Effect of Adenosine on Atrioventricular Conduction. I: Site and Characterization of Adenosine Action in the Guinea Pig Atrioventricular Node

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Adenosine has a negative dromotropic effect and modulates hypoxia-induced atrioventricular (AV) conduction delay. To further characterize the negative dromotropic effect of adenosine in the guinea pig heart, we determined a) the site of adenosine-induced AV conduction block; b) the effect of uptake and deamination of adenosine on its concentration-negative dromotropic effect, and c) the adenosine receptor that mediates this action. In isolated AV node preparations (n = 16), adenosine in a dose-dependent manner decreased significantly the duration and amplitude of the action potential of atrial and nodal cells and, in addition, markedly depressed the maximum rate of rise of the action potential of nodal cells. At high concentrations (> 20 μM), adenosine rendered nodal cells inexcitable. In isolated perfused hearts (n = 7), adenosine (5.7 μM) prolonged total AV conduction time by 21 ± 2 msec. Of this prolongation, 83% was due to an increase in the nodal-to-His-bundle interval and the remaining 17% to an increase in the atrial-to-nodal interval. Infusion of adenosine to cause a 50% increase (EC50) in atria-to-His bundle (AH) interval prolongation resulted in a perfusate (arterial) adenosine concentration of 5.0 ± 0.6 μM and effluent (venous) adenosine concentrations of 2.8 ± 0.4 μM, i.e., an arteriovenous difference of 44% (n = 4). When adenosine uptake and deamination were inhibited with dipyridamole (0.5 μM) plus erythro-9-(2-hydroxy-3-nonyl)adenine (5 μM), respectively, the EC50s were 0.28 ± 0.02 (perfusate) and 0.32 ± 0.03 μM (effluent). These data indicate that when nucleoside metabolism is inhibited, arterial and venous concentrations of adenosine reach equilibrium. In an additional 10 hearts, the following rank order of potency of adenosine agonists in causing AH interval prolongation was found: N'-cyclopentyladenosine > N'-(L-2-phenyl-isopropyl)adenosine > 5'-N-ethylcarboxamidoadenosine > 2-chloroadenosine > adenosine, which is compatible with activation of an A1-type receptor. In summary: 1) the site of adenosine-induced AV conduction block is the nodal zone of the AV node, 2) when adenosine uptake and deamination are inhibited, adenosine in concentrations similar to that released by hypoxia causes significant AV interval prolongation, and 3) the adenosine receptor mediating the negative dromotropic effect of adenosine is of the A1-type. (Circulation Research; 1986;59:427-436)

Although the above studies have established that adenosine has a negative dromotropic effect, several important questions concerning this action of the nucleoside remain unanswered. For example, a specific receptor that mediates the negative dromotropic effect of adenosine has not been demonstrated. Based on electrophysiological and morphological studies, the AV node has been shown to consist of three different groups of cells, which are the atrionodal (AN), nodal (N), and nodal–His bundle (NH) cells.9,10 Until now, it has not been shown what effect adenosine has on these cells and whether the ability of this nucleoside to induce AV block is related to its action on these cells. Second, although the amount of adenosine released (measured in the effluent) during hypoxia is in the submicromolar range,11-13 the amount of exogenous adenosine needed to induce AV conduction delays of similar degree to those seen during hypoxia appears to be an order of greater magnitude.14 This difference could be due to uptake and subsequent deamination of adenosine by endothelial and cardiac cells, but such a possibility has yet to be examined. Furthermore, while it is apparent that the negative dromotropic effect of adenosine is receptor mediated, the characterization of this receptor has not been carried out.
The present study, therefore, was designed to address some of the above questions. Specifically, the following are the objectives of the study: 1) to demonstrate a site of adenosine-induced AV block in the AV node; 2) to examine the effects of adenosine uptake and deamination on the negative dromotropic action of adenosine; and 3) to characterize the receptor that mediates the negative dromotropic effect of adenosine. In addition, in the accompanying study, we sought to quantify the role of adenosine as mediator of AV conduction delay seen during hypoxia.

**Materials and Methods**

**Isolated Perfused Heart Preparation**

Guinea pig hearts were isolated and perfused with a modified Krebs-Henseleit solution gassed with 95% O₂ and 5% CO₂ as previously described. The sinoatrial node and part of the right atrium were excised to facilitate electrical pacing of the hearts and to expose the AV node so that recording electrodes could be placed in the bundle of His region (see below). Hearts were stimulated at a rate of 3 Hz via bipolar electrodes placed on either the left atrium or interventricular septum for evaluation of antegrade (A to V) and retrograde (V to A) conduction, respectively.

After instrumentation, hearts were allowed to equilibrate for 20–30 minutes before starting the experiments. All experimental interventions were preceded and followed by control measurements. In some hearts in which multiple experimental interventions were carried out, the order of the interventions was varied so that the effects of the order could be evaluated. The atria-to-His-bundle (AH) interval prolongation caused by any given intervention was determined by subtracting the average of pre- and postintervention AH interval from the AH interval obtained during the intervention. If pre- and postintervention times varied by more than 15%, the data were discarded. Based on this criterion, approximately 5% of the experiments were discarded. All baseline electrophysiological parameters (i.e., action potential characteristics and conduction times) remained stable for up to 3 hours.

**Isolated AV Node Preparation**

Guinea pig hearts were rapidly removed as described above, rinsed, and immediately placed in a dissecting chamber superfused with oxygenated Krebs-Henseleit solution. The right atrium (RA) with both superior and inferior venae cavae (SVC and IVC, respectively), the interatrial septum (IAS) and upper part of the interventricular septum (IVS), septal cusp of the tricuspid valve (TV) with part of the base of the right ventricle were dissected out. The preparation, which contained the AV node, was transferred to a tissue bath and superfused at 10 ml/min with Krebs-Henseleit solution. The temperature of the superfusion fluid was 35°C. Figure 1 illustrates one such preparation that includes most of RA, the ostium of the coronary sinus (CS), IVC, IAS, His bundle (HB), and part of the right ventricle. The preparation was stimulated at 3 Hz via a bipolar electrode placed in the region of the crista terminalis. After instrumentation, the preparations were allowed to stabilize for 20 minutes before experiments were commenced. Action potential parameters and AV node conduction times remained stable for up to 1 hour.

**Chemicals**

Adenosine, DL-propranolol HCl (Sigma); dipyridamole (gift of Boehringer Ingelheim); and EHNA (gift of Burroughs-Wellcome) were dissolved in perfusion medium and infused to achieve final perfusion concentrations. N⁶-cyclopentyladenosine (CPA), 2-chloroadenosine (2-CADO), 5'-N-ethylcarboxamidoadenosine (NECA) (Research Biochemicals); and N⁶-(L-2-phenylisopropyl)adenosine (l-PIA, Boehringer Mannheim) were dissolved in dimethylsulfoxide (DMSO,

**Figure 1. Effect of adenosine (15 μM) on the action potentials of atrial (A), atrionodal (AN), nodal (N), and nodal-His bundle (NH) cells.** The photograph in the center of the figure is from a guinea pig isolated AV node preparation and shows the approximate sites from which the action potentials were recorded. RA = right atrium, CS = ostium of coronary sinus, IV = inferior vena cava, IAS = interatrial septum, HB = His bundle, TV = septal cusp of tricuspid valve. Action potentials labelled (1) are controls and (2) are in the presence of adenosine. Action potential tracings have been retouched.
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Sigma) to make stock solutions which were then infused to achieve the desired final perfusate concentrate. Final concentration of DMSO was 0.5% v/v or less in the perfusion fluid. DMSO in concentrations of up to 1% v/v had no effect on any of the parameters measured.

Electrophysiological Techniques

In all isolated perfused hearts, unipolar extracellular electrodes were placed in the region of the AV node in order to record His bundle electrogram (HBE). In some hearts, another electrode was placed on the left atrium to obtain a left atrial electrogram (LAE). Transmembrane potentials were recorded by the hanging microelectrode technique, using the tips of glass capillary micropipettes filled with 3 M KCl. These electrodes had tip resistances of 20–30 MΩ. Ag–AgCl wires served as reversible half-cells. The hanging microelectrode technique allowed for stable intracellular recordings of up to 15 minutes from cells in the AV node region of vigorously contracting isolated perfused hearts. Intracellular recordings were obtained from right atrium (RA), atrionodal (AN), and nodal (N) cells so that interatrial (LA–RA), right atrial to atrionodal (RA–AN), and atrionodal to nodal (AN–N) conduction times could be measured. In addition, nodal-to-His-bundle (N–H) and His-bundle-to-ventricle (H–V) conduction times could also be determined.

In isolated AV node preparations, transmembrane potentials of atrial (A), AN, N, and NH cells were recorded with the hanging microelectrode technique as described above. Since the isolated AV node contracts less forcefully than the isolated perfused hearts, stable impalements with hanging microelectrodes could often be maintained for periods over 30 minutes. In a few experiments, two simultaneous stable impalements could be successfully obtained and hence action potentials from two different cells (e.g., A and N, Figure 3) could be recorded simultaneously. In all isolated AV node preparations, electrograms were continuously recorded with unipolar glass-insulated tungsten electrodes (resistance of 9 to 12 MΩ) placed in the right atrium, in the region of the coronary sinus and interatrial septum.

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. All samples were assayed within one week of collection. Samples were assayed for adenosine using reverse phase high-performance liquid chromatography (HPLC) in the isocratic mode according to the method employed by Hartwick et al.

Data Analysis

All values are reported as mean ± standard error of the mean (SEM). The Student’s t-distribution was used for paired data analysis. The Student’s t-distribution with Welch correction was used for comparison of unpaired data. Dunnett’s test was used to determine the concentration dependence of adenosine’s negative dromotropic effect.

Protocols

Experiments to determine the site of AV block induced by adenosine in the AV node were carried out in both isolated perfused hearts and isolated AV node preparations.

1. ISOLATED PERFUSED HEARTS. In this series of experiments (n = 7), hearts were instrumented for both intracellular and extracellular recordings. After the recording of control action potentials as well as atrial and His bundle electrograms, either 5 or 8 μM adenosine was infused. When a steady state effect of adenosine was achieved, action potentials were again recorded. In two preparations, the effects of adenosine on the action potentials and AV conduction times were examined during both antegrade and retrograde conduction.

2. ISOLATED AV NODE PREPARATION. In this series of experiments (n = 6), A, AN, N, and NH cells were first identified according to their electrophysiological properties as well as their locations (based on anatomical landmarks) in the preparation. Although this subdivision of the AV node into AN, N, and NH zones is somewhat arbitrary, it is useful for defining the various conduction times that constitute the total AV conduction time. In every preparation, after control action potentials were obtained, adenosine (8 or 20 μM) was added to the superfusion medium. When maximal effect of adenosine was reached, recordings were again obtained. This procedure was repeated for A, AN, N, and NH cells in each preparation, and often each cell was exposed twice to adenosine. In some preparations, as illustrated in Figure 2, simultaneous action potentials were recorded for AN and N cells. In other experiments, in addition to the intracellular recordings, electrograms from different sites of the preparation (e.g., right atrium, IAS) were continuously recorded throughout the experiment.

Effect of Uptake and Deamination of Adenosine on its Negative Dromotropic Effect

In a series of 4 hearts, 0.5 μM dipyridamole, and 5 μM EHNA were used to determine the effects of uptake and deamination of adenosine on its concentration–response relationship. First, a control adenosine concentration–response curve was determined by infusion of increasingly higher concentrations of the nucleoside and recording the resultant prolongation of the AH interval. The AH interval measurements in the steady-state conditions were made during control and adenosine infusions. During control conditions and at each dose of adenosine, an effluent sample was obtained for determination of adenosine content. After a 10-minute washout period, the hearts were pretreated with dipyridamole and EHNA for 5 minutes. With these substances still present, another adenosine concentration–response curve was then determined in the same manner.
Characterization of the Adenosine Receptor

In this series of experiments (n = 10), the adenosine agonists CPA, 2-CADO, NECA and L-PIA were used to characterize the adenosine receptor that mediates its dromotropic action. In each heart, an adenosine dose–response curve was first obtained, followed by dose–response curves of the various agonists. Washout periods of 15 minutes separated each intervention. Some hearts were pretreated with 5 μM EHNA and 0.05 μM dipyridamole after which the above protocol was repeated. To ensure that catecholamines did not influence AV conduction and the effects of adenosine or its analogs, 1 μM propranolol was present in the perfusion fluid. Propranolol was found to have no effect on the negative dromotropic action of adenosine or in any of its analogs.

Results

Site of AV Block Induced by Adenosine

A. ISOLATED AV NODE PREPARATION. As demonstrated by Figure 1 and Table 1, the effect of adenosine on action potentials from A, AN, N, and NH cells varied according to cell type. For example, adenosine caused an overall depression of the excitability of the N cell by significantly reducing amplitude, duration, and rate of rise of phase 0 \( V_m \). In A and AN cells, adenosine shortened the duration of the action potential and, finally, failed to excite, resulting in AV block. Upon washout, the N cell excitability and the duration and amplitude of A cell action potential were restored.

<table>
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<tr>
<th>Table 1. Effect of Adenosine on Action Potential Parameters</th>
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<tr>
<td><strong>Atrial (A)</strong></td>
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<td><strong>Control</strong></td>
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<td>Amplitude ( \text{mV} )</td>
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<td>Resting Potential ( \text{mV} )</td>
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<td>Maximum Rate of Rise ( V_m )</td>
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<td>Action Potential Duration at 90% Repolarization ( \text{msec} )</td>
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<td>Action Potential Duration at 50% Repolarization ( \text{msec} )</td>
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\*Values are mean ± SEM for 16 preparations. Amplitude = amplitude of action potential in \( \text{mV} \); Resting Potential = resting potential in \( \text{mV} \); Maximum Rate of Rise = maximum rate of rise of action potential in volts per second; Action Potential Duration at 50% Repolarization = action potential duration at 50% and 90% of repolarization in \( \text{msec} \), respectively.

†Significantly \( p < 0.05 \) different from control.

**Figure 2. Effect of adenosine on the action potential of an atrial (A) and nodal (N) cell during antegrade conduction.** Action potentials are control, in the presence of adenosine, and after its washout. Note that in the A cell adenosine caused only a reduction in action potential duration and a small decrease in its amplitude whereas in the N cell adenosine progressively increased the delay for its activation, caused an overall depression of the action potential and, finally, failed to excite, resulting in AV block. Upon washout, the N cell excitability and the duration and amplitude of A cell action potential were restored.
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B. Adenosine (15 μM)

FIGURE 3. Effect of adenosine on intratral conduction time and on the action potential of a nodal (N) cell. Shown in the upper right is a schematic of the preparation and the locations of extracellular electrodes (A1-A3) and site from which the nodal (N) action potential was recorded. 1 = crista terminalis, 2 = inferior vena cava, 3 = coronary sinus, 4 = AV node, 5 = His bundle, 6 = tricuspid valve. Panel A: intraatrial electrogram and action potential from an N cell recorded during the control period; Panel B: recordings made during adenosine infusion; Panels C and D: recordings made after 1 and 3 minutes of washout of adenosine, respectively. Inset shown on lower right depicts the events of Panel B at 10 × faster sweep speed. Note that adenosine caused no change in the excitation timing of the 3 atrial sites from which electrograms were recorded but it markedly depressed the action potential of the N cell. Calibrations in Panel D apply to Panels A–C.

were recorded. On washout of adenosine, the N cell action potential parameters returned to baseline.

B. ISOLATED PERFUSED HEART. In isolated perfused hearts, adenosine induced delays in AV conduction time (Figures 4–6). The site of action of adenosine was determined by analysis of His bundle electrograms and intracellular recordings from A, AN, and N cells. As shown in Figure 4, adenosine caused a pronounced reduction in the AN cell action potential duration which was associated with a prolongation of the AH interval (Figure 4, panels B and C). Still in the presence of adenosine, a small increase in the rate of atrial pacing resulted in 2:1 AV conduction block (Figure 4, panel D). Note that during 2:1 AV block, impulse propagation to the AN cell was maintained at 1:1, thus demonstrating that the site of AV block was distal to the AN cell. The conduction time from atrial to AN cell was not affected by adenosine. Washout of adenosine was accompanied by prompt resumption of 1:1 AV conduction and recovery of the AN cell action potential (Figure 4, Panel E).

Adenosine causes greater changes in the action potential of N than A or AN cells which are accompanied by either marked prolongation of the AH interval or second or higher degree of AV block, depending on the concentration of the nucleoside and rate of pacing. In the example shown in Figure 5, adenosine caused an initial prolongation of the AH interval (Panel B) followed by AV block (Panel C). Concomitant with this increase in AV conduction time and block, adenosine markedly depressed and then abolished the N cell action potential, respectively (Figure 5, Panels B and C). Thus, in contrast to AN cells (Figure 4, Panel D), second-degree AV block was associated with abolition of the N cell action potential during the blocked beats. After washout of adenosine, the AV conduction times as well as the N cell action potential returned to baseline parameters. Not shown, during retrograde conduction, the site of VA conduction block caused by adenosine was also the N zone. That is, during infusion of adenosine there was no increase in conduction time between V and H (12–14 msec) but H to AN or A markedly prolonged. Whenever VA block occurred, the N cells' action potentials were abolished whereas the V-to-H interval remained unaltered and the atria could no longer be captured by the ventricular pacing, i.e., there was complete VA dissociation.

Figure 6 summarizes the effect of 5.7 ± 0.3 μM...
FIGURE 4. Negative dromotropic effect of adenosine in the isolated perfused guinea pig heart. Shown are recordings of His bundle electrogram (HBE), left atrial electrogram (LAE) and action potential of an atrionodal cell (AN-AP). Panel A is control, panels B, C, and D are records obtained at various times during infusion of adenosine, and Panel E after washout of adenosine. S = stimulus artifact, A, H, V = atrial, His bundle, and ventricular depolarizations, respectively. As shown in Panels B and C, in the presence of adenosine, the action potential duration and amplitude of the AN cell were reduced and the AH interval prolonged. Still in the presence of adenosine (Panel D), a 15-msec decrease in cycle length (from 330 msec, Panels A, B, C, and E to 315 msec, Panel D) resulted in conduction block that was distal to the AN cell. Calibrations in Panel E apply to all other panels.

FIGURE 5. Adenosine-induced prolongation in AH interval, AV block, and depression of the action potential recorded from a nodal (N) cell of an isolated perfused guinea pig heart. Depicted are His bundle electrogram (HBE) and N cell action potential (N-AP) records obtained during control (Panel A); Panels B, C, and D were obtained at 10, 15, and 50 seconds, respectively, after application of a 10-second pressure pulse of adenosine (1 mM) delivered via a catheter positioned in the aortic root. Note that as the N cell action potential was depressed (Panel B) the AH interval prolonged and AV block ensued when the N cell action potential was abolished (Panel C). During washout both the N cell action potential and AH interval returned to control. A, H, and V = atrial, His bundle, and ventricular depolarizations, respectively. Calibrations in Panel D apply to all other panels.
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Influence of Metabolism of Adenosine Dose–Response Curves

In order to determine the influence of adenosine uptake and deamination on its negative dromotropic action, the effect of dipyridamole and EHNA was investigated. As shown in Figure 7, adenosine uptake and deamination removed much of the exogenously applied adenosine, i.e., the concentration of adenosine present in the effluent was significantly less than that present in the perfusate. The concentration of adenosine needed to prolong the AH interval by 50% of maximal response (EC50), as determined from the perfusate adenosine concentration–response curve, was 5.0 ± 0.6 μM, whereas the EC50 calculated from the adenosine levels measured in the effluent was significantly less, i.e., 2.8 ± 0.4 μM. However, when 0.5 μM dipyridamole and 5.0 μM EHNA were present, the amount of adenosine recovered in the effluent was the same as its concentration in the perfusate. The EC50s for the perfusate and effluent concentration–response curves when uptake and deamination were inhibited were 0.28 ± 0.02 μM and 0.32 ± 0.03 μM, respectively. These values were not significantly different from each other, but were significantly different from the EC50 values reported for "perfusion" and "effluent" concentration–response curves when uptake and deamination had not been taken into account. Thus, when adenosine uptake and deamination were inhibited, the adenosine concentration–response relation was shifted by an order of magnitude to the left.

Identification of the Adenosine Receptor

The objective of this series of experiments was to classify, by using various adenosine analogs, the receptor that mediates the negative dromotropic action of adenosine. Figure 8 and Table 2 summarize the potency of adenosine and its various analogs to prolong the AH interval. As shown in Figure 8, like adenosine, its analogs caused AV conduction delays in a dose-dependent manner. The rank order of potency of these agonists to increase AV conduction time was: CPA > L-PIA > NECA > 2-CADO > adenosine. The dose–response curves of the analogs are parallel to but shifted to the left of the adenosine concentration–response curve. In addition, as shown in Table 2, uptake and deamination of adenosine did not influence the negative dromotropic effect of the adenosine analogs. This is demonstrated by the lack of significant difference between EC50 values calculated from concentration–response curves obtained either in the absence or presence of dipyridamole plus EHNA (Table 2). In contrast, as expected, dipyridamole plus EHNA did reduce the adenosine EC50 by an order of magni-

Figure 6. Summary of the effect of adenosine on atrioventricular (AV) conduction in isolated perfused guinea pig hearts. In each bar, the total AV conduction time has been divided into various subintervals, i.e., stimulus to left atrial (S–LA), left atrial to right atrial (LA–RA), right atrial to atrionodal (RA–AN), atrionodal to nodal (AN–N), nodal to His bundle (N–H), and His bundle to ventricular (H–V) intervals. Measurements of the intervals were made during control, in the presence of adenosine (Ado, 5.7 ± 0.3 μM), and after its washout. Note that the prolongation of the N to H interval accounted for 83% of the total increase in AV conduction time. Data are mean ± SEM of 7 hearts.

Figure 7. Concentration–response relations for the negative dromotropic effect of adenosine (% response = % of maximal AH interval prolongation) in the absence and presence of dipyridamole (Dip, 0.5 μM) plus EHNA (5 μM). During control and dipyridamole plus EHNA (Dip + EHNA) interventions, adenosine concentrations in both the perfusate and effluent were measured. Each concentration–response curve was plotted using concentration of adenosine in the perfusate (closed symbols) and in the effluent (open symbols) versus % response. In control, the effluent adenosine curve is to the left of the perfusate curve, whereas in the presence of dipyridamole plus EHNA, the effluent and perfusate adenosine curves are superimposable and both fall to the left of the control curves. Each data point is mean ± SEM of 4 preparations.
Figure 7. This finding is consistent with data presented in Figure 8.

Discussion

This study is the first to present direct and quantitative evidence that a) the site of AV conduction block induced by adenosine is the AV node, specifically the N zone of the AV node, b) uptake and deamination of adenosine significantly influences the concentration-response curve of its negative dromotropic effect, and c) the receptor mediating this action of the nucleoside adenosine conforms with the currently established characteristics of the A1 adenosine receptor.

Site of Action of Adenosine

Based on morphological markers and electrophysiological parameters, three distinct groups of cells (AN, N, and NH) have been recognized in the AV node region of rabbit hearts. In the present study in guinea pig hearts, atrial, AN, N, and NH cells were identified according to their electrophysiological characteristics (i.e., action potential parameters and excitation timing), which were comparable to those reported for the rabbit heart.10,17-21

Although it has been previously postulated that the AV node is the probable site of adenosine-induced AV block,2 a specific site(s) within the AV node has not been identified. In this study, the N zone of the AV node in particular is identified as the site of adenosine-induced AV block. This identification is based on the following findings: 1) in the isolated perfused hearts, the increase in N to H conduction time accounted for 83% of the total prolongation of AV conduction time caused by adenosine, 2) in both isolated perfused hearts and AV nodal preparations, concentrations of adenosine that cause AV conduction delay and block markedly depressed or abolished the action potentials in N cells whereas it only shortened the action potential but did not affect \( V_{\text{max}} \) of the other cell types in the AV node region, 3) in cases where adenosine caused AV or VA conduction block, the site of block was distal to the AN cells, whereas during retrograde conduction, the site of block was distal to the NH cells. The ability of an electrical impulse to propagate through the AV node is dependent on both excitability and the amplitude and maximal rate of rise \( (+V_{\text{max}}) \) of the upstroke of the action potential of the underlying tissue.22,23 It is expected, then, that reduction of the amplitude and \( V_{\text{max}} \) of the nodal action potential by adenosine will cause AV conduction delay or block. The marked reduction of the AN action potential duration by adenosine could have also contributed to the loss of excitability of the N cell and hence AV conduction delay and block. In addition, the prolongation of the AN to N conduction time could as well be due in part to the reduction of the AN cell action potential duration. A study by Masuda et al24 suggested that the duration and plateau of a normal fast action potential (e.g., atrial) may determine whether or not an impulse will propagate into a region where the underlying action potentials are "slow responses" such as the N zone of the AV node. However, it is unlikely that the reduction of the AN cell action potential duration observed in the present study can fully account for the AV block caused by adenosine since during retrograde conduction adenosine-induced VA block occurs without any demonstrable effect on the action potential of the NH cells.

Hypoxia and ischemia impair AV conduction.1,25-28 For example, Sengers et al29 have shown that excitability of N cells of the rabbit AV node are depressed during hypoxia, and this is accompanied by prolongation of the AH interval. Similarly, as shown in this study, adenosine causes an overall decrease in the duration and amplitude of N cell action potentials and concomitantly prolongs the AH interval or causes second degree AV block. Thus, since adenosine has been implicated as an important modulator of hypoxia-induced AV conduction delay and block,29 it is possible that adenosine produced and released during hypoxia could account for the depression of excitability of the N cells and associated AV conduction disturbances.

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<tr>
<th>Table 2. Concentrations of Various Adenosine Agonists to Cause 50% (EC50) Increase in AV Conduction Delay</th>
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<tbody>
<tr>
<td>Agonist</td>
</tr>
<tr>
<td>Ado</td>
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<tr>
<td>2-CADO</td>
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<td>NECA</td>
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<td>L-PIA</td>
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<td>CPA</td>
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Values are mean ± SEM for n as indicated in parentheses. Ado = adenosine; DIP = dipyridamole, other abbreviations as in text. no DIP/EHNA indicates that no dipyridamole or EHNA was present in the perfusion fluid. DIP/EHNA indicates a combination of 0.5 μM dipyridamole and 5 μM EHNA, which was present in the perfusion fluid.
Effect of Adenosine Uptake and Deamination on Its Negative Dromotropic Action

It has been shown that uptake and deamination of extracellular adenosine by endothelial cells and cardiac myocytes plays an important role in the regulation of interstitial adenosine concentration. In the present study, dipyridamole and EHNA, when used in combination, shifted the adenosine concentration-response curve by an order of magnitude to the left, thus potentiating the effect of exogenously applied adenosine. Furthermore, in the presence of dipyridamole and EHNA the perfusate and effluent concentration of adenosine were the same, which suggests that adenosine metabolism was completely inhibited. Since the hearts were perfused at a constant flow, an increase in coronary flow induced by dipyridamole or EHNA is unlikely to be the explanation for similar perfusate and effluent adenosine concentrations. Rather, this finding suggests that the concentration of adenosine in the vascular space and interstitium reached equilibrium. Thus, under these conditions, effluent levels of adenosine may probably reflect the actual interstitial concentration of the nucleoside. This latter finding is consistent with recent studies in isolated perfused guinea pig hearts and in situ dog kidney using multiple-indicator-diffusion techniques that showed that a) uptake of adenosine by endothelial cells is significant, and hence interstitial and intravascular levels of adenosine can be regulated independently, and b) during blockade of adenosine uptake with 10 μM dipyridamole, the outflow dilution curves for adenosine and its analog [3H]D-arabinofuranosyl hypoxanthine, which is not transported by the nucleoside carrier, were superimposable.

Adenosine Receptor

Two distinct subtypes of extracellular adenosine receptors that can be differentiated both functionally and pharmacologically are believed to mediate the actions of adenosine. In functional terms, the A1 (R1) receptor mediates inhibition of adenylyl cyclase and, hence, lowers cellular cyclic AMP (cAMP) whereas the A2 (R2) receptor has the opposite effect. In rat adipocyte membranes, Londo et al. determined that L-PIA > adenosine > NECA in the ability to inhibit adenylyl cyclase whereas in rat liver and Leydig cell tumor membranes the order of potency of these analogs to inhibit adenylyl cyclase is reversed, i.e., NECA > adenosine ≥ L-PIA. Also, Bruns et al. reported the following order of potency of various adenosine analogs to displace [3H]NECA binding from A1 receptors in rat neural membranes: CPA > L-PIA > NECA ≥ 2-CADO. In the present study, the rank order of potency for the negative dromotropic effect of adenosine and its analogs was the same as the one reported by Bruns et al. and, hence, suggests activation of an A1-type receptor. Similarly, the negative chronotropic and inotropic effect of adenosine in mammalian atria has been attributed to activation of A1 receptors. The negative chronotropic, inotropic (in atrial tissue), and dromotropic effects of adenosine have been termed "direct" effects of adenosine while the ability of this nucleoside to inhibit the stimulatory action of catecholamines on cardiac tissue has been termed the "indirect" or "antiadrenergic" effect of adenosine. Of note is that while the original classification of the A1 adenosine receptor 27 was based on the ability of adenosine and its analogs to inhibit adenylyl cyclase activity, the direct effects of adenosine have not been linked thus far to this inhibitory action. In contrast, there is reasonable evidence that the indirect effect of adenosine in various tissues, including ventricular myocardium, is modulated by inhibition of adenylyl cyclase. The ionic basis of the antiadrenergic effect of adenosine has been attributed to its ability to antagonize the catecholamine-induced increase in calcium inward current. On the other hand, in atrial myocytes, the direct action of adenosine (e.g., shortening and hyperpolarization of the action potential) is associated with a large increase in outward potassium current and efflux of K. Although experiments in the present study were not designed to determine whether the negative dromotropic action of adenosine and its analogs is associated with inhibition of adenylyl cyclase, stimulation of this enzyme is not a prerequisite for the negative dromotropic effects of adenosine. This is supported by the finding of similar concentration-response curves for the negative dromotropic effect of adenosine in the absence and presence of beta receptor blockade. Although it is possible that the mechanism of action in atrial cells, the negative dromotropic effect of adenosine could also be due to an increase in outward potassium current, it remains to be elucidated.

Acknowledgment

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