached maximal dilatation, a greater degree of $O_2$ deprivation elicits increased production of Ado [6]. The resulting moderately high myocardial Ado concentration (due to a moderate decrease in the global $O_2$ supply) gives rise to AV conduction delay between the atrium and the bundle of His, while an even higher Ado concentration (resulting from still greater reduction in $O_2$ supply) may result in AV block [38]. This negative dromotropic effect therefore represents another mechanism by which Ado helps to redress the $O_2$ supply–demand imbalance.

The negative dromotropic effect mediated by Ado has been studied extensively in the isolated perfused guinea
pig heart. It has been demonstrated that the degree of AV conduction impairment is approximately proportional to the amount of Ado formed in the hypoxic or ischemic myocardium [38]. Ado has also been shown to have similar effects on the human AV node [102].

Severe reduction in $O_2$ delivery to the heart may result in very high concentrations of Ado, which in turn depress the SA node and result in bradycardia [6]. This effect on the SA node has been demonstrated both in guinea pig hearts and in man [6,102]. Ado therefore further reduces the $O_2$ requirements of the heart, offering additional protection against ischemic damage [6].

Ado has a direct negative inotropic effect in the atria, where it shortens the action potential duration [38]. In ventricular muscle, however, negative inotropic response is observed in most species, including the human ventricle [103,104], only if adenylate cyclase is activated.

5.3. Attenuation of vascular ischemic injury

5.3.1. Vascular dilatation and protection from oxygen-derived free radicals

During hypoxic or ischemic conditions, myocardial perfusion may be reduced by two main mechanisms: release of an endothelial vasoconstrictor, or impairment of endothelium-mediated relaxation by the action of superoxide anions released from activated neutrophils or from the endothelium itself [105–108]. It has been proposed that Ado released from the myocytes may help to prevent more extensive ischemic damage, not only by inducing relaxation of the vascular muscle, but also by protecting the endothelium and the vascular smooth muscle against toxic effects of $O_2$-derived free radicals [108,109]. However, since the toxic effects of ischemia and reperfusion appear to override the beneficial effects of endogenously produced Ado, higher concentrations of exogenously administered Ado may help to combat these detrimental vascular effects [6].

5.3.2. Neutrophil activation

A second mechanism whereby ischemia can result in microvascular damage is by causing the capillaries to become plugged with activated neutrophils [6,105,110,111]. Cronstein et al. [112] have shown that Ado may in fact contribute to the obstruction of capillaries by facilitating the chemotaxis of activated neutrophils (an $A_2$-receptor-mediated action). However, in contrast to this $A_2$-mediated effect, $A_1$-receptor activation prevents not only neutrophil adherence to the vascular endothelium, but also the release of superoxide anions by activated neutrophils [110]. Since neutrophil $A_1$-receptor stimulation occurs at much lower concentrations of Ado than the $A_2$-receptor effect [112], the endogenous Ado may be sufficient to promote neutrophil chemotaxis but not to prevent superoxide anion release. Nevertheless, the exogenous administration of pharmacological concentrations of Ado may prevent free radical release and thereby counter the neutrophil-mediated injury [6].

5.3.3. Platelet adherence and aggregation

A third factor that contributes to vascular damage and reduced tissue perfusion during ischemia is the adherence of platelets to injured endothelial cells and the aggregation of platelets at these sites [6,75]. Ado opposes platelet aggregation and prevents microthrombosis [113]. Exogenous administration of Ado may again provide additional protection.

5.4. Role of adenosine in angiogenesis

Chronic ischemia associated with coronary artery stenosis may lead to the development of a collateral circulation in the heart and skeletal muscle. Adair et al. [114] have demonstrated that Ado plays a role in this angiogenic process. Other researchers showed that when cultured endothelial cells were placed in conditions of low $O_2$ concentration (2%), cell proliferation was increased; culture medium from hypoxic cells also stimulated the proliferation of cells grown in 20% $O_2$ [115]. The addition of an Ado receptor antagonist to the hypoxic medium prevented this increase in cell proliferation. Furthermore, dipyrudamole, an Ado transport blocker, increased the formation of new capillaries in the rat heart [116]. These observations therefore indicate that Ado serves as a protective agent against prolonged periods of reduced blood flow by stimulating the development of new blood vessels.

6. Therapeutic potential of adenosine

The many beneficial actions of Ado, together with its favorable pharmacological profile, make it an attractive therapeutic and diagnostic tool and leave little doubt that agents that can harness these effects while minimizing adverse events, will become established in the treatment and diagnosis of cardiovascular disease and the protection of the ischemic myocardium [6,7].

The clinical use of Ado is currently restricted to the treatment of paroxysmal supraventricular tachycardias, with additional diagnostic indications in broad- or narrow-QRS complex tachycardias and, in combination with myocardial perfusion scintigraphy, in the detection of coronary artery disease [54,117]. The emergence of analogues, regulators, or transport and metabolic inhibitors of Ado, may, however, extend its therapeutic and diagnostic applications and provide significant new advances in various clinical conditions. It now remains to be determined whether Ado has any benefits in the treatment of peri-operative supraventricular tachycardia, acute arterial hypertension, and pulmonary hypertension, and in the treatment and diagnosis of arrhythmias [54]. Additionally, its potential role in regional perfusion injury, preconditioning and infarction, cardio-

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plegic solutions, preservation solutions for heart transplantation, coronary artery bypass surgery, and other diagnostic applications has exciting prospects [6,7,54].

6.1. Antiarrhythmic therapy

Ado is well suited for the treatment of paroxysmal supraventricular tachycardias [118-120] given that it depresses AV nodal conduction and that 90% of supraventricular tachycardia episodes with a ventricular rate greater than 150 involve re-entry in the AV node [102]. The safety and efficacy of Ado in the diagnosis and treatment of supraventricular tachycardia are now well established, and Ado has been documented to have over 90% success rate [17] within 30 s of administration [119]. In patients with sustained atrial tachycardia, isoproterenol facilitation of a spontaneous or inducible tachycardia showed marked correlation with high sensitivity of the arrhythmia to Ado [121]. Additionally, Ado has been noted to have several advantages over verapamil in the treatment of supraventricular tachycardia [118]: although both agents successfully terminate arrhythmias in 90% of cases, Ado has a faster onset of action, and does not alter the hemodynamic status of the patients [4,122]. Furthermore the short half-life of Ado ensures rapid termination of adverse effects if they occur [118].

Ado-induced hyperpolarization and antagonism of the catecholamine effects on the pacemaker current accounts for the termination of tachycardia due to sinus node re-entry [123]. The potential for Ado to produce sinus arrest or AV block when used in the clinical setting for supraventricular tachycardia is very low, since twice the dose of Ado is required to produce a greater than 50% slowing in the sinus rate or heart block than that needed to terminate supraventricular tachycardia [102]. Since the negative chronotropic effect of Ado on the sinus node is minor, it has been concluded that Ado is unlikely to be very effective in the treatment of sinus node tachycardia [124].

Although the majority of ventricular tachycardias are insensitive to Ado, it has been reported that Ado is effective in tachycardias induced by catecholamines [17] or by exercise [125].

6.2. Myocardial protection and preservation

6.2.1. Regional reperfusion injury

Considerable attention has recently focused on the pathophysiology of myocardial ischemia and of the reversible postischemic dysfunction, also termed myocardial stunning [126], associated with subsequent reperfusion. It has been proposed that intra arterial infusion of Ado may offer cardioprotective benefits by attenuating reperfusion injury and the extent of myocardial stunning. Intracoronary infusion of Ado in a canine model of coronary artery occlusion results in vasodilatation and a consequent marked improvement in segmental ventricular systolic shortening [127,128]. The beneficial effects of Ado on the recovery of regional ventricular function were prevented with an Ado receptor antagonist [129].

Additional evidence to support the cardioprotective role of Ado was provided from experiments in closed-chest dogs which involved coronary infusion of Ado during the first hour of reperfusion following periods of regional ischemia lasting 40–180 min [130–132]. Ado infusion was found to result in a better functional recovery and in a considerable reduction in infarct size. The benefit from Ado was, however, lost when the ischemic period was extended to 3 h. Although the mechanism by which Ado may help ventricular recovery following ischemia is not clear, the therapeutic potential of Ado in this area is an exciting prospect.

6.2.2. Preconditioning and infarct size limitation

A large body of evidence suggests that Ado plays a role in ischemic preconditioning with respect to its initiation and mediation [127,128,133–139]. Downey and coworkers have proposed that Ado, released during a preconditioning occlusion, stimulates cardiac A1-receptors, leaving the heart protected against infarction even after the nucleoside has been withdrawn [133,134]. Ado has also been implicated in the protection afforded by ischemic preconditioning against myocardial stunning [135–137] as well as in the protection against infarction, that is observed ≥ 24 h after the short, preconditioning ischemia [138] (‘second window’ of protection). Ado receptor blockade, before or after the conditioning stimulus, has been shown to suppress the cardioprotective effects of preconditioning [133,139]. Further studies have indicated that preconditioning is mediated by the opening of KATP channels [62,140,141], probably initiated by the A1-receptor activation of a G-protein. In addition, recent data have implicated the activation of PKC in the protective effect of preconditioning [142]. However, the roles of Ado, A1-receptor, PKC and KATP channels are still controversial, [143–146] and the mechanisms whereby preconditioning exerts a protective effect require further investigation. Based on the effect of selective A1 agonist and A1 antagonist, it has also been recently proposed that preconditioning is mediated via A1 receptors [147].

Although studies on the role of Ado in reducing infarct size are not all in agreement, a general conclusion can be drawn that Ado infusion can reduce infarct size significantly if the length of occlusion is less than 3 h [130,133]. However, some other recent data indicate that the administration of exogenous Ado may only confer cardioprotection during reperfusion after periods of myocardial ischemia when administered in combination with lidocaine [148]. The therapeutic potential of Ado in this area is currently attracting a great deal of interest.

6.2.3. Cardioplegia and cardiac transplantation

The ability of Ado to induce cardiac arrest or hasten K+–induced arrest has led to investigation of the cardio-
plegic potential of the nucleoside. In a study conducted by Wyatt et al. [149], standard K+ cardioplegia was compared with Ado-enriched K+ cardioplegia in an in vivo canine model subjected to 1 h of global ischemic arrest. The degradation of ATP was significantly reduced, and postischemic recovery of ventricular function was significantly improved, in the Ado-enriched cardioplegic group compared with the standard cardioplegic medium [149]. This and other studies with Ado, used alone or in combination with K+ cardioplegia, suggest that Ado provides additional cardioprotection during global ischemia [149-152] and provides yet another therapeutic potential for Ado.

Recently, the use of Ado in cardiac transplant preservation has attracted much attention. Several studies have reported a marked improvement in the preservation of ventricular function of canine or rat hearts when Ado is added to the standard Krebs-Henseleit or to the Euro-Collins transplant perfusion fluid [153,154]. Ado was also found to preserve ventricular function of cardiac transplants after a storage period of up to 24 h in a hypothermic perfusion system [155]. Moreover, in clinical studies involving human heart transplant recipients, storage of donor hearts in an Ado-enriched solution resulted in improved end-ischemic ATP and creatine phosphate levels, and reduced intra-operative defibrillation and pacing requirements [156].

6.2.4. Coronary artery bypass grafts
Ado may also be of value in the prophylaxis of bypass graft occlusion: infusions of Ado have been reported to produce a stable coronary vasodilatation in patients undergoing aortocoronary bypass surgery [118].

6.3. Control of blood pressure

6.3.1. Controlled hypotension
Ado has been used during neurosurgical procedures to induce [54,118] and maintain a low blood pressure and thus reduce bleeding [97]. The Ado-induced hypotension is not associated with changes in heart rate, plasma catecholamine levels, and cardiac lactic acid uptake [157], and levels of plasma renin activity remain unaltered [158]. These results compare favorably with those observed for hypotension induced by sodium nitroprusside and nitroglycerin [157,158]. The absence of an increase in catecholamine levels and renin activity reflects three distinct factors associated with Ado use: absence of reflex tachycardia, absence of tachyphylaxis, and absence of rebound hypertension [157,158].

Additional studies have also shown Ado to evoke a rapid and safe decline in blood pressure [159]. Moreover, surgical hypotension was maintained at a steady level, again without tachycardia and with significantly less reflex tachycardia than that observed with nitroglycerin or nitroprusside [159]. Ado also produced an increase in cardiac output in patients with low output due to high peripheral vascular resistance [159], thus prompting the current investigation of the use of Ado as an afterload reducing agent in low output states [118].

In various other clinical studies, continuous infusion of Ado produced a stable and easily controllable hypotension within 1–3 min [160,161] and, following discontinuation of Ado, mean arterial pressure was restored within 1–5 min. Furthermore, it has also been shown that continuous Ado infusion can control postoperative hypertension in patients following coronary artery bypass surgery [162]. More clinical work is required to define the role of Ado in the treatment of acute episodes of perioperative arterial hypertension [54].

6.3.2. Pulmonary hypertension
Continuous infusion of Ado in patients with pulmonary hypertension caused a 40% decline in pulmonary vascular resistance and over a 50% increase in cardiac output [163-165]. Additionally, direct infusion into the pulmonary artery resulted in a decrease in pulmonary and systemic vascular resistance. Nevertheless, the systemic arterial pressure remained unaltered, and the ratio of pulmonary to systemic vascular resistance declined significantly [163]. Since Ado has only moderate effects on the systemic circulation when infused into the pulmonary artery, it may have a specific role in the management of perioperative pulmonary hypertension and in the assessment of pulmonary vasodilator reserve in intensive care [54].

7. Diagnostic uses

In addition to its therapeutic benefits, Ado has considerable diagnostic utility due to its selective antiarrhythmic activity, short half-life, and minimal hemodynamic consequences [19,118].

7.1. Coronary artery disease
Pharmacologically induced increases in coronary flow, in combination with perfusion scintigraphy or echocardiography, have been used successfully to investigate the degree of coronary artery disease [54], and there is abundant literature on the use of Ado in this context [166–168]. Since Ado produces coronary steal in patients who are unable to undergo conventional exercise testing, Ado thallium imaging is used to assess the extent of coronary artery disease in such patients [54,97].

7.2. Arrhythmias

7.2.1. Tachycardias
The origin of some wide-complex tachycardias can be revealed by the combination of AV block and anti-adrenergic effects induced by Ado [54]. Due to its differential
Fig. 5. Effects of adenosine in narrow-complex tachycardia [4]. AV, atrioventricular; AVRT, atrioventricular reciprocating tachycardia; PJRT, permanent form of junctional reciprocating tachycardia; JET, junctional atrioventricular; AVRT, atrioventricular reciprocating tachycardia; PJRT, permanent form of junctional reciprocating tachycardia; JET, junctional atrioventricular; MAT, multifocal atrial tachycardia; AAT, automatic atrial tachycardia.

effects on supraventricular tissue and ventricular tissue, Ado has proved to be a useful and safe tool to distinguish between ventricular tachycardia and supraventricular tachycardia with rate-dependent QRS complex aberration [17,169]. The efficacy and safety of Ado as a therapeutic and/or diagnostic adjunct for wide-complex tachycardias have been confirmed [170].

In addition, in narrow-complex tachycardia for example, Ado is a useful diagnostic tool to distinguish between atrial tachycardia and tachycardia episodes that require the AV node as part of the re-entrant circuit (Fig. 5). In cases in which atrial flutter waves are difficult to discern on a surface electrocardiogram, an incorrect diagnosis of AV nodal re-entry may result. In these patients, therefore, Ado-induced transient AV block, with perpetuation of the atrial arrhythmia, can facilitate the correct diagnosis and thus guide appropriate therapy [19].

7.2.2. Pre-excitation

Ado can be used to unmask ventricular or latent pre-excitation [117]. When used as a diagnostic tool for latent pre-excitation in a small number of patients with supraventricular tachycardia and a normal electrocardiogram during sinus rhythm, Ado was noted to be 100% sensitive and specific, provided that AV block or PR prolongation was induced [171].

8. Adverse effects

An extensive safety review of Ado found facial flushing, dyspnoea, and chest pain and/or pressure to be the most common side effects associated with its use [117], with an incidence of 11–18% [119]. Such effects are reported to be dose-related [119]. However, although these adverse effects were transient, with a median duration of 50 s [119], and do not require clinical intervention, they can become intolerable. In addition, caution should be exercised when administering Ado to asthmatic patients as it has been reported to cause bronchoconstriction. Other less commonly reported side-effects of Ado include nausea, headache, lightheadedness [19], dizziness, sweating, metallic taste, hyperventilation, and palpitations [118].

Since exogenous Ado is reported to produce angina pectoris-like pain [172,173] (an effect attributed to direct stimulation of cardiac afferent vessels) it has been suggested that endogenous Ado may play a role in the genesis and mediation of anginal chest pain [54,172,173]. Enhanced Ado release during ventricular fibrillation is also suspected to be responsible for the increase in defibrillation threshold with time [174].

9. Potential therapeutic agents related to adenosine

In parallel with the recognition of the cardioprotective role of Ado in various settings, and since the exogenous administration of Ado is associated with a number of systemic side-effects, recent efforts have focused on developing novel agents that act to modulate endogenous Ado.

9.1. Adenosine-regulating agents

Considerable effort has been made to develop novel Ado-regulating agents (ARAs) which modulate the endogenous production and availability of Ado. The prototype of this new class of agents is acadesine (5-amino-4-imidazole carboxamide riboside; also called AICA-riboside), a natural precursor of Ado, which has recently attracted a great deal of interest since it has been shown to increase the availability of Ado only in ischemic tissues [175]. Although the exact mechanism by which acadesine increases Ado levels is not known, it appears to enhance the net conversion of AMP to Ado in energy-deprived cells and, consequently, its effects are evident in ischemic, but not in normally perfused, healthy tissues. Hence, acadesine may be viewed as a novel site- and event-specific ARA; such novel ARAs are expected to be of clinical benefit in attenuating postoperative cardiac events.

A study by Leung et al. [176] indicated that acadesine may have a favorable effect on decreasing the frequency of perioperative myocardial infarction and the extent of myocardial injury in patients undergoing coronary artery bypass grafting. Acadesine was also shown to improve the posts ischemic recovery of contractile function both in vitro and in vivo and, in both instances, the co-administration of an Ado receptor antagonist inhibited this improvement [177]. These and other studies provided strong support for acadesine induced alleviation of myocardial stunning, and indicated that endogenous cardioprotective mechanisms can be stimulated pharmacologically to provide benefits. In addition, Tsuchida et al. [178] demonstrated that acadesine significantly lowers the threshold for the protective preconditioning phenomenon in rabbits to less than 2 min of
ischemia. These results suggested that acadesine might be used in the therapeutic setting to enhance the preconditioning phenomenon in patients undergoing percutaneous transluminal coronary angioplasty, as well as in those with unstable angina [178]. More recently, randomized multicenter clinical trials, carried out in patients undergoing coronary artery bypass grafting have yielded results that are less convincing about the protective effect of acadesine: acadesine did not significantly influence the incidence of perioperative myocardial infarction or of all adverse cardiovascular outcomes, defined using prespecified criteria. [179,180].

9.2. Adenosine enhancers

Ado enhancers increase the availability of the nucleoside by inhibiting its uptake into metabolizing cells (nucleoside transport inhibitors or NTIs) [34] or by directly inhibiting enzymes responsible for its degradation. These Ado enhancers modify the actions of Ado by substantially increasing and prolonging its cardiovascular effects [17]. A novel type of Ado enhancers has been recently described, which cause an allosteric modification of Ado binding to receptors and induce a potentiation of Ado effects [181].

Lidoflazine is an NT1 and has been shown to enhance cardioprotection during myocardial global normothermic ischemia [182]. Dogs treated with lidoflazine during cardiopulmonary bypass and reperfusion survived and recovered 66-90% of the preischemic hemodynamic function. In contrast, no control (untreated) survived after 30 min of reperfusion. Myocardial ultrastructural damage at the end of the bypass was more marked in untreated dogs than in treated ones [182]. While lidoflazine may possess a variety of pharmacological properties in addition to its NTI property (e.g., local anesthetic action), nucleoside transport inhibition is likely to be the mechanism mediating the beneficial action of administering lidoflazine during cardiac surgery performed under extracorporeal circulation with repetitive aortic cross-clamping in humans or in dogs [183,184]. During reperfusion after the last ischemic period, functional recovery was complete in lidoflazine treated dogs, but < 30% in control, untreated ones. The improvement of function by lidoflazine could be inhibited by theophylline [183]. Myoflazine, an NT1 related to lidoflazine but devoid of local anesthetic action, also caused cardioprotection [185].

Recently, R75231 has been proposed as a potent and specific NTI. The NTI action of R75231 is also present in human hearts where the drug does not change the rate of ATP degradation, but causes an increased Ado accumulation and a decreased purine base accumulation during 2 h of normothermic ischemia [186]. R75231 was shown to induce a protective effect similar to the one obtained with continuous infusion of exogenous Ado in rabbit hearts to which the drug was administered prior to 20-min ischemia [187]. In dog hearts kept in ice-cold cardioplegic solution for 24 h before reperfusion, R75231 inhibited hypoxanthine accumulation during ischemia, and inhibited contracture and myocardial enzyme (creatine kinase and lactate dehydrogenase) release on reperfusion [188]. R75231 also enhances the protective effects of ischemic preconditioning, an observation that is consistent with the hypothesis that Ado mediates this process [189].

Dipyridamole is a well-known potent Ado uptake blocker which potentiates the actions of Ado [17]. Benzo- diazepine and its derivatives are also reported to have the same effect, although to a lesser extent. High concentrations of Ca\(^{2+}\)-channel blockers have been found to inhibit the nucleoside transport system in human erythrocytes and cardiac membranes, and to potentiate the cardiac actions of Ado in the dog, but the relevance of these effects remains to be determined [17]. Finally, the NTI P-nitrobenzylthioinosine was shown to reduce the depletion of cellular ATP, when added to cardioplegically preserved human ventricular myocytes or rat hearts [190].

Inhibitors of Ado deaminase, such as 2'-deoxycoformycin (dcF, pentostatin) and erythrohydroxynonyladenine (EHNA), have also been demonstrated to enhance the effects of Ado [17,191].

Although Ado enhancers appear to hold potential clinical importance, more work with these agents is required to establish their precise role in the clinical setting.

9.3. Adenosine receptor antagonists

Other agents that can modify the actions of Ado are Ado receptor antagonists. Methylxanthines such as theophylline and caffeine bind on Ado receptors in cardiac membranes and competitively antagonize the specific binding of agonists to these receptors [17]. They are, however, relatively weak and nonselective, and depress the electrophysiological and vasodilator actions of Ado. Other xanthine derivatives have, however, been developed which are reported to be considerably more potent and selective (for the A1-receptor) than theophylline and caffeine [17]. Additionally, Ado antagonists that are not derivatives of methylxanthines and which are highly selective for A1-receptors have also been synthesized [17].

Although it has been suggested that Ado receptor antagonists, specifically of the A1-receptor, may be effective antihypertensive agents [67] and that potent and selective antagonists may have potential therapeutic value in the treatment of cardiac rhythm disturbances such as ischemic AV block, in general the clinical usefulness of Ado antagonists remains to be determined [17].

10. Conclusions

In the last decade or so, an abundance of evidence has emerged to support the role of Ado as an important endogenous modulator of cardiac function and myocardial
O₂ supply–demand balance, thus suggesting that it plays a multifaceted role in the protection of the ischemic myocardium. Ado is released from the myocardium in response to a decreased O₂ supply/demand ratio and acts both to decrease O₂ consumption by depressing cardiac activity, and to increase O₂ supply by eliciting coronary vasodilatation. Additionally, Ado appears to enhance energy production during periods of O₂ deprivation by increasing the glycolytic flux, and by acting as a substrate for purine salvage to restore energy charge during reperfusion.

Many animal and human studies have also indicated that Ado may limit the degree of vascular injury during ischemia and reperfusion by the inhibition of release or actions of O₂ radicals, or by inhibition of platelet aggregation or neutrophil activation, thereby helping to preserve endothelial cell function and microvascular perfusion. In addition, Ado has been implicated in coronary angiogenesis during sustained ischemia associated with coronary artery stenosis.

With the ever-increasing understanding of the pharmacology and metabolism of Ado, which has been paralleled with a growing interest in the role of Ado with respect to its cardioprotective antiarrhythmic and vasodilator actions, considerable attention is now being focused on the therapeutic and diagnostic potential of the nucleoside, as well as of its enhancers and antagonists, in a number of clinical situations. Indeed, due its potential benefits, Ado may assume an increasingly prominent position in the first-line management of arrhythmias in future years, and could also be of value as a cardiac diagnostic agent. Moreover, given the paramount implications of drug-receptor interactions, considerable attention is now being focused on the isolated guinea pig heart in response to isoproterenol, acetylcholine, and adenosine: The minimal role of vascular endothelium. Circ Res 1987;61:594–600.

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