Adenosine as well as hypoxia and ischemia are known to cause atrioventricular conduction block. To test the hypothesis that adenosine is the primary mediator of hypoxia-induced atrioventricular conduction delay in isolated perfused guinea pig hearts, we characterized: (a) the time courses of hypoxia-induced adenosine release and delay in atrioventricular conduction, (b) the relationships between oxygen tension, adenosine concentration in the effluent, and atria-to-His-bundle interval, and (c) the adenosine receptor mediating the negative dromotropic effect of hypoxia. Oxygen tension and effluent adenosine levels were linearly related with a correlation coefficient (r) of -0.85 and a slope of -6.3 ± 0.37 pmol/min/g/torr. Likewise, oxygen tension and atria-to-His-bundle interval prolongation were linearly related with r = -0.85 and a slope of -0.180 ± 0.013 msec/torr. The EC50 of effluent adenosine in causing atria-to-His-bundle prolongation was 0.26 ± 0.02 µM. Adenosine deaminase, an enzyme that deaminates adenosine to inosine and is limited to the extracellular space, significantly attenuated (61%) the atria-to-His-bundle interval prolongation caused by hypoxia. This prolongation was further reduced (81%) by a combination of adenosine deaminase and theophylline, an adenosine receptor blocker. Adenosine deaminase also reduced (by 95%) the atria-to-His-bundle interval prolongation in normoxic recipient hearts caused by the effluent of hypoxic donor hearts. Several adenosine antagonists, i.e., theophylline, 8-phenyltheophylline, and 8-(p-sulfophenyl)theophylline antagonized in a dose-dependent manner the negative dromotropic effect of exogenous adenosine and hypoxia. Schild analysis of the antagonism of hypoxia-induced atria-to-His-bundle interval prolongation by 8-(p-sulfophenyl)theophylline yielded the following pA2 values: 5.30 ± 0.25 and 5.28 ± 0.31 using oxygen tension and effluent adenosine vs. AH interval prolongation, respectively. 8-(p-Sulphophenyl)theophylline also antagonized to an equal extent atria-to-His-bundle interval prolongations of similar magnitude caused either by adenosine or hypoxia. We conclude that 1) adenosine is the primary mediator of hypoxia-induced atrioventricular conduction delay, and 2) the adenosine receptor that mediates the negative dromotropic effect of hypoxia is similar to that of exogenous adenosine. (Circulation Research 1986;59:437–446)
gift of Burroughs-Wellcome) were dissolved in perfusion medium and infused to achieve final perfusion concentrations. 8-Phenyltheophylline (8-PT, Sigma) was dissolved in dimethylsulfoxide (DMSO, Sigma) to make stock solutions that were then infused to achieve the desired final perfusate concentration. Final concentration of DMSO was 0.5% (v/v) or less in the perfusion fluid. Concentrations of DMSO of up to 1% (v/v) had no effect on the control AH interval. Adenosine deaminase (type VIII, Sigma) was dialyzed over a Spectropor membrane with a molecular weight cutoff of 6,000–8,000. Adenosine deaminase at 5 U/ml caused a 3 msec or less shortening in control AH interval.

Oxygen Pressure Determination
Perfusate oxygen pressure (Po2) was measured via a Clark-type polarographic electrode (Diamond Electrotek) placed in the perfusate line at a point 5 cm proximal to the aortic cannula. At the end of each experiment a calibration curve was obtained by plotting the electrode output current against oxygen tension (mm Hg) measured in fluid samples of known Po2. A linear plot of electrode response vs. oxygen tension was obtained.

Adenosine Assay
Samples of 4 ml of the effluent were collected in tubes and immediately frozen at −70°C for later analysis of adenosine content. Samples were analyzed as described previously.4 In the experiments involving adenosine deaminase, samples were collected into tubes containing enough ice cold perchloric acid to give a final concentration of 0.2 M. Immediately after collection, samples were centrifuged at 2000g at 4°C for 15 minutes to remove any precipitate. The supernatant was titrated to pH 5.5 with NaOH, filtered using 0.22 μm Millipore filters and assayed for adenosine and inosine.

Data Analysis
All values are reported as mean ± standard error of the mean (SEM). Student's t-distribution for paired data was used for most data analysis and Student's t-distribution with Welch approximation was used for comparison of unpaired data. Linear regression analysis was used to determine lines of best fit.

Schild analysis of the dose–response curves for 8-PST was performed according to the method of Arunlakshana and Schild.5 For the calculation of pA2 (− log Kc, Kc = equilibrium coefficient) using oxygen pressure instead of adenosine concentration, the dose–ratio term of the Schild equation was inverted. The significance of any deviation from unity in the slopes of Schild plots and the 95% confidence intervals of pA2 values were determined according to Tallarida and Jacob.6

Protocol
Hypoxia
Hearts were allowed to equilibrate for 20 minutes with normoxic perfusion solution (Po2 = 608 ± 15 mm Hg) before being subjected to 3 successive periods of hypoxia. Hypoxia was initiated by switching from normoxic to hypoxic solution (Po2 = 46 ± 3 mm Hg) for 4 minutes. After each period of hypoxia, hearts were reoxygenated with normoxic solution for 20 minutes. Each heart was perfused with hypoxic solution for a total of 3 periods. A pilot study of 6 hearts showed that AH interval prolongation as well as adenosine release measured were comparable in 3 successive periods of hypoxia (Figure 1). Typically, the effect of hypoxia alone was evaluated in the first period, the influence of a given pharmacological intervention on the hypoxia effect was evaluated in the second period, and the reversibility of the pharmacologic intervention was assessed in the third hypoxic period. Pharmacologic interventions were initiated 5 minutes prior to initiation of hypoxia and stopped 2 minutes into the reoxygenation period. There was a 54-second transit time in the perfusion system. These series of experiments were divided into 4 groups. In Group A (n = 6), the effects of hypoxia alone were evaluated. In Group B (n = 16), the ability of adenosine receptor blockade with theo-
phylline, 8-PT, and 8-PST to antagonize the hypoxia-induced AH prolongation was determined. In Group C (n = 12), experiments were designed to determine whether deamination of adenosine to inosine by the enzyme adenosine deaminase (5 U/ml) could attenuate hypoxia-induced AH prolongation. In Group D (n = 6), experiments were performed to determine whether enzymatic degradation of adenosine and adenosine receptor blockade were additive as inhibitors of hypoxia-induced AH prolongation.

Hearts in Series

In a total of 6 hearts, the effect of effluent from hypoxic donor hearts on the AV conduction of normoxic recipient hearts was determined. Donor hearts were made hypoxic for a period of 10 minutes and then reoxygenated for 10 minutes. A total of 5 periods of hypoxia were performed per donor heart. Collection of venous effluent was started 2 minutes into the hypoxia period and stopped 2 minutes into the reoxygenation period. The effluent from the first two periods of hypoxia was then treated with 5 U/ml of adenosine deaminase. In the last 3 periods of hypoxia, donor hearts were treated with 5 μM EHNA. All effluent (i.e., either hypoxic or normoxic) from the donor hearts was filtered (Millipore, 0.22 μm) and gassed with 95% O₂, 5% CO₂ prior to infusion into normoxic recipient hearts. In order to prevent uptake and deamination of adenosine, the recipient hearts were treated with 0.05 μM dipyridamole and 5 μM EHNA. To assess the effect of effluent from donor hearts in AV conduction time of recipient hearts, the following protocol was used. Effluent from donor hearts was perfused through recipient hearts until a steady-state AH interval prolongation was achieved at which time 10 μM 8-PST was infused into the recipient hearts. After washout (9–15 minutes) of donor effluent and 8-PST, the adenosine deaminase treated donor effluent was infused into the recipient hearts and its effect observed.

Graded Hypoxia

Graded hypoxia was produced by infusing perfusion solution that was gassed with 5% CO₂ and varying percentages of O₂ and N₂. Oxygen concentrations were 54%, 38%, 25%, and 10%. The general protocol was as follows: First, the heart was perfused for 20 minutes with normoxic perfusion solution. At this point, infusion of hypoxic medium was started and continued for a period of 7–10 minutes, after which the heart was reoxygenated for 15 minutes before infusion of another hypoxic solution. This procedure was repeated 3 to 4 times using perfusate with successively lower O₂ s.

Relation of P₀₂, Adenosine Release, and AH Interval

Graded hypoxia was induced as described above for 10 minutes. At 4, 6, 8, and 10 minutes into the hypoxic period, AH interval prolongation and perfusate oxygen pressure were recorded, and effluent samples were collected for later analysis of adenosine content. A total of 4 hypoxic periods were performed in each heart.

Results

Hypoxia

Group A. In this series of experiments, the reproducibility of hypoxia-induced AH interval prolongation and adenosine release during three successive periods of hypoxia was evaluated. As shown in Figure 1, hypoxia caused significant and comparable increases in effluent adenosine levels as well as prolongations in AH interval in all three successive periods of hypoxia. It should be noted that the AH interval and effluent adenosine values returned to control within 4 minutes of reoxygenation thus indicating the reversibility of the effects of hypoxia. The onset of hypoxia-induced changes in AH interval occurred approximately 2 minutes after switching from normoxic to hypoxic solution (Figure 1). Most of this delay was due to the transit time of the system which was approximately 1.5 minutes. There was a delay of approximately 30 seconds between the onset of AH prolongation and the rise of adenosine concentration in the effluent adenosine during hypoxia. This delay was due to two factors: a) the typical effluent sample was 4 ml in volume, which represents a 30-second collection time, and b) there was a delay of approximately 1 to 3 seconds between appearance of effluent in the venous bed and the point of collection. When these delays are accounted for the increase in concentration of adenosine in the effluent precedes the increase in AH prolongation seen during hypoxia.

Determination of the Potency of 8-PST in Antagonizing the Hypoxia-Induced Prolongation of AH Interval

Graded hypoxia was induced as described above and 8-PST was then used to antagonize its effects. The AH interval prolongation was recorded at 5 minutes into the hypoxic period; at this point 1, 3, and 10 μM 8-PST were infused in that order into the perfusate line. Effluent samples were taken and perfusion fluid oxygen pressures were determined before, during, and after each hypoxic period.

Alkylxanthine Antagonism of Hypoxia and Adenosine-Induced AH Interval Prolongation

In 6 hearts, 8-PST was used to antagonize AH interval prolongations of equal magnitude caused by either hypoxia or adenosine. Initially, the hearts were made hypoxic by infusing Krebs-Henseleit solution equilibrated with 20% O₂. At 5 minutes into the hypoxic period, the HBE was recorded and then 3 μM 8-PST was added to the perfusate. On reaching a steady-state effect of 8-PST, the HBE was recorded again. After a 15-minute washout, the heart was treated with enough adenosine (5–7 μM) to achieve the same magnitude of AH interval prolongation seen during the previous episode of hypoxia. At 5 minutes into the adenosine infusion period, the effect of 3 μM 8-PST was again evaluated. Adenosine and 8-PST were washed out for 15 minutes after which the hypoxia and adenosine protocols were repeated using 10 μM instead of 3 μM 8-PST.
FIGURE 2. Antagonism of hypoxia-induced atria-to-His-bundle (AH) interval prolongation by 8-PST. The average time courses of changes in both AH interval prolongation (Panel A) and effluent adenosine (Ado, Panel B) during hypoxia (N2) and reoxygenation (O2) are shown for the following interventions: control hypoxia, and hypoxia in the presence of 10 μM 8-PST. Interventions were done in the order listed. Effluent adenosine levels are reported as pmol of adenosine appearing in the effluent per minute per gram wet weight heart tissue. The AH interval prolongations for the control and 8-PST hypoxia interventions that were noted between 2.5 and 5 minutes were significantly different from each other, whereas the effluent adenosine values for both the control and 8-PST hypoxia were not significantly different from each other. Values represent mean ± SEM of six hearts.

GROUP B. The alkylxanthines theophylline, 8-PT, and 8-PST all antagonized in a concentration-dependent manner the hypoxia-induced AH prolongation. For example, 0.5 μM 8-PT reduced the AH interval prolongation noted at 4.75 minutes of hypoxia from 47 ± 6 to 27 ± 5 msec (43%); 10 μM 8-PT further reduced this prolongation to 15 ± 3 msec (68%). Similar results were obtained with the polar adenosine antagonist 8-PST, which has been shown by Heller and Olsson not to cross cell membranes. As shown in Figure 2A, at 4.75 minutes, 10 μM 8-PST reduced hypoxia-induced AH interval prolongation from 41 ± 5 to 13 ± 1 msec (68% reduction). 8-PST did not significantly change hypoxia-induced adenosine release (Figure 2B). This result suggests that 8-PST and other alkylxanthines do not mediate their effects via reducing the amount of adenosine released during hypoxia. Finally, theophylline caused a significant reduction in hypoxia-induced AH interval prolongation in hearts that were treated with 2 μM propranolol. AH prolongation observed at 4.75 minutes of hypoxia was reduced by 30 μM theophylline from 56 ± 3 to 18 ± 2 msec (68%).

GROUP C. In another series of experiments it was determined whether adenosine deaminase, via deamination of adenosine to inosine, could attenuate hypoxia-induced AH interval prolongation. As shown in Figure 3, adenosine deaminase significantly attenuated the AH prolongation (Figure 3A) and concomitantly reduced adenosine levels in the effluent (Figure 3B). At 4.25 minutes, adenosine deaminase reduced the effluent levels of adenosine from 2244 ± 218 to 106 ± 43 pmol/min/g (95% reduction). Associated with this marked reduction in adenosine levels, there was an increase in inosine as expected (not shown). Concomitant with the decrease in effluent levels of adenosine was a 61% reduction in the AH prolongation, i.e., from 46 ± 5 to 18 ± 2 msec. Thus, adenosine deaminase caused a greater reduction in adenosine levels (95%) than in AH interval (61%).

GROUP D. Although little or no adenosine was found in the effluent of adenosine deaminase treated hypoxic hearts, there was still some prolongation of the AH interval. To further investigate this finding, adenosine deaminase treatment was combined with adenosine receptor blockade by theophylline. Adenosine deami-
Hearts in Series

Caused a 25-fold increase in release of adenosine from this prolongation by 81% i.e., to 8 ± 2 msec. 43 ± 5 to 18 ± 2 msec (58%), and the combination of Clemo and Belardinelli Effect of Adenosine on AV Conduction 441

Hypoxia also these AV conduction abnormalities, hypoxia also prolonged in normoxic recipient hearts. In donor hypoxic donor hearts caused significant AH interval postponement in normoxic recipients. In donor hearts, five successive periods of hypoxia all caused second degree or greater AV conduction block within the first five minutes of hypoxia. Concomitant with these AV conduction abnormalities, hypoxia also caused a 25-fold increase in release of adenosine from

Table 1. Adenosine and Inosine Released by Normoxic and Hypoxic Guinea Pig Hearts

<table>
<thead>
<tr>
<th>Intervention</th>
<th>[Adenosine] μM</th>
<th>[Inosine] μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent, normoxia</td>
<td>0.02 ± 0.01</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Effluent, hypoxia</td>
<td>0.49 ± 0.05</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>Effluent, hypoxia, ADA</td>
<td>0.05 ± 0.01</td>
<td>1.16 ± 0.04</td>
</tr>
<tr>
<td>Effluent, hypoxia, EHNA</td>
<td>1.41 ± 0.07</td>
<td>0.07 ± 0.02</td>
</tr>
</tbody>
</table>

Values are expressed as the means ± SEM, n = 4; ADA = adenosine deaminase (5 U/ml), EHNA (5 μM).

FIGURE 5. Effect of reoxygenated effluent from hypoxic donor hearts on the AH interval prolongation of normoxic recipient hearts. C-1 = atria-to-His-bundle (AH) interval prolongation induced in untreated normoxic recipients caused by effluent from hypoxic donor hearts; C-2 = that induced by effluent from normoxic donors in normoxic recipient hearts that had been treated with a combination of 0.05 μM dipyridamole (DIP) and 5 μM EHNA. C-3 = that induced by effluent from hypoxic donors in normoxic recipient hearts that had been treated with 0.05 μM DIP plus 5 μM EHNA. The + 8-PST and + ADA bars depict the antagonism of the AH interval prolongation seen in C-3 by 10 μM 8-PST and adenosine deaminase (ADA), respectively. N and O represent effluent that came from either hypoxic or normoxic donor hearts, respectively. The AH interval prolongation in bar C-3 is significantly greater than that of C-1 or C-2. The reduction in AH interval prolongation caused by either 8-PST or adenosine deaminase were significant. The residual prolongation in AH interval in the presence of + ADA was not significantly different from that of either C1 or C2. Values are mean ± SEM of four hearts.
block caused by the effluent from hypoxic donors (Figure 5, + ADA bar).

**Graded Hypoxia**

In the experiments illustrated in Figures 1–4, neither adenosine release nor AH prolongation induced by hypoxia were constant over time. Hence, in order to compare the reversal of either hypoxia- or adenosine-induced AV conduction disturbances by alkylxanthines, a graded hypoxia protocol was employed. In a series of 6 hearts, near steady-state conditions for both hypoxia-induced adenosine release and AH prolongation were achieved during periods of graded hypoxia of up to 8 minutes. The oxygen tension threshold for hypoxia-induced AH interval prolongation and adenosine release was 367 mm Hg. As illustrated in Figure 6, significant linear correlations existed between oxygen tension and both effluent adenosine and AH interval prolongation. Also, as expected, a significant linear correlation existed between effluent adenosine and AH interval prolongation; this is shown in Figure 7 (curve labeled hypoxia). In addition, Figure 7 contains the exogenous adenosine concentration-response curve in the presence of dipyridamole plus EHNA (see also Figure 7 in preceding article). The EC50 for the hypoxia curve was 0.26 ± 0.02 μM which was not significantly different from the EC50 value of 0.28 ± 0.02 μM reported for the exogenous adenosine concentration–response curves in the presence of EHNA plus dipyridamole.

**Alkylxanthine Antagonism of Hypoxia-Induced AH Interval Prolongation**

The ability of the polar adenosine antagonist 8-PST to inhibit hypoxia-induced changes in AH interval in a concentration-dependent manner suggests the central role that adenosine plays in this phenomenon. As shown in Figure 8A, each increase in concentration of 8-PST caused successively larger reductions in AH interval prolongation that was induced by a given degree of hypoxia. Schild analysis of this family of dose–response curves resulted in a pA2 value of 5.30 ± 0.25 and a slope of the Schild plot of −0.98 ± 0.27. In Figure 8B, effluent adenosine was substituted for oxygen tension and plotted against AH interval prolongation. Schild analysis of these plots gave a pA2 value of 5.28 ± 0.31 and a slope of −0.85 ± 0.27. There was no significant difference between the pA2 values determined either using oxygen tension or effluent adenosine.

**Reversal of Similar AH Interval Prolongation Caused Either by Hypoxia or Exogenous Adenosine**

Receptor theory predicts that response is correlated with receptor occupancy. Thus, when a certain fraction of the adenosine receptor population in the AV node is activated by exogenously applied adenosine or released endogenously by hypoxia, a proportional prolongation in AH interval is expected. In either case, the prolongation should then be equally inhibited by an adenosine antagonist. This is demonstrated in Figure 9, where the polar adenosine antagonist 8-PST antagonized to the same extent the AH interval prolongations of similar magnitude caused either by hypoxia (P0.170 ± 4 mm Hg) or exogenous adenosine (5.9 ± 0.5 μM). For example, 3 μM 8-PST reduced the AH prolongation caused by hypoxia by 24% and that induced by exogenous adenosine.
by adenosine by 28%. At a higher concentration, 8-PST (10 μM) reduced the AH prolongation induced by hypoxia and adenosine by 69% and 66%, respectively.

Discussion

The present study contains the strongest evidence shown thus far that adenosine is the primary mediator of hypoxia-induced AV conduction delay in isolated guinea pig hearts. This study documents the role of adenosine in hypoxia-induced AH interval prolongation by demonstrating that a) both adenosine release and AH prolongation have similar time courses, b) hypoxia-induced AV conduction delay is significantly attenuated by either adenosine antagonists or with adenosine deaminase, c) hypoxia produces adenosine in sufficient quantities to account for its effects, d) linear relations exist between oxygen tension, effluent adenosine levels, and AH prolongation, and e) the adenosine receptor mediating the negative dromotropic action of hypoxia is similar to that mediating the negative dromotropic action of exogenous adenosine.

Adenosine Produced During Hypoxia

In this study, hypoxia is shown to cause a significant release of adenosine, which is associated with an increase in the AH interval. Of importance is whether this amount of adenosine produced and released is sufficient to account for the degree of AV conduction delay caused by hypoxia, i.e., is the amount of adeno-
sine formed and released during hypoxia sufficient to account for its effects? The evidence that the amount of adenosine produced by hypoxia can account for its dromotropic effect is supported by the following findings: a) the dose–response curve for exogenous adenosine and adenosine released by hypoxia were superimposable (Figure 7), and b) the effluent from hypoxic donor hearts produced enough adenosine to cause significant AH interval prolongations in normoxic recipient hearts. However, these findings were only observed when uptake and subsequent deamination of adenosine by coronary endothelium, vascular smooth muscle, and cardiac cells were inhibited by dipyridamole and EHNA. Thus, when adenosine uptake and deamination were inhibited in the recipient hearts, the adenosine produced during hypoxia by the donor hearts was sufficient to induce AV conduction disturbances in normoxic recipient hearts similar to those seen in hypoxic donors. EHNA, an adenosine deaminase inhibitor, significantly increased the amount of adenosine in the effluent of hypoxic donor hearts. This indicates that adenosine deaminase may play an important role in regulating the interstitial concentration of adenosine during hypoxia. This finding is in keeping with the ability of EHNA alone to potentiate a) the negative inotropic effect of adenosine on isolated rat left atria and b) the negative dromotropic effect of adenosine and ATP in isolated guinea pig heart.

**Attenuation of Hypoxia-Induced AH Interval Prolongation**

**ALKYLXANTHINES.** In the present study, our findings that theophylline, 8-PST, and 8-PT inhibit hypoxia-induced AH interval prolongation is in agreement with our earlier reports that these alkylxanthines inhibit adenosine-induced AH interval prolongation. The present series of experiments demonstrate that theophylline’s inhibition of hypoxia-induced AH interval prolongation was not influenced by its possible induction of release of catecholamines because propranolol was present in the perfusion fluid. Also, the results with 8-PT demonstrate that hypoxia-induced AV conduction delay is inhibited in a dose-dependent fashion. Since 8-PT is a very poor phosphodiesterase inhibitor, the possibility that the effect of these alkylxanthines is due to elevation of intracellular cyclic AMP via inhibition of phosphodiesterase can be ruled out. Finally, an extracellular adenosine receptor is probably involved in the mediation of hypoxia-induced AV conduction disturbances because 8-PST, a polar and thus non-lipid permeable adenosine antagonist, inhibited hypoxia-induced AH interval prolongation.

**ADENOSINE DEAMINASE.** As shown in the results, while adenosine deaminase deaminated nearly all (e.g., 95%) of the effluent adenosine, it did not attenuate the AH interval prolongation to the same extent. This apparent discrepancy is probably not due to some other substance with negative dromotropic properties being released into the effluent during hypoxia because adenosine deaminase both reduced AH interval prolongation and deaminated effluent adenosine to an equal extent in the donor-recipient experiments. More likely, as recently proposed by Proctor and Duling, this discrepancy could be that endogenous adenosine is protected in some way from deamination by infused adenosine deaminase. However, irrespective of the explanation, adenosine must at least in part be the cause of this residual AH prolongation because the AH interval prolongation was inhibited further by the addition of theophylline.

**Identification of Adenosine Receptor Mediating Effects of Hypoxia**

Similar equilibrium coefficients for an antagonist in different tissues or different experimental conditions suggest that the receptors being occupied by the antagonist is the same. This has been demonstrated by Birnbaumer et al, who found similar Ks for propranolol in heart tissue binding studies and in inhibition of both the positive chronotropic property of isoproterenol as well as the isoproterenol-stimulated cardiac adenylate cyclase. In the present study, Schild analysis of antagonism of the negative dromotropic effects of either adenosine or hypoxia by 8-PST allows quantitative comparison of the receptors mediating each phenomenon. Using Schild analysis, a pA2 of 5.30 ± 0.25 was determined for the antagonism by 8-PST of hypoxia-induced AV conduction delay. We reported a pA2 of 5.55 ± 0.09 for 8-PST’s antagonism of adenosine-induced AH prolongation in guinea pig hearts, which is not significantly different from that reported in this study for 8-PST antagonism of hypoxia-induced AV conduction delay. In order to demonstrate differences in receptors, a difference of three-fold or greater should exist between Ks for each receptor. Given that only a 1.8-fold difference exists, it is likely that the receptors in both cases have similar characteristics.

**Effluent vs. Interstitial Adenosine Concentrations**

We have previously shown that unless isolated hearts are treated with a nucleoside transport blocker plus an adenosine deaminase inhibitor, the adenosine recovered in the effluent (during dose–response experiments) is far less than the amount of adenosine infused. The recent demonstration by Sparks et al that the in situ coronary endothelium can rapidly and significantly remove adenosine indicates that effluent adenosine may not be a true index of interstitial fluid adenosine. Thus, adenosine dose–response curves from intact hearts may underestimate the true sensitivity of cardiac cells to adenosine. Therefore, in order to circumvent this potential problem in the pA2 experiments of the present study, oxygen tension instead of effluent adenosine was used. Our study and others have shown that oxygen tension is linearly related in a negative manner to effluent adenosine. When oxygen tension is substituted for effluent adenosine in the Schild analysis of 8-PST antagonism of hypoxia-induced AV conduction delay, conclusions can be drawn that are independent of any assumptions concerning adenosine metabolism. The similarity of pA2’s for oxygen tension and effluent adenosine demonstrates...
that effluent adenosine concentration under the conditions of the present experiments is proportional to interstitial adenosine concentration. This conclusion is further supported by the fact that the EC50 for the “dose–response” curve of adenosine released by hypoxia was comparable to that of the exogenous adenosine dose–response curve when metabolism of adenosine had been inhibited.

The finding that 8-PST was equally effective in antagonizing the AH interval prolongation caused either by adenosine or hypoxia demonstrates that the receptors mediating these phenomena are likely to be the same. Furthermore, it indicates that adenosine is the primary, if not the only, mediator of hypoxia-induced AH interval prolongation under the present experimental conditions. If other mechanisms were involved in hypoxia-induced AV conduction delay, 8-PST would have inhibited the AH interval prolongation to a lesser extent than it inhibited the adenosine-induced AH interval prolongation. Thus, since the antagonism by 8-PST in both cases was equal, the involvement of other mechanisms in the negative dromotropic effect of hypoxia is unlikely.

Potential Clinical Implications

Provided the findings of this study can be extrapolated to the clinical setting, it could be speculated that interventions known to modify the negative dromotropic action of adenosine would also modulate the conduction disturbances during ischemia of the AV node region. For example, any agent with anti-adenosine properties (i.e., receptor blockade or enzymatic removal) may prove efficacious in ameliorating the AV conduction disturbances accompanying acute inferior myocardial infarction. However, it should be noted that in the present study hypoxia and not ischemia was studied and hence direct extrapolation from one to another cannot be made. Nevertheless, since adenosine has been shown to produce similar dromotropic effects in the human heart, the laboratory observations of the present study may assume greater clinical relevance than previously recognized.

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