Receptor and non-receptor-dependent mechanisms of cardioprotection with adenosine

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Receptor and non-receptor-dependent mechanisms of cardioprotection with adenosine. Am J Physiol Heart Circ Physiol 284: H519–H527, 2003. First published September 19, 2002; 10.1152/ajpheart.00717.2002.—The relative roles of mitochondrial (mito) ATP-sensitive K⁺ (mitoK⁺ATP) channels, protein kinase C (PKC), and adenosine kinase (AK) in adenosine-mediated protection were assessed in Langendorff-perfused mouse hearts subjected to 20-min ischemia and 45-min reperfusion. Control hearts recovered 72 ± 3 mmHg of ventricular pressure (50% preischemia) and released 23 ± 2 IU/g lactate dehydrogenase (LDH). Adenosine (50 μM) during ischemia-reperfusion improved recovery (149 ± 8 mmHg) and reduced LDH efflux (5 ± 1 IU/g). Treatment during ischemia alone was less effective. Treatment with 50 μM diazoxide (mitoK⁺ATP opener) during ischemia and reperfusion enhanced recovery and was equally effective during ischemia alone. A₂ agonism [100 nM 2-chloro-N⁶-(3-iodobenzyl)-adenosine-5'-N'-methyluronamide], A₁ agonism (N⁶-cyclohexyladenosine), and AK inhibition (10 μM iodotubercidin) all reduced necrosis to the same extent as adenosine, but less effectively reduced contractile dysfunction. These responses were abolished by 100 μM 5-hydroxydecanoate (5-HD, mitoK⁺ATP channel blocker) or 3 μM chelerythrine (PKC inhibitor). However, the protective effects of adenosine during ischemia-reperfusion were resistant to 5-HD and chelerythrine and only abolished when inhibitors were coinfused with iodotubercidin. Data indicate adenosine-mediated protection via A₁/A₂ adenosine receptors is mitoK⁺ATP channel and PKC dependent, with evidence for a downstream location of PKC. Adenosine provides additional and substantial protection via phosphorylation to 5'-AMP, primarily during reperfusion.

Adenosine can protect the myocardium from ischemic insult and initiate the protective phenomenon known as preconditioning. The molecular mechanisms involved are incompletely understood, although varied investigations implicate receptor-dependent activation of mitochondrial (mito) ATP-sensitive K⁺ (mitoK⁺ATP) channels (3, 13, 47) and protein kinase C (PKC) (20, 28, 31). Evidence also supports involvement of mitoK⁺ATP channels in adenosine receptor (AR)-mediated “delayed” cardioprotection (55). Non-receptor-mediated or metabolic effects of adenosine are not generally considered when protection against ischemia is examined, despite evidence for significant effects of these paths (1). Indeed, we (33, 35) recently acquired evidence of multiple mechanisms mediating protection in response to adenosine. The relative importance of receptor-dependent and -independent pathways in adenosine-mediated protection in ischemic hearts has not been adequately addressed. Furthermore, the protective roles of both mitoK⁺ATP channels and PKC have been questioned in recent studies. Cohen and colleagues (4) found that whereas G protein-coupled preconditioning stimuli protect via a mitoK⁺ATP channel-dependent process, adenosine is an exception. Additionally, there is evidence against an essential role for PKC in cardioprotective responses (25, 30), and even evidence that adenosine can inhibit rather than activate PKC translocation in ischemic myocardium (8). Conflicting data also exist regarding the location of PKC upstream (20, 39, 54) versus downstream (23, 43, 50–52) of mitoK⁺ATP channels in protective signaling cascades. These varied contradictory findings may reflect contributions of multiple parallel pathways to cardioprotection (19, 44, 45).

Given these uncertainties, the purpose of this study was to characterize protective effects of adenosine in ischemic-reperfused hearts, identify the roles of mitoK⁺ATP channels and PKC, and determine the importance of non-receptor-mediated “substrate” effects of adenosine (phosphorylation to 5'-AMP). Because conflicting data exist regarding the ability of adenosine to protect when supplied pre- (24, 37, 46) versus postischemia (6, 9, 34), we compared the effects of adenosine before and during ischemia versus during ischemia and reperfusion.

METHODS
All investigations described in this study conformed to the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Langendorff-perfused heart preparation. Hearts were isolated from 16- to 20-wk-old male C57/B16 mice (body wt =
CaCl₂, 1.2 MgSO₄, 11 glucose, and 0.6 EDTA. The buffer was equilibrated with 95% O₂-5% CO₂ at 37 °C. The level of chelerythrine was also assessed. This concentration of 5-HD selectively blocks mitoKATP activity with little or no effect on sarcolemmal KATP channels (11, 22).}

Effects of chelerythrine on receptor-mediated effects of adenosine were studied in a separate series of experiments. Specifically, hearts were stabilized and were either untreated (n = 8), treated for 5 min with 50 μM adenosine alone (n = 8), treated with 3 μM chelerythrine for 15 min (n = 7), or treated with 3 μM chelerythrine for 15 min with 50 μM adenosine infusion initiated after 10 min (n = 8). Maximal reductions in heart rate (A₁AR-mediated bradycardia) and elevations in coronary flow (A₂AR-mediated dilation) were assessed.

To examine the role of adenosine phosphorylation via adenosine kinase (AK), hearts were treated with 10 μM of the AK inhibitor iodotubercidin alone (n = 9), or in the presence of 100 μM 5-HD (n = 9) or 3 μM chelerythrine (n = 8). A 10 μM concentration of iodotubercidin will maximally block AK activity (18, 35). Protection with 50 μM adenosine during ischemia and reperfusion was also assessed in the presence of 10 μM iodotubercidin alone (n = 9), 10 μM iodotubercidin plus either 100 μM 5-HD (n = 8), or 3 μM chelerythrine (n = 8).

Determination of myocardial injury via enzyme efflux. To assess necrosis, effluent was collected throughout reperfusion for LDH quantitation. Samples were stored at −80°C until analyzed enzymatically (Sigma; St. Louis, MO). Postischemic LDH efflux over 45-min reperfusion (IU/g) was determined by multiplying concentration (IU/ml) by effluent volume (ml/g).

Statistical analyses. Data are presented as means ± SE of individual experiments. Functional responses to ischemia-reperfusion and drug treatments were compared by multway ANOVA with repeated measures. Postischemic LDH efflux was compared with one-way ANOVA. When differences were detected by ANOVA, a Tukey’s post hoc test was employed for specific comparisons. In all tests, a value of P < 0.05 was considered significant.

RESULTS

Functional response to ischemia-reperfusion. Ventricular pressure development was high, and coronary flow was submaximal under normoxic conditions (Fig. 1). Contractile function failed to recover to preischemic levels after 45-min reperfusion. In control hearts diastolic pressure remained elevated at ~20 mmHg, and developed pressure recovered <50% of preischemic levels (Fig. 1). After an initial hyperemic response, coronary flow recovered to ~90% of preischemic levels in all groups (Fig. 1C).

Protection with adenosine, diazoxide, and AR agonism. No differences in normoxic contractile function were detected between untreated hearts and those treated with diazoxide, adenosine, CHA, or CI-IB-MECA (Figs. 1 and 2). Adenosine predictably elevated preischemic coronary flow (Fig. 1C). Diazoxide provided cardioprotection when present during ischemia and reperfusion (Fig. 1). An almost identical protection was evident with the treatment during ischemia alone. Treatment with adenosine substantially improved postischemic contractile function, with a much greater extent.
degree of protection evident when supplied during ischemia and reperfusion versus ischemia alone (Fig. 1). The effects of adenosine supplied during ischemia and reperfusion exceeded those for diazoxide. Protection was also evident with A1AR agonism with CHA, and A3AR agonism with Cl-IB-MECA (Fig. 2). Coronary flow was enhanced in adenosine-treated (Fig. 1C) and Cl-IB-MECA-treated hearts (Fig. 2C) during reperfusion. This dilation was not evident in any other group.

Myocardial LDH efflux in control hearts was ~23 IU/g during 45-min reperfusion (Fig. 3). Adenosine, diazoxide, CHA, and Cl-IB-MECA all significantly reduced postischemic LDH efflux, with the greatest reductions evident for adenosine and Cl-IB-MECA. In contrast to the marked difference in functional recov-

Fig. 1. Effects of adenosine and mitochondrial (mito) ATP-sensitive K+ (mitoKATP) channel activation with diazoxide on recovery from 20-min global normothermic ischemia. Values are shown for postischemic left ventricular diastolic pressure (A), developed pressure (B), and coronary flow (C). Data are provided for control hearts (n = 15) and hearts treated with 50 μM adenosine during ischemia alone (Isch, n = 8), 50 μM adenosine during ischemia and reperfusion (Isch-Rep, n = 9), 50 μM diazoxide during ischemia alone (Isch, n = 8), and 50 μM diazoxide during ischemia and reperfusion (Isch-Rep, n = 9). Values are means ± SE of individual experiments. *P < 0.05 vs. control.

Fig. 2. Effects of A1 agonism with N6-cyclohexyladenosine (CHA) and A3 agonism with 2-chloro-N6-(3-iodobenzyl)-adenosine-5'-N-methyluronamid (Cl-IB-MECA) on recovery from 20-min global normothermic ischemia. Values are shown for postischemic left ventricular diastolic pressure (A), developed pressure (B), and coronary flow (C). Data are provided for control hearts (n = 8) and hearts treated with 100 nM CHA (n = 8) or 100 nM Cl-IB-MECA (n = 8) during ischemia and reperfusion. Values are means ± SE of individual experiments. *P < 0.05 vs. control.
ery with adenosine treatment during ischemia and reperfusion versus ischemia alone (Fig. 1), there was a modest difference in LDH efflux between these two groups (Fig. 3).

Roles of mitoKATP channels, PKC, and AK in cardio-protection. Infusion of the PKC inhibitor chelerythrine did not modify intrinsic ischemic tolerance (Fig. 4) and failed to modify receptor-mediated functional effects of 50 μM adenosine treatment (Table 1). Chelerythrine abrogated cardioprotection with diazoxide, ischemic adenosine, CHA, and CI-IB-MECA (Figs. 3 and 4). Chelerythrine inhibited both anti-necrotic (Fig. 3) and functional effects (Fig. 4). Whereas chelerythrine also eliminated the anti-necrotic effects of adenosine treatment during ischemia and reperfusion, functional protection was only modestly reduced (Fig. 4). Postischemic contractile recovery remained substantially elevated above that for untreated hearts. Enhanced postischemic coronary flows observed with adenosine and CI-IB-MECA treatments were abolished by chelerythrine (Fig. 4C). Reflow in all other groups was unaltered.

The effects of mitoKATP channel blockade with 5-HD essentially mirrored those of chelerythrine. Treatment with 5-HD failed to modify intrinsic ischemic tolerance (Figs. 3 and 4) and abrogated cardioprotection with diazoxide, ischemic adenosine, CHA, and CI-IB-MECA. Infusion of 5-HD eliminated the anti-necrotic effects of adenosine treatment during ischemia and reperfusion (Fig. 3), whereas substantial contractile protection was still evident (Fig. 4). As opposed to chelerythrine, 5-HD failed to alter postischemic reflow in adenosine and CI-IB-MECA-treated hearts (Fig. 4C).

Fig. 3. Postischemic efflux of lactate dehydrogenase (LDH) in hearts treated with 50 μM adenosine during ischemia alone (Isch) or ischemia and reperfusion (Isch-Rep), and 50 μM diazoxide, 100 nM CHA or 100 nM CI-IB-MECA during ischemia and reperfusion. Data are shown for efflux over the 45-min reperfusion period for hearts from Figs. 1 and 2. Effects of protein kinase C (PKC) inhibition with 3 μM chelerythrine and mitoKATP channel inhibition with 100 μM 5-HD were assessed. Values are means ± SE of individual experiments. *P < 0.05 vs. control; †P < 0.05 vs. no inhibitor; ‡P < 0.05 vs. adenosine (Isch).

Fig. 4. Effects of PKC inhibition and mitoKATP channel inhibition on protective responses to adenosine, diazoxide, CHA, and CI-IB-MECA. Values are shown for final recoveries (at 45-min reperfusion) for left ventricular diastolic pressure (A), developed pressure (B), and coronary flow (C). Data for hearts not treated with the inhibitors chelerythrine or 5-HD are from Figs. 1 and 2. Effects of protein kinase C (PKC) inhibition with 3 μM chelerythrine and mitoKATP channel inhibition with 100 μM 5-HD were assessed. Values are means ± SE of individual experiments. *P < 0.05 vs. control; †P < 0.05 vs. no inhibitor; ‡P < 0.05 vs. adenosine (Isch).
Inhibition of adenosine kinase with 10 μM iodotubercidin exerted cardioprotection, and this effect was eliminated by 5-HD and chelerythrine (Fig. 5). Coinfusion of iodotubercidin with adenosine reduced the level of functional protection observed with adenosine alone but did not reduce the anti-necrotic effect (Fig. 5). Coinfusion of 5-HD or chelerythrine with iodotubercidin totally eliminated functional protection observed in adenosine-treated hearts (Fig. 5). The elevation in postischemic coronary flow with iodotubercidin was inhibited by PKC inhibition with chelerythrine (Fig. 5), as was the increase in flow with adenosine (Fig. 4).

**DISCUSSION**

The chief goals of this study were to characterize cardioprotective effects of exogenous adenosine and identify the relative importance of mitoKATP channels, PKC, and AK in observed protection. We assessed responses to exogenous adenosine and A1AR and A3AR agonism, testing the effects of mitoKATP channel inhibition and activation with a well-characterized blocker (5-HD) and opener (diazoxide) (11, 27, 22, 39), PKC inhibition with the isoform nonspecific blocker chelerythrine (15, 43, 54), and AK inhibition with iodotubercidin (18, 35). Data reveal that ischemic tolerance in the murine heart is enhanced by adenosine, A1AR and A3AR agonism, AK inhibition, and mitoK ATP channel activation. Effects of adenosine on necrosis appear primarily mediated during or shortly after the ischemic insult or shortly after the ischemic insult.

### Table 1. Effects of chelerythrine on adenosine-mediated functional responses

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Heart Rate, beats/min</th>
<th>Coronary Flow, ml·min⁻¹·g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>8</td>
<td>384 ± 13</td>
<td>28.7 ± 1.5</td>
</tr>
<tr>
<td>Chelerythrine</td>
<td>7</td>
<td>393 ± 14</td>
<td>30.7 ± 1.4</td>
</tr>
<tr>
<td>Adenosine</td>
<td>7</td>
<td>186 ± 36*</td>
<td>38.6 ± 1.5*</td>
</tr>
<tr>
<td>Adenosine + chelerythrine</td>
<td>7</td>
<td>194 ± 21*</td>
<td>39.0 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE of n individual experiments. Heart rate and coronary flow were assessed in normoxic hearts that were either untreated or treated with 3 μM chelerythrine, 50 μM adenosine, or 50 μM adenosine in the presence of chelerythrine. Chelerythrine was infused for 10 min before any measurements or adenosine infusion. Maximal heart rate or flow changes in response to adenosine were assessed during 5 min of continuous infusion. *P < 0.05 vs. untreated. No difference was observed between adenosine responses in the presence or absence of chelerythrine.

**Fig. 5.** Effects of blockade of adenosine kinase on protection with adenosine. Values are shown for final postischemic recoveries (at 45-min reperfusion) for left ventricular diastolic pressure (A), developed pressure (B), and coronary flow (C), and for total LDH efflux throughout reperfusion (D). Data for control and adenosine-treated hearts are taken from Figs. 1 and 3. Data are also shown for hearts treated with 10 μM iodotubercidin (n = 9), 10 μM iodotubercidin plus 100 μM 5-HD (n = 1), 10 μM iodotubercidin plus 3 μM chelerythrine (n = 8), 50 μM adenosine plus 10 μM iodotubercidin (n = 9), 50 μM plus 10 μM iodotubercidin plus 100 μM 5-HD (n = 8), and 50 μM adenosine plus 10 μM iodotubercidin and 3 μM chelerythrine (n = 8). In all cases, drugs were infused before and after the ischemic insult. Values are means ± SE of individual experiments. *P < 0.05 vs. control; †P < 0.05 vs. no inhibitor; ‡P < 0.05 vs. adenosine.
insult and occur via a mitoKATP channel and PKC-dependent process. Cardioprotection with A1AR and A3AR agonism, and AK inhibition is also mitoKATP and PKC dependent. However, adenosine treatment during ischemia and reperfusion also enhances outcome via a mitoKATP/PKC-independent path involving the activity of AK.

**Characteristics of adenosine-mediated cardioprotection and role of AK.** To identify the effects of adenosine and ARs, we studied responses to exogenous adenosine, exogenous A1AR and A3AR agonism, and enhanced endogenous adenosine at the expense of phosphorylation to 5′-AMP. Data reveal exogenous adenosine protects against necrosis and contractile dysfunction when supplied during ischemia alone and provides further protection when supplied during reperfusion (Figs. 1 and 3). These observations contrast the effects of the mitoKATP channel activator diazoxide, which does not further enhance final recovery of function when supplied during ischemia and reperfusion versus ischemia alone (Fig. 1). Interestingly, posts ischemic diazoxide does accelerate initial functional recovery during the first 10-20 min of reperfusion. This more rapid recovery supports some short-term benefit from mitoKATP channel activation postischemia, potentially involving a reduction in stunning. We (13) previously observed beneficial effects of intrinsically activated mitoKATP channels in posts ischemic myocardium.

Previous studies (37, 41, 46, 53) suggest receptor activation before and during ischemia is essential in reducing stunning in vivo and in vitro. Conversely, other studies (6, 9, 34) demonstrate specific protection during reperfusion. Our data indicate that the addition of adenosine during reperfusion does enhance recovery beyond that observed with adenosine during ischemia alone (Figs. 1 and 3), consistent with the observations of Gao et al. (9) in isolated mouse hearts. The final level of functional protection with adenosine supplied during ischemia and reperfusion markedly exceeds that with diazoxide, A1AR agonism, or A3AR agonism (Fig. 4), although these all comparably enhanced recovery of function during the initial 20 min of reperfusion (Figs. 1 and 2). Given reasonably high levels of CHA and Cl-IB-MECA employed, coupled with ischemic elevations in endogenous adenosine (34), it is likely A1AR and A3ARs are maximally activated during ischemia in the CHA and Cl-IB-MECA groups. Thus the greater functional protection with adenosine suggests protective processes additional to A1AR and A3AR activation. This effect does not involve A2AR activation because these do not reduce injury in the current model (33). A non-receptor-mediated protective response is supported by our prior observation that antagonism of extracellular receptors does not eliminate adenosine-mediated functional protection (33). Additionally, Finegan et al. (6) showed that protection with adenosine is not eliminated by receptor antagonism whereas protection with selective receptor agonists can be abrogated.

Effects of iodotubercidin indicate that adenosine mediates some cardioprotection through phosphorylation to 5′-AMP (Fig. 5). When supplied alone, this AK inhibitor protects against ischemia-reperfusion, in agreement with earlier findings (33, 35). The protective response to iodotubercidin alone is related to enhancement of endogenous adenosine levels and resultant receptor activation, as demonstrated recently (33). An adenosine-receptor-mediated effect is also consistent with the comparable effects of PKC and mitoKATP channel inhibitors on responses to iodotubercidin and A1AR and A3AR agonists (Figs. 4 and 5). Despite protection with iodotubercidin alone, the drug significantly reduces protection with adenosine (Fig. 5). This somewhat paradoxical observation is explained by the fact that adenosine not only activates cell surface receptors but is also substrate for metabolic reactions, including phosphorylation by AK. Provision of iodotubercidin, either alone or in the presence of exogenous adenosine, enhances AR-mediated protection (33) while simultaneously blocking adenosine phosphorylation. The iodotubercidin-dependent reduction in protection with exogenous adenosine therefore indicates phosphorylation by AK contributes to protection. This agrees with the early findings of Bolling and colleagues (1), who concluded that metabolic substrate effects of adenosine are important in protection against ischemia.

**Importance of mitoKATP channels and PKC in adenosine-mediated cardioprotection.** Although there is evidence that adenosine-mediated protection involves PKC and/or mitoKATP channels (20, 28, 31, 42, 47, 49), the protective roles of these signaling elements have been questioned (4, 25, 30). In support of a role for mitoKATP channels, diazoxide provided protection similar to that with A1AR and A3AR agonism (Figs. 1–3). More convincingly, the protective responses to CHA (an A1-selective agonist) (17, 24) and Cl-IB-MECA (an A3-selective agonist) (16, 21, 47) were entirely abrogated by 5-HD and chelerythrine (Figs. 3 and 4), and protection with iodotubercidin was also sensitive to these inhibitors (Fig. 5). These data collectively indicate that receptor-mediated protection is dependent on mitoKATP channels and PKC. These data also verify that levels of 5-HD and chelerythrine employed are sufficient to fully eliminate protection mediated via A1 and A3 receptor agonism.

It is generally considered PKC lies upstream of mitoKATP channels in the signaling cascade (20, 39, 54). However, there is also evidence mitoKATP channel-mediated protection is PKC dependent (23, 43, 50–52). We find that the PKC inhibitor chelerythrine blocks cardioprotection with the mitoKATP channel opener diazoxide (Figs. 3 and 4). This supports a downstream location of PKC distal to the target activated by diazoxide (i.e., mitoKATP channels). This observation agrees with findings of Ashraf and colleagues (43, 50–52) in different models. Although this contrasts the conventional path in which PKC is upstream of mitoKATP channels (20, 39, 54), our findings and those of Ashraf et al. do not exclude an upstream function of PKC. Either A1 or A3AR may modify mitoKATP channel activity via a PKC-dependent process, with activated mitoKATP channels subsequently initiating a protective cascade involving modulation of PKC. This is con-
sistent with evidence for a “codependent” protective process requiring both mitoK\(_{\text{ATP}}\) channel and PKC activities (12).

**Non-receptor-mediated protection with adenosine.** As already discussed, protection with adenosine markedly exceeds that with mitoK\(_{\text{ATP}}\) channel activation and A\(_2\)AR or A\(_3\)AR agonism. Furthermore, protection with adenosine during ischemia and reperfusion is not eliminated by 5-HD or chelerythrine, despite abrogation of responses to CHA, CI-IB-MECA, ischemic adenosine, and diazoxide (Figs. 3 and 4). Reduced adenosine-mediated protection with iodotubercidin and elimination of protection after cotreatment with iodotubercidin and either 5-HD or chelerythrine indicates the response involves phosphorylation to 5′-AMP. The 5-HD and chelerythrine-resistant protective effect, evident with postischemic adenosine only, may reflect improved nucleotide pool repletion, as suggested by Bolling et al. (1), and/or modulation of AMPK activity. Activation of AMPK has been shown to protect against ischemic insult (5, 36). Cardioprotection via AMPK appears related to glucose metabolism (5), and studies show AMPK stimulates glucose transport and metabolism (38), potentially via modulation of ERK activity (2, 38). Iodotubercidin can inhibit effects of activated AMPK on glucose metabolism (38) and also inhibits ERK activation (7). The effects of iodotubercidin on AMPK are likely mediated via AK inhibition because AMPK is regulated by 5′-AMP levels, and AMP mimetics must also be phosphorylated by AK to modulate the enzyme. Thus the effects of iodotubercidin are consistent with a role for AMPK in adenosine-mediated protection. Irrespective of precise mechanism, our data collectively demonstrate that adenosine mediates substantial protection, which is not entirely explained by AR activation, is partly independent of mitoK\(_{\text{ATP}}\) and PKC activities, and is inhibited by a putative AK inhibitor.

**Study limitations.** An important limitation in the present study relates to the recent observation chelerythrine can inhibit ligand binding at, and activation of, ARs (40). Although inhibitory concentrations of chelerythrine exceed those employed here, we nonetheless tested this possibility. Because we found that chlortropic and vasodilatory effects of 50 \(\mu\)M adenosine are unaltered by 3 \(\mu\)M chelerythrine (Table 1), nonspecific antagonism of ARs does not appear to be a major confounding factor in the present study. Nonetheless, this may contribute to effects of chelerythrine in other studies, particularly when levels of inhibitor are >3–5 \(\mu\)M.

Another complicating factor relates to potential non-specific effects of iodotubercidin, which may activate fatty acid oxidation (10) or inhibit protein kinases (26). However, nonspecific effects cannot explain responses to iodotubercidin in our model: effects of iodotubercidin are blocked by PKC and mitoK\(_{\text{ATP}}\) channel inhibition with chelerythrine and 5-HD (Fig. 5) and are inhibited by AR antagonism (35). These data support an AR-mediated action of iodotubercidin alone.

A final point to highlight is that protection observed with CHA is counter to prior studies in which we found no protection with the alternate A\(_1\)AR agonist N\(_6\)-cyclopentyladenosine (33, 34). The explanation for this difference is not obvious. One possible confounding factor is that CHA may be less specific than N\(_6\)-cyclopentyladenosine and therefore activate protective A\(_3\)ARs. Alternatively (or in addition), there is evidence N\(_6\)-cyclopentyladenosine (but not CHA) is rapidly transported and catabolized by mammalian cells (32). This might limit efficacy of the drug as a cardioprotectant. Furthermore, uptake and phosphorylation of these types of adenosine analogs rapidly induces apoptosis (29). Such effects of N\(_6\)-cyclopentyladenosine could counteract beneficial effects of A\(_1\)AR activation. These possibilities remain to be directly tested.

In conclusion, our observations reveal that A\(_1\) and A\(_3\)AR agonists mediate protection via a mitoK\(_{\text{ATP}}\) channel and PKC-dependent process. Data support location of PKC downstream of mitoK\(_{\text{ATP}}\) channel activation in the protective cascade. This mitoK\(_{\text{ATP}}\) channel/PKC-dependent protection appears to occur primarily (but not exclusively) during ischemia versus reperfusion. Adenosine itself additionally mediates substantial protection, which is neither mitoK\(_{\text{ATP}}\) channel nor PKC dependent and is inhibited by treatment with iodotubercidin, supporting a role for phosphorylation to 5′-AMP via AK. This response appears to occur primarily during the reperfusion period, and is consistent with the early studies of Bolling and colleagues (1) supporting protection via non-receptor-mediated effects of the purine nucleoside.

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