Endogenous Adenosine Does Not Activate ATP-Sensitive Potassium Channels in the Hypoxic Guinea Pig Ventricle In Vivo

Jiang Xu, MD; Li Wang, MD; Carl M. Hurt, BA; Amir Pelleg, PhD

Background. The activation of ATP-sensitive K⁺ (K⁺ATP) channels by K⁺ATP openers, eg, pinacidil, hypoxia, and ischemia, is known to shorten the ventricular action potential. Since adenosine is released in increased amounts during cardiac hypoxia and ischemia, the hypothesis that endogenous adenosine activates K⁺ATP channels was tested in vivo in a guinea pig model.

Methods and Results. Anesthetized animals (n=37) were subjected to transient acute global hypoxia by ventilation with 100% N₂. Monophasic action potentials (MAP) were recorded in ventricular and atrial myocardium by use of custom-made Ag/AgCl electrode catheters. In addition, right atrial and left ventricular electrograms as well as systemic arterial blood pressure were monitored throughout the experiments. Under normoxic conditions, pinacidil (1.8 µg/kg IV, n=8), a K⁺ATP channel opener, shortened ventricular MAP duration (APD); this effect was reversed by glibenclamide, a potent K⁺ATP channel blocker, but not by 8-cyclopentyl-1,3-dimethylxanthine (CPT), a potent A₁-selective adenosine antagonist. Global hypoxia shortened atrial and ventricular APD. Glibenclamide but not CPT reversed this effect of hypoxia on ventricular but not atrial MAP. CPT but not glibenclamide reversed the effect of hypoxia on atrial MAP. In addition, CPT delayed the appearance of the atrioventricular (AV) nodal conduction block associated with global hypoxia. Finally, the ability of CPT to selectively attenuate A₂-adenosine receptor-mediated effects of adenosine agonists in ventricular and supraventricular tissues was confirmed in 17 animals. CPT reversed the negative dromotropic effect of adenosine on AV nodal conduction and the antiadrenergic effect of N⁶-cyclopentyladenosine (CPA) mediated by A₁-adenosine receptor but not the adenosine-induced decrease in systemic blood pressure caused by the vasodilatory action of the nucleoside mediated by A₂-adenosine receptor.

Conclusions. (1) Endogenous adenosine released during global cardiac hypoxia mediates, in part, AV nodal conduction delay and shortening of atrial but not ventricular APD. (2) The action of adenosine on atrial APD is mediated by A₁-adenosine receptors, probably via Iₖ,ATP. (3) Endogenous adenosine apparently does not play an important role in the early stages of acute global hypoxia–induced activation of K⁺ATP channels. The present results are consistent with the hypothesis that the shortening of ventricular APD in the hypoxic heart is due, in part, to activation of K⁺ATP channels. (Circulation. 1994;89:1209-1216.)

Key Words: adenosine • potassium • hypoxia • action potentials • receptors

The purine nucleoside adenosine is released into the extracellular spaces during physiological and pathophysiological conditions associated with altered oxygen supply/demand ratio.1 Once outside of cells, adenosine exerts pronounced effects on the mammalian heart, including a negative chronotropic effect on cardiac pacemakers, a negative dromotropic effect on atrioventricular (AV) nodal conduction, a negative inotropic effect on atrial myocardium, a vasodilatory effect on the coronary arteries, an antiadrenergic effect, an inhibitory effect on neutrophil activation, an anti–platelet aggregation effect, and a modulatory effect on cardiac metabolism. Taken together, these effects characterize adenosine as an endogenous cardioprotective agent.2

Recently, it has been shown that adenosine can activate ATP-sensitive potassium (K⁺ATP) channels and that this action is mediated by A₁-adenosine receptors (A₁-AdoR).3 4 In addition, the activation of K⁺ATP channels by adenosine was suggested to play a mechanistic role in the vasodilatory effect of adenosine in porcine coronary arteries.5 Since the activation of K⁺ATP channels has been proposed to exert a protective effect in the ischemic/hypoxic myocardium,6-11 it is tempting to speculate that activation of these channels by adenosine is another manifestation of its cardioprotective effects.

Thus, the present experiments were aimed at testing the hypothesis that endogenous adenosine activates K⁺ATP channels during acute global myocardial hypoxia in vivo. For this purpose, a guinea pig model was used in which the release of endogenous adenosine and activation of A₁-AdoR during global myocardial hypoxia have been well documented.12 13

Methods

Animal Preparation and Instrumentation

Guinea pigs (n=62) anesthetized with 98% ketamine plus 2% acepromazine 1.65 mL/kg IV and intubated with an endotracheal tube were ventilated with room air supplemented as necessary with oxygen using a Harvard respirator (model 665). The heart was exposed via longitudinal sternotomy. Bipolar electrode catheters were introduced via the right jugular vein and positioned in the right atrium (RA) and right ventricle (RV) for RA and RV pacing. The left brachial artery was cannulated for recording of blood pressure (BP). A small-bore (0.58-mm) catheter was inserted into the left jugular vein and positioned in the right atrium for the administration of drugs. A custom-made monophasic action potential (MAP) electrode

Received June 26, 1993; revision accepted November 11, 1993.
From the Likoff Cardiovascular Institute, Hahnemann University Department of Medicine, Philadelphia, Pa.
Correspondence to Amir Pelleg, PhD, Hahnemann University, Broad and Vine Streets, MS 110, Philadelphia, PA 19102-1192.
catheter was introduced through the left carotid artery and positioned in the left ventricle for recording of left ventricular endocardial MAP. RA epicardial MAPs were recorded with a custom-made suction catheter.

**Protocols**

Animals were randomly divided into eight groups. In all groups, protocols were performed after a stabilization period of 20 minutes. In group 1 (n=8), the effects of pinacilid (1.8 μmol/kg IV) on sinus cycle length, BP, and ventricular MAP amplitude (APA) and duration (APD) were determined before and after glibenclamide (15 μmol/kg IV). In groups 2 (n=17; 10 without and 7 with cardiac pacing) and 3 (n=6), transient global hypoxia was induced by ventilating the animals with 100% nitrogen for 130 seconds followed by a recovery period of 10 minutes. This protocol was repeated in the presence of either 8-cyclopentyl-1,3-dimethylxanthine (CPT; 0.3 mg/kg IV), a selective A1-AdoR antagonist, or glibenclamide (15 μmol/kg IV), a blocker of KATP channels.

To eliminate potential complications from the antiadrenergic action of adenosine and to determine the temporal pattern of APD, group 4 animals (n=7) were subjected to global hypoxia as described above after administration of propranolol (1 mg/kg IV). In group 5 (n=7), CPT (0.3 mg/kg IV) was also administered before hypoxia. In both groups (ie, 4 and 5), RV pacing was operated (paced cycle length, 240 milliseconds) throughout experimentation.

**Characterization of CPT Actions**

To verify that CPT is a selective A1-AdoR antagonist in ventricular and supraventricular tissues of the guinea pig heart in vivo, the following experiments were performed. First, the effects of CPT on the negative dromotropic and vasodilatory actions of adenosine mediated by A1-AdoR and A2a-AdoR, respectively, were determined. In group 6 (n=6), exogenous adenosine (0.5, 1.0, and 1.5 μmol/kg) was given during right atrial pacing in the absence and presence of CPT (0.3 mg/kg IV), and AV nodal conduction was quantified. In five other animals (group 7), the vasodilatory action of adenosine (1.5 μmol/kg) on peripheral resistive blood vessels quantified as systemic arterial blood pressure was determined during sequential AV pacing (to exclude interference by AV conduction delays caused by adenosine) in the absence (control) and presence of CPT (0.3 mg/kg IV).

Finally, to determine the effect of CPT on ventricular A1-AdoR, the antiadrenergic action of A1-AdoR agonist N6-cyclopentyladenosine (CPA, 2 μmol/kg IV) was determined in group 8 (n=6) as follows: The positive inotropic action of isoproterenol (0.1 μg/kg IV) quantified as left ventricular dP/dt was determined during AV sequential pacing in the absence (control) and presence of CPA followed by CPT (0.3 mg/kg IV).

**MAP Recordings**

Custom-made electrodes were used for MAP recordings. For epicardial recording, a mini suction electrode was constructed from polyethylene tubing 2 mm in diameter and two isolated silver wires (Ag, 5T, Medwire Co, New York, NY), the tip of which was chlorinated to create a Ag/AgCl junction. Then silver wires were inserted into the polyethylene tubing, and glued in place such that their tips were 0.5 mm proximal to the tip of the tubing and 1 mm away from each other. Negative pressure was applied to the polyethylene tubing using a 3-mL syringe fitted with a stopcock.

For intramycocardial MAP recording, one silver wire, the same as described above, was plunged by use of a needle (23 gauge), and the other wire was positioned epicardially next to the sludge site by use of saline-soaked cotton.

MAP electrodes were connected to a high-impedance amplifier with negative capacitance.
TABLE 2. Effects of CPT and GLI During Normoxia and Hypoxia (Baseline)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>CPT</th>
<th></th>
<th>GLI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂</td>
<td>N₂</td>
<td>P</td>
<td>O₂</td>
<td>N₂</td>
<td>P</td>
</tr>
<tr>
<td>APD₀, ms</td>
<td>106±7</td>
<td>66±5</td>
<td>.05</td>
<td>113±6</td>
<td>65±5</td>
<td>.05</td>
</tr>
<tr>
<td>APAV, mV</td>
<td>21±2</td>
<td>12±2</td>
<td>.05</td>
<td>19±2</td>
<td>11±2</td>
<td>.05</td>
</tr>
<tr>
<td>AVΒ₀, s</td>
<td>...</td>
<td>83±8</td>
<td></td>
<td>132±16</td>
<td>.05*</td>
<td></td>
</tr>
<tr>
<td>SCL, ms</td>
<td>227±9</td>
<td>220±7</td>
<td>NS</td>
<td>242±7</td>
<td>219±7</td>
<td>.05</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>68±2</td>
<td>51±2</td>
<td>.05</td>
<td>64±2</td>
<td>50±2</td>
<td>.05</td>
</tr>
</tbody>
</table>

CPT indicates 8-cyclopentyl-1,3-dimethylxanthine (0.3 mg/kg IV); GLI, glibenclamide (15 μmol/kg IV); APD₀, ventricular monophasic action potential duration; APAV, ventricular monophasic action potential amplitude; AVΒ₀, time to atrioventricular nodal conduction block; SCL, sinus cycle length; and BP, blood pressure. n=10.

*C versus control and GLI.

CPT Versus Glibenclamide

The actions of CPT versus glibenclamide are summarized in Figs 3 and 4. As can be seen in these figures, the actions of CPT and glibenclamide in the atria were the opposite of those in the ventricles. Although CPT reversed the shortening of atrial APD induced by global hypoxia, it was ineffective in the ventricles. In contrast, glibenclamide effectively reversed the ventricular APD shortening caused by global hypoxia but was ineffective in the atria. In addition, CPT but not glibenclamide significantly delayed the appearance of a complete AV nodal conduction block during global myocardial hypoxia (Figs 3 and 4).

Temporal Pattern of APD During β-Adrenergic Receptor Blockade

Fig 5 shows data obtained in groups 4 and 5 during RV pacing and in the presence of propranolol. As can be seen, APD₀ was shortened in a time-dependent manner. There was no difference between the temporal pattern of APD shortening in the control group (group 4) versus that obtained in animals treated with the A₁-AdoR blocker CPT (group 5).

The fact that both groups pretreated with propranolol did not show altered effects of hypoxia on APD indicates that potential modulation of the effect of endogenous catecholamines on APD by endogenous adenosine did not play a major role in hypoxia-induced APD shortening in this model.

Characterization of CPT Action

As can be seen in Table 5, CPT acted as a selective A₁-AdoR antagonist in this guinea pig model. Specifically, CPT markedly attenuated the negative dromotropic action of exogenous adenosine (mediated by A₁-AdoR) on AV nodal conduction expressed as prolongation of adenosine of PR and atrio-His intervals and the number of adenosine-induced nonconducted atrial beats (Table 5, A). In contrast, CPT did not significantly affect the reduction of systemic arterial blood pressure caused by the vasodilatory action of adenosine (mediated by A₂-AdoR) on peripheral resistive vessels (Table 5, B). Data in Table 5, C indicate that CPT effectively antagonizes the antiadrenergic action of the adenosine derivative CPA mediated by ventricular A₁-AdoR. Indeed, CPA markedly attenuated the positive inotropic action of isoproterenol expressed as left ventricular dp/dt, and CPT reversed this action of CPA (Table 5, C).

Discussion

Present Findings

The major findings of the present study were that (1) CPT, a selective A₁-AdoR antagonist, did not alter the effects of global cardiac hypoxia on ventricular myocardial MAP, nor did it alter their temporal pattern; (2) in contrast, CPT prevented the shortening of the atrial myocardial MAP duration caused by hypoxia; (3) CPT also antagonized hypoxia-induced AV nodal conduction delay; (4) glibenclamide markedly attenuated the pina-

TABLE 3. Effects of CPT and GLI During Normoxia and Hypoxia (Cardiac Pacing)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>CPT</th>
<th></th>
<th>GLI</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂</td>
<td>N₂</td>
<td>P</td>
<td>O₂</td>
<td>N₂</td>
<td>P</td>
</tr>
<tr>
<td>APD₀, ms</td>
<td>90±4</td>
<td>67±4</td>
<td>.05</td>
<td>97±6</td>
<td>69±4</td>
<td>.05</td>
</tr>
<tr>
<td>APAV, mV</td>
<td>26±4</td>
<td>15±1</td>
<td>.05</td>
<td>20±3</td>
<td>12±2</td>
<td>.05</td>
</tr>
<tr>
<td>AVΒ₀, s</td>
<td>...</td>
<td>73±8</td>
<td></td>
<td>108±14</td>
<td>.05*</td>
<td></td>
</tr>
<tr>
<td>SCL, ms</td>
<td>196±5</td>
<td>196±5</td>
<td>NS</td>
<td>200±7</td>
<td>200±7</td>
<td>NS</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>65±2</td>
<td>47±2</td>
<td>.05</td>
<td>64±3</td>
<td>47±2</td>
<td>.05</td>
</tr>
</tbody>
</table>

CPT indicates 8-cyclopentyl-1,3-dimethylxanthine (0.3 mg/kg IV); GLI, glibenclamide (15 μmol/kg IV); APD₀, ventricular monophasic action potential duration; APAV, monophasic action potential amplitude; AVΒ₀, time to atrioventricular nodal conduction block; SCL, sinus cycle length; and BP, blood pressure. n=7.

*C versus control and GLI.
cidil- as well as hypoxia-induced shortening of ventricular but not the hypoxia-induced shortening of atrial myocardial MAP; and (5) glibenclamide did not alter the effects of hypoxia on AV nodal conduction. In addition, the present results are consistent with previous observations that hypoxia is associated with increased release of endogenous adenosine, reduced amplitude and shortened duration of atrial and ventricular action potentials, and depressed AV nodal conduction.

**CPT as a Pharmacological Tool**

In the present study, CPT was used as a selective A1-AdoR antagonist in the ventricular and supraventricular tissues of the guinea pig heart in vivo. Data of Table 5 confirm previous studies14 that indicated that CPT is a selective A1-AdoR antagonist. Indeed, CPT antagonized the negative dromotropic action of adenosine on AV nodal conduction and the antiadrenergic action of CPA, both of which are known to be mediated by A1-AdoR without significantly altering the vasodilatory action of adenosine, which is known to be mediated by A2-AdoR.

**Relation to Previous Studies**

Numerous in vitro and in vivo studies using cellular electrophysiological techniques have documented the pronounced alterations in cell membrane action potential caused by acute hypoxia/ischemia. These include cell membrane depolarization leading to reduced am-

---

**TABLE 4. Effects of CPT and GLI During Normoxia and Hypoxia**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLI</th>
<th>CPT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O2</td>
<td>N2</td>
<td>P</td>
</tr>
<tr>
<td>APDA, ms</td>
<td>37±1</td>
<td>24±2</td>
<td>.05</td>
</tr>
<tr>
<td>APA, mV</td>
<td>13±1</td>
<td>11±1</td>
<td>NS</td>
</tr>
<tr>
<td>AVBt, s</td>
<td>...</td>
<td>64±11</td>
<td>NS</td>
</tr>
<tr>
<td>SCL, ms</td>
<td>222±13</td>
<td>213±9</td>
<td>NS</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>62±2</td>
<td>57±2</td>
<td>.05</td>
</tr>
</tbody>
</table>

CPT indicates 8-cyclopentyl-1,3-dimethylxanthine (0.3 mg/kg IV); GLI, glibenclamide (15 μmol/kg IV); APDA, atrial monophasic action potential duration; APA, atrial monophasic action potential amplitude; AVBt, time to atrioventricular nodal conduction block; SCL, sinus cycle length; and BP, blood pressure. n=6.

*Vs control and GLI.

---

**Fig. 1.** Example of recordings obtained during baseline conditions. Tracings are, from top to bottom, standard leads I and II ECGs (ECG I, II), left ventricular electrogram (LV), atrial (MAPa) and ventricular (MAPv) monophasic action potentials, and systemic arterial blood pressure (BP).

**Fig. 2.** Example of ventricular (V) and atrial (A) monophasic action potential recordings during control (C) and after pinacidil (PIN, 1.8 μmol/kg IV) as well as during global hypoxia (Hypo) before and after 8-cyclopentyl-1,3-dimethylxanthine (CPT, 0.3 mg/kg IV) and glibenclamide (GLI, 15 μmol/kg IV). Horizontal bar=200 milliseconds; vertical bar=10 mV and 5 mV for ventricular and atrial recordings, respectively.
plitude of the action potential and shortening of the APD. Similar observations were obtained with MAP recordings in vitro and in vivo. The present data confirm these findings; i.e., the induction of global hypoxia was associated with reduction in the amplitude and the duration of atrial and ventricular MAP. It has been proposed that the shortening of the APD during hypoxia/ischemia is the result of decreased intracellular concentrations of ATP and the subsequent activation of glibenclamide-sensitive K⁺ATP channels. The fact that glibenclamide markedly attenuated the effect of pinacidil and hypoxia on APD in the ventricular myocardium supports this hypothesis. However, glibenclamide did not affect the response of atrial APD to hypoxia, suggesting that a mechanism other than K⁺ATP channel activation is responsible for the hypoxia-induced shortening of atrial APD. This is in agreement with the view that the density of K⁺ATP channels in atrial myocytes is relatively low compared with ventricular myocytes.

The shortening of the atrial APD and increased AV conduction delay caused by adenosine are attributed to the activation of I_{K,ATP}. Moreover, these effects are mediated by A₁-AdoR. Thus, the data obtained in the presence of CPT are in congruence with A₁-AdoR-mediated induction of I_{K,ATP} as a probable cause of the altered atrial MAP. This is based on the following well-established facts: (1) acute myocardial hypoxia is associated with the release of endogenous adenosine to the extracellular fluids; (2) extracellular adenosine exerts pronounced electrophysiological effects, includ-
ing negative chronotropic and dromotropic actions on sinus node automaticity and AV nodal conduction, respectively, as well as shortening of atrial myocardial refractoriness; and (3) these actions are mediated by A1-AdoR and pertussis toxin–sensitive G protein.

It can be concluded that CPT prevented the shortening of atrial APD by blocking A1-AdoR in this tissue. This conclusion is supported by the finding that in the present experimental model, CPT antagonized the detrimental effect of hypoxia on AV nodal conduction. This latter observation indicates that substantial amounts of endogenous adenosine were released into the extracellular fluid, which altered AV nodal conduction. Similar conclusions were obtained in a previous study using a different selective A1-AdoR antagonist. Moreover, since CPT did not alter the effects of hypoxia on ventricular APD, it can be concluded that activation of K\textsuperscript{+ATP} channels by adenosine does not play a major role in the mechanism of hypoxia-induced shortening of ventricular APD. This accords with the fact that no significant I\textsubscript{K,AdoA1} is found in guinea pig ventricular myocytes. Furthermore, since CPT did not alter the temporal pattern of APD shortening, this argues against an early effect of adenosine on K\textsuperscript{+ATP} channels that is masked by subsequent activation of these channels via change in intracellular ATP levels.

### Adenosine and Cardioprotection

Adenosine has been shown to act as an endogenous cardioprotective agent and a mediator of ischemic/hypoxic preconditioning. Since adenosine was suggested to activate K\textsuperscript{+ATP} channels, the activation of which is believed to play a mechanistic role in preconditioning, it has been assumed that K\textsuperscript{+ATP} channels mediate, at least in part, the cardioprotective effects of adenosine. In the present study, glibenclamide did not alter the electrophysiological effects of endogenous adenosine. These data could explain the inability of glibenclamide to prevent preconditioning in rabbit and rat hearts. The lack of effect of glibenclamide on preconditioning is in accord with the present conclusion that endogenous adenosine

---

**Table 5. Characterization of CPT Actions**

**A. Effect of CPT on the negative dromotropic action of exogenous adenosine (n=6)**

<table>
<thead>
<tr>
<th></th>
<th>Control Saline</th>
<th>Control ADO, (\mu)mol/kg</th>
<th>CPT Saline</th>
<th>CPT ADO, (\mu)mol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR, ms</td>
<td>60±0</td>
<td>87±6</td>
<td>98±5</td>
<td>108±4</td>
</tr>
<tr>
<td>AH, ms</td>
<td>49±1</td>
<td>74±8</td>
<td>85±5</td>
<td>98±4</td>
</tr>
<tr>
<td>NAB, n</td>
<td>0</td>
<td>4±1</td>
<td>15±2</td>
<td>30±3</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>56±3</td>
<td>46±3</td>
<td>40±4</td>
<td>35±4</td>
</tr>
</tbody>
</table>

**B. Effect of CPT on adenosine-induced decrease in systemic blood pressure (n=5)**

<table>
<thead>
<tr>
<th></th>
<th>Control ADO, 1.5 (\mu)mol/kg</th>
<th>CPT ADO+CP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mm Hg</td>
<td>55±3</td>
<td>52±3*</td>
<td></td>
</tr>
<tr>
<td>AV PCL, ms</td>
<td>178±2</td>
<td>166±4</td>
<td></td>
</tr>
</tbody>
</table>

**C. Effect of CPT on the antiadrenergic action of CPA (n=6)**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+ISO</th>
<th>ISO+CPA</th>
<th>ISO+CPA+CP T</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV dP/dt, mm Hg/s</td>
<td>590±53</td>
<td>1139±174</td>
<td>694±84*</td>
<td>1028±179</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>56±2</td>
<td>60±2</td>
<td>49±3*</td>
<td>58±3</td>
</tr>
<tr>
<td>AV PCL</td>
<td>179±1</td>
<td>179±1</td>
<td>179±1</td>
<td>179±1</td>
</tr>
</tbody>
</table>

CPT indicates 8-cyclopentyl-1,3-dimethylxanthine (0.3 \(\mu\)g/kg IV); ADO, adenosine; PR, P wave to R deflection interval; AH, atrial to His bundle interval; NAB, nonconducted atrial beats over 120 seconds after adenosine; BP, blood pressure; AV PCL, atrioventricular sequential pacing cycle length; CPA, N\textsuperscript{6}-cyclopentyladenosine (2 \(\mu\)mol/kg IV); ISO, isoproterenol (0.1 \(\mu\)g/kg IV); and LV dP/dt, time derivation of left ventricular pressure.

\(^{*}P<.05\).
does not activate K$_{\text{ATP}}$ channels. Although most earlier studies used localized ischemia and not global hypoxia as the preconditioning perturbation, recent reports have shown that hypoxic and ischemic preconditioning were equipotent in the rat heart$^{21}$ and in preventing infarction in the dog$^{22}$ and that hypoxia-induced APD shortening is mediated in part by the activation of K$_{\text{ATP}}$ channels.$^{23}$ Thus, the cardioprotective role of adenosine in preconditioning is probably mediated by a mechanism other than K$_{\text{ATP}}$ channel activation. The dog could be an exception, however, since glibenclamide antagonized the cardioprotective effect of an A$_{1}$-adenosine agonist in this species.$^{24}$ It should be noted, however, that the interaction between glibenclamide and adenosine in the canine heart is complex and not well understood.$^{25,26}$

There is no immediate explanation for the discrepancy between the two in vitro studies that used patch clamp techniques and showed adenosine-induced activation of K$_{\text{ATP}}$ channels$^{3,4}$ and the present in vivo study. It can be speculated that the conditions of global hypoxia did not cause high enough elevation of extracellular adenosine to activate K$_{\text{ATP}}$ Channels. This is not likely, however, because these levels must have been sufficiently high to cause AV nodal conduction block and to shorten atrial MAP duration.

A recent study using a blood-perfused rabbit papillary muscle has raised serious questions regarding the role of K$_{\text{ATP}}$ channels in the action potential shortening during ischemia or hypoxia and further emphasized the multiple effects of glibenclamide on ischemic/hypoxic myocytes, which complicate interpretation of relevant experimental data.$^{27}$ Thus, from the present results, the mechanism of action potential shortening in the hypoxic ventricular myocardium cannot be conclusively derived. However, the fact that CPT did not reverse the effect of hypoxia on the APD argues strongly against the involvement of A$_{1}$-AdoR activation in these effects of hypoxia. Furthermore, the effects of CPT on hypoxia-induced AV nodal conduction delay and atrial MAP duration shortening indicate that endogenous adenosine released during this period mediated these effects. This is in agreement with previous reports demonstrating that hypoxia is a potent stimulus of myocardial formation of adenosine$^{28,29}$ and its induction of AV block.$^{30}$

**Antiadrenergic Action of Adenosine**

Catecholamines can either shorten or prolong APD, depending on their concentration at the receptor site.$^{31}$ Since adenosine theoretically antagonizes the actions of catecholamine in the ventricular myocardium,$^{1}$ this effect of endogenous adenosine could have modified the changes in APD caused by K$_{\text{ATP}}$ channel activation. However, the present data obtained in animals treated with propranolol argue against this possibility.

**Conclusions**

Activation of K$_{\text{ATP}}$ channels has recently been shown to exert a cardioprotective effect on the ischemic/reperfused mammalian heart. The present data could be interpreted to indicate that the cardioprotective effects of adenosine are not mediated by activation of K$_{\text{ATP}}$, as has been shown in two in vitro studies.$^{3,4}$ There is no immediate explanation for the apparent discrepancy between what was expected from these in vitro studies and the present results. It could well be that in the hypoxic model, the washout of extracellular adenosine prevents its accumulation at the receptor site. Further studies are required before the physiological role of the activation of K$_{\text{ATP}}$ channels by adenosine via A$_{1}$-AdoR in cardiac myocytes is determined.

**Acknowledgments**

Dr Luiz Belardinelli suggested testing of the present working hypothesis. We thank Dwendolyn Dillard for her assistance in preparing the manuscript. Dr Pelleg is supported by grant HL-43006 from the National Institutes of Health.

**References**


Endogenous adenosine does not activate ATP-sensitive potassium channels in the hypoxic guinea pig ventricle in vivo.

J Xu, L Wang, C M Hurt and A Pelleg

_Circulation_. 1994;89:1209-1216
doi: 10.1161/01.CIR.89.3.1209

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/89/3/1209