Review

Adenosine, adenosine receptors and myocardial protection: An updated overview

Kanigula Mubagwa*, Willem Flameng

Centre for Experimental Surgery and Anaesthesiology, University of Leuven, 3000 Leuven, Belgium

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Abstract

Adenosine (Ado) accumulates in tissues under metabolic stress. On myocardial cells, the nucleoside interacts with various receptor subtypes (A₁, A₂₃, and probably A₃A and A₁B) that are coupled, via G proteins, to multiple effectors, including enzymes, channels, transporters and cytoskeletal components. Studies using Ado receptor agonists and antagonists, as well as animals overexpressing the A₁ receptor indicate that Ado exerts anti-ischemic action. Ado released during preconditioning (PC) by short periods of ischemia followed by reperfusion induces cardioprotection to a subsequent sustained ischemia. This protective action is mediated by A₁ and A₃ receptor subtypes and involves the activation and translocation of PKC to sarcolemmal and to mitochondrial membranes. PKC activation leads to an increased opening of ATP-sensitive K⁺ (K<sub>ATP</sub>) channels. Recent studies implicate mitochondrial rather than sarcolemmal K<sub>ATP</sub> channels in the protective action of PC. Other effectors possibly contributing to cardioprotection by Ado or PC, and which seem particularly involved in the delayed (second window of) protection, include MAP kinases, heat shock proteins and iNOS. Because of its anti-ischemic effects, Ado has been tested as a protective agent in clinical interventions such as PTCA, CABG and tissue preservation, and was found in most cases to enhance the post-ischemic recovery of function. The mechanisms underlying the role of Ado and of mitochondrial function in PC are not completely clear, and uncertainties remain concerning the role played by newly identified potential effectors such as free radicals, the sarcoplasmic reticulum, etc. In addition, more studies are needed to clarify the signalling mechanisms by which A₁ receptor activation or overexpression may promote apoptosis and cellular injury, as reported by a few recent studies.

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1. Introduction

The purine nucleoside adenosine (Ado; see Table 1 for abbreviations and acronyms) is recognized as a major local (autocrine and paracrine) regulator of tissue function, especially when energy supply acutely fails to meet cellular energy demand. The nucleoside is produced by the tissue under stress, in order to increase the energy supply (by vasodilatation) and to decrease the energy demand in the same tissue (‘negative feedback regulator’ or ‘retaliatory metabolite’; see Ref. [1]). In addition to this regulatory role to prevent ischemia, Ado also exerts a protective role against cell injury caused by established ischemia. Ado fulfills these roles mainly in tissues such as the heart, the brain and the kidney, that are especially prone to ischemic injury. In the heart the effects of Ado are exerted via direct action on cardiomyocytes, vascular smooth muscle cells and endothelial cells, as well as indirectly via actions on synaptic transmission in the autonomic nervous system [2] or on inflammatory cells (e.g. neutrophils [3]). In the past we have presented an overview of the effects of Ado on cardiac function and on circulation [4]. Since intense...
Table 1
List of abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AC</td>
<td>Adenylate cyclase</td>
</tr>
<tr>
<td>ACh; AChR</td>
<td>Acetylcholine; acetylcholine receptor</td>
</tr>
<tr>
<td>Ado; Ado-R</td>
<td>Adenosine; adenosine receptor</td>
</tr>
<tr>
<td>AVNRT</td>
<td>Atrioventricular node reentrant tachycardia</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>ERK</td>
<td>Extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat shock protein</td>
</tr>
<tr>
<td>$I_{\text{Ca,L}}$</td>
<td>L-type Ca$^{2+}$ current</td>
</tr>
<tr>
<td>JNK</td>
<td>Jun N-terminal kinase</td>
</tr>
<tr>
<td>$K_{\text{ACh}}, K_{\text{Ado}}, K_{\text{ATP}}$</td>
<td>Acetylcholine-activated, adenosine-activated, ATP-inhibited (channels)</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactic dehydrogenase</td>
</tr>
<tr>
<td>MAPK; MARKAPK</td>
<td>Mitogen-activated protein kinase; mitogen-activated kinase activated protein kinase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>PC</td>
<td>Preconditioning</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>PLC; PLD</td>
<td>Phospholipase C; phospholipase D</td>
</tr>
<tr>
<td>PTCA</td>
<td>Percutaneous transluminal coronary angioplasty</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
</tr>
<tr>
<td>SAPK</td>
<td>Stress-activated protein kinase</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>CPDPX=DPCPX</td>
<td>1,3-dipropyl-8-cyclopentylxanthine</td>
</tr>
<tr>
<td>CI-IB-MECA</td>
<td>Chloro-$N$-(3-iodobenzyl)-$N$-methyl-5$'$-carboxyladenosine</td>
</tr>
<tr>
<td>LNAMMA; LNNA</td>
<td>$N$-$N$'-monomethylarginine; $N$-$N$'-nitroarginine</td>
</tr>
<tr>
<td>SPT</td>
<td>Sulfophenyltheophylline</td>
</tr>
<tr>
<td>EHNA</td>
<td>Erythro-$9$-(2-hydroxy-3-nonyl)adenosine</td>
</tr>
<tr>
<td>NECA</td>
<td>5$'$-(N-ethylcarboxamido)adenosine</td>
</tr>
<tr>
<td>NBMPR</td>
<td>5$'$-(4-nitrobenzyl)-6-thinoine</td>
</tr>
<tr>
<td>R-PIA</td>
<td>(R)$-N$'$-phenylisopropyladenosine</td>
</tr>
<tr>
<td>S-PIA</td>
<td>(S)$-N$'$-phenylisopropyladenosine</td>
</tr>
</tbody>
</table>

Drugs (mentioned in the text but not included in Table 2)

- CPDPX=PCPX
- CI-IB-MECA
- LNAMMA; LNNA
- SPT
- EHNA
- NECA
- NBMPR
- R-PIA
- S-PIA

Research has continued in this field, knowledge on the mechanisms underlying Ado effects in the various cell types or tissues has accumulated, and in the meantime many other reviews or more comprehensive works on the subject have been published (e.g. Refs. [1,5–12]). Recent progress has been made mainly regarding the role of Ado as cardioprotective agent, especially its implication in ischemic preconditioning (PC), a process by which short periods of ischemia followed by reperfusion protect against otherwise lethal injury induced by sustained ischemia [13]. In this context, the following major aspects have been examined: (1) the roles of various Ado receptors and their coupling mechanisms with enzyme or channel effectors, (2) the mechanisms by which cardioprotection is achieved, and (3) the usefulness of Ado as a myocardial therapeutic agent. Therefore, these topics will constitute the focus of this update review, in which we will restrict ourselves to direct effects involving cardiomyocytes and will refer mainly to studies published during the last 5 years.

2. Adenosine formation, binding on receptors and activation of effectors

2.1. Adenosine formation during ischemia

Ado accumulates during ischemia or hypoxia (see Refs. [1,4]). Increased Ado formation is due to the imbalance between O$_2$ supply and demand [14], resulting in an imbalance between ATP formation and consumption. One important factor contributing to the Ado accumulation is an increase in the substrate (mainly AMP) from which the nucleoside is formed. Studies in isolated hearts as well as in dissociated cardiac myocytes have shown that Ado formation is linked to the accumulation of free ADP and to the formation (by myokinase) of AMP from ADP [15–17]. However, the increase of AMP may not be sufficient and may need to be associated with an increase in the activity of the Ado forming enzymes (nucleotidases). Recently, an important role for the Ado salvage enzyme Ado-kinase in
### Table 2

**Adenosine receptor classification**

<table>
<thead>
<tr>
<th>Receptor subtype</th>
<th>Number of amino-acids</th>
<th>Effect on AC</th>
<th>Typical selective agonists</th>
<th>Typical selective antagonists</th>
<th>Coupling G protein</th>
<th>Effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1$</td>
<td>328</td>
<td>Inhibition</td>
<td>High agonist affinity</td>
<td>CPA=N$^5$-cyclopentyladenosine</td>
<td>DPCPX=8-cyclopentyl-1,3-dipropylxanthine</td>
<td>$G_{i/o}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R-PIA&gt;NECA&gt;S-PIA</td>
<td>CCPA=2-chloro-N$^5$-cyclopentyladenosine</td>
<td>WRC-0571=C8-N-methylisopropylamino-N6-5'-endothryony-N0861</td>
<td>$G_\beta\delta$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CHA=N$^5$-cyclohexadenosine</td>
<td></td>
<td>G?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GR79236=N-[(15,trans)-2-hydroxycyclopentyl]adenosine</td>
<td></td>
<td>G?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SPA=N$^5$-p-sulphophenyladenosine</td>
<td></td>
<td>G?</td>
</tr>
<tr>
<td>$A_{2A}$</td>
<td>410</td>
<td>Stimulation</td>
<td>High agonist affinity</td>
<td>CGS21680=2-p-(2-carboxyethyl)phenethylnamino-5'-N-ethylcarboxamidoadenosine</td>
<td>ZM241365=4-2-[7-amino-2-(2-furyl)-1,2,4-triazol[1,5-a][1,3,5]triazin-5-yl-amino]ethylphenol</td>
<td>$G_\delta$</td>
</tr>
<tr>
<td></td>
<td>(long COOH tail)</td>
<td></td>
<td></td>
<td>NECA&gt;R-PIA&gt;S-PIA</td>
<td>SCH58261=5-amino-7-[2-phenylethyl]-2-(2-furyl)pyrazolo[4,3-c]-1,2,4-triazol[1,5-e] pyrimidine</td>
<td>$G_\delta$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DMYP=(N$^{0}$-2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl)adenosine</td>
<td>CGS-15943=chloroerfuranyldihydrotetraenoaziquinazolinimine</td>
<td>PLC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NECA=ethylcarboxamidoadenosine</td>
<td></td>
<td>PKC</td>
</tr>
<tr>
<td>$A_{2B}$</td>
<td>320</td>
<td>Stimulation</td>
<td>Low agonist affinity</td>
<td>?</td>
<td>Enprofylline</td>
<td>$G_\delta$</td>
</tr>
<tr>
<td>$A_3$</td>
<td>320</td>
<td>Inhibition</td>
<td>High agonist affinity</td>
<td>IB-MECA=(N$^6$-iodobenzyl-5-N'-methylcarboxamidoadenosine)</td>
<td>BWA-1433=8-(4-carboxyethylphenyl)-1,3-dipropylxanthine</td>
<td>$G_{1/o}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R-PIA=NECA&gt;S-PIA</td>
<td></td>
<td>MRS1191=3-ethyl-5-benzyl-2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydropyridine-3,5-dicarboxylate</td>
<td>$I_{K(Ado)}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MRS1523=(5-propyl-2-ethyl-4-propyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate)</td>
<td>$G_\delta$</td>
</tr>
</tbody>
</table>
the accumulation and release of the nucleoside has been stressed [16,18,19]. Inhibition of this enzyme by increased inorganic phosphate (Pi) increases Ado formation independently of any change in AMP [19]. The inhibition of Ado-kinase during hypoxia, and probably during ischemia, favors Ado release against Ado salvage by resynthesis into AMP [16].

New techniques have allowed the measurement of Ado in microdialysates of the myocardial interstitial fluid. Most studies have examined the pattern of Ado accumulation during ischemic PC. Interstitial Ado increases during the brief periods of ischemia used to preconditition the heart and can remain elevated during the ensuing reperfusion. As observed in previous studies [20], the new results show that interstitial or venous effluent Ado levels decrease with repetitive brief ischemia. As a consequence, Ado levels during sustained ischemia are lower in preconditioned hearts than in control hearts [21–23]. The decrease of Ado accumulation during sustained ischemia in preconditioned myocardium could not be reproduced by replacing the preconditioning ischemia by a short intracoronary injection of Ado [24]. This indicates that the lower Ado accumulation during sustained ischemia in preconditioned myocardium must be due to an altered purine metabolism and not to an effect mediated by receptors. Given the similarity in the protection afforded by PC or by Ado injection, the above findings also indicate that it is the increased Ado level during the preconditioning, short ischemia (and not the level during the subsequent sustained ischemia) that is critical in determining the extent of myocardial protection afforded by PC. Studies in which the interstitial Ado levels were increased by giving dipyridamole either before or after the preconditioning short ischemia also support the critical role of the Ado level during PC, instead of the levels during the sustained ischemia [25].

2.2. Binding to receptors and activation of effectors

The released Ado interacts with sarcolemmal membrane receptors. Ado receptors (AdoRs) exist in at least four different subtypes: A1, A2A, A2B, and A3 (see Refs. [26–29]). These receptors were initially defined pharmacologically based on the effect (inhibition or stimulation) on adenylate cyclase (AC) and on the potency sequence or selectivity of agonists and antagonists (see Table 2). They have also been found to have different primary amino-acid sequences and molecular weights. Subtle interspecies differences exist in the primary structure of each receptor subtype (see Refs. [28,30]). All the receptor subtypes appear to be expressed in cardiomyocytes (see Ref. [29]), although in the heart A2-AdoRs are known to be most prevalent in coronary vessels. Controversy still exists regarding the presence and function of A2A-AdoRs in cardiomyocytes. A2A-AdoR mRNA has been detected in the rat heart by reverse transcription (RT)-PCR [31] and in the human ventricle by Northern blot [32]. However, in these studies the contribution of myocytes vs. non-myocytes was not determined. The presence of A2A-AdoR mRNA in cardiac myocytes and a functional coupling of A2A-AdoRs to cAMP accumulation and positive inotropy have been demonstrated in rat [33]. Contrast, others, using RT-PCR and in-situ hybridization techniques in porcine myocardium, detected A2A-AdoR mRNA only in coronary arterioles but failed to detect A2A-AdoRs on cardiomyocytes [34]. Early studies in ventricular myocytes from different species failed to obtain any A2-AdoR-mediated effect on action potential, cell shortening or cAMP accumulation [35]. However, a recent study found that activation of A2A-AdoRs in the rat heart induces effects opposing the anti-adrenergic actions of A1-AdoRs [36]. Even more intriguing is the observation that the A2A-AdoR agonist CGS21680, while causing an increase in cAMP (that could be inhibited by the A2A-AdoR antagonist CGS15943), failed to induce any inotropic action in cardiac muscle, hence suggesting a tissue/cellular compartmentation of the A2A-AdoR-mediated effects [37]. Controversy also exists regarding the presence and function of A2B-AdoRs in mammalian cardiomyocytes, but functional evidence for their presence has been obtained in avian cells [38]. More data are needed to resolve these issues but it is possible that some of these discrepancies are due to differences in the expression of the receptor subtypes in various species.

All the AdoRs are coupled to their effectors via G proteins. The A1 and A3 subtypes are coupled to G proteins (Gi, Go) mediating the inhibition of AC and to G proteins mediating the catabolism of phospholipids. In the rat ventricular myocardium A1-AdoRs can be co-immunoprecipitated with Gi3 and Go, and the percentage of receptors co-precipitated in this way or showing high agonist affinity decreases in old animals [39], hence suggesting that age-related losses in Ado effects (see also Refs. [40,41]) are due, at least in part, to an uncoupling between receptors and G proteins. The A2 subtypes are coupled to AC via the stimulatory protein Gi and (at least for A2B-AdoRs) to the phosphoinositide metabolism via Giq. Thus the effectors coupled to AdoRs are multiple, due to divergence in the coupling between each receptor and various G proteins, which are in turn linked to different enzymes, channels or transporters. Therefore a single stimulus (the binding of Ado to a given receptor) can elicit different responses in different tissues or cells, or elicit multiple or complex responses in a given tissue or cell. The multiplicity of receptor subtypes in a given cell or tissue, the species-related structural properties of each subtype, and the above-mentioned divergence in the coupling of each receptor to various effectors make it difficult to determine the subtype underlying a particular response. However, with increasing availability of subtype-selective ligands and of AdoR-subtype knock-out animals, it is becoming feasible to delineate the role of each particular receptor.
The coupling of $A_1$ and $A_3$-AdoR subtypes to AC has been extensively demonstrated and reviewed. The functional importance of the stimulatory coupling of $A_1$-AdoRs to AC remains unclear. In the vascular system, these receptors mediate vasodilatation, an effect attributed largely to $A_2a$-AdoR subtypes [42]. As mentioned earlier, in the myocardium, $A_3$-AdoRs activation can be associated with a positive inotropic action. It has been shown that this positive inotropic action is due not only to the coronary vasodilatation and the resulting stretching of the myocardium (‘garden hose effect’), but also to direct, cAMP-dependent and cAMP-independent actions on myocytes [43]. The potential ‘positive’ (chronotropic, dromotropic and inotropic) effects due to this coupling of $A_3$-AdoRs to AC are unmasked when the inhibitory pathways via $A_1$- and $A_3$-AdoRs are suppressed by treating cells with selective antagonists or by inactivating $G_s/G_i$ with pertussis toxin [44,45].

Among the other enzymes coupled to AdoRs, phospholipases and PKC have been the focus of a lot of interest given their possible role in cardioprotection (see below). Recent studies by Liang et al. [46] indicate that in the avian heart the $A_1$- and $A_3$-AdoR subtypes utilize different pathways to couple to PKC. While the activation of both receptor types induces an accumulation of diacylglycerol (DAG), this effect is more sustained after $A_3$-AdoR stimulation. $A_1$-AdoRs cause a concomitant accumulation of phosphoinositides, indicating that these receptors are coupled to phospholipase C (PLC). Indeed the DAG production after $A_1$-AdoR activation is reversed by the PLC inhibitor U-73122, which also suppresses $A_1$-AdoR-mediated cardioprotection [46]. In contrast, $A_3$-AdoRs preferentially induce the formation of phosphatidylethanolamine, and their cardioprotective action can be inhibited by either ethanol or propranolol, which prevent the conversion of phosphatidic acid into DAG [46]. Thus, at least in the avian heart, $A_3$-AdoRs are selectively or preferentially coupled to PLD.

AdoRs are also coupled to other kinases. The activation of G-protein coupled receptor tyrosine kinases is responsible for the phosphorylation and homologous desensitization of $A_1$ and $A_3$ AdoRs associated with agonist binding [47,48]. A similar situation probably prevails for $A_3$-AdoRs since transfection of cell lines with dominant-negative G protein-coupled receptor kinase-2 has been shown to attenuate their desensitization [49]. The activation of AdoRs causes a phosphorylation and an activation of p38-mitogen-activated protein kinase (p38-MAPK), of its substrate, the MAPK-activated protein kinase 2 (MAPKAPK2), of stress activated protein kinases/jun N terminal kinases (SAPKs/JNKs) and of extracellular signal-regulated kinases (ERKs) [50]. The functional consequences of the changes in these kinases have not been clarified.

Evidence for a coupling of AdoRs to nitric oxide synthase (NOS) has been obtained in nodal tissues. In guinea-pig and rabbit AV node, the anti-adrenergic negative
dromotropic action of Ado or its suppression of $\beta$-adrenergically stimulated L-type Ca$^{2+}$ current ($I_{Ca-L}$) is abolished by drugs that inhibit NOS (LNNNA, LNMMA) or the cytosolic guanylate cyclase (LY83588) [51].

Except for the coupling of $A_1$-AdoRs to specific K$^+$ (called $K_{Ado}$, identical to $K_{AC}$) channels, which is achieved directly through G protein $\beta Y$ subunits, the coupling to other channels is through second messengers, such as cAMP, DAG and probably NO/cGMP. Via $G_s$ and AC Ado is able to affect all channels that are regulated by cAMP, including L-type Ca$^{2+}$ channels, delayed rectifier K$^+$ channels, CFTR Cl$^-$ channels, $I_K$ channels, etc. (see Refs. [1,4]). The effects of Ado on most of these channels are seen only when cAMP was previously elevated, e.g. following $\beta$-adrenergic or histaminergic receptor stimulation. However, a change by Ado of the voltage-dependent activation of $I_K$ under basal conditions has been reported in human atrial myocytes [52]. Via the phosphoinositide pathway, Ado acts on channels by activating PKC. This is the case for AdoR-mediated activation of sarcolemmal ATP-sensitive K$^+$ ($K_{ATP}$) channels. PKC activation shortens the delay for $K_{ATP}$ channel activation during myocardial ischemia [53]. AdoRs may also modulate L-type Ca$^{2+}$ channels via PKC, in addition to the recognized negative coupling via AC. In guinea-pig cardiac myocytes, the H$_2$O$_2$-induced increase in $I_{Ca-L}$ recorded under perforated-patch clamp conditions was suppressed by $A_1$-AdoR (but not $A_2a$- or $A_3$-AdoR) agonists, and this effect was mimicked by the PKC inhibitor bisindolmaleimide [54].

The signal amplification between the binding of the agonist to its receptor and the activation of effectors has been analyzed for the AC pathway. Ado is found to be more potent (about 10 times) at antagonizing cAMP-dependent effects (e.g. inhibition of $\beta$-adrenergically stimulated L-type Ca$^{2+}$ current ($I_{Ca-L}$)) than at inducing directly $G_s/G_i$-coupled, cAMP-independent responses (e.g. increase of $I_{K(Ado)}$) (A similar difference in potency for the two types of responses exists for the ‘accentuated antagonism’ exerted by muscarinic ACh receptors; see Refs. [55,56].) The presence of a large receptor reserve for the cAMP-dependent effects accounts for such a difference. Occupancy of less than 50% of $A_1$-AdoRs is sufficient to cause maximal cAMP-dependent response. In contrast, no receptor reserve is available for the $A_1$-AdoR-mediated stimulation of G proteins (measured as GTP$\gamma$S binding) [57]. Similarly, full receptor occupancy is needed for the maximal induction of $I_{K(Ado)}$. The irreversible inactivation of $A_1$-AdoRs in guinea-pig myocytes reduces the efficacy of Ado to induce $I_{K(Ado)}$ while having little influence on the inhibitory effects of Ado on AC [58]. These results are consistent with the following amplification processes between stimulus and response: a first amplification involves the coupling of each receptor to a specific pool of G proteins; a second amplification occurs between G proteins and AC, but allows convergence of different G protein molecules to common or intersecting AC pools.
3. Cardioprotection mediated via adenosine receptors

In addition to its role as physiologic regulator of the matching between O2 supply and demand, Ado exerts a protective role against ischemic injury. Ado also protects against other causes of injury, including the sarcoplasmic reticulum (SR) dysfunction induced by Ca2+ paradox, an effect that could be abolished by the receptor antagonist 8-sulfophenyltheophylline (SPT) [59]. Below we examine the role of various elements of the Ado signaling pathway in the anti-ischemic myocardial protection.

3.1. Roles of A1- and A2-AdoR subtypes

The role of A1-AdoRs in mediating PC-induced myocardial protection against infarction, arrhythmias or post-ischemic contractile dysfunction is well recognized (see Refs. [60–62]) (however, the role of Ado for the improvement of contractile function by PC is not unanimously accepted; see Refs. [63–67]). Recent studies suggest that in addition to the A1-AdoR subtype, the A2-AdoRs also are involved in cardiomyocyte protection. Similarly, protection of coronary function could be mediated by both A1- and A3 receptors [68].

Data in avian and rabbit myocytes suggest a synergistic effect of A1- and A2-AdoRs in mediating cardioprotection, in contrast to the antagonistic action between the two subtypes (A1 protective, A3 deleterious) noted for the kidney [69]. Although equal protective effects can be obtained via activation of either A1- or A2-AdoRs in rabbit hearts [70], it has been shown that the affinity of Ado to A2-AdoRs is lower than to A1-AdoRs and that protection by preconditioning with ischemia or with Ado could be fully prevented by a selective inhibition of A1-AdoRs with low concentrations of WBA1433. While these results suggest a prevalence of the protective role mediated via A1-AdoRs [71], in a rabbit myocyte model, the A1-AdoR selective antagonist DPCPX was unable to fully suppress the protective effect of PC and had to be associated with an A2-AdoR antagonist [72].

Data from transgenic mice show that A1-AdoR overexpression is associated with increased resistance toward ischemia (increased posts ischemic contractile function, decreased lactate dehydrogenase release, and decreased infarct size) [73]. In this case, ischemic PC had no additional beneficial effect (in contrast to wild-type mice where PC allowed cardioprotection), and treatment with an AdoR antagonist suppressed protection in transgenic mice. Similarly, in a chick myocyte model of simulated ischemia, overexpression of either A1- or A2-AdoRs led to an increased resistance to subsequent sustained ischemia and enhanced the protective effect of ischemic PC [74].

In some studies A1-AdoR selective (e.g. CPDPX [75,76]) or nonselective antagonists (e.g. PD-115199 [76]) failed to abolish the protective affect of ischemic PC. One possible explanation is that multiple AdoR subtypes contribute to cardioprotection and that not all subtypes were fully inhibited by the antagonists. Alternatively, additional mechanisms, not involving Ado, could also be involved.

Most of the work in support of the role of A1,-AdoRs in myocyte cardioprotection has been carried out in avian cells, and it is important to know whether the same applies to mammalian cardiomyocytes. Improved post-ischemic recovery of mechanical function mediated by A1,-AdoRs has been found in rabbit isolated cardiomyocytes [72], in human atrial muscle [77] and in the chronically instrumented conscious rabbit [78], thus supporting the view that mammalian A1,-AdoRs can also be involved in cardioprotection against reperfusion-induced contractile dysfunction. In contrast, preliminary studies using A1,-AdoR knock-out mice suggest the opposite role, i.e. that the A1,-AdoR can enhance ischemic injury, since deletion of this receptor increases the resistance to contractile dysfunction or cell necrosis caused by myocardial ischemia [79,80]. Thus the role of A1,-AdoRs in cardioprotection in the mammalian myocardium remains highly controversial and more experiments will be needed to delineate the critical switch towards beneficial vs. deleterious effects in the signaling from these receptors. Similarly, the role of cardiac A2,-AdoRs in cardioprotection also remains unclear. A2,-AdoRs do not mediate myocardial PC (and may even enhance hypoxic/ischemic injury [81]), contrary to their protective role in other tissues, e.g. in the liver where A2,-AdoR activation and the resulting increase in cAMP production [82] or NO formation [83] may be involved. However, beneficial effects mediated by A2,-AdoRs might be achieved indirectly via the known effects of these receptors on neutrophils [3].

In summary, the majority of studies support a protective role mediated by A1,-AdoRs and the involvement of these receptors in the cardioprotection afforded by ischemic PC. In contrast, discordant results have been reported for the role of A2,-AdoRs, and more work is needed to determine the extent to which the effects reported for A2,-AdoRs depend on the animal species and the experimental conditions used.

3.2. Mechanisms of cardioprotection by adenosine

Multiple processes are involved in the protection of the myocardium against injury by ischemia and reperfusion. These include antiadrenergic effects that oppose the enhanced sympathetic stimulation associated with ischemia, as well as effects on non-myocyte components within the myocardium. The following discussion will be concerned only with direct processes that are not due to the accentuated antagonism against adrenergic stimulation at the level of cardiomyocytes.

3.2.1. Role of PKC

The mechanisms underlying Ado-mediated protection are not fully clear, but seem to involve PKC, since
inhibitors of this kinase (e.g. chelerythrine, staurosporine, bisindolmaleimide, polymyxin B) suppress the Ado-induced beneficial effects. Protection by ischemic PC also seems to involve PKC, but the mechanisms up- or downstream of PKC activation are not fully resolved [10].

Upstream of the activation of this enzyme, PC may favor the formation of Ado and of PKC activators by stimulating phosphatidylinositol-3-kinase [84]. Downstream of PKC activation, PC or AdoR activation may involve multiple possible effectors, including the activation of $K_\text{ATP}$ channels [85], the activation of other kinases (e.g. MAP kinases; [86,87]), or a translocation of heat shock proteins [88]. The cascade of events activated by short preconditioning ischemia or by Ado is proposed to be as follows: (1) the brief period of preconditioning ischemia and reperfusion releases Ado and other potential cardioprotective substances (e.g. bradykinin); (2) Ado and these substances activate receptors coupled to phospholipases via G proteins; (3) DAG released from the action of the PLC and/or PLD induces the activation and translocation of PKC: while $\varepsilon$- and $\alpha$-PKC are translocated to the sarcolemma, $\delta$-PKC may be translocated to mitochondrial membranes [89,90]; (4) other kinases (particularly the p38-MAPK) become activated (see Refs. [91,92]) and induce the phosphorylation of MAPKAPK2; (5) the latter enzyme phosphorylates HSP27, a heat shock protein that controls cytoskeletal actin filament polymerization; (6) at some point along this cascade there is an increased opening of $K_\text{ATP}$ channels, supposed to be the final mediator of myocardial protection [93].

Thus a large majority of studies support a critical role for PKC in cardioprotection, but the way this protection is achieved remains unclear and could involve multiple effectors downstream of PKC activation, including a modification of sarcolemmal and mitochondrial ion transport, of cytoskeletal structures, or of the intracellular signaling.

3.2.2. Role of ATP-sensitive $K^+$ channels. Sarcolemmal vs. mitochondrial channels

ATP-sensitive $K^+$ ($K_\text{ATP}$) channels are present in cardiomyocytes and in vascular smooth muscle cells. Vascular $K_\text{ATP}$ channel opening regulates blood flow (see Ref. [4]). Vascular function is better preserved in Ado or ischemic preconditioned hearts [66], but Ado activation of these channels in coronary vessels does not seem to be the principal mediator of the cardioprotective action. Instead, myocyte $K_\text{ATP}$ channels play a key role in protection.

The involvement of $K_\text{ATP}$ channels in ischemic PC has been a subject of controversy for a long time, with some studies showing that $K_\text{ATP}$ channel blockers such as glibenclamide (=glyburide) were able to prevent the protective effect of PC, while other studies either found no influence of glibenclamide or of other blockers (e.g. HMR1883 [59] or HMR1098 [94]), or obtained species-related effects (e.g. with the blocker 5-hydroxydecanoate [95,96]). Even when the involvement of $K_\text{ATP}$ during ischemia or hypoxia was recognized, a role for AdoRs in their activation could not always be demonstrated. For example in the guinea-pig heart in vivo, the shortening of ventricular action potential duration during acute myocardial hypoxia could be blocked by glibenclamide but was not mediated via the activation of $\Lambda_1$-AdoRs [97], thereby indicating that these receptors were not responsible for the activation of sarcolemmal ATP-sensitive $K^+$ channels under these experimental conditions. With the discovery that $K_\text{ATP}$ channels are present in mitochondrial membranes [98] (see also Refs. [99–101]) there is debate as to the relative contribution of sarcolemmal vs. mitochondrial $K_\text{ATP}$ channels in the myocardial protection afforded by PC [102–104]. Increasing evidence supports a more important role of mitochondrial $K_\text{ATP}$ channels [105]. Specific blockers of sarcolemmal $K_\text{ATP}$ channels do not inhibit protection [94,106,107], while cardioprotection often can be induced by $K_\text{ATP}$ channel openers at concentrations that do not affect the sarcolemmal channels (e.g. nicoxanil below 100 $\mu$M [108], diazoxide [106,109]). Protection of rat hearts stored for 10 h in cardioplegic solution could be enhanced by pre-storage ischemic PC or by diazoxide, and was abolished by the mitochondrial-selective $K_\text{ATP}$ channel blocker 5-hydroxydecanoate [109]. In a cellular model of simulated ischemia, Ado decreased cell injury and was shown to enhance mitochondrial $K_\text{ATP}$ channel activity. This effect could be prevented by the AdoR antagonist SPT or by PKC inhibitors, and could be blocked by 5-hydroxydecanoate, but not by HMR1098, a selective blocker of sarcolemmal $K_\text{ATP}$ channels [110]. Similar results were obtained in whole-heart models of PC or Ado application [111]. In a rat model of regional ischemia in Langendorff-perfused hearts, inhibitors of mitochondrial function such as dinitrophenol and cyclosporin have been shown to induce PC [112]. The protection afforded by these agents or by Ado could be prevented by 5-hydroxydecanoate or by trimetazidine, a known mitochondrial ‘protector’, hence supporting a mitochondrial site for the mechanisms underlying cardioprotection. These results do not exclude a role of sarcolemmal $K_\text{ATP}$ channels but indicate that the opening of these channels is not critical for protection. The mechanism of activation of mitochondrial $K_\text{ATP}$ channels is not known but could involve a priming action toward increased open probability caused by activated $\delta$-PKC. In guinea-pig myocytes, preconditioning by anoxia decreases the delay of activation of sarcolemmal $K_\text{ATP}$ channels and increases the open probability of these channels following metabolic inhibition [113]. This effect on sarcolemmal channels also depended on PKC activity. A similar mechanism could activate mitochondrial $K_\text{ATP}$ channels.

In summary, mitochondrial function, and specifically the opening of mitochondrial $K_\text{ATP}$ channels, has emerged as a major player in cardioprotection. However, more data are needed to elucidate on the one hand the link between
AdoRs, protein kinase and mitochondrial channel activation, and on the other hand the link between increased channel opening and myocardial protection.

### 3.2.3. Role of the ecto-5′-nucleotidase

Increased ecto-nucleotidase activity has also been proposed as a mechanism underlying the cardioprotection afforded by PC. In rat hearts overexpressing HSP, both the ecto-5′-nucleotidase activity and the recovery from ischemic injury were higher than in control hearts [114]. Increased synthesis of Ado could therefore be one of the mechanisms by which HSP mediates cardioprotection. However, some data suggest that ischemic PC is associated with a decreased rather than an increased accumulation of purine metabolites [22,115,116]. In a study in rabbits, inhibiting the ecto-5′-nucleotidase by α,β-methyleneadenosine diphosphate (AOPCP) did not prevent the beneficial effect of ischemic PC [117]. In addition, no positive correlation was found between myocardial 5′-nucleotidase activity and PC-induced protection in dogs, and in preconditioned animals the highest enzyme activities were associated with the largest infarcts [118]. These data suggest that increased 5′-nucleotidase activity may not be a critical event in PC. On the other hand, it has been proposed that the decreased purine metabolite accumulation induced by ischemic PC could protect the myocardium by preventing extracellular ATP (ATP$_p$)-induced arrhythmias [115], but more experimental evidence is needed to confirm that ATP$_p$ contributes to arrhythmogenesis in vivo.

### 3.2.4. Delayed protection

Ischemic PC not only protects against injury by prolonged ischemia imposed within minutes after the short preconditioning episodes but has also been shown to cause protection against delayed injury by sustained ischemia imposed after 24 h. The mechanisms underlying this delayed protection afforded by PC (the so-called ‘second window of protection’) (see Ref. [119]) remains unclear. Ado, A$_1$-AdoRs [120] and K$_{ATP}$ channels [121–123] have also been implicated as mediators of this phenomenon. A recent study shows that injection of mice with the A$_1$-AdoR agonist CPA decreases the size of infarction induced 24 h later [124]. This effect was ablated by the antagonist DPCPX and was absent in mice in which iNOS was pharmacologically inhibited or had been genetically knocked-out [124].

Treatment with the A$_1$-AdoR agonist CCPA caused a large increase in p38 mitogen-activated protein kinase (p38 MAPK) activity and an increase in the phosphorylated isoforms of the heat shock protein HSP27 in myocardial samples procured 24 h post treatment [125] (however, see Ref. [120] where no increased expression of heat shock proteins was found in CCPA-protected myocardium). Prior inhibition of either PKC or tyrosine kinase prevented the increase in p38 MAPK activity and HSP27 phosphorylation [125]. In a cellular model using human cardiomyocytes, short simulated ischemia or Ado application conferred protection against cell death (less LDH release and higher propidium iodide exclusion) upon sustained simulated ischemia imposed 24 h after the nucleoside application. The protective action was suppressed by SB203580, a p38-MAP-kinase inhibitor, given prior to the preconditioning ischemia or to Ado, or by 5-hydroxydecanoate given immediately prior to lethal ischemia [126].

Thus the signaling pathway for the delayed cardioprotection by PC appears more complex but less clearly defined compared to the pathway for the acute protection. The delayed protection probably involves changes in the expression of MAPKs and of iNOS.

### 3.2.5. Remote protection

Brief ischemia in one organ may not only precondition the organ itself but can also confer protection against sustained ischemia in a remote organ. This implies either a modification of the nervous input to the remote organ by a stimulus in the preconditioned organ, or a release of a hormone. Since a transfer of the effluent from donor-preconditioned hearts to virgin acceptor hearts will elicit cardioprotection against injury caused by 40-min global ischemia and 60-min reperfusion, this implicates a humoral mechanism for the remote protection [127]. Although no consensus exists on the nature of the mediator (see Ref. [127]), some studies suggest that Ado may be the humoral mediator of the remote protection. In rabbits, either a short coronary artery occlusion (cardiac or local preconditioning) or a short renal artery occlusion (renal or remote preconditioning), each followed by brief reperfusion, caused a decrease in infarct size, a delay in the decrease in ATP levels, and a preservation of intracellular pH during subsequent sustained (40-min) myocardial ischemia, and resulted in better recovery of ATP and PCr during reperfusion [128,129]. SPT or 5-hydroxydecanoate administered before PC abolished the protection [129]. Surprisingly, SPT given intravenously before the 40-min myocardial ischemia (but after PC) has been reported to abolish the improvement in myocardial energy metabolism and the infarct reduction caused by either preconditioning method [128].

### 3.2.6. Sequence of events during preconditioning

The relationship between the events taking place during cardioprotection by PC or their sequence in the cascade described above is not fully clear. Activated PKC enhances the protective effect of subsequent Ado treatment but is able to precondition the heart in the absence of Ado [130] or in the presence of AdoR antagonists, indicating that PKC activation is downstream of AdoR activation [131]. Although it is generally accepted that K$_{ATP}$ channels also act downstream of the Ado receptors and of PKC in mediating the protective effect [130,132], in some studies...
the protection by mitochondrial K\textsubscript{ATP} channel openers has been reported to depend on PKC [133] whereas the above cascade scheme suggests that the effect of channel openers should not be suppressed by PKC inhibitors. In addition, it has been proposed that K\textsubscript{ATP} channel opening and AdoR activation are coupled independently to PKC activation in a cellular model of hypoxia, but that they synergistically contribute to protection [134]. The role of PKC or K\textsubscript{ATP} channels in mediating the antiarrhythmic effect of PC in the rat has been questioned, since the protective effect was resistant to 5-hydroxydecanoate and to PKC inhibition by staurosporine or calphostin C [135]. Furthermore, Ado activates PKC, but PKC activation has been shown to stimulate formation of Ado by activating the enzyme ecto-5'-nucleotidase [136]. If AdoR activation precedes PKC translocation and in addition PKC activation leads to increased Ado formation by activating ecto-5'-nucleotidase [8], a positive feedback would be expected. Equally unclear is the protective action of activating MAPK during PC, which is in contrast to the deleterious effect of increased TNF, also supposed to be mediated by the p38-MAPK, as occurs in chronic heart failure [137]. However, Ado and PC have been reported to decrease TNF during ischemia/reperfusion [138,139]. Finally it should be noted that some studies have shown that PKC activation can cause deleterious effects [140].

3.2.7. Other effectors could participate in protection by adenosine

Since oxygen free radicals may be involved in the cell injury associated with ischemia/reperfusion, suppression of these oxidants has also been invoked as a component of PC. Applying hypoxia or Ado in a chick isolated cardiomyocyte model has been reported to confer protection by attenuating oxidant generation during subsequent ischemia/reperfusion [141]. Similarly A\textsubscript{1}-AdoR activation causes a reduction in OH radical generation following reperfusion [142]. Mitochondrial SOD was also shown to be better preserved in Ado-treated hearts 24 h post treatment [143]. However, unambiguously resolving the role of oxygen radical may be difficult, since their lower production in preconditioned myocardium could well be a consequence rather than the cause of myocardial protection. In addition, whereas free radicals are considered to contribute to cell injury during myocardial reperfusion, their formation during short ischemia could be beneficial: free radical generation by mitochondria may be enhanced by the opening of mitochondrial K\textsubscript{ATP} channels and contribute to the signaling pathway (e.g. PKC activation) of PC (see Ref. [105]).

Ischemic PC has been shown to induce changes in the SR function. In homogenates or microsomal fractions from preconditioned rat myocardium, the number of ryanodine binding sites and the rate of Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release were decreased [144,145]. The same effects can also be induced by activating A\textsubscript{3}-AdoRs [146].

Other factors reported to mediate the protection by PC include Ado-induced changes in glucose uptake and in energy metabolism, in the intracellular acidosis present during sustained ischemia, and an inhibition of the Na\textsuperscript{+} / H\textsuperscript{+} exchanger, etc. However, Ado receptor activation was not associated with improved exogenous glucose uptake during subsequent low-flow ischemia [147]. A recent study suggests that A\textsubscript{1}-AdoRs can inhibit the stimulation of Na\textsuperscript{+} /H\textsuperscript{+} exchange by alpha-adrenergic agonists [148], but the significance of this effect for myocardial ischemia has not yet been examined.

4. Clinical importance of the cardioprotective effect of Ado

4.1. Protection against ischemic injury

In vitro studies suggest that Ado also protects human cardiac myocytes submitted to hypoxia [149–151] or to simulated ischemia [77,152], and that this effect is mediated via PKC [150,151]. Thus human cardiomyocytes respond to Ado in the same way as cardiomyocytes from other species.

The use of Ado to prevent or attenuate the consequence of myocardial ischemia in patients has been proposed (e.g. Ref. [153]). Treatment with low doses of dipyridamole, which causes accumulation of Ado, improved tolerance during exercise-stress test (less frequently induced chest pain, increased asymptomatic exercise time, lower ST segment depression on ECG, and less frequent wall motion abnormalities) compared to a placebo group [154]. Chronic treatment with the Ado uptake inhibitor also caused sustained cardioprotection against ischemia/reperfusion injury [155]. However, chronic elevation of Ado levels may be associated with a loss of response, probably due to desensitization at the receptor level [156,157]. Slow desensitization to A\textsubscript{1}-AdoR activation following chronic treatment with Ado is caused by a decrease of receptor density accompanied by a downregulation of G\textsubscript{i}/G\textsubscript{o} [158].

Administration of Ado may also be beneficial during percutaneous transluminal coronary angioplasty (PTCA). In patients undergoing PTCA of the left anterior descend- ing coronary artery, intracoronary administration of Ado was associated with less marked signs of ischemia, and exhibited less deterioration of isovolumetric contraction or of the left ventricular ejection fraction [159]. Ado administration during PTCA can be targeted to the infarct-related artery, in order to selectively act on the ischemic territory [160]. Such a treatment is associated with improved recovery of flow and of contractile function, without side effects [160]. Intracoronary infusion of Ado before PTCA was more effective than ischemic preconditioning with balloon inflation in rendering the myocardium resistant to subsequent ischemia [161]. Dipyridamole has similar beneficial action [162]. In the ‘Acute Myocardial Infarc-
tion STudy of ADenosine (AMISTAD)’ trial [163], Ado was tested as an adjunct to thrombolysis performed within a few hours after myocardial infarction: infarct size was reduced in patients with anterior wall infarction who received Ado [163].

The beneficial effects of short Ado application during cardiac surgery have been examined in patients undergoing coronary artery bypass surgery (CABG). Short treatment with Ado did not decrease the release of troponin I, i.e. preconditioning by the agonist did not result in reduced cell necrosis over the first 48 post-operative hours [164], but reduced the degradation of high-energy phosphates during CABG [165]. A retrospective analysis of postoperative cardiac function in patients undergoing CABG with or without PC with Ado indicated a greater recovery of function in the patients who received Ado [166]. Ado added to intraoperative cold blood cardioplegia, and infused pre- and post-aortic crossclamping, decreased the need for post-operative inotropic support in patients undergoing CABG [167,168].

Ado may also be useful for tissue preservation. Although Ado-enhanced ischemic preconditioning has been reported to be as effective as magnesium-supplemented potassium short cardioplegia [169], adding Ado to the cardioplegic solution might augment the protective effect. Rat hearts preserved with diadenosine-tetraphosphate show a higher recovery of mechanical and coronary function as well as a lower release of lactate dehydrogenase and creatine kinase upon reperfusion after 8 h of storage, and this increased preservation can be prevented by 5-hydroxy-decanoate [170]. Similarly, in isolated cells, Ado application during cardioplegia preserved the inotropic response to β-adrenergic receptor stimulation [171]. In contrast, addition of the A2-AdoR agonist CI-IB-MECA to the cardioplegia medium failed to improve post-cardioplegia mechanical and coronary function or to decrease creatine kinase release, while receptor activation before the cardioplegia was able to confer protection of function post-cardioplegia [172].

5. Does adenosine exert cardiotoxic effects?

Despite its common antiarrhythmic action on sinatorial and atrioventricular reentry, Ado can exert pro-arrhythmic action. This action of Ado occurs mostly in the atrium (for example see Ref. [173]) and is secondary to its physiological shortening of atrial action potential duration and refractory period. Ado pro-arrhythmic action may also originate in other structures and result in sinus pauses, sinus block, AV block, etc. These arrhythmias can be induced by a bolus injection of Ado. While this calls for caution and patient surveillance when administering Ado or related compounds, the nucleoside remains relatively safe because of its short half-life and of considerable efficiency in the diagnosis and treatment of AVNRT.

Ado has been reported to exert toxic, pro-apoptotic effects on neonatal cardiomyocytes, when given at high concentrations (>200 μM). The effects consisted of a sustained increase in intracellular Ca^{2+} concentration, an activation of caspase-3 protease, a dissolution of contractile myofilaments, nuclear alterations or breakdown, and a modification of the cell shape [174,175]. Selective activation of A1-AdoRs by high agonist concentrations (IB-MECA>10 μM), but not of A2-AdoRs, also induced apoptosis. The effects could also be prevented by the A1-AdoR antagonist MRS1253 or by β-adrenoceptor activation [175]. Similarly, an inhibition of A1-AdoRs has been reported to be beneficial against injury (measured as infarct size) induced by regional myocardial ischemia in the anesthetized cat, hence suggesting the participation of these receptors in the ischemic injury [176]. In isolated rat hearts the A1-AdoR antagonist DPCPX completely blocked, whereas Ado increased the β-adrenergic receptor-independent sensitization of AC, which may contribute to arrhythmias and necrosis during ischemia [177]. PKC activation also prevents this sensitization. Thus it has been proposed that sensitization of AC in acute myocardial ischemia could be dependent on activation of A1-AdoRs and might be mediated by an activation of PKC [177].

6. Concluding remarks

Ado is a true retaliatory agent that regulates oxygen supply to the myocardium and mediates myocardial protection by acting on A1-AdoRs and probably A2-AdoRs. The cascade of events involved in the cardioprotective effects against established ischemia is complex and appears to involve many kinases (PKC and tyrosine kinases) as well as heat shock proteins and mitochondrial (and to some extent sarcenomemal) K_{ATP} channels. The protective action of endogenous Ado is manifested during ischemic PC, and exogenous Ado can be used as a pharmacological agent to protect or precondition the myocardium. However, in contrast to the proposal that for ‘preconditioning: we do not need more experiments, because our current knowledge already permits us to develop pharmacological agents’ [178], recent results indicate a complexity in the mechanisms recruited by PC. These need to be clarified by more research work before they can be fully put to use to produce myocardial protection.

More work is needed to evaluate the controversial role of A1-AdoRs in cardioprotection, the link between receptor activation and opening of mitochondrial K_{ATP} channels, and the contribution of MAP kinases. Novel mechanisms to protect the myocardium are being currently studied. Some of these mechanisms appear to use the Ado pathway. For example, anesthetics such as isoflurane can precondition the heart, and since this effect is abolished by SPT and glibenclamide, it has been proposed to be mediated by AdoRs and K_{ATP} channels [179]. In another study a similar
dependence on AdoRs and PKC was obtained [180]. How these substances activate AdoRs has not been clarified. Other new protective mechanisms do not use Ado but may converge on the same signaling pathways. Among them is the activation of other receptors, such as muscarinic AChRs [181] or opioid receptors [60], which is able to mimic short Ado application or ischemia in protecting or preconditioning the heart against subsequent long ischemia.

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