

**Novel Approach for Enhancing Atrioventricular Nodal Conduction Delay Mediated by Endogenous Adenosine**

C. Kollias-Baker, J. Xu, A. Pelleg, L. Belardinelli

**Abstract** The 2-amino-3-benzothiophene derivative PD 81,723 potentiates the A<sub>1</sub> receptor-mediated negative dromotropic effect of exogenous adenosine and adenosine receptor agonists in guinea pig isolated perfused and in situ hearts. The objective of this study was to determine whether PD 81,723 could amplify the cardiac actions of endogenous adenosine. Two approaches known to increase the myocardial interstitial concentration of adenosine—hypoxia, which increases the production of adenosine and the inhibition of adenosine kinase, which decreases its metabolism—were used to test this hypothesis. In guinea pig hearts in situ, PD 81,723 (2 mg/kg IV) potentiated the atrioventricular (AV) nodal conduction delay caused by hypoxemia (P<sub>aco2</sub>, 14 to 19 mm Hg). In guinea pig isolated hearts, PD 81,723 (5 μmol/L) increased by twofold the stimulus–to–His bundle (S-H) interval prolongations induced by both a 5-minute period of hypoxia (25% O<sub>2</sub>/70% N<sub>2</sub>/5% CO<sub>2</sub>) and the administration of the adenosine kinase inhibitor idoxotubercidin (40 to 70 mmol/L) but had no effect on coronary conductance. Hypoxia and hypoxia plus PD 81,723 (5 μmol/L) caused equivalent increases in the concentration of adenosine in epicardial transudate, from 0.13±0.10 to 0.49±0.05 μmol/L, respectively. Similar to the allosteric enhancer, the nucleoside uptake blocker drafalazine (0.1 μmol/L) also increased by twofold the S-H interval prolongation caused by hypoxia. In contrast to the allosteric enhancer, drafalazine increased the concentration of adenosine in epicardial transudate during hypoxia from 0.48±0.01 to 1.5±0.4 μmol/L. Drafalazine also increased coronary conductance by approximately twofold in guinea pig normoxic constant-flow perfused hearts. In conclusion, PD 81,723 enhances the AV nodal conduction delay, but not the coronary vasodilation, caused by interventions known to increase the concentration of adenosine in myocardial interstitial fluid. Thus, allosteric potentiation of the cardiac actions of adenosine by PD 81,723 is receptor subtype selective and event specific.

**Key Words** hypoxia • idoxotubercidin • His bundle • coronary conductance

There is increasing interest in the development of agents that augment the response to adenosine in a site- and event-specific manner.1,2 PD 81,723, a 2-amino-3-benzothiophene derivative, is a member of a new class of agents that allosterically enhance the A<sub>1</sub> receptor-mediated responses of adenosine.3,6 Recently, our laboratory reported that PD 81,723 specifically potentiates the negative dromotropic effects of exogenous adenosine and of the adenosine receptor agonist N<sup>6</sup>-cyclopentyladenosine.4 In the preceding article in this issue of Circulation Research,6 the potentiation of the negative dromotropic effect of adenosine by PD 81,723 was shown to be concentration dependent, selective for the A<sub>1</sub> receptor, and not accompanied by an increase in the content of adenosine in cardiac effluent. In the absence of adenosine or adenosine agonists, the cardiac effects of PD 81,723 were shown to be minimal.4,6 Consistent with the results of these functional studies, PD 81,723 selectively enhanced the binding of adenosine agonists to A<sub>1</sub> receptors in human and guinea pig cardiac and brain membranes but did not affect the binding of the nucleoside transporter ligand [benzyl-<sup>3</sup>H]nitrobenzylthioninosine.6 PD 81,723 did not, however, potentiate A<sub>2a</sub> adenosine receptor-mediated coronary vasodilation or enhance the binding of an agonist to A<sub>2a</sub> receptors in guinea pig brain membranes.6 Thus, PD 81,723, which has been shown to potentiate only A<sub>1</sub> receptor-mediated responses in the presence of adenosine or adenosine agonists, may provide not only site- and event-specific potentiation of the effects of adenosine but also receptor-selective enhancement (ie, A<sub>1</sub> versus A<sub>2a</sub>). However, it has yet to be determined whether this 2-amino-3-benzothiophene derivative can amplify the cardiac actions of endogenously produced adenosine.

The myocardial interstitial concentration of adenosine can be elevated by interventions that either increase production (eg, hypoxia or ischemia) or decrease degradation (ie, inhibition of cellular uptake and enzymatic metabolism of adenosine) of the nucleoside. Inhibitors of the metabolism of adenosine, such as adenosine kinase and adenosine deaminase inhibitors, and nucleoside uptake blockers have been shown to increase the accumulation of interstitial adenosine.7,9 Likewise, hypoxia and ischemia are potent stimuli for the production of endogenous adenosine.10,14 Endogenously released adenosine has been proposed to play a major role in the atrioventricular (AV) nodal conduction delay observed during hypoxia and ischemia in guinea pig isolated and in situ hearts.13,15,16 The objectives of the present study were (1) to determine the
The myocardial interstitial concentration of adenosine was elevated by decreasing degradation of adenosine with the kinase inhibitor iodotubercidin and by stimulating production of adenosine by hypoxia and hypoxemia in guinea pig isolated perfused and in situ hearts, respectively.

### Materials and Methods

#### Chemicals

(2-Amino-4,5-dimethyl-3-thienyl)-[3-(trifluoromethyl)phenyl] methanone, known as PD 81,723, was a generous gift from Dr R.L. Bruns, Eli Lilly & Co. The nucleoside uptake inhibitor drafazlaine was a gift from Dr H. Van Belle of Janssen Pharmaceutica. 8-Cyclopentylthophylline (CPT), adenosine deaminase, and iodotubercidin were purchased from Research Biochemicals Inc. For in vitro experiments, 10 mmol/L stock solutions of PD 81,723 and CPT were prepared in dimethyl sulfoxide (DMSO). The final concentration of DMSO was ≤0.1% (vol/vol) in the perfusion fluid. Concentrations of DMSO up to 0.5% (vol/vol) had no effect on the stimulus-to-His bundle (S-H) interval. For in vivo experiments, PD 81,723 (25 μmol/L) and CPT (0.3 μg/mL) were dissolved in 48% and 20% Moleculos (Pharmatec), respectively.

#### Experimental Preparations

##### Isolated Hearts

Guinea pigs (Hartley) of either sex weighing between 250 and 300 g were anesthetized with methoxyflurane and killed by cervical dislocation. The hearts were quickly excised, rinsed in ice-cold Krebs-Henseleit solution, and perfused through the aorta at a constant flow of 10 mL/min. Hearts were instrumented for recording of His bundle electrograms and electrically stimulated as described in the preceding article. The perfusion medium (Krebs-Henseleit solution) was gassed either with 95% O₂/5% CO₂ (P₀2, 56±15 mm Hg) or with 25% O₂/70% N₂/5% CO₂ (P₀2, 224±11 mm Hg). Per fusate oxygen pressure (P₀2) was determined with an arterial blood gas analyzer (IL model 1312, Instrument Labs). Because hearts were perfused at constant flow, coronary conductance (in milliliters per minute per millimeter of mercury) was calculated as the ratio between the coronary perfusion rate (10 mL/min) and the perfusion pressure (in millimeters of mercury).

##### In Situ Hearts

Experiments were performed in guinea pigs of either sex, weighing between 480 and 700 g. Guinea pigs (n=14) were anesthetized with 98% ketamine plus 2% acepromazine (1.6 to 1.7 mL/kg IM) and harnepirized with heparin sulfate (750 μg/kg IV). The animals were ventilated with room air by use of a small animal respirator (model 885, Harvard Apparatus). A custom-made bipolar electrode catheter was introduced through the left carotid artery and positioned in the aortic root for recording of the His bundle electrogram. Another bipolar electrode catheter was introduced through the jugular vein and positioned in the right atrium for electrical pacing of the heart. The left brachial artery was cannulated with a polyethylene catheter and connected to a pressure transducer for continuous monitoring and recording of systemic arterial pressure and collection of blood samples for determination of arterial oxygen pressure (Pao₂). Pao₂ was determined by use of a blood gas analyzer (model 178, Corning). 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.

His bundle electrograms, ECG surface leads I and II, and the systemic arterial pressure signals were amplified by Gould biomedical amplifiers and recorded on a strip-chart recorder (model MT 9500, Astro Med Corp).

### Determination of the Concentration of Adenosine in Epicardial Transudate

Epicardial transudate samples were collected by using porous disks placed on the epicardial surface as described previously. Disks (Magna nylon membrane filters, MS1) 6 mm in diameter with a pore size of 0.45 μm were preweighed dry in storage vials before soaking in Krebs-Henseleit solution. For each sample, two dry disks were applied to the epicardial surface of the left ventricle and left in place for 1 minute to allow equilibration of the epicardial transudate with the disk fluid. This was repeated for a total collection period of 2 minutes and a total of four disks per sample. Once removed from the surface of the left ventricle, the disks were placed in the storage vials, and at the termination of each experiment, the vials were reweighed and stored at −80°C for later analysis of adenosine content.

#### Adenosine Assay

Each sample of epicardial transudate, consisting of four disks, was soaked for 30 minutes in two sequential 200-μL aliquots of buffer (100 mmol/L K₃PO₄ and 1% methanol). Samples were filtered by centrifugation (Spinex 0.22-μm filter, Costar), and the concentration of adenosine in the samples was determined by reversed-phase high-performance liquid chromatography according to a previously published isocratic method. Adenosine in the samples was identified by retention time and verified by enzymatic degradation to inosine using adenosine deaminase. The content of adenosine in the samples was determined by comparison of peak areas of each sample to peak areas of samples containing known quantities of adenosine.

#### Statistical Analysis

All values are expressed as mean±SEM. For experimental protocols having multiple comparison groups, statistical analysis was based on ANOVA followed by Bonferroni testing (in STAT MAC, GraphPad Software Inc). Differences between group means and control versus interventions were considered significant at P<.05.

### Protocols

#### Effects of PD 81,723 and Drafazlaine on Hypoxia-Induced AV Nodal Conduction Delay and Epicardial Transudate Adenosine Content

Isolated hearts (n=12) were allowed to equilibrate for 20 minutes under normoxic conditions (P₀2, 563±15 mm Hg) before being subjected to three successive periods of hypoxia. A 5-minute hypoxic perfusion period was initiated by switching from normoxic to hypoxic solution (P₀2, 224±11 mm Hg). After each 5-minute period of hypoxia, the hearts were reoxygenated with normoxic solution (P₀2, 563±15 mm Hg) for at least 30 minutes. During the first hypoxic period, the S-H interval prolongation was determined in the absence of any drug. During the second hypoxic period, the effect of either PD 81,723 (5 μmol/L) or drafazlaine (0.1 μmol/L) on the S-H interval prolongation was evaluated. In the third hypoxic period, the reversibility of the effect of PD 81,723 or drafazlaine was assessed by use of the adenosine receptor antagonist CPT (10 μmol/L). The potentiating agents (ie, PD 81,723 or drafazlaine) and the antagonist CPT were infused into the perfusate line at rates to achieve the desired final perfusate concentration. Infusion of each agent was begun 15 minutes before initiation of hypoxia and stopped 1 minute after the reoxygenation period. There was a 54-second transit time in the perfusion system. The results of pilot studies in two hearts...
showed that the magnitude of the S-H interval prolongation was similar in three successive periods of hypoxia (no drug present), with intervening 30-minute periods of reoxygenation.

In these same hearts, the effects of PD 81,723 and draflazine on the adenosine content of epicardial transudate during normoxia and hypoxia were determined. Epicardial transudate samples were collected, as described above, at two time points: (1) after 20 minutes of normoxia and (2) during the last minute of hypoxia and the first minute of reoxygenation, in the absence and presence of either PD 81,723 (5 μmol/L) or draflazine (0.1 μmol/L).

**Effects of PD 81,723 and Draflazine on Coronary Vasodilation During Normoxia and Hypoxia**

**Normoxia.** In this series of experiments, the effects of PD 81,723 (n=5) and draflazine (n=5) on the coronary perfusion pressure were evaluated in hearts perfused at constant flow (10 mL/min) with normoxic (P02, 563±15 mm Hg) Krebs-Henseleit solution. Isolated hearts were allowed to equilibrate for 30 minutes before the administration of either PD 81,723 (5 μmol/L) or draflazine (0.1 μmol/L). The infusions of PD 81,723 and draflazine were maintained for a total of 10 minutes. The effect of either the adenosine antagonist CPT (n=2) or adenosine deaminase (n=3) was determined in the presence of draflazine (0.1 μmol/L). During the last 5 minutes of draflazine infusion, either CPT (10 μmol/L) or adenosine deaminase (10 U/mL) was infused concurrently. The coronary perfusion pressure was measured continuously throughout the experiment.

**Hypoxia.** In a separate series of experiments, isolated hearts (n=5) were perfused at constant flow (10 mL/min) with normoxic Krebs-Henseleit solution (P02, 563±15 mm Hg) for 30 minutes before being subjected to two successive transient 30-second periods of perfusion with hypoxic solution (P02, 224±11 mm Hg). After each 30-second period of hypoxic perfusion, hearts were reoxygenated with normoxic solution for at least 30 minutes. Administration of PD 81,723 was begun at least 15 minutes before the second period of hypoxic perfusion. The effect of hypoxia on coronary perfusion pressure was determined in the absence and presence of PD 81,723 (5.0 μmol/L). Coronary perfusion pressure was continuously recorded throughout the experiment.

**Effects of PD 81,723 on Iodotubercidin-Induced Increases in S-H Interval and Coronary Conduction**

**S-H interval.** In this series of experiments carried out in six isolated constant-flow perfused hearts, the effect of the adenosine kinase inhibitor iodotubercidin (40 nmol/L) on the S-H interval was determined in the absence and presence of PD 81,723 (5 μmol/L) and PD 81,723 plus the adenosine antagonist CPT (10 μmol/L). Each drug alone or in combination (ie, iodotubercidin alone, iodotubercidin plus PD 81,723, and iodotubercidin plus PD 81,723 plus CPT) was consecutively infused for 15 minutes before recording the steady-state S-H interval. In a separate heart, the S-H interval was continuously recorded during consecutive infusions of iodotubercidin (perfusion concentration, 70 nmol/L), iodotubercidin plus PD 81,723 (perfusion concentration, 20 μmol/L), and iodotubercidin plus PD 81,723 plus CPT (perfusion concentration, 10 μmol/L).

**Coronary conduction.** In a separate series of experiments carried out in six isolated constant-flow perfused hearts, the effect of iodotubercidin (17 nmol/L) on coronary conduction was determined in the absence and presence of PD 81,723 (5 μmol/L) and PD 81,723 plus either CPT (perfusion concentration, 10 μmol/L; n=3) or adenosine deaminase (perfusion concentration, 7 μU/mL; n=3). Each drug alone or in combination (ie, iodotubercidin alone, iodotubercidin plus PD 81,723, and iodotubercidin plus PD 81,723 plus either CPT or adenosine deaminase) was consecutively infused for 15 minutes, during which time the coronary perfusion pressure was continuously recorded. Before completion of the experiment, adenosine was infused (perfusate concentration, 3 μmol/L) to determine the maximum coronary vasodilation attainable in each heart. Coronary conductance was calculated as the ratio between the coronary perfusion rate (10 mL/min) and the perfusion pressure.

**Effect of Allosteric Enhancer PD 81,723 on In Situ Hearts**

In this series of experiments (n=14), hypoxemia was induced in anesthetized guinea pigs by ventilation with one of two hypoxic gas mixtures. Animals of group I (n=7) were ventilated with a gas mixture that contained 8% O2, 92% N2. Animals of group II (n=7) were ventilated with a gas mixture that contained 5% O2, 95% N2. After a 20-minute stabilization period, during which time the animals were ventilated with room air, the animals were subjected to two successive 10-minute periods of ventilation with hypoxic gas. Each hypoxic period was followed by a reoxygenation period (ie, ventilation with room air) of at least 30 minutes. The P-wave-to-R-wave interval (PR interval), the atria-to-His bundle interval (AH interval), the number of nonconducted atrial beats, and arterial PO2 (PaO2) and pH were determined at the end of each normoxic and hypoxic period. In groups I and II, the hearts were atrially paced at rates of 320 and 270 beats per minute, respectively, throughout the experimental period. Cardiovascular and blood gas parameters of animals of both groups I and II were determined during normoxemia, hypoxemia, and hypoxia in the presence of PD 81,723 (2 mg/kg IV). In addition, the effect of the adenosine receptor antagonist CPT (0.3 mg/kg IV) on cardiovascular responses to hypoxemia in the presence of PD 81,723 was also evaluated in animals of group I.

**Results**

**Potentiation of Hypoxia-Induced AV Nodal Conduction Delay**

In six isolated perfused hearts, the S-H interval in response to a 5-minute period of hypoxia increased to a maximum value of 62±1 milliseconds (ΔS-H prolongation, 12±1 milliseconds) and returned to the baseline value (ie, prehypoxic value) of 50±2 milliseconds within 4 minutes of reoxygenation (Fig 1). In the same hearts, the allosteric enhancer PD 81,723 (5 μmol/L), although prolonging the S-H interval during normoxia by only 2 to 3 milliseconds, significantly increased the maximum S-H interval prolongation observed during hypoxia to 75±5 milliseconds (Fig 1; ΔS-H prolongation, 25±5 milliseconds). The adenosine antagonist CPT (10 μmol/L) significantly attenuated the maximum S-H interval prolongation caused by hypoxia in the presence of PD 81,723 to 57±1 milliseconds (Fig 1; ΔS-H prolongation, 7±1 milliseconds). In a separate group of six hearts, draflazine (0.1 μmol/L) also potentiated the maximum S-H interval prolongation caused by hypoxia but had little effect on the baseline S-H interval. That is, during normoxia, draflazine increased the S-H interval from 51±2 to 54±2 milliseconds. As shown in Fig 2, during a 5-minute period of hypoxia, the maximum S-H interval prolongation significantly increased from 64±2 milliseconds (ΔS-H prolongation, 13±2 milliseconds) in the absence to 81±6 milliseconds (ΔS-H prolongation, 27±6 milliseconds) in the presence of draflazine (0.1 μmol/L). The adenosine receptor antagonist CPT (10 μmol/L) in the presence of draflazine (0.1 μmol/L) significantly attenuated the maximum S-H interval pro-
longation caused by hypoxia to 59±1 milliseconds (Fig 2; ∆S-H prolongation, 5±1 milliseconds).

**Adenosine Content in Epicardial Transudate During Hypoxia**

Although both the allosteric enhancer PD 81,723 and the nucleoside uptake inhibitor draflazine potentiated the hypoxia-induced AV nodal conduction delay, only the potentiation by draflazine was associated with a concomitant increase in the concentration of adenosine in the epicardial transudate during hypoxia (Fig 3). Under normoxic conditions, the concentration of adenosine in the epicardial transudate was 0.13±0.15 μmol/L (n=12). Under normoxic conditions in the presence of PD 81,723 (5 μmol/L, n=6) and draflazine (0.1 μmol/L, n=6), the concentration of adenosine in the epicardial transudate did not significantly increase (0.2±0.1 and 0.3±0.1 μmol/L, respectively). A 5-minute period of hypoxia in the absence of any drug (ie, control conditions) was associated with an increase in the concentration of adenosine in the epicardial transudate to 0.48±0.1 μmol/L (Fig 3). An equal period of hypoxia (ie, 5 minutes) in the presence of PD 81,723 (5 μmol/L) was associated with an equivalent (P>0.05) increase in the concentration of adenosine in the epicardial transudate to 0.45±0.3 μmol/L (Fig 3). In contrast, in the presence of draflazine (0.1 μmol/L), hypoxia was accompanied by a significant increase in the concentration of adenosine in the epicardial transudate to 1.5±0.4 μmol/L (Fig 3).

**Draflazine, but Not PD 81,723, Causes Coronary Vasodilation**

**Normoxia**

The nucleoside uptake inhibitor draflazine, but not the allosteric enhancer PD 81,723, caused coronary vasodilation during perfusion with a normoxic Krebs-Henseleit solution (Fig 4). As shown in Fig 4, infusion of PD 81,723 (5 μmol/L) did not cause coronary vasodilation. The mean coronary perfusion pressure in the absence and presence of PD 81,723 was 47±5 mm Hg (n=5). In contrast, draflazine (0.1 μmol/L) caused a marked coronary vasodilation (Fig 4). The mean coronary perfusion pressure decreased from 45±2 mm Hg in the absence to 27±5 mm Hg in the presence of draflazine (0.1 μmol/L, n=5). In the continued presence of draflazine (0.1 μmol/L), adenosine deaminase (10 U/mL) reversed the coronary vasodilation caused by the nucleoside uptake inhibitor (Fig 4). Adenosine deaminase (n=3) and the adenosine antagonist CPT (10 μmol/L, n=2) significantly increased the mean coronary perfusion pressure from a nadir of 27±5 mm Hg during infusion of draflazine alone to 44±4 mm Hg.
Hypoxia

In a separate group of hearts (n=5), the effect of the allosteric enhancer PD 81,723 on hypoxia-induced coronary vasodilation was evaluated (not shown). PD 81,723 did not potentiate the coronary vasodilation caused by hypoxia. The maximum increase in coronary conduction caused by a transient 30-second period of hypoxia was 0.026±0.01 mL/min per millimeter of mercury in the absence and 0.023±0.01 mL/min per millimeter of mercury in the presence of PD 81,723 (5 μmol/L).

Effects of Hypoxemia and PD 81,723 on AV Nodal Conduction in Hearts In Situ

To investigate the effects of PD 81,723 on changes in AV nodal conduction time intervals (PR and AH) and arterial pressure and pH during hypoxemia, two groups of anesthetized guinea pigs were subjected to successive periods of ventilation with one of two hypoxic gas mixtures: group I, with PO2 of 8%, and group II, with PO2 of 5%. In groups I and II during the first and second periods of hypoxemia, the PR interval, the AH interval, the number of nonconducted atrial beats, and the arterial pressure and pH were determined in the absence and presence of PD 81,723 (2 mg/kg IV). In addition, in animals of group I these same parameters were measured during a third period of hypoxemia in the presence of PD 81,723 plus the adenosine receptor antagonist CPT (0.3 mg/kg IV). During ventilation with room air (ie, normoxemia), the PO2 and pH values of both groups I and II were 95±6 mm Hg and 7.4±0.03, respectively. The arterial pH during hypoxemia (which varied between 7.4±0.01 and 7.25±0.04) was not significantly different from the pH during normoxemia. Similarly, neither of the drugs used (ie, PD 81,723 and CPT) altered the arterial pH during normoxemia or hypoxemia.

In animals of group I, ventilation with hypoxic gas (PO2, 8%) caused hypoxemia (PO2, 19±1 mm Hg) but no overt prolongation of AV nodal conduction time (Table 1). In the presence of PD 81,723 (2 mg/kg IV), however, the same degree of hypoxemia (PO2, 20±1 mm Hg) caused significant slowing of AV nodal conduction and the development of second-degree AV block in seven of seven animals (Table 1). For example, the PR and AH intervals increased by 88% and 100%, respectively (Table 1). Similarly, although 1:1 AV nodal conduction was observed in animals of group I during hypoxemia alone, in the presence of PD 81,723 (2 mg/kg IV) hypoxemia caused second-degree AV block, with a mean of 28±7 nonconducted atrial beats during the hypoxic period (Table 1). The adenosine receptor antagonist CPT (0.3 mg/kg IV) did not affect the degree of hypoxemia (PO2, 19±1 mm Hg) but significantly attenuated the slowing of AV nodal conduction observed in the presence of PD 81,723 (Table 1). The PR and AH intervals were reduced by 29% and 32%, respectively, from intervals recorded during hypoxemia in the presence of PD 81,723 (Table 1). Similarly, second-degree AV block, observed during hypoxemia in the presence of PD 81,723, was completely absent after administration of CPT (Table 1).

In the animals of group II, ventilation with hypoxic gas (PO2, 5%) caused hypoxemia (PO2, 13.7±0.8 mm Hg) and overt prolongation of AV nodal conduction time. The PR and AH intervals increased by 54% and 61%, respectively, and second-degree AV block developed in seven of seven animals, with an average of 2±7 nonconducted atrial beats during the hypoxic period (Fig 5 and Table 2). In the presence of PD 81,723

Table 1. Effect of Ventilation of Anesthetized Guinea Pigs With Hypoxic Gas (8% O2) on Cardiovascular Parameters in the Absence and Presence of PD 81,723 and the A, Adenosine Receptor Antagonist Cyclopentyltheophylline

<table>
<thead>
<tr>
<th></th>
<th>Normoxemia</th>
<th>Hypoxemia</th>
<th>Hypoxemia + PD</th>
<th>Hypoxemia + PD + CPT</th>
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<tbody>
<tr>
<td>PO2, mm Hg</td>
<td>95.0±4.6</td>
<td>19.0±1.0</td>
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<td>PCL, ms</td>
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<td>190±6</td>
<td>190±6</td>
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<tr>
<td>PR, ms</td>
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<td>60±2</td>
<td>113±7*</td>
<td>80±4*</td>
</tr>
<tr>
<td>AH, ms</td>
<td>50±2</td>
<td>50±2</td>
<td>103±8*</td>
<td>70±4*</td>
</tr>
<tr>
<td>NAB</td>
<td>0</td>
<td>0</td>
<td>28±7*</td>
<td>0</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>50±1</td>
<td>46±1</td>
<td>44±1</td>
<td>45±1</td>
</tr>
</tbody>
</table>

PD indicates PD 81,723; CPT, cyclopentyltheophylline; PCL, atrial pacing cycle length; PR, P-wave–to–R-wave interval; AH, atrial–to–His bundle interval; NAB, number of nonconducted atrial beats; and BP, arterial blood pressure. Values are mean±SEM from seven animals. *P<.05 vs values obtained during hypoxemia alone; †P<.05 vs values obtained during hypoxemia plus PD.
(2 mg/kg IV), the same degree of hypoxemia (PaO₂, 14.4±0.02 mm Hg) caused a more severe prolongation of AV nodal conduction time (Fig 5 and Table 2). That is, the PR and AH intervals increased by 52% and 60%, respectively, from intervals recorded during hypoxemia alone (Table 2). Similarly, in the presence of PD 81,723 (2 mg/kg IV), during the hypoxic period the number of nonconducted atrial beats increased from 2±7 to 216±13 beats (Fig 5 and Table 2).

**PD 81,723 Selectively Potentiates A₁ but Not A₂ Adenosine Receptor–Mediated Effects of Iodotubercidin**

In isolated hearts (n=6), the allosteric enhancer PD 81,723 potentiated the A₁ receptor–mediated S-H interval prolongation caused by the adenosine kinase inhibitor iodotubercidin (Fig 6). Iodotubercidin alone (40 nmol/L) prolonged the S-H interval from 55±1 to 59±1 milliseconds (Fig 6; ΔS-H interval prolongation, 4±1 milliseconds). Concurrent infusion of iodotubercidin (perfusate concentration, 40 nmol/L) and PD 81,723 (perfusate concentration, 5 μmol/L) caused a significantly greater prolongation of the S-H interval to 65±2 milliseconds (Fig 6; ΔS-H interval prolongation, 10±2 milliseconds). The adenosine antagonist CPT (10 μmol/L) reversed the S-H interval prolongation caused by iodotubercidin and PD 81,723 to 57±1 milliseconds (ΔS-H interval prolongation, 2±0 milliseconds), a value not significantly different from the baseline S-H interval duration of 55±1 milliseconds (Fig 6). A typical example of the effect of iodotubercidin (70 nmol/L), PD 81,723 (20 μmol/L), and CPT (10 μmol/L) on the S-H interval is shown in Fig 7.

In a separate group of constant-flow perfused hearts (n=6), PD 81,723 did not potentiate the A₂ receptor–mediated increase in coronary conductance caused by iodotubercidin (Fig 6). As shown in Fig 6, iodotubercidin (17 nmol/L) alone increased the coronary conductance from 0.20±0.01 to 0.33±0.01 mL/min per millimeter of mercury (change in conductance, 0.13±0.02 mL/min per millimeter of mercury), and concurrent infusion of iodotubercidin (17 nmol/L) and PD 81,723 (5 μmol/L) caused an equivalent (P>.05) coronary vasodilation, with a mean coronary conductance of 0.36±0.02 mL/min per millimeter of mercury (change in conductance, 0.16±0.03 mL/min per millimeter of mercury). Both the adenosine antagonist CPT (10 μmol/L, n=3) and adenosine deaminase (7 U/mL, n=3) significantly reduced the increase in coronary conductance caused by iodotubercidin to 0.20±0.02 and 0.24±0.03 mL/min per millimeter of mercury, respectively (Fig 6; change in conductance, 0.03±0.00 mL/min per millimeter of mercury). A typical example of the effect of iodotubercidin (17 nmol/L), PD 81,723 (5 μmol/L), and adenosine deaminase (7 U/mL) on the coronary perfusion pressure is shown in Fig 7. To determine the maximal coronary vasodilation attainable in these hearts, adenosine was infused at the end of each hypoxic period (216±33 mm Hg).

**Table 2. Effect of Ventilation of Anesthetized Guinea Pigs With Hypoxic Gas (5% O₂) on Cardiovascular Parameters in the Absence and Presence of PD 81,723**

<table>
<thead>
<tr>
<th></th>
<th>Normoxemia</th>
<th>Hypoxemia</th>
<th>Hypoxemia + PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO₂, mm Hg</td>
<td>95.0±4.6</td>
<td>13.7±0.8</td>
<td>14.4±0.7</td>
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<td>PCL, ms</td>
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<td>PR, ms</td>
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<td>AH, ms</td>
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<td>133±8*</td>
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<td>0</td>
<td>2±7</td>
<td>216±33</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>56±2</td>
<td>51±1</td>
<td>44±2</td>
</tr>
</tbody>
</table>

PD indicates PD 81,723; PCL, atrial pacing cycle length; PR, P-wave–to–R-wave interval; AH, atrial–to–His bundle interval; NAB, number of nonconducted atrial beats; and BP, arterial blood pressure. Values are mean±SEM from seven animals. During normoxemia, animals were ventilated with room air. The dose of PD was 2 mg/kg IV.

*P<.05 vs values obtained during hypoxemia alone.
The results of the present study demonstrate that PD 81,723 potentiates the AV nodal conduction delay, but not the coronary vasodilation, caused by endogenously released adenosine in guinea pig isolated and in situ hearts. In isolated hearts, hypoxia alone increased the S-H interval (Fig 1), the coronary conductance (Fig 5), and the concentration of adenosine in the epicardial transudate (Fig 3). PD 81,723 potentiated by twofold the hypoxia-induced S-H interval prolongation (Fig 1) but had no effect on increases in coronary vasodilation (data not shown) or in the concentration of adenosine in the epicardial transudate (Fig 3) caused by hypoxia. Similarly, in anesthetized guinea pigs, PD 81,723 unmasked the AV nodal conduction delay caused by moderate hypoxemia (Table 1) and exacerbated the AV nodal conduction delay associated with severe hypoxemia (Table 2). Similar to the effects of hypoxia, the adenosine kinase inhibitor Iodotubercidin alone increased the S-H interval and the coronary conductance in isolated guinea pig hearts (Figs 6 and 7). Iodotubercidin has been shown to increase the release of adenosine into cardiac effluent by inhibiting the conversion of adenosine to AMP.\(^7\) Associated with the increased release of adenosine in cardiac effluent and the estimated increase in the interstitial concentration of adenosine, Iodotubercidin caused a concentration-dependent increase in coronary vasodilation.\(^7\) In the present study, the effects of two concentrations of Iodotubercidin on the S-H interval and the coronary conductance were evaluated. The lower concentration of Iodotubercidin (17 nmol/L) increased the coronary conductance (Fig 6) without prolonging the S-H interval (not shown), whereas the higher concentration of Iodotubercidin (40 nmol/L) caused maximum coronary vasodilation (not shown) and prolonged the S-H interval (Fig 6). These findings are consistent with previous reports that in guinea pig isolated hearts the EC\(_{50}\) value of adenosine necessary to prolong the S-H interval is severalfold higher than that necessary to cause coronary vasodilatation.\(^20\) Consistent with the effects of PD 81,723 on hypoxia-induced increases in the S-H interval and in coronary conductance, PD 81,723 potentiated only the Iodotubercidin-induced prolongation of the S-H interval but did not affect the increase in the coronary

**Discussion**

experiment (not shown). Adenosine (3 \(\mu\)mol/L) increased the coronary conductance to 0.57±0.04 mL/min per millimeter of mercury (change in conductance, 0.38±0.11 mL/min per millimeter of mercury).

![Fig 6. Bar graphs showing potentiation by PD 81,723 of the A1, but not A2a receptor-mediated effects of the adenosine kinase inhibitor Iodotubercidin (ITC) in guinea pig isolated perfused hearts. PD 81,723 (5 \(\mu\)mol/L) potentiated the stimulus-to-His bundle (S-H) interval prolongation (A1 response, top) but not the increase in coronary conductance (A2a response, bottom), caused by ITC. The adenosine antagonist 8-cyclopentyltheophylline (CPT, 10 \(\mu\)mol/L) reversed the increases in S-H interval duration and coronary conductance caused by ITC alone and by ITC plus PD 81,723 (top) and ITC alone (bottom). Not shown, adenosine (3 \(\mu\)mol/L) caused maximum coronary vasodilation with a mean coronary conductance of 0.57±0.04 mL/min per millimeter of mercury (change in conductance, 0.38±0.11 mL/min per millimeter of mercury). Values are mean±SEM from six and three hearts for the top and bottom panels, respectively. *P<.05 vs ITC alone.]

![Fig 7. Recordings of stimulus-to-His bundle (S-H) interval duration and coronary perfusion pressure from guinea pig isolated constant-flow perfused hearts (n=2) in the absence and presence of Iodotubercidin, PD 81,723, and either the adenosine antagonist 8-cyclopentyltheophylline (CPT, top) or adenosine deaminase (ADA, bottom). Top. Whereas Iodotubercidin caused a small increase in the S-H interval, concurrent infusion of Iodotubercidin and PD 81,723 resulted in a substantially greater prolongation of the S-H interval, which was reversed by CPT. Bottom, PD 81,723 did not potentiate the coronary vasodilatory effect of Iodotubercidin. ADA reversed the coronary vasodilation caused by Iodotubercidin. PD 81,723 (20 \(\mu\)mol/L) alone caused a maximum S-H interval prolongation of 5 milliseconds (data not shown).]
Vasodilation caused by the adenosine kinase inhibitor (Figs 6 and 7). Whereas the prolongation of the S-H interval is mediated through activation of the A<sub>1</sub> receptor, the coronary vasodilatory effect of adenosine is mediated through activation of the A<sub>2a</sub> receptor. The lack of potentiation of the hypoxia- and iodotubercidin-induced increases in coronary conductance by PD 81,723 provides further evidence of the A<sub>2a</sub> receptor-selective effect of this allosteric enhancer.

Similar to PD 81,723, the nucleoside uptake inhibitor drafalzine also increased the maximum S-H interval prolongation caused by hypoxia in isolated hearts by twofold (Fig 2). However, in contrast to the allosteric enhancer, the effect of drafalzine was associated with a threefold increase in the concentration of adenosine in the epicardial transudate (Fig 3). Although both PD 81,723 and drafalzine exacerbated the AV nodal conductance delay caused by hypoxia, their effects on the coronary perfusion pressure (ie, coronary conductance) were markedly different. During normoxic constant-flow perfusion, drafalzine caused marked coronary vasodilation, whereas PD 81,723 had no significant coronary vasodilatory effect under the same conditions (Fig 4). The attenuation of the drafalzine-induced coronary vasodilation by adenosine deaminase and the adenosine antagonist CPT indicates that this effect of the nucleoside uptake blocker is mediated through endogenous adenosine and activation of the A<sub>2a</sub> receptor, respectively.

In the present study, the findings that both PD 81,723 and drafalzine potentiated the hypoxia-induced prolongation of the S-H interval provide additional support for the role of endogenous adenosine as a mediator of hypoxia-induced prolongation of AV nodal conduction time. There is substantial evidence in support of this hypothesis. For instance, exogenous adenosine causes prolongation of AV nodal conduction time similar to that induced by hypoxia. The time course of adenosine production and the amount of the nucleoside released during hypoxia are sufficient to account for the observed increase in AV nodal conduction time. Furthermore, agents that attenuate the negative dromotropic actions of adenosine, such as adenosine receptor antagonists and agents that increase the degradation of adenosine (eg, adenosine deaminase), have been shown to significantly reduce the AV nodal conduction delay caused by hypoxia. In the present study, PD 81,723 and drafalzine, two agents that potentiate the A<sub>1</sub> receptor-mediated effects of adenosine by different mechanisms (ie, allosteric enhancement and nucleoside uptake inhibition, respectively), markedly increased the prolongation of AV nodal conduction time caused by hypoxia. The allosteric enhancer PD 81,723 augmented the hypoxia-induced AV nodal conduction delay in guinea pig isolated (Fig 1) and in situ hearts (Fig 5). Similar to PD 81,723, the nucleoside uptake inhibitor drafalzine further impaired AV nodal conduction during hypoxia in guinea pig isolated hearts (Fig 2). The inhibition of the potentiating effects of these agents by the adenosine receptor antagonist CPT (Figs 1 and 2) suggests that their effects are mediated through activation of the A<sub>1</sub> adenosine receptor. Altogether, these findings provide strong corroborating evidence that adenosine is a primary mediator of hypoxia-induced AV nodal conduction delay in the guinea pig heart.

Implications

Agents that enhance the effects of adenosine in a site- and event-specific manner offer many therapeutic advantages. For instance, the effects of these compounds would be limited to a particular location, such as an ischemic region of the myocardium and/or a particular time period, such as upon coronary artery occlusion, hypoxic perfusion, or any condition when interstitial adenosine accumulates either because of an increase in its production or a decrease in its metabolism. The results of the present study indicate that the allosteric enhancer PD 81,723 and the nucleoside uptake inhibitor drafalzine may act as event-specific enhancers of the A<sub>2a</sub>-mediated effects of endogenously released adenosine. Both the allosteric enhancer PD 81,723 and the nucleoside uptake blocker drafalzine potentiated the hypoxia-induced AV nodal conduction delay but had negligible effects on AV nodal conduction during normoxia. Because global hypoxia was chosen as the intervention to increase the formation of adenosine, the potential site-specific properties of these agents could not be directly evaluated. Regardless, because the effects of PD 81,723 were limited to a response mediated by the A<sub>1</sub> receptor, allosteric enhancement by this compound is receptor-subtype selective. The marked vasodilation caused by drafalzine, even under normoxic conditions, demonstrates that this nucleoside uptake blocker, like others, potentiates all receptor-mediated responses of adenosine regardless of receptor subtype.

In conclusion, PD 81,723 enhances the A<sub>1</sub> receptor-mediated responses of exogenous and endogenously produced adenosine. Allosteric enhancement of the actions of adenosine may prove an ideal approach to exploit the antiarrhythmic and cardioprotective properties of this nucleoside. Recently, the antiarrhythmic potential of PD 81,723 was demonstrated in anesthetized rabbits subjected to acute transient myocardial ischemia. PD 81,723 significantly decreased the incidence of ventricular tachycardia and fibrillation in this ischemic model. Moreover, in humans, dipyridamole, an agent that potentiates the actions of endogenous adenosine, has been shown to effectively terminate catecholamine-facilitated nonreentrant ventricular tachyarrhythmias. This anti-β-adrenergic action of adenosine is due to activation of A<sub>1</sub> receptors; thus, allosteric enhancement of A<sub>1</sub> adenosine receptor-mediated responses may prove to be a valuable alternative to β-adrenergic blockade in the treatment of catecholamine-dependent ventricular arrhythmias in humans. As to the cardioprotective action of adenosine, which has yet to be shown clinically, if it is determined to be mediated through the A<sub>1</sub> receptor, then PD 81,723 should potentiate this action of adenosine as well. For further studies involving the site- and event-specific characteristics of PD 81,723, a model of localized ischemia or hypoxia will be required for a more conclusive demonstration of the site-specific properties of this allosteric enhancer.

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