Species-Dependent Effects of Adenosine on Heart Rate and Atrioventricular Nodal Conduction

Mechanism and Physiological Implications

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This study 1) compares the negative chronotropic and dromotropic actions of adenosine in guinea pig, rat, and rabbit hearts; 2) investigates the mechanism(s) for the different responses; and 3) determines the physiological implications. Isolated perfused hearts were instrumented for measurement of atrial rate and atroventricular (AV) nodal conduction time. Differences in metabolism of adenosine were determined in the absence and presence of dipyridamole (nucleoside uptake blocker) and erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, adenosine deaminase inhibitor). Dipyridamole plus EHNA decreased adenosine's EC50 for the negative dromotropic effect by 14-fold in guinea pig heart and 1.6-fold in rat heart. This is consistent with the greater number of [3H]nitrobenzylthioinosine binding sites measured in membranes from guinea pig (1,231±68 fmol/mg protein) compared with rat (302±31 fmol/mg protein) and rabbit (260±28 fmol/mg protein) atria. The potency of adenosine to slow atrial rate and prolong AV nodal conduction time was greater in guinea pig than in rat or rabbit hearts. This rank order of potency correlated well with the number of binding sites for the specific adenosine receptor radioligand 125I-aminobenzyladenosine in guinea pig (102±13 fmol/mg protein), rat (11±0.5 fmol/mg protein), and rabbit (8±1 fmol/mg protein) atrial membranes. Hypoxia increased the rate of adenosine release by severalfold and caused slowing of heart rate and AV block. In spontaneously beating hearts, the main effect of hypoxia was a slowing of ventricular rate, which in the guinea pig heart was due to AV block and in the rat heart to atrial slowing. In atrial paced hearts, hypoxia caused a marked prolongation of AV nodal conduction time in guinea pig (39±4 msec) and rabbit (29±5 msec) hearts, but only small effect in rat hearts (10±2 msec). The differences in response to hypoxia could be accounted for by the species-dependent differences in the 1) amount of adenosine released and metabolized, 2) sensitivity of the hearts to adenosine, and 3) dependency of AV nodal conduction on atrial rate. The findings indicate that the results from physiological or pharmacological studies on adenosine in one species may not be applicable to others, and the ultimate effect of adenosine and hypoxia is to slow ventricular rate. (Circulation Research 1990;67:960–978)

Since the early studies on the cardiovascular actions of adenosine and adenine nucleotides, it has been noted that marked species differences in both magnitude and type of response to administration of this nucleoside exist.1–5 For example, the depressant effect of adenosine on atrioventricular (AV) nodal conduction (i.e., AV block) in guinea pig heart is greater than in dog, cat, and rabbit hearts, whereas the reverse seems to apply to the sinoatrial slowing.1–5 In addition, in the same species, one action of adenosine, such as AV block, may predominate over another (e.g., sinus slowing).1–5 Although none of these studies dealt with the subject of species-dependent differences to adenosine and their implications in a systematic manner, they have led to the widespread acceptance that the cardiac effects of adenosine are species dependent. Similarly, neither the mechanism nor the implications of such differences have been a matter of investigation.

Sources of variations that may explain the species-dependent differences in response to adenosine include the following: 1) metabolism (uptake and deamination), 2) receptor density and affinity, 3) receptor-effector coupling mechanism and its efficacy, and 4) influence of one cardiac function
(heart rate) on another (AV nodal conduction) while both or one of them is affected by adenosine. In addition, because the actions of adenosine can be influenced by the parasympathetic/sympathetic tone, the functional status of the autonomic nervous system could conceivably play a role in the difference in response to adenosine among species and experimental models. Therefore, the present study is designed to take into account many of the potential sources of variation to explain the species-dependent differences in response to adenosine. An additional objective of this study was to determine whether the species and tissue (sinoatrial versus AV node) dependence in response to exogenously applied adenosine could explain the differences in response of guinea pig, rat, and rabbit hearts to a stimulus such as hypoxia, known to increase formation of adenosine. In short, the objectives of this study are 1) to characterize the species-dependent differences of the negative chronotropic and dromotropic actions of adenosine, 2) to determine the potential mechanism (sources) for the differences, and 3) to elucidate the physiological implication(s).

Materials and Methods

Experiments were carried out in isolated hearts of adult guinea pigs (Hartley), rats (Sprague-Dawley), and rabbits (New Zealand White) of either sex weighing 250–300 g, 200–250 g, and 2–2.5 kg, respectively. Guinea pigs and rats were anesthetized with methoxyflurane (by inhalation) and rabbits with a mixture of acepromazine (1 mg/kg) plus ketamine (60 mg/kg) given intramuscularly. Once anesthetized, the animals were killed by cervical dislocation, and the hearts were quickly excised and rinsed in ice-cold Krebs-Henseleit solution. Hearts were retrogradely perfused via the aorta at a constant flow rate of 8 ml/min (guinea pig and rat) and 20 ml/min (rabbit) (i.e., 4–5 ml/min/g). The perfusate was a modified Krebs-Henseleit solution with the following composition in millimoles per liter: NaCl 117.9, KCl 4.5, CaCl₂ 2.5, MgSO₄ 1.18, KH₂PO₄ 1.18, pyruvate 2.0, glucose 5.5, Na₂EDTA 0.57, ascorbic acid 0.007, and NaHCO₃ 25.0. The Krebs-Henseleit solution was gassed with 95% O₂ plus 5% CO₂, which results in a PO₂ of 500–600 mm Hg and pH of 7.3–7.4. The temperature of the perfusate was maintained at 35±0.5°C throughout the experiment.

In experiments designed to study the effect of adenosine and hypoxia on atrial rate (i.e., spontaneously beating heart preparations), the entire right atrium, including part of the superior and inferior venae cavae were left undissected, whereas extraneous tissue was trimmed away. A unipolar extracellular electrogram was recorded from the surface of the right atrium with a Teflon-coated stainless-steel electrode, as previously described. Atrial rate was determined from measurements of atrial cycle lengths or directly from a tachometer (BioTACH, Gould Inc., Cleveland) triggered by the right atrial electrogram.

In hearts in which AV nodal conduction time was measured at a constant rate of atrial pacing, the sinoatrial nodal region (including vena cava) and part of the right atrium were excised to facilitate electrical stimulation and provide exposure to the AV nodal area. A unipolar extracellular electrode was positioned in the AV nodal area to record the His-bundle electrogram according to a previously described method.12 Hearts were electrically paced at an atrial cycle length of 330 msec (unless otherwise indicated) via a bipolar electrode placed on either the remaining part of right atrium or the intra-atrial septum. The stimulator, an interval generator (model 1830, WPI, New Haven, Conn.) delivered the stimuli through a stimulus isolation unit (model 1880, WPI) as square-wave pulses of 2–3 msec in duration at twice the threshold intensity. Measurements of AV nodal conduction times were made from His-bundle electrograms during constant atrial pacing. Because the depressant effects of adenosine and hypoxia (4–5 minutes) are confined to the proximal area of the AV node, the atrial-to-His bundle or stimulus-to-His bundle (S-H) interval can be used as a measure of the effects of adenosine and hypoxia on AV nodal conduction. The S-H interval was measured visually from an oscilloscope display at a sweep speed of 10 msec/cm or from recordings obtained with a four-channel Gould strip-chart recorder (model R53400) at speeds of 100 or 250 mm/sec.

After completion of dissection, trimming, and instrumentation (i.e., placement of stimulating and recording electrodes), the hearts were allowed to equilibrate for 30–45 minutes before the experiments were begun. Experimental interventions were always preceded and followed by measurements of atrial rate (spontaneously beating preparations) and of S-H intervals (atrial paced preparations). Whenever the precontrol and postcontrol values differed by more than 15%, the intervening experimental data were discarded. Likewise, if any of the interventions caused a prolongation of the stimulus-to-atrial interval from baseline (i.e., 2–5 msec in guinea pig and rat and 7–12 msec in rabbit hearts) by more than 3 msec, the data were discarded. Unless otherwise noted, whenever any of the interventions caused second- or third-degree AV block, the longest stable S-H interval before onset of AV block was the value used for statistical analysis.

Chemicals

Adenosine, atropine, and dl-propranolol HCl were purchased from Sigma Chemical Co., St. Louis. The adenosine competitive antagonist BW A1433U, a para-phenyl acylate derivative of 1,3-dipropyl-8-phenylkanthine,14 and the adenosine deaminase inhibitor erythro-9-(2-hydroxy-3-nonyl)adenine HCl (EHNA) were kindly donated by Dr. S. Daluge from Burroughs Wellcome, Research Triangle Park, N.C.; dipyridamole was a gift from Boehringer Ingelheim, Ingelheim, FRG. N⁶-Cyclopentyl-adenosine (CPA) was purchased from Research Bio-
TABLE 1. Adenosine Concentration in the Effluent of Isolated Perfused Hearts

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Guinea pig</th>
<th>Rat</th>
<th>Rabbit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9±0.1</td>
<td>2.8±0.1</td>
<td>0.4±0.1*</td>
</tr>
<tr>
<td>DIP+EHNA</td>
<td>2.1±0.2</td>
<td>6.0±0.1</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM of concentrations of adenosine in μM from four guinea pig, five rat, and six rabbit hearts. Effluent levels of adenosine were measured during steady-state effects of infused adenosine (2 and 6 μM) before (control) and after treatment of the hearts with 1 μM dipyridamole (DIP) and 5 μM erythro-9-(2-hydroxy-3-nonyl)adenine HCl (EHNA). Rat hearts were treated with 10 μM DIP and 50 μM EHNA. *Value significantly different (p<0.05) from that of guinea pig and rabbit hearts.

Adenosine Assay

Samples of 2 ml of the heart’s effluent were collected in chilled tubes and frozen at −80°C until analysis for adenosine content. A 100-μl aliquot of each sample was assayed for adenosine by reverse-phase high-performance liquid chromatography (HPLC-Spectra-Physics, San Jose, Calif.) in the isocratic mode, according to the method of Hartwick et al. With this method, adenosine concentrations as low as 1 pmol could be detected and measured accurately.

Radioligand-Binding Assays

Binding assays were performed in crude cell membranes from atrial tissue of 10 guinea pig, five rat, and eight rabbit hearts. Because of their small size, guinea pig and rat atria from two or three hearts had to be combined to yield sufficient membranes for the binding assay. The hearts were first perfused for 5–10 minutes with oxygenated Krebs-Henseleit solution to wash out the blood, and then both atria were removed and homogenized in ice-cold buffer, as described previously.17 The binding assay for the cardiac adenosine receptor was carried out as described in previous studies that documented that 125I-ABA binds with high affinity and specificity to adenosine A1 receptors in atrial and ventricular myocardium.15,17,18 The [3H]NBMPR (a radioligand that specifically labels the nucleoside transporter) binding assay was conducted according to the method described by Williams et al.19 Nonspecific binding of 125I-ABA and [3H]NBMPR was defined as binding not displaced by 10 μM N6-(L-2-phenylisopropyl)adenosine and 100 μM nonradioactive NBMPR, respectively. Specific binding was determined by subtracting nonspecific from total binding.

Data Analysis

All data are expressed as mean±SEM. Statistical analysis for most of the data was based on analysis of variance (ANOVA) followed by t tests corrected for multiple comparisons, for example, intervention (adenosine, hypoxia) versus precontrol or postcontrol and control hypoxia versus hypoxia plus BW A1433U. Student’s t distribution with Welch approximation was used for comparison of unpaired data, that is, comparison of rates of release of adenosine caused by hypoxia among the species studied. The EC50 values (concentration of adenosine required to cause 50% of maximal effect) were determined according to the method described by Tallarida and Murray.20 Differences between group mean data were considered significant at p<0.05, unless otherwise indicated. The lines drawn through the data points representing the relations between changes in AV nodal conduction time and atrial rate during hypoxia were fitted by eye (see Figures 11 and 12).

Experimental Protocols

Concentration-response relations for adenosine. Concentration-response relations for the negative chronotropic (atrial rate slowing) and dromotropic (S-H interval prolongation) effects of adenosine were obtained in the absence and presence of dipyridamole (an adenosine uptake blocker)21 and EHNA (adenosine deaminase inhibitor).22 Because extracellular adenosine (e.g., infused intra-arterially) is subjected to rapid uptake and deamination, its concentration in the vascular and interstitial space may reach equilibrium only when metabolism of the nucleoside has been fully inhibited.13 Because the degree of adenosine metabolism in the isolated heart preparations varies among the species studied (Table 1), the experiments designed to compare the adenosine concentration-response curves in guinea pig, rat, and rabbit hearts were carried out in the presence of dipyridamole plus EHNA. The concentrations of dipyridamole and EHNA used were adjusted so that perfusate and effluent levels of adenosine reached equilibrium, that is, were the same (Table 1). In the case of guinea pig and rabbit hearts, 1 μM dipyridamole and 5 μM EHNA were sufficient, whereas in...
rat hearts 10 μM diprydamole and 50 μM EHNA were required to fully inhibit adenosine metabolism. In all experiments (except for CPA, see Figure 3), during control conditions and at each dose of adenosine an effluent sample was collected for measurement of adenosine concentration. After a washout period of 10–15 minutes, the hearts were treated with diprydamole plus EHNA for at least 10 minutes before a second concentration-response curve was determined. Hence, in all plots [Ado] effluent means the concentration of adenosine measured in the heart’s effluent during the steady-state adenosine infusion.

Concentration-response relations for adenosine-induced atrial slowing were obtained in spontaneously beating heart preparations from six guinea pigs and 10 rats. Cumulative doses of adenosine were infused at concentrations starting from 3×10^{-8} M in the case of guinea pig and 1×10^{-7} M in rat hearts up to a concentration that caused greater than 60% slowing of baseline rate. Atrial rate was determined 3–5 minutes after the infusion of each adenosine dose was started. Under the conditions of our experiments, the steady-state effects of adenosine are usually attained within 2–3 minutes after start of the infusion. Atrial-to-ventricular conduction time was also determined at each dose of adenosine.

AV nodal conduction (S-H interval) is modulated by atrial rate (atrial cycle length) and is dependent on tissue oxygenation (i.e., perfusion flow and perfusate PO_{2}). Therefore, we determined the effect of atrial cycle length on baseline S-H interval, on the adenosine-induced S-H interval prolongation. The effect of coronary perfusion pressure and flow rate on the S-H prolongation caused by adenosine was also investigated in four guinea pig and three rat hearts paced at a constant atrial cycle length of 330 msec. Neither coronary perfusion pressure (between 72±1.5 and 21±3 mm Hg) nor flow rate (between 8 and 14 ml/min) altered the magnitude of S-H prolongation caused by adenosine (0.1–20 μM).

Baseline S-H intervals were determined in guinea pig (n=6), rat (n=4), and rabbit (n=5) hearts at atrial cycle lengths of 320, 220, and 180 msec. Measurements of S-H intervals were made at each one of the above cycle lengths during steady state, that is, at the end of 1–2 minutes of pacing at each atrial cycle length.

The effect of the rate of atrial pacing on the adenosine-induced S-H prolongation was investigated only in rat hearts (n=4) because 1) similar experiments in guinea pig and rabbit hearts have been previously reported; 2) the baseline S-H intervals in guinea pig and rabbit hearts were not significantly different at the atrial rate used in the present study; and 3) under the conditions of the present experiments, the baseline spontaneous atrial rate of isolated perfused guinea pig and rabbit hearts is similar and slower than that of the atrial pacing cycle length used (i.e., 330 msec).

Because AV nodal conduction is modulated by atrial rate, which in turn is affected by adenosine, the effect of adenosine and CPA on AV nodal conduction was determined in hearts paced at a constant atrial cycle length of 330 msec. Concentration-response curves for adenosine and CPA were obtained in three separate groups of hearts of each species by infusion of increasingly higher concentrations of the negative dromotropic agents. In the case of adenosine, two concentration-response curves were determined in the same heart, that is, one in the absence and the other in the presence of dipyridamole plus EHNA.

Hypoxia. This series of experiments was designed to investigate the effect of hypoxia on atrial rate and on AV nodal conduction under conditions in which atrial rate was allowed to vary (i.e., spontaneously beating preparations [group A] and during constant atrial pacing [group B]). To obtain significant atrial slowing (i.e., to maximize the changes in atrial rate), the duration of the hypoxic perfusion was 10 minutes (group A) instead of 4 minutes (group B).

In spontaneously beating hearts (group A) from 24 guinea pigs and 11 rats, the effect of 10 minutes of hypoxia on atrial rate and AV conduction time in the absence and presence of the adenosine antagonist BW A1433U was investigated. A 30-minute period of normoxia (95% O_{2}+5% CO_{2}) was allowed before control measurements of atrial cycle lengths and AV conduction times were obtained. Hypoxia was initiated by switching from perfusion of normoxic to hypoxic solution (equilibrated with 95% N_{2}+5% CO_{2}; PO_{2}, 40–50 mm Hg). Measurements of atrial cycle lengths and AV conduction times were made at 60-second intervals after starting the hypoxic perfusion. Two 10-minute hypoxic perfusions, a control hypoxic period followed by a second hypoxic period in the presence of BW A1433U, were performed. After the first hypoxic period (control hypoxia) the hearts were reoxygenated for at least 20 minutes. The hearts were then treated with the adenosine antagonist BW A1433U (20 μM) starting 10 minutes before and throughout the second period of hypoxia and reoxygenation. Samples of 2 ml effluent were collected for measurement of adenosine concentrations before and after 2.5, 5, 7.5, and 10 minutes of hypoxic perfusion and after 5 minutes of reoxygenation. A subset of three rat hearts were exposed to brief periods of atrial pacing (e.g., Figure 9) at cycle lengths 10–20 msec shorter than that of the spontaneous rate during control hypoxia.

The effect of 4 minutes of hypoxia (95% N_{2}+5% CO_{2}; PO_{2}, 40–50 mm Hg) on S-H interval prolongation was examined in another series (group B) of nine guinea pig, 11 rat, and 10 rabbit hearts paced at a fixed atrial cycle length of 330 msec. A first 4-minute control hypoxic period was followed by a second 4-minute hypoxia in the presence of 20 μM BW A1433U with an intervening reoxygenation period of 20 minutes. In six of 10 rabbit hearts the dose of BW A1433U used was 8 μM. Infusion of the adenosine
antagonist BW A1433U was started 10 minutes before the second hypoxic period and lasted throughout the hypoxia and reoxygenation periods. Measurements of S-H interval were made every 15 seconds after the initial 2 minutes of hypoxia and every 30 seconds during the reoxygenation period. Effluent samples (2 ml) were obtained during normoxia, at 2.5 and 4 minutes of hypoxia, and at 1.5 and 6 minutes of reoxygenation. In a separate subset of 17 rabbit hearts, the hypoxic perfusate was equilibrated with a gas mixture containing 30% O₂, 5% CO₂, and 65% N₂ (PO₂, 80–100 mm Hg) instead of 95% N₂. The remainder of the protocol for this group of hearts was the same as the previous one.

Results

Adenosine Metabolism in the Perfused Heart

When hearts were perfused with a solution containing adenosine, 50–80% of the administered dose was not recovered in the effluent (Table 1). The amount of intra-arterially infused adenosine recovered in the effluent of the isolated hearts varied among the three species studied. At the highest perfusate concentration (6 μM), the differences among the species were not significant, whereas at the lowest concentration (2 μM) the amount of adenosine recovered in the effluent of rat heart was significantly less than that of guinea pig and rabbit hearts. In the presence of dipyridamole and EHNA, the adenosine levels measured in the heart’s effluent were significantly higher than control but more importantly were essentially the same as in the perfusion fluid, except in the rat heart, in which the effluent concentration of adenosine remained 15% less than that administered. That is, when adenosine uptake and deamination were inhibited, the recovery of intra-arterially infused adenosine in the effluent was complete or near complete in all three species. This finding formed the basis for the use of dipyridamole and EHNA in the determination of concentration-response curves for the negative chronotropic and dromotropic effect of adenosine.

Adenosine and Nucleoside Transport Binding Sites

To compare the number and affinities of adenosine receptors and nucleoside transport binding sites among species, the binding of appropriate radioligands to atrial membranes was studied. Binding of 125I-ABA was greater (p<0.05) in guinea pig than in rat and rabbit atria (Table 2). There were approximately 10-fold more high-affinity 125I-ABA binding sites in atrial membranes from guinea pigs than in atrial membranes from either rats or rabbits. Likewise, the specific binding of [3H]NBMPR to nucleoside transport sites was greater (approximately four-fold) in atrial membranes from guinea pig than in atrial membranes from rat or rabbit. In contrast, the rank order of affinities for adenosine A₁ receptor binding sites for 125I-ABA was rat>guinea pig>rabbit. With regard to nucleoside transport binding sites, the rank order of affinities of NBMPR for these sites was guinea pig=rat>rabbit.

Chronotropic Effect

Adenosine caused a concentration-dependent slowing of atrial rate (Figure 1) and prolongation of AV conduction time (not shown) in spontaneously beating isolated guinea pig and rat hearts. The baseline heart rate was faster in rat than in guinea pig hearts. Both the threshold and EC₅₀ for adenosine-induced slowing of atrial rate was lower in guinea pig than in rat hearts, that is, 0.09 and 0.48±0.03 μM in guinea pig versus 0.5 and 6.7±2.7 μM in rat. Determination of the threshold and EC₅₀ concentrations of adenosine’s effect on AV conduction time is hampered by the fact that atrial rate is not constant.

Dromotropic Effect

To characterize the negative dromotropic action of adenosine and its species dependency, atrial rate was held constant by pacing. Adenosine prolonged AV conduction time (Figure 2) in the hearts of all three species studied. The effect was concentration dependent (Figure 2) and was greater as the rate of pacing increased (not shown). However, the magnitude of this effect was markedly species dependent, that is, greater in guinea pig than rat and rabbit hearts (Figure 2). The baseline values of the S-H interval at all three rates of pacing (i.e., atrial cycle lengths of 320, 220, and 180 msec) were significantly (p<0.05) shorter in rat (S-H=31±1, 35±0.5, and 40±1 msec, respectively) than in either guinea pig (S-H=42±1,
52±2, and >80 msec, respectively) or rabbit hearts (see Reference 23). On the other hand, the baseline S-H values were not different between guinea pig and rabbit hearts.

**Adenosine Concentration-Response Curves: Comparison Among Species**

To compare the sensitivity of guinea pig, rat, and rabbit hearts to the negative dromotropic action of adenosine and CPA, concentration-response curves were obtained. Guinea pig hearts were by far the most responsive to adenosine, whereas rat and rabbit hearts were approximately equally responsive to the nucleoside (Figure 2A). Inhibition of uptake and deamination of adenosine by dipyridamole and EHNA, respectively, caused an approximately 14-fold shift to the left in the adenosine concentration-response curve in guinea pig hearts (i.e., EC50 decreased from 3.2±1.1 to 0.23±0.1 µM), whereas it caused only a relatively small shift in the concentration-response curves of rat and rabbit hearts (Figure 2B). The EC50 in rat heart decreased from 9.7±2.3 to 7.2±1.4 µM. In rabbit hearts the EC50 values could not be determined, because the maximal response was not achieved with the concentrations of adenosine used. In both guinea pig and rat hearts, second-degree AV block occurred more frequently and at lower concentrations of adenosine when the hearts were treated with dipyridamole plus EHNA. In contrast, in rabbit hearts, at the atrial rate of pacing used (i.e., cycle length, 330 msec), adenosine at a concentration as high as 1 mM (Figure 2B) did not cause second-degree AV block.

To rule out adenosine metabolism as an explanation for the species-dependent magnitude of the prolongation of AV nodal conduction by the adenosine, concentration-response curves to the nonmetabolizable adenosine analogue CPA were obtained. CPA (Figure 3) caused a dose-dependent prolongation of the S-H interval. Guinea pig hearts were more responsive to CPA than were rat or rabbit hearts. Rabbit hearts were more responsive than rat hearts. The EC50s for prolongation of the S-H interval by CPA in guinea pig, rat, and rabbit hearts were ~9.1, 91±3.5, and 57±19 nM, respectively. CPA at its highest concentrations caused second-degree AV block in guinea pig and rat but not in rabbit heart.

**Comparison of Chronotropic and Dromotropic Response**

To compare the sensitivity of atrial rate and AV nodal conduction to adenosine in hearts from each species, the atrial rate (Figure 1), atrioventricular interval (I-A-I) (Figure 2A), and stimulus-to-His interval (S-H) (Figure 2B) were measured in the absence (control), in the presence of dipyridamole (1 µM) plus erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) (5 µM) and CPA (100 µM). Rat hearts were treated with higher concentrations of dipyridamole and EHNA (i.e., 10 and 50 µM, respectively). In the absence of dipyridamole plus EHNA, the effluent concentration of adenosine was significantly less than that of the perfusate, whereas in their presence, both perfusate and effluent concentrations of adenosine were equal (see Table 1). Each data point is mean±SEM of six guinea pig, five rat, and five rabbit preparations. *Occurrence of second-degree atrioventricular block.
species, the data from Figures 1 and 2B were recalculated as percent decrease (atrial rate) and increase (S-H interval), and are presented in Figure 4. Guinea pig hearts were more responsive than rat hearts to adenosine. That is, the potency of adenosine to induce both atrial rate slowing and S-H prolongation was greater in guinea pig than in rat heart. The threshold concentrations of adenosine to cause atrial rate slowing and S-H prolongation were approximately sevenfold and 15-fold lower in guinea pig than in rat heart, respectively. Also, in both species the concentration of adenosine that caused a significant slowing of atrial rate was lower than that which caused prolongation of the S-H interval. The EC$_{50}$ for atrial slowing and for S-H prolongation were 0.48±0.03 and 0.23±0.1 μM (p<0.05), respectively, in guinea pig hearts. In rat hearts the EC$_{50}$ values for slowing of atrial rate and S-H prolongation were not significantly different (i.e., 6.7±2.7 versus 7.2±1.4 μM, respectively).

**Hypoxia**

The effects of hypoxia on atrial rate and on AV nodal conduction were studied in two distinct experimental models. Group A experiments were performed in spontaneously beating heart preparations from guinea pigs and rats, and group B experiments were carried out in isolated guinea pig, rat, and rabbit heart preparations paced at a constant atrial rate (see "Materials and Methods").

**Group A (Spontaneously Beating Hearts)**

As illustrated in Figures 5–8, the changes in atrial rate and AV conduction caused by 10 minutes of hypoxia were markedly different between guinea pig and rat hearts. In guinea pig hearts, two distinct patterns of response to hypoxia were noted (compare Figures 5–7). In 14 of 24 hearts (Figures 5A and 6A) there was no significant atrial slowing during the 10-minute hypoxic period, whereas in 10 hearts (Figures 5B and 8B), whereas in guinea pig hearts...
AV conduction time prolonged and invariably progressed to second-degree AV block (Figures 5B, 6B, and 7B). The rate of adenosine release by hearts during hypoxia increased significantly in both species, but the peak rate of release was significantly greater in guinea pig than in rat hearts (Figure 5C). After reoxygenation, all parameters returned to baseline. Not shown, the effects of 10 minutes of hypoxia in spontaneously beating rabbit hearts (n=3) were somewhat similar to those of guinea pig hearts (sub-group 1). The rate of adenosine release (nanomoles of adenosine in the effluent per minute per gram wet weight of heart tissue) was significantly higher (p<0.05) during hypoxia in the presence of BW A1433U than control. Values are mean±SEM of nine guinea pig hearts.
group 1, see below). In all three rabbit hearts studied, second-degree AV block occurred between 2 and 4 minutes of hypoxia. In two of the three hearts, at 5–6.5 minutes into the hypoxic period, when atrial rate significantly slowed, there was a transient resumption of 1:1 AV conduction, but AV conduction time remained prolonged (AV > 80 msec).

**FIGURE 7.** The effect of the adenosine antagonist BW A1433U (20 μM) on the changes in atrial rate (panel A), atrioventricular (AV) conduction time (panel B), and effluent levels of adenosine (Ado, panel C) during hypoxia (N2) and normoxia (O2) in spontaneously beating guinea pig hearts (subgroup 2). The decrease in atrial rate and prolongation of AV conduction time caused by hypoxia during control and BW A1433U between 4 and 10 minutes were significantly (p < 0.05) different from each other. All hearts developed second-degree AV block during control hypoxia but none when BW A1433U was present. *The rate of adenosine release (nanomoles of adenosine in the effluent per minute per gram wet weight of heart tissue) was significantly higher (p < 0.001) during hypoxia in the presence of BW A1433U than control. Values are mean ± SEM of 10 guinea pig hearts.

**Effect of BW A1433U on Hypoxia-Induced Atrial Slowing and Prolongation of Atrioventricular Conduction Time**

**Guinea pig hearts.** Figures 6 and 7 illustrate the effects of 20 μM BW A1433U on the changes in atrial and ventricular rate (panel A), AV conduction time (panel B), and rate of adenosine release (panel C) caused by hypoxia. Figure 6 summarizes the findings from the subgroup of guinea pig hearts in which atrial rate did not slow (subgroup 1), whereas Figure 7 summarizes results from those hearts (subgroup 2) in which a significant decrease in atrial rate was observed. Because in subgroup 1 (Figure 6) atrial rate did not slow but AV block occurred (10 of 11 hearts, i.e., 91%), the changes in ventricular rate caused by hypoxia alone (control hypoxia) and hypoxia plus BW A1433U were determined. The decrease in ventricular rate in the presence of 20 μM BW A1433U was significantly less (between 5 and 11 minutes) than during control hypoxia (Figure 6A). This attenuated decrease in ventricular rate was due to the fact that second-degree AV block was less frequent (5 of 11 hearts, i.e., 45%) and lasted only a couple of minutes in the presence of BW A1433U. In contrast, during control hypoxia, hearts developed second-degree AV block (between 3.5 to 5 minutes), and their ventricular rate became dependent on the escape rhythm rate. The rate of adenosine release (Figure 6C) during hypoxia plus BW A1433U was significantly greater than during control hypoxia. It should be noted that the rate of adenosine released was significantly elevated after 4.5 minutes of hypoxia at a time when the control hearts developed second-degree AV block. After 5 minutes of reoxygenation, all parameters had returned to baseline. During normoxia, BW A1433U did not cause significant changes in any of the parameters measured.

In subgroup 2 (Figure 7), that is, hearts in which atrial rate slowed during hypoxia, BW A1433U significantly blunted the hypoxia-induced decrease of atrial rate (panel A). Likewise, the AV conduction prolongation and second-degree AV block caused by hypoxia were markedly reduced by 20 μM BW A1433U. In fact, the prolongation of AV conduction time during hypoxia plus BW A1433U was much less in this series of hearts (subgroup 2) than in the hearts of subgroup 1. Also, rate of adenosine release in subgroup 2 (approximately 1 nmol/min/g) was significantly (p < 0.05) less than that of subgroup 1 (approximately 3 nmol/min/g). On the other hand, in both subgroups the rate of adenosine release was greater during hypoxia in the presence of BW A1433U than during control hypoxia. After reoxygenation (except for atrial rate during control hypoxia), all parameters returned to baseline.

**Rat hearts.** In contrast to guinea pig, hypoxia consistently slowed the atrial rate in rat hearts but did not increase AV conduction time (Figure 8). Concomitant with these changes, the rate of adenosine release increased significantly to a maximum of 5.7 ± 1.1 nmol/
The adenosine antagonist BW A1433U (20 μM) significantly altered the hypoxia-induced changes in atrial rate (panel A), AV conduction time (panel B), and rate of adenosine release (panel C). For example, the atrial slowing caused by hypoxia was significantly attenuated in the presence of BW A1433U, whereas the prolongation of AV conduction time was greater. In fact, in the presence of BW A1433U, hypoxia led to second-degree AV block, which was not observed during control hypoxia. As a consequence, the ventricular rate (Figure 8A, inset) was significantly slower (between 9 and 11 minutes) during hypoxia plus BW A1433U than during control hypoxia. As in the guinea pig heart, hypoxia plus BW A1433U in the rat heart produced a greater adenosine release than control hypoxia, and the adenosine levels were still elevated after 5 minutes of reoxygenation.

To demonstrate that second-degree AV block could occur in rat hearts during control hypoxia, a separate series of three spontaneously beating hearts were subjected to transient constant atrial pacing, an example of which is given in Figure 9. As illustrated in Figure 9, at 5 minutes of hypoxia atrial rate had slowed from 250 beats/min (control hypoxia) to 214 beats/min. Atrial pacing at a constant rate of 240 beats/min (i.e., 12% higher than the spontaneous
rate during hypoxia) led to a second-degree AV block. Cessation of atrial pacing (Figure 9C) promptly restored 1:1 AV conduction, and spontaneous atrial rhythm resumed at a rate similar to that before pacing. Similar results were obtained in two additional hearts.

Role of Adenosine in Atrial Slowing During Hypoxia

The relative contribution of endogenously released adenosine to the slowing of atrial rate caused by hypoxia in guinea pig and rat hearts is shown in Figure 10. The data depicted in this figure were derived from the results shown in Figures 7 (guinea pig) and 8 (rat). The adenosine-sensitive component of atrial rate slowing caused by hypoxia (ordinate of Figure 10) was calculated by subtracting the changes in atrial rate during control hypoxia from that observed during hypoxia plus BW A1433U. This adenosine-sensitive component of atrial slowing was plotted against adenosine concentrations determined from the rates of adenosine release during hypoxia. In guinea pig and rat hearts, the magnitude of atrial slowing ascribed to endogenously released adenosine amounted to 39±9 and 41±14 beats/min, respectively. This magnitude of atrial slowing is comparable to that caused by similar concentrations of exogenous adenosine (Figure 1). Further analysis and interpretation of these data are considered in “Discussion.”

Relation of Atrioventricular Conduction Time to Atrial Rate

Because atrial rate modulates AV nodal conduction time, the relation between these two functions was determined in both guinea pig (Figure 11) and rat (Figure 12) hearts with the following three experimental conditions: 1) during infusions of adenosine, 2) during control hypoxia, and 3) during hypoxia plus BW A1433U. The data from Figures 1, 7, and 8 are replotted as the magnitude of the increase in AV conduction time on the ordinate and magnitude of the decrease in atrial rate on the abscissa (Figures 11 and 12). During infusions of adenosine in guinea pig hearts (Figure 11A), slowing of the atrial rate was paralleled by a prolongation of the AV conduction time, most prominently once the atrial rate had slowed by 30 beats/min. In sharp contrast, during infusions of adenosine in rat hearts, atrial rate slowed by as much as 100 beats/min before any noticeable increase occurred in AV conduction time (Figure 12A). During hypoxia, the relation between AV conduction time and atrial rate was similar to that obtained during infusions of adenosine. However, in guinea pig hearts, the slope of the relation between AV conduction time and atrial rate was steeper during hypoxia (Figure 11B) than during the infusion of adenosine (Figure 11A). In contrast, in rat hearts the relations were strikingly similar during hypoxia and infusion of adenosine (Figures 12A and 12B), except for the AV block observed during infusion of adenosine but not during hypoxia. Thus, changes in atrial rate and AV conduction during infusions of adenosine and hypoxia were more similar in rat than in guinea pig heart (compare Figures 11 and 12). The adenosine antagonist BW
A1433U markedly altered the relations between AV conduction time and atrial rate during hypoxia. In guinea pig hearts (Figure 11B), BW A1433U significantly blunted the hypoxia-induced atrial slowing and concomitantly prevented the prolongation of AV conduction time. In rat hearts (Figure 12B), BW A1433U likewise attenuated the hypoxia-induced atrial slowing, but in contrast to guinea pig hearts, it led to a marked prolongation of AV conduction time that resulted in second-degree AV block. Thus, in rat hearts BW A1433U produced a large shift to the left and upward of the curve relating AV conduction time to atrial rate.

**Group B (Atrial Paced Hearts)**

In this series of experiments the effects of hypoxia on S-H interval prolongation and rate of adenosine release in isolated guinea pig, rat, and rabbit hearts paced at a constant atrial rate were determined. In addition, the relative contribution of endogenously released adenosine to the negative dromotropic response during hypoxia was investigated.

In all three species (but to different degrees) hypoxia induced a significant prolongation of the S-H interval and an increase in the rate of adenosine release (Figure 13). The time course and magnitude of the S-H prolongation were similar in guinea pig and rabbit hearts, but rat hearts were markedly less responsive (Figure 13A). The maximum prolongation of S-H interval in rat hearts during hypoxia was 10±2 msec, whereas both in guinea pig and rabbit hearts S-H was prolonged by 39±4 and 29±5 msec, respectively. In addition, second-degree AV block occurred in 90% and 12% of guinea pig and rabbit hearts, respectively, but did not occur in rat hearts. The rate of adenosine release in rabbit hearts was 4.4-fold greater than from guinea pig hearts and was least from rat hearts (7.7-fold less than rabbit). The effect of 4 minutes of hypoxia on S-H interval prolongation in rat hearts paced at a faster atrial rate (cycle length, 180 msec) was somewhat greater (i.e., 15±5 versus 9±2 msec) than when the hearts were paced at a cycle length of 290–330 msec, but it was still markedly less than that observed in either guinea pig or rabbit hearts (not shown).

**BW A1433U Antagonism of Hypoxia-Induced Stimulus-to-His Bundle Prolongation**

Figure 14 illustrates the effect of the adenosine antagonist BW A1433U on hypoxia-induced S-H interval prolongation (panels A) and rate of adenosine release (panels B) in guinea pig, rat, and rabbit hearts. The concentration of BW A1433U used (i.e., 20 μM) is likely to almost completely block the actions of adenosine. In guinea pig and rabbit hearts, BW A1433U significantly attenuated the

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**Figures 11 and 12**

**Figure 11.** Relation between atrial rate and atrioventricular (AV) conduction time in the presence of exogenously applied adenosine (panel A) and during hypoxia (panel B) in guinea pig hearts. Data are from Figures 1 and 7. Details of the interpretation of the data are given in the text.

**Figure 12.** Relation between atrial rate and atrioventricular (AV) conduction time in the presence of exogenously applied adenosine (panel A) and during hypoxia (panel B) in rat hearts. Data are from Figures 1 and 8. Details of the interpretation of the data are given in the text.
Role of Adenosine in Stimulus–to–His Bundle Prolongation During Hypoxia

To determine the relative contribution of adenosine to the hypoxia-induced S-H prolongation in guinea pig, rat, and rabbit hearts, the adenosine-sensitive component of S-H prolongation caused by hypoxia was plotted against adenosine concentrations calculated from the rates of adenosine release during hypoxia (Figure 15). The adenosine-sensitive component is the difference between the S-H prolongation during hypoxia alone (control hypoxia) and hypoxia plus 20 μM BW A1433U. The results, illustrated in Figure 15, indicate that the relative contribution of endogenously released adenosine to the hypoxia-induced S-H prolongation is species dependent. Adenosine appeared to play a greater role in the hypoxia-induced AV nodal conduction time prolongation in guinea pig than in rabbit hearts, but has no effect in rat hearts.
FIGURE 14. The effect of the adenosine antagonist BW A1433U on hypoxia-induced stimulus-to-His bundle (S-H) prolongation (panel A) and effluent adenosine (Ado, panel B) during hypoxia (N2) and normoxia (O2) in guinea pig, rat, and rabbit hearts. The average time courses of the changes in S-H interval and rate of adenosine release (nanomoles per minute per gram wet weight heart tissue) were determined during control hypoxia (filled symbols) and in the presence of BW A1433U (open symbols). The S-H interval prolongations during hypoxia plus BW A1433U (between 2 and 5.5 minutes) were significantly different (p<0.05) from control hypoxia in guinea pig and rabbit but not in rat hearts. During control hypoxia, 90% of guinea pig and 12% of rabbit hearts developed second-degree atrioventricular block, whereas during hypoxia plus BW A1433U, block did not occur. Values represent mean±SEM of nine guinea pig, 11 rat, and eight rabbit hearts. *Values significantly (p<0.05) different from control hypoxia.

Discussion

The present study shows that adenosine causes concentration-dependent slowing of atrial rate (i.e., negative chronotropic effect) and AV nodal conduction (i.e., negative dromotropic effect), confirming earlier work with adenosine. In addition, it demonstrates that 1) the magnitude of the negative chronotropic and dromotropic effects of adenosine are species dependent, that is, the guinea pig heart is more sensitive than either the rat or rabbit heart; 2) in guinea pig hearts adenosine was more potent in slowing AV nodal conduction than atrial rate, although the threshold concentration was lower for the latter; 3) in rat hearts the threshold concentration of adenosine to cause atrial slowing was lower than that to prolong AV nodal conduction time, but its potency for both effects was approximately the same; and 4) the response to hypoxia in guinea pig and rabbit hearts was characterized by AV block, whereas slowing of atrial rate without AV block was the main response of hypoxia in rat hearts. These differences in response to hypoxia could in part be accounted for by species-dependent differences in the amount of adenosine released and metabolized during hypoxia, the sensitivity of each heart to the actions of adenosine, and the intrinsic properties of the sinoatrial and AV nodes (e.g., dependency of AV nodal conduction on atrial rate).

Variables That Potentially Influence the Effects of Adenosine

The validity of the above conclusions rests on how well the many variables affecting heart rate and AV nodal conduction were controlled. Electrophysiological characteristics of the sinoatrial and the AV nodes and modulators of nodal function such as the autonomic nervous system may have a significant impact on the effects of adenosine. Because AV nodal conduction time is dependent on rate (i.e., faster rate...
equal longer AV conduction time and vice versa), to
determine the true potency of adenosine to depress
AV conduction, atrial rate must be held constant. In
addition, when comparing species with different
baseline heart rates and AV nodal conduction times
(including relation between atrial pacing cycle length
and AV nodal conduction time), it is crucial to
determine to what extent the effect of adenosine on
AV conduction is affected by such variables. Baseline
S-H intervals determined at three different atrial
pacing cycle lengths were similar in guinea pig and
rabbit hearts but shorter in rat hearts. Because the
AV nodal conduction prolongation caused by aden-
osine has been shown in guinea pig and rabbit hearts
to be greater at faster pacing rates,23,24 a finding now
confirmed in rat hearts as well, the comparison of the
negative dromotropic effect among these three spe-
cies must be carried out at the same (or nearly the
same) atrial pacing rate. Pacing the rat hearts at an
atrial cycle length of 290–330 msec may have led to
an underestimation of the magnitude of the S-H
prolongation caused by adenosine. However, because
the rate dependency of the S-H prolongation induced
by adenosine seems to be less in rat than in rabbit
(Figure 1 of Reference 23) or guinea pig (Figures 3
and 4 of References 23 and 24, respectively), the
magnitude of the underestimation is minimal. Thus,
a potential error in determining the true sensitivity of
rat hearts to adenosine should be small even when
hearts are paced at atrial cycle lengths greater than
220 msec. The dependency of the S-H prolongation
caued by adenosine on sinus rate is proportionally
greater at higher concentrations of adenosine and
must also be considered.23,24

Adenosine Metabolism

Uptake and deamination of extracellular adeno-
sine by endothelial cells and cardiomyocytes are
important determinants of interstitial levels of this
nucleoside.25,26 Removal of exogenously applied
adenosine in perfused hearts, in addition to washout,
can be accomplished by cellular uptake and enzy-
matic processes.27,28 Inhibition of these processes can
account for the potentiation of the cardiac actions
of adenosine caused by dipyridamole (adenosine trans-
port blocker) and EHNA (adenosine deaminase
inhibitor). We previously reported (which is con-
firmed in the present study) that during constant
infusions of adenosine in the presence of dipyri-
damole and EHNA, the perfusate (arterial) and effl-
uent (venous) concentrations of adenosine were
the same.13 This indicates that the metabolism of
adenosine is inhibited by dipyridamole and EHNA,
in agreement with the results of recent studies of
isolated guinea pig hearts by multiple-indicator diffu-
sion techniques.28–30 Furthermore, the results of
these latter studies28–30 and our previous13 and pre-
sent observations emphasize that in a multicellular
preparation such as the isolated perfused heart,
metabolism of adenosine must be blocked to deter-
mine the true potency of adenosine in evoking a
given cardiac response.

The influence of adenosine uptake and deami-
nation on adenosine’s potency can be demonstrated
by the approximately 14-fold decrease in the EC50
value for the negative dromotropic effect in guinea
pig hearts caused by the addition of dipyridamole
plus EHNA (Figure 2). In comparison, in rat hearts
the EC50 decreased by 1.6-fold, whereas in rabbit
hearts there was no significant change in the EC50
value. Because both an uptake blocker (dipyri-
damole) and an inhibitor of adenosine deaminase
(EHNA) were both present, one cannot determine
the relative contribution of uptake and deamination
to the increase of adenosine’s potency. Nevertheless,
the finding that atrial membranes from guinea pig
hearts contain 6.8- and 7.6-fold more transport bind-
ing sites than do rat and rabbit hearts, respectively,
is consistent with the greater leftward shift of the
adenosine concentration–negative dromotropic
response curve in guinea pig hearts than in the other
two species. It is important to mention that NBMPR
binding sites have been shown to identify transport-
inhibitory sites in intact cardiomyocytes; hence, the
number of NBMPR binding sites is predictive of
nucleoside transport capacity.31 Our findings
regarding species differences in the number and
affinity of NBMPR binding sites (Table 2) are in
keeping with previous reports that guinea pig hearts
have an eightfold greater number of NBMPR trans-
port binding sites than do rat hearts.19 NBMPR
binding affinity was similar in atrial membranes from
all three species and did not correlate with the
increased potency of adenosine caused by dipyridam-
ole plus EHNA. Whether this is due to the fact that
in rat hearts, in contrast to guinea pig hearts, extra-
cellular deamination is the principal mechanism for
removal (inactivation) of adenosine52 and not nucle-/oside uptake via a dipyridamole-sensitive transport
system, remains to be demonstrated. The nucleoside
transporter in rat in comparison to guinea pig myo-
cytes has a lower affinity for dipyridamole,31 and that
may account for the small shift (1.6-fold) in
EC50 value of the cardiac actions of adenosine caused
by dipyridamole in rat hearts, a finding also reported
by others.31,33

The dose-response curves for the nonmetaboliz-
able and relatively selective adenosine A1 receptor
CPA further rule out nucleoside metabolism as the
mechanism for the species difference in the magni-
tude of the S-H prolongation caused by adenosine.
In a previous study it was shown that dipyridamole
and EHNA do not increase the potency of CPA, which
satisfies the premise that this adenosine analogue
is neither transported nor deaminated to a significant
degree by the same transport system and enzymatic
processes as is adenosine.13 The EC50 values for CPA
were smaller in guinea pig hearts than either rat or
rabbit hearts; this species difference in response to
CPA was similar to that seen with adenosine.
Adenosine Receptor-Effect System

It is now well established that the cardiac actions of adenosine are initiated by activation of specific cell surface receptors (see Reference 34 and references therein).

The density and affinity of cardiac adenosine \( A_1 \) receptors reported in the literature varies widely depending on the radioligand (agonist or antagonist), the source of tissue (atria versus ventricle), and the preparation (crude or purified membranes, or intact myocytes). There are, however, only few studies of adenosine receptors in cardiac atria and none that compares density and affinity of these receptors among species.\(^3\) Regardless of the absolute values of \( B_{\text{max}} \) and \( K_d \), the presence of approximately 10-fold greater density of adenosine receptors in guinea pig versus rat and rabbit atria is in agreement with the rank order of sensitivity to adenosine among the three species studied. On the other hand, the lower affinity found in rat in comparison to guinea pig and rabbit atria is not consistent with the adenosine potency being greater in guinea pig than in rat or rabbit hearts. This suggests that receptor density is a more important determinant of the overall sensitivity of the heart to the actions of adenosine than affinity of these receptors. Alternatively, the high-affinity binding sites are not the "functional" receptors, and atrial \( A_1 \) receptors cannot be taken as an index of the receptors in sinoatrial and AV nodal tissue. Against these latter two possibilities are the findings that 1) upregulation and downregulation of these high-affinity binding sites are associated with increased and decreased cardiac responses to adenosine, respectively, and 2) a high correlation exists between adenosine receptor antagonist effects on dromotropic actions of adenosine and the affinity of the same antagonists to compete with \(^{125}\text{I-ABA} \) for binding to adenosine receptors on ventricular membranes.\(^1,14,17,36\)

The lower \( E_{\text{C}0} \) for the adenosine \( A_1 \) receptor analogue CPA in rabbit than in rat hearts cannot be readily explained. Rabbit atrial membranes have the lowest number of \(^{125}\text{I-ABA} \) binding sites and twofold higher affinity than found in rat heart membranes. Thus, some other yet unrecognized binding characteristic of CPA to rat cardiac \( A_1 \) receptors could account for the low potency of this analogue to slow AV nodal conduction. This phenomenon is not unique to the rat because similarly in dog heart, the \( N^6 \)-substituted adenosine analogues (including CPA) have significantly lower potency than \( 5'-\text{(N-ethylcarboxamido)} \) adenosine and adenosine itself to cause slowing of heart rate.\(^37\) This latter finding is consistent with the observation that in cardiac membranes from dog heart, \( N^6 \)-derivative adenosine analogues bind poorly to adenosine \( A_1 \) receptors (J. Linden, personal communication, 1989).

In addition to receptor number and affinity, another potential mechanism for the species-dependent sensitivity to adenosine is the coupling system (including G-protein) of the adenosine \( A_1 \) receptor to the ionic conductances and, hence, cellular responses.

Role of Adenosine in Hypoxia

Evidence that endogenously released adenosine contributes to bradyarrhythmias (i.e., slowing of heart rate and AV conduction disturbances) caused by hypoxia and ischemia comes from studies in various species, including human (see Reference 34 and references therein). However, most of the evidence, especially in the case of AV conduction disturbances, is derived from experiments in guinea pig hearts. In fact, in rabbit hearts the findings of Young et al.\(^38\) indicate that the role of adenosine as mediator of the negative dromotropic effects of hypoxia is negligible. Similarly, in spontaneously beating rat hearts, ischemia causes only moderate prolongation of AV conduction time, which suggests (although indirectly) that adenosine may play a minimal role, as in the case of the rabbit heart.\(^39\) However, since in this latter study heart rate was not held constant during the ischemic period, the impairment in AV nodal conduction could have been masked by the significant slowing of the atrial rate. Evidence in support of a role of adenosine in ischemia- or hypoxia-induced sinus bradycardia is derived in part from experiments in isolated perfused canine right atrium and rat hearts.\(^39-41\)

In contrast to the data in rabbit and rat hearts, the evidence that adenosine plays a major role in the AV conduction disturbances associated with hypoxia and ischemia in guinea pig hearts is overwhelming.\(^34\) Thus, important species differences exist not only in response to adenosine but also in response to stimuli such as hypoxia. Hypoxia and ischemia have been shown in many experimental preparations to cause a significant increase in the formation of adenosine.\(^42-45\) The activities of the enzymes that determine the rates of formation and degradation of adenosine (e.g., \( 5'-\text{nucleotidase} \) and adenosine deaminase) are different among species.\(^44\) Hence, it is not surprising, as shown in this (Figures 13 and 14) and other studies,\(^44\) that during hypoxia and ischemia the rate of adenosine formation varies among species.\(^44,46\) To this end, the effects of hypoxia were investigated in two experimental models (i.e., spontaneously beating and paced hearts) with the intent to determine the relative contribution of endogenous adenosine to the changes in heart rate and AV nodal conduction and whether species differences in the response to hypoxia could be ascribed to adenosine.

Hypoxia in Spontaneously Beating Hearts

Hypoxia and ischemia have been reported to cause sinus slowing, sinoatrial nodal block, and pacemaker shift.\(^11,47,48\) Endogenously released adenosine has been implicated in the sinus slowing observed during hypoxia and ischemia in various species and experimental models.\(^34\) However, except for the recent study by Headrick and Willis,\(^39\) none of the previous
reports determined either to what extent adenosine contributes to sinus slowing or the relation between the amount of adenosine release and bradycardia. The present study not only confirms the findings of Headrick and Willis39 but extends it to other species, identifies major differences between rat and guinea pig hearts, and provides explanation for interspecies and intraspecies differences in response to hypoxia.

In spontaneously beating rat hearts, hypoxia was characterized by a significant slowing of atrial rate without prolongation in AV conduction time. In contrast, in guinea pig hearts two somewhat distinct patterns of response to hypoxia emerged. In one series of hearts (subgroup 1), atrial rate did not slow, whereas in another (subgroup 2), it did. However, AV conduction delay developed in both subgroups during hypoxia. Although the reason(s) for this major difference in response is not understood, in both subgroups the ultimate effect of hypoxia was slowing of ventricular rate.

Rat hearts did not develop AV block during hypoxia. The most likely explanation for this finding is that in rat hearts the amount of adenosine released during hypoxia was below the threshold concentration for causing AV conduction time prolongation (compare Figures 10 and 8 with 4). In addition, the relation between AV conduction time and atrial rate had a gradual slope; atrial rate slowed as much as 100 beats/min before AV conduction time started to increase (Figure 12). On the other hand, if atrial rate did not slow, either because of the presence of the adenosine antagonist BW A1433U (Figure 12B) or atrial pacing (Figure 9), AV block developed. These findings are consistent with the interpretation that in rat hearts AV conduction is not significantly impaired by hypoxia as long as atrial rate decreases.

Unlike rat hearts, guinea pig hearts developed AV block even though in some of them atrial rate significantly slowed. The steep slope of the AV conduction time versus atrial rate relation during hypoxia (Figure 11B) can explain the high occurrence of AV block in guinea pig hearts. This is also in keeping with the similar EC50 values of adenosine’s negative chronotropic and dromotropic actions (Figure 4A). However, this should not imply that in hearts in which AV block occurred despite atrial slowing the dependency of AV nodal conduction on rate is absent. In fact, the response to BW A1433U clearly shows that in hearts in which atrial rate slowed, there was significantly less prolongation of AV conduction time (compare Figures 6B and 7B).

The importance of adenosine’s contribution to atrial rate slowing during hypoxia in the present study appears to be greater than that found by Headrick and Willis.39 Based on the rates of endogenously released adenosine and the effects of BW A1433U, adenosine can account for an atrial rate decrease of 40 beats/min on average (Figure 10), which is approximately 40% and 70% of the total atrial slowing caused by hypoxia in rat and guinea pig hearts, respectively. According to the Headrick and Willis39 study of rat hearts, the approximately 2 nmol/min/g adenosine released during ischemia caused the heart rate to fall 48 beats/min, a value that is remarkably similar to the results reported here. However, in the study of Headrick and Willis,39 the relative contribution of adenosine to the bradycardia caused by ischemia amounted to approximately 25%, which is less than the 40% found in the present study, but there are important differences between these two studies. For example, in their study,39 ischemia and not hypoxia was the stimulus for production of adenosine, and the adenosine antagonist used was 8-phenyltheophylline (PA2=6.37), which is less potent than BW A1433U (PA2=7.7)14; hence, the contribution of adenosine to the bradycardia could have been underestimated.

In rat hearts, exogenously applied adenosine (in the presence of dipyridamole plus EHNA) and hypoxia yielded similar relations between AV conduction time and atrial rate which was not the case in guinea pig hearts (compare Figures 11 and 12). The reason for the discrepancy in the effect of adenosine and hypoxia on the relation between AV conduction time and atrial rate in guinea pig hearts is not clear; however, it is worth mentioning that similar relations were obtained when adenosine was administered in the absence of dipyridamole plus EHNA (not shown). That is, in the absence of dipyridamole plus EHNA, a slowing of atrial rate of 41±11 beats/min caused by adenosine was accompanied by a prolongation of 33±5 msec in the S-H interval; these changes in AV conduction and atrial rate are similar to those caused by hypoxia (Figure 11B). This finding suggests that the combination of dipyridamole plus EHNA causes a greater potentiation of the negative chronotropic than dromotropic action of adenosine. Although speculative, this interpretation is consistent with the fact that the threshold concentration of adenosine for its negative chronotropic action is lower than for its negative dromotropic effect.

Hypoxia in Atrial Paced Hearts

In rat hearts, the relatively small S-H prolongation caused by hypoxia and the lack of effect of the adenosine antagonist BW A1433U leads to the conclusion that in this species adenosine plays little or no role in the hypoxia-induced AV conduction disturbances. According to the data depicted in Figure 15, the most likely explanation for this response is that the amount of adenosine released during hypoxia in rat heart is approximately 10-fold lower than the threshold level required to cause S-H prolongation (compare Figures 2B and 15). On the other hand, in guinea pig hearts, the amount of adenosine released during hypoxia reached levels that are in the range of those that cause S-H prolongation. In fact, comparing the data of Figure 2B with that of Figure 15, it becomes evident that the adenosine levels during hypoxia lie in the steepest range of the adenosine concentration-response curve. Although hypoxic rabbit hearts release severalfold greater amounts of
adventitious than rat and guinea pig hearts, the contribution of endogenous adenosine to hypoxia-induced S-H prolongation is relatively small (approximately 10 msec according to data of Figure 15). This finding can be accounted for by the data of Figure 2B that shows that in rabbit hearts endogenous concentrations in the range of 7–100 μM prolonged S-H interval by only 8–10 msec. Thus, differences in amounts of endogenously released adenosine and in AV nodal conduction sensitivity to this nucleoside can account for the differences in the degree (or lack) of contribution of adenosine as mediator of hypoxia-induced AV conduction time prolongation.

The present study was not designed to investigate what other mediators (or mechanisms), in addition to adenosine, contribute to the effects of hypoxia on AV conduction time and heart rate. However, the observation that neither atropine nor propranolol affects the hypoxia-induced AV conduction time suggests that acetylcholine and catecholamines may play little or no role in the effects of hypoxia on AV conduction in the isolated perfused heart. The observation of Young et al18 that aminophylline fails to antagonize the depressant effects of hypoxia on AV conduction time and Wenckebach cycle length in rabbit hearts could be interpreted to conflict with the present results. However, it should be noted that xanthine derivatives such as aminophylline (PA2 = 5) are much less potent adenosine antagonists than BW A1433U (PA2 = 7.7).14 Similarly, theophylline is the least, and BW A1433U the most, potent adenosine antagonist to compete with 125I-ABA for binding adenosine A1 receptors in guinea pig ventricular membranes.14

Conclusions

The major implication of this study is that direct extrapolation of results from adenosine studies in one species to others, including humans, cannot be made. Caution should be exercised in extrapolation of pharmacological testing of adenosine analogues and antagonists from one species to others and when establishing both potency and selectivity of these agents based solely on a single cardiac action (e.g., heart rate) of adenosine.

The results of this study indicate that the ultimate effect of adenosine and hypoxia is to slow ventricular rate either by causing AV block (guinea pig) or slowing atrial rate without AV block (rat). This would have the effect of sparing the ventricular myocardium from work, and hence retarding cell injury, when O2 supply is limited. Because adenosine acts as an inhibitory feedback signal that protects the myocardium from excessive work by reducing ventricular rate (directly or indirectly by causing AV block), inhibition of its effects leads to further imbalance between O2 supply-demand, and as a consequence, adenosine production increases.12 In fact, the heart’s effluent concentration of adenosine during hypoxia was greater in the presence of the adenosine antagonist BW A1433U than in its absence, that is, during control hypoxia. In guinea pig and rabbit hearts, ventricular rate during hypoxia was significantly faster and oxygen consumption presumably greater in the presence of BW A1433U than in its absence.

In conclusion, further investigation of species-dependent sensitivity and mechanism of action of adenosine may prove useful in helping to define the role of this nucleoside as modulator of cardiovascular functions.

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