Effect of Adenosine on Histamine Release and Atrioventricular Conduction During Guinea Pig Cardiac Anaphylaxis

Lois J. Heller and Jean F. Regal

Anaphylactic events occurring in cardiac tissue can result in severe metabolic imbalances. The present study addresses the question of whether adenosine, produced in response to this stress, influences either the antigen-antibody-induced alterations in cardiac function or the release of histamine, which is known to be one of the important mediators of the anaphylactic reaction. Isolated hearts of passively sensitized guinea pigs were perfused at constant flow in a Langendorff preparation with physiological salt solution. Under control conditions, antigen challenge evoked a rapid transient release of histamine, an increase in coronary vascular resistance and beating rate, and an increase followed by a decrease in left ventricular systolic pressure. The antigen-induced transient increase in adenosine release from 0.26±0.07 to 4.66±0.48 nmol/min/g was associated with a 75±9% increase in the PR interval in all hearts and atrioventricular blocks in six of 17 hearts. Antigen challenge was also conducted in the presence of theophylline, 8-(4-sulphenyl) theophylline (SP-T), erythro-9-(2-hydroxy-3-nonyl) adenosine hydrochloride (EHNA), or exogenous adenosine. The major findings were that 1) the antigen-induced prolongation of the PR interval was attenuated by the adenosine receptor blockers theophylline (to 23±6%) and SP-T (to 15±4%); 2) the incidence of antigen-induced atrioventricular blocks tended to be decreased by theophylline (to three of 10 hearts) and SP-T (to zero of seven hearts) and to be increased by the adenosine deaminase inhibitor, EHNA (to six of 10 hearts); 3) none of the interventions had major influences upon antigen-induced alterations in vascular resistance, atrial automaticity, or systolic pressure; and 4) EHNA and adenosine both significantly increased adenosine levels before anaphylaxis and also enhanced the total histamine release induced by antigen challenge from a control value of 2,321±244 ng/g to 3,424±307 ng/g and 4,298±616 ng/g, respectively. We conclude from our data that increases in levels of endogenous adenosine during cardiac anaphylaxis may contribute to the development of atrioventricular conduction delays and blocks and that increases in levels of adenosine before antigen challenge may increase the amount of histamine released during cardiac anaphylactic reactions. (Circulation Research 1988;62:1147-1158)
to answer these questions. These interventions included addition to the perfusion media of theophylline, an adenosine receptor antagonist, 27 8-(4-sulfophenyl) theophylline (SP-T), a theophylline analogue confined to the extracellular space 20 and possessing potent adenosine-receptor antagonistic action, 20, 27 erythro-9-(2-hydroxy-3-nonyl)-adenosine hydrochloride (EHNA), an adenosine deaminase inhibitor 28 that has been shown to significantly increase estimates of interstitial levels of adenosine in isolated rat hearts, 29 or exogenous adenosine. The latter intervention was designed to examine the effect of significant elevation of tissue adenosine before antigen challenge upon the antigen-induced histamine release. Anaphylactic reactions produced in the presence of these interventions were compared with those produced under control conditions.

The major findings of this study were that endogenous adenosine produced during anaphylactic reactions 1) contributes significantly to PR interval prolongation and atrioventricular conduction blocks, 2) has minimal influence upon antigen-induced alterations in coronary vascular resistance, atrial automaticity, and left ventricular systolic pressure, and 3) has little influence upon the amount of histamine released by the antigen challenge. However, if adenosine levels are elevated before antigen challenge, especially by perfusion with exogenous adenosine, histamine release is significantly enhanced.

Materials and Methods
Production of Antibody and Passive Sensitization of Guinea Pigs
Male guinea pigs (300–500 g, Dunkin-Hartley descendants from Bio-Lab, St. Paul, Minnesota) were immunized with ovalbumin according to a regimen that produced both IgG and IgE antibodies. The serum from 12 animals was pooled and passed over a protein A-Sepharose affinity column to separate IgG from other serum constituents. The ovalbumin-specific content of this IgG preparation, assessed by a passive cutaneous anaphylactic reaction of anesthetized nonsensitized guinea pigs, was such that 245 ng IgG gave a 5-mm bluing reaction in three of five guinea pigs. Aliquots of this preparation were stored at −20°C for later use. Details of these procedures are included in a previous study. 30 For the experiments described in this report, untreated ether-anesthetized guinea pigs were passively sensitized to ovalbumin by intracardiac injections of 0.34 mg of the IgG preparation, 12–24 hours before removing hearts for perfusion. The few hearts (<5%) that showed visible evidence of damage from the intracardiac injection were not included in the study.

Isolated Perfused Heart Preparation
Hearts removed from passively sensitized guinea pigs that had been injected with heparin (2.5 mg i.p.) and anesthetized with sodium pentobarbital (35 mg/kg i.p.) were placed in ice-cold modified Krebs-Henseleit solution. This solution contained (mM) NaCl 118.0, KCl 4.7, NaHCO3 25.0, CaCl2 3.0, MgSO4 1.2, KH2PO4 1.2, glucose 10.0, and Na2-EDTA, 0.5; and insulin, 10 units/l 31 and heparin 1,000 units/l. After trimming extraneous tissue from the heart, the aorta was attached to the perfusion apparatus, and the coronary bed was perfused in a Langendorff preparation with modified Krebs-Henseleit solution bubbled with 95% O2-5% CO2, pH 7.4. A probe placed in the right ventricle through the atrioventricular valve was used to record the intraventricular temperature, and by feedback regulation of the temperature of the water jackets surrounding the perfusion lines, the intracardiac temperature was maintained at 32°C. A peristaltic pump was used to hold coronary flow constant at approximately 10 ml/min, a rate that produced an initial perfusion pressure of about 50 mm Hg. Perfusion pressure was measured from a side arm in the perfusion line located at heart level. Coronary vascular resistance was calculated by dividing the perfusion pressure by the flow rate per gram of wet heart weight. The wet weight of the heart was determined at the end of each experiment. A partially inflated fluid-filled balloon attached to a pressure transducer was placed in the left ventricle through the mitral valve to record ventricular pressure. Balloon volume was adjusted to obtain a diastolic pressure (5–10 mm Hg) that produced maximum systolic pressure. A drain was placed in a puncture hole at the apex of the left ventricle to remove any fluid that might accumulate in the chamber. Bipolar surface electrocardiograms were continuously recorded from electrodes placed on the right atrium and left ventricle. Atrial and ventricular rates were determined from the frequency of P waves and QRS complexes on the ECG records. PR intervals were determined at timed intervals from ECG records obtained at paper speeds of 125 mm/sec. Hearts were perfused for 45 minutes before antigen challenge to allow stabilization of the measured variables. The procedures described above were used for previous studies of cardiac anaphylaxis. 12–14

Protocol for Antigen Challenge
After equilibration in control preparations (n = 17), 1.34 mg ovalbumin in 0.2 ml normal saline was injected into the aortic cannula. Changes in perfusion pressure, left ventricular pressure, atrial and ventricular beating rates, and PR intervals were monitored for 10 minutes after antigen challenge. Preliminary experiments indicated that challenge with these levels of antigen and antibody resulted in consistent anaphylactic responses with maximal increases in heart rate, coronary vascular resistance, and left ventricular systolic pressure. Because of tachyphylaxis to ovalbumin, hearts were challenged only once. Previous experiments had shown that guinea pig hearts passively sensitized to ovalbumin reacted to challenge with bovine serum albumin with less than a 5% change in heart rate, perfusion pressure, PR interval, and left ventricular systolic pressure. 12 Samples of venous effluent were collected on ice before and at timed intervals after antigen challenge. One-milliliter aliquots were acidified with 0.1 ml 2N

Downloaded from http://circres.ahajournals.org/ by guest on November 12, 2017
perchloric acid to prevent enzymatic degradation of histamine, adenosine, and inosine. Samples were then stored at -20°C until assayed. Results were expressed as either the concentration in the venous effluent or the release into the venous effluent. Release was calculated as the concentration in the venous effluent multiplied by the coronary flow rate per gram wet weight of heart. The total amount of histamine, adenosine, or inosine released during anaphylaxis was estimated by summation of the average content of the substance in the effluent within each collection interval. In the case of adenosine and inosine, basal release determined from preantigen levels was subtracted from the total release to obtain an estimate of the total change in release of adenosine and inosine induced by antigen challenge.

To determine to what extent the antigen-induced alterations in coronary vascular resistance and left ventricular systolic pressure were influenced by antigen-induced alterations in heart rate, atrial tissue was removed from five passively sensitized hearts, and ventricular rate was held constant by external pacing at 4.0 Hz. This pacing rate was chosen because it was above the maximum spontaneous ventricular rate achieved during cardiac anaphylaxis. Pacing was initiated 10 minutes before antigen challenge and continued for the remainder of the experiment.

Four different approaches were used to assess the effects of adenosine on cardiac anaphylaxis. In each case, hearts were perfused with a substance that might influence either the effects or levels of adenosine in the hearts. Perfusion with these substances began 10 minutes before the antigen challenge and was continued for the remainder of the experiment. These substances included 1) 100 μM theophylline (n = 10), an adenosine receptor antagonist,27 which at this concentration has been shown to significantly attenuate adenosine-induced effects upon isolated perfused rat5,28 and guinea pig22 hearts; 2) 10 μM SP-T (n = 7), an adenosine receptor antagonist,27 which at this concentration was found in five preliminary experiments to attenuate the decrease in coronary vascular resistance induced by perfusion of guinea pig hearts with 10 μM exogenous adenosine (from -17 ± 2% to -2 ± 5%, p < 0.03) and to eliminate the adenosine-induced development of atrioventricular nodal conduction blocks (from 100% to 0%, p < 0.00); 3) 10 μM EHNA (n = 10), an adenosine deaminase inhibitor,28 which at this concentration has been shown to significantly increase estimates of interstitial levels of adenosine in isolated perfused rat hearts29; and 4) 10 μM exogenous adenosine (n = 8).

Histamine Analysis

Histamine content of the samples of coronary effluent was determined by a manual fluorometric method.35,36 Histamine standards were included in each assay and carried through the extraction procedure. Addition of 10 μM adenosine did not alter the histamine standard curve. The total histamine content of eight individual guinea pig hearts (which were neither sensitized with antibody nor challenged with antigen), perfused as described above for 45 minutes, was determined in the following manner. Individual hearts were homogenized in 2–3 ml 0.4N perchloric acid. The homogenate was then centrifuged at 30,000g at 4°C for 15 minutes, and the supernatant was diluted with an equal volume of water and was assayed according to the methods identified above.

Adenosine and Inosine Analysis

Adenosine and inosine content of the acidified samples of venous effluent was determined by high-performance liquid chromatography (HPLC) techniques previously described.12 Briefly, 250-μl samples of acid-treated venous effluent were injected directly onto a reverse-phase, 5-μm C-18 HPLC column. The mobile solvent was programmed as a linear gradient changing from 95% 5 mM KH2PO4 and 5% of 100% methanol to 75% 5 mM KH2PO4 and 25% of 100% methanol during 25 minutes at a flow rate of 0.5 ml/min. Absorbance of the column eluate was continuously monitored at 254 nM. Absorbance peaks were identified by comparison with retention times and peak magnitudes of acidified known samples of adenosine and inosine.

Data Analysis

Results are expressed as mean ± SEM. Baseline values of the variables determined before interventions were made are designated as pre values. Values of the variables determined 10 minutes after addition of theophylline, SP-T, EHNA, or exogenous adenosine (just before antigen challenge) are designated as initial values. The maximum value of the variable achieved during the anaphylactic reaction is designated as the peak value. Paired t tests were used to compare pre values with initial values with determine whether any of the four interventions altered baseline levels of the variables being monitored. Paired t tests were also used to compare initial values with peak values to determine whether antigen challenge caused a significant change in the variable. Determination of differences in peak values between control and treated groups was assessed in two ways: 1) conservatively by analysis of variance (ANOVA) with Scheffe’s test for multiple comparisons between groups or 2) less conservatively with an unpaired t test with Bonferroni’s correction for multiple comparisons. Since statistical analysis may be controversial in some cases, levels of significance from both assessments are reported. In all cases, significant differences were unequivocally declared for p values less than 0.05 obtained with the conservative ANOVA test. Fisher’s exact test was used to determine whether the difference in incidence of arrhythmias was significant.

Results

Antigen-Induced Alterations in Control Experiments

Addition of antigen to the perfusate of isolated, passively sensitized, guinea pig hearts resulted in release of histamine, significant alterations in various functional characteristics, and substantial changes in aden-
osine and inosine levels in the venous effluent. The
time course of these events as they occurred in the
control experiments is indicated in Figure 1. Histamine
release from one of the preparations and coronary vas-
cular resistance determinations from three of the prep-
arations were not reliably obtained and therefore not
included in the data analyses. All data were included in
the analyses of the other variables.

Note that the release of histamine was very rapid and
was completed within 4 minutes of the antigen chal-
lenge (Figure 1A). The maximum concentration of
histamine achieved in the venous effluent after antigen
challenge was 246 ± 38 ng/ml. Increases in coronary
vascular resistance (Figure 1B) and atrial rate (Figure
1C) reached their maximum values at approximately 2
minutes after the antigen challenge, and although there
was a tendency to decline somewhat, both coronary
vascular resistance and atrial rate remained elevated
above initial values throughout the 10-minute observa-
tion period. Left ventricular systolic pressure in-
creased initially and then decreased below initial val-
ues to a minimum at 7 minutes (Figure 1D).

There was a significant increase in the PR interval
that was transient, reaching a peak at about 3 minutes
after antigen challenge and returning toward initial
levels near the end of the 10 minutes (Figure 1E).
Second- and third-degree atroventricular nodal blocks
were evident in a total of six of the 17 preparations
(Figure 1F) and appeared between 1.5 and 7.0 minutes
after antigen challenge. PR intervals were not deter-
mined in these preparations when conduction blocks
occurred, and therefore, the sample number in each
PR-interval data point of Figure 1E between 1.5 and
7.0 minutes is reduced from the total of 17 by the
number of preparations with conduction blocks at
this time.

Adenosine and inosine release also increased tran-
siently after antigen challenge and had returned to
initial levels by about 8 minutes after antigen challenge
(Figures 1G and 1H). The concentrations of adenosine
and inosine in the venous effluent before antigen
challenge were 33 ± 8 nM and 275 ± 38 nM, respec-
tively, and reached maximums of 578 ± 56 nM and
3,105 ± 394 nM, respectively, during the anaphylactic
reaction.

The peak venous adenosine and inosine concentra-
tions achieved during cardiac anaphylaxis of the six
preparations that did develop conduction blocks were
566 ± 74 nM and 3,904 ± 777 nM, respectively, while
those of the 11 preparations that did not develop con-

---

**Figure 1.** Time course of antigen-induced alterations in various characteristics of isolated, passively sensitized, perfused guinea pig hearts obtained under control conditions. (See text for details.)
duction blocks were 585 ± 79 nM and 2,660 ± 407 nM, respectively. Although the mean value of venous inosine achieved in hearts developing conduction blocks was somewhat higher than that of hearts without conduction block, the difference was not significant (p = 0.14).

The effect of pacing upon coronary vascular resistance and left ventricular systolic pressure and upon the changes that occur during anaphylaxis is indicated in Table 1. Changes in these variables that occurred during anaphylaxis of the spontaneously beating preparations are included in Table 1 for comparison. Note first that the initial coronary vascular resistance of the preparations to be paced was slightly lower than that of the spontaneously beating preparations, perhaps reflecting the fact that the atria were removed at the beginning of the equilibration period. Note also that initiation of pacing had no significant effect upon the coronary vascular resistance but did result in a significant decrease in systolic pressure. Finally, note that the antigen-induced responses of the paced preparations were similar to those of the unpaced preparations in that in both groups, significant increases in vascular resistance and systolic pressure occurred, although the magnitude of the vascular resistance change in the paced preparations was somewhat less than in the unpaced preparations. The increase in systolic pressure in the paced preparations was transient and returned to the preantigen challenge levels within 3–4 minutes but, unlike the spontaneously beating preparations, did not fall below these initial values. These data indicate that antigen-induced increases in coronary vascular resistance and systolic pressure in the spontaneously beating hearts are not the results of an increase in beating rate but rather the results of the direct effects of the various mediators of the anaphylactic reaction.

The delayed depression in systolic pressure, however, may reflect the effect of the sustained antigen-induced tachycardia.

**Effects of Adenosine-Modulating Interventions Upon Antigen-Induced Alterations**

Adenosine and inosine release. The effects of the various interventions upon initial concentrations of adenosine and inosine in the venous effluent are indicated in Table 2. Note that addition of theophylline and the theophylline analogue, SP-T, did not significantly alter the initial concentrations of adenosine, but for unknown reasons, SP-T did decrease the initial concentration of inosine. Addition of EHNA increased the adenosine concentration and decreased inosine concentration, which is consistent with its action as an adenosine deaminase inhibitor. Addition of 10 μM exogenous adenosine increased venous levels of adenosine to 5.56 μM before antigen challenge. Since venous levels of inosine also increased substantially, we conclude that the infused adenosine was exposed to an endogenous adenosine deaminase.

---

**Table 1. Coronary Vascular Resistance and Left Ventricular Systolic Pressure Before and After Antigen Challenge of Paced (4.0 Hz) and Unpaced Isolated, Passively Sensitized, Guinea Pig Hearts**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Paced preparations</th>
<th>Unpaced preparations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Initial</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>135 ± 14</td>
<td>240</td>
</tr>
<tr>
<td>CVR (mm Hg/ ml/min/g)</td>
<td>4.31 ± 0.33</td>
<td>4.41 ± 0.34</td>
</tr>
<tr>
<td>LVSP (mm Hg)</td>
<td>109 ± 8</td>
<td>78 ± 3*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Paced preparations, n = 5; unpaced preparations, n = 17 for heart rate and LVSP. n = 14 for CVR. Values in parentheses represent minimum LVSP obtained during the 10 minutes after antigen challenge. Pre values obtained just before pacing and 10 minutes before antigen challenge; Initial values obtained just before antigen challenge; Peak values obtained in response to antigen challenge.

CVR, coronary vascular resistance; LVSP, left ventricular systolic pressure.

†p<0.05 as compared with Pre value. †p<0.05 as compared with Initial value.

---

**Table 2. Effect of Various Adenosine-Modulating Interventions on the Concentration of Adenosine and Inosine in Venous Effluent of Isolated Perfused Guinea Pig Hearts**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>[Adenosine], nM</th>
<th>[Inosine], nM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Initial</td>
</tr>
<tr>
<td>Control (n = 17)</td>
<td>. . .</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>+ THEO, 100 μM (n = 10)</td>
<td>36 ± 17</td>
<td>43 ± 14</td>
</tr>
<tr>
<td>+ SP-T, 10 μM (n = 7)</td>
<td>28 ± 4</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>+ EHNA, 10 μM (n = 10)</td>
<td>37 ± 7</td>
<td>71 ± 17*</td>
</tr>
<tr>
<td>+ ADO, 10 μM (n = 8)</td>
<td>27 ± 5</td>
<td>5,559 ± 266*</td>
</tr>
</tbody>
</table>

Adenosine and inosine levels were determined in the venous effluent before addition of the adenosine-modulating substances (Pre) and 10 minutes later just before antigen challenge (Initial).

THEO, theophylline; SP-T, 8-(4-sulfophenyl) theophylline; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenosine hydrochloride; and ADO, adenosine.

*p<0.05, as compared with Pre value (paired t test).
Antigen challenge evoked a significant increase in release of adenosine and inosine in all groups \((p<0.05, \text{paired } t \text{ test})\). The antigen-induced release of adenosine and inosine followed approximately the same time course in all experimental groups as in the control group (Figures 2A and 2B). The data in Figure 2C represent the total change in adenosine and inosine release from the hearts induced by antigen challenge. Note that neither theophylline nor its analogue affected the total change in antigen-induced release of adenosine or inosine. Addition of EHNA, however, increased the total change in antigen-induced adenosine release (Scheffe \(p<0.03\), Bonferroni \(p<0.001\)) and tended to decrease the total change in antigen-induced inosine release (Scheffe \(p<0.20\), Bonferroni \(p<0.04\)). These results are consistent with an EHNA-induced inhibition of adenosine deaminase. The sum of the total change in antigen-induced adenosine and inosine release in the presence of EHNA was not different from the control experiments. Addition of exogenous adenosine resulted in an increase in the total change in antigen-induced release of both adenosine (Scheffe \(p<0.03\), Bonferroni \(p<0.05\)) and inosine (Scheffe \(p<0.03\), Bonferroni \(p<0.02\)).

**Histamine release.** The time course of release of histamine after antigen challenge in the treated groups was similar to that of the control group as shown in Figure 3A. The average total histamine released by antigen challenge in any of the groups represented a range from 30% to 63% of the average total histamine content of guinea pig hearts (Figure 3B). The total amount of histamine released by antigen challenge was increased significantly above that of the control group by addition of exogenous adenosine (Scheffe \(p<0.05\), Bonferroni \(p<0.01\)) and tended to be increased by addition of EHNA (Scheffe \(p<0.20\), Bonferroni \(p<0.04\)). The reason for the high variability in the SP-T group was not apparent.

**Atrial rate.** The time course of changes in atrial rate induced by antigen challenge in all groups was similar to that of the control group as shown in Figure 4A. None of the interventions had a significant effect upon the initial atrial rate or upon the maximum antigen-induced increase in atrial rate (Figure 4B). Except for the preparations perfused with exogenous adenosine or those that developed atrioventricular conduction blocks during anaphylaxis, ventricular rate was the same as the atrial rate shown in Figure 4A. Addition of exogenous adenosine produced second- and third-degree blocks in all preparations before antigen challenge so that the ventricular rate often became irregular and, at an average rate of 74 ±4 beats/min, was significant.

---

**Figure 2.** Effects of antigen challenge on adenosine and inosine release from isolated, passively sensitized, perfused guinea pig hearts obtained under control conditions (CTL) and in the presence of 100 \(\mu M\) theophylline (THEO), 10 \(\mu M\) 8-(4-sulfophenyl) theophylline (SP-T), 10 \(\mu M\) erythro-9-(2-hydroxy-3-nonyl)adenosine hydrochloride (EHNA), or 10 \(\mu M\) adenosine (ADO). Panel A: Time course of adenosine release after antigen challenge. Panel B: Time course of inosine release after antigen challenge. Panel C: Total change in release of adenosine (ADO), inosine (INO), and adenosine + inosine (ADO + INO) obtained over the 10-minute interval after antigen challenge. * and ** \(p<0.05\) as compared with value obtained under control conditions (\(t\) test with Bonferroni correction and ANOVA followed by Scheffe test, respectively).
Heller and Regal

Adenosine's Role in Cardiac Anaphylaxis 1153

Minutes After Antigen Challenge

FIGURE 3. Effect of antigen challenge on histamine release from isolated, passively sensitized, perfused guinea pig hearts obtained under control conditions (CTL) and during perfusion with 100 μM theophylline (THEO), 10 μM 8-(4-sulfophenyl) theophylline (SP-T), 10 μM erythro-9-(2-hydroxy-3-nonyl)adenosine hydrochloride (EHNA), or 10 μM adenosine (ADO). Panel A: Time course of changes in histamine release after antigen challenge. Panel B: Total histamine release obtained over the 10-minute interval after antigen challenge (solid bars) in the various groups and total histamine content of perfused unsensitized and unchallenged guinea pig hearts (hatched bar). * and ** p<0.05 as compared with value obtained under control conditions (t test with Bonferroni correction and ANOVA followed by Scheffe test, respectively). Percent designations above each solid bar represent comparison of mean value to average total histamine content of unchallenged hearts.

FIGURE 4. Effect of antigen challenge on atrial rate of isolated, passively sensitized, perfused guinea pig hearts obtained under control conditions (CTL) and during perfusion with 100 μM theophylline (THEO), 10 μM 8-(4-sulfophenyl) theophylline (SP-T), 10 μM erythro-9-(2-hydroxy-3-nonyl)adenosine hydrochloride (EHNA), or 10 μM adenosine (ADO). Panel A: Time course of changes in atrial rate after antigen challenge. Panel B: Atrial rate before addition of the substance (PRE), atrial rate 10 minutes after addition of the substance and just before antigen challenge (INITIAL), peak atrial rate achieved in response to antigen challenge (PEAK). *p<0.05 as compared with INITIAL value within that group (paired t test).

ly slower than the atrial rate. In these preparations treated with exogenous adenosine, the conduction blocks remained throughout the anaphylactic reaction during which time the ventricular rate increased to a maximum of 226±6 beats/min.

PR intervals and atrioventricular conduction blocks. The time course of antigen-induced changes in the PR interval in preparations treated with theophylline, SP-T, and EHNA was generally similar to that of the control group, as shown in Figure 5A. Because of the development of conduction blocks after antigen challenge in some preparations, the actual sample number in the data points from 1.5 to 7.0 minutes after antigen challenge will be less than the total in that group as indicated in Table 3. Addition of theophylline, theophylline analogue, or EHNA had no significant effects upon initial PR intervals as indicated in Figure 5B and did not evoke any arrhythmias before antigen challenge. As noted above, addition of exogenous adenosine resulted in second- or third-degree conduction blocks in all eight preparations before antigen challenge. The maximum antigen-induced prolongation in the PR interval of the preparations (which, in preparations with conduction blocks, occurred either just before the block began or just after it ended) was significantly attenuated in the presence of theophylline (Scheffe p<0.05, Bonferroni p<0.02) or SP-T (Scheffe p<0.03, Bonferroni p<0.01) but was not influenced by EHNA. The antigen-induced incidence and average duration of second- or third-degree conduction blocks in all eight preparations before antigen challenge. The maximum antigen-induced prolongation in the PR interval of the preparations (which, in preparations with conduction blocks, occurred either just before the block began or just after it ended) was significantly attenuated in the presence of theophylline (Scheffe p<0.05, Bonferroni p<0.02) or SP-T (Scheffe p<0.03, Bonferroni p<0.01) but was not influenced by EHNA. The antigen-induced incidence and average duration of second- or third-degree conduction blocks is given in Table 2. (All experiments were included for determination of the average duration of the block.) There was no statistically significant difference in incidence or average duration of these arrhythmias between groups. However, the com-
The time course of changes in left ventricular systolic pressure after antigen challenge in all but one of the experimental groups was similar to that of the control group (Figure 7A). As can be seen from Figure 7B, the only intervention that influenced the initial value of systolic pressure was addition of theophylline, which resulted in a slight but significant increase in pressure development (paired t test). The antigen-induced increase in systolic pressure was attenuated in the presence of the SP-T (Scheffe p<0.01, Bonferroni p<0.01) but was not

### Table 3. Incidence and Duration of Atrioventricular Conduction Blocks Induced by Antigen Challenge of Isolated, Passively Sensitized, Guinea Pig Hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>+THEO</th>
<th>+SP-T</th>
<th>+EHNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence (n/total n)</td>
<td>6/17</td>
<td>3/10</td>
<td>0/7</td>
<td>6/10</td>
</tr>
<tr>
<td>Duration* (minutes)</td>
<td>1.1±0.5</td>
<td>0.5±0.3</td>
<td>0</td>
<td>1.2±0.4</td>
</tr>
</tbody>
</table>

*Duration of block is calculated from the total n in each group.
THEO, theophylline; SP-T, 8-(4-sulphophenyl) theophylline; and EHNA, erythro-9-(2-hydroxy-3-nonyl)adenosine hydrochloride.
altered by the other interventions. The magnitude of the delayed decrease in systolic pressure was not altered by any of the interventions.

Discussion

Responses of the isolated, sensitized guinea pig hearts to antigen challenge under control conditions (shown in Figure 1) are similar to those reported in a previous study from this laboratory and by others. The present study comparing these control responses (shown in Figure 1) are similar to those reported in a previous study from this laboratory and by others. The effectiveness of SP-T in blocking the antigen-induced alterations in impulse conduction in the heart may be primarily due to released histamine. They based this conclusion upon three lines of evidence: 1) arrhythmias produced by exogenous histamine are qualitatively similar to those produced by immunological challenge, 2) the alterations are directly proportional to the amount of histamine released from the isolated heart during anaphylaxis, and 3) antihistamines can prevent the development of the arrhythmias. Studies by Wiedmeier and Spell indicate that exogenous histamine results in significant increases in adenosine released from isolated perfused hearts. This information, combined with the present observation that theophylline and SP-T attenuated the atrioventricular conduction delays and blocks during cardiac anaphylaxis without altering the histamine release, suggests that it is not the histamine that is actually producing the atrioventricular conduction defects, but rather it is the endogenous adenosine produced in response to the other effects of histamine (and probably other mediators as well).

Endogenous Adenosine Promotes Development of Atrioventricular Conduction Delays and Blocks During Cardiac Anaphylaxis

This conclusion is based upon the previous observations that exogenous adenosine produces atrioventricular nodal conduction delays and blocks in guinea pig hearts and the present observations that 1) substantial amounts of adenosine are released during anaphylaxis with a time course that is similar to the PR-interval prolongation and incidence of atrioventricular blocks (Figure 1); 2) theophylline and SP-T, which significantly attenuate the effect of exogenous adenosine on development of conduction block in this preparation, also attenuate the antigen-induced increase in PR intervals (Figures 5A and 5B); 3) SP-T eliminated the development of atrioventricular conduction blocks (Table 3); and 4) EHNA tended to increase the incidence of conduction blocks (Table 3). These findings are consistent with previous studies suggesting that endogenous adenosine may be an important modulator of atrioventricular nodal function under hypoxic conditions and confirm preliminary reports that adenosine may also contribute to atrioventricular conduction blocks during cardiac anaphylaxis.
Endogenous Adenosine Has Little or No Effect on Changes That Occur in Atrial Automaticity, Left Ventricular Systolic Pressure, or Coronary Vascular Resistance During Cardiac Anaphylaxis

We originally hypothesized that the high adenosine levels that were achieved during the anaphylactic reaction might have some modulating influence upon the antigen-induced alterations in other cardiac functional variables. Specifically, since adenosine is a vasodilator, and has negative chronotropic effects, we expected that the antigen-induced increase in coronary vascular resistance, systolic pressure, and atrial rate would be enhanced by the presence of the adenosine receptor antagonists theophylline and SP-T. We also expected that the increased adenosine levels achieved in the presence of EHNA and exogenous adenosine during anaphylaxis (Figure 2) might have attenuated the antigen-evoked increase in coronary vascular resistance, systolic pressure, and sinus rate.

In the present studies, the predicted effects of the adenosine receptor antagonists on antigen-induced alterations in beating rate, coronary vascular resistance, and left ventricular systolic pressure clearly were not obtained. The antigen-induced increases in atrial rate, vascular resistance, and systolic pressure were not enhanced by theophylline or SP-T (Figures 4B, 6B, and 7B). In fact, in the presence of theophylline, the antigen-induced increase in coronary vascular resistance was significantly attenuated (Figure 6B), and in the presence of SP-T, the antigen-induced increases in left ventricular systolic pressure were actually eliminated (Figure 7B). Since antigen-induced changes in coronary vascular resistance and systolic pressure in the spontaneously beating preparations are similar to those achieved in paced preparations (comparing data from Figure 1 and Table 1), it is unlikely that the slight differences in the ventricular beating rate between the control preparations and those treated with theophylline and SP-T that were due to the variable appearance of conduction blocks during anaphylaxis could account for the unexpected effects of theophylline and SP-T. These attenuating effects of theophylline and SP-T upon antigen-induced alterations in coronary vascular resistance and systolic pressure, respectively, are interesting, but they are unexplainable findings that may well be related to other effects of these agents (e.g., an increase in cyclic AMP by theophylline) that are not directly related to their antagonistic influence upon adenosine receptors.

The enhanced adenosine levels observed during anaphylaxis in the presence of EHNA and exogenous adenosine also did not produce the expected effects. Neither of the interventions produced the expected attenuations of the antigen-induced increases in atrial rate, coronary vascular resistance, or left ventricular systolic pressure (Figures 4B, 6B, and 7B). It should be noted, however, that some (or all) of these proposed adenosine-induced attenuations might have been cancelled out by the adenosine-induced augmentation of histamine release that occurred with EHNA and exogenous adenosine perfusion as discussed below.

These results might suggest that under anaphylactic conditions, theophylline and SP-T do not block the effects of endogenous adenosine and that EHNA does not increase interstitial adenosine concentration in the vicinity of the effector cells. However, we think it more likely that the powerful vasoconstrictive, positive chronotropic and initial positive inotropic effects of various mediators of cardiac anaphylaxis (e.g., histamine, thromboxane, prostaglandins, and leukotrienes) simply overwhelm the vasodilatory, negative chronotropic and indirect negative inotropic effects of the increased adenosine levels. The only significant observable effect of the elevated adenosine levels during anaphylaxis is the pronounced negative dromotropic action.

Adenosine Production During Anaphylaxis Does Not Influence Histamine Release, but Elevation of Adenosine Levels Before Antigen Challenge Enhances Histamine Release

The first part of this conclusion is based upon the observations that maximum histamine release after antigen challenge always preceded maximum adenosine release and that the time course of histamine release after antigen challenge was not altered by any of the interventions. The second part of the conclusion is based upon the observations that treatment with EHNA and exogenous adenosine that increased initial prechallenge concentrations of adenosine in the venous effluent (Table 2) also was associated with enhanced histamine release upon antigen challenge (Figures 3A and 3B). These findings are consistent with previous studies indicating that exogenous adenosine enhanced antigen-induced release of histamine from rat mast cells, from guinea pig lung tissue, and when added at the time of antigen challenge, from human basophil cells and human lung mast cells. It should be noted, however, that the presence of adenosine before antigen challenge has also been found to inhibit histamine release from the human cells.

The total histamine content of guinea pig hearts was similar to that reported by others. The proportional amount of histamine released under control conditions in these preparations (34% of the total) is similar to that reported by Capurro and Levi to be released from guinea pig hearts during in vivo fatal systemic anaphylaxis (31% of the total). The adenosine-induced enhancement of histamine release with EHNA and exogenous adenosine to 51% and 63% of the total, respectively, suggest that there may be substantial modulation of this variable.

Our study also suggests that adenosine's potentialization of histamine release in this model is probably not mediated through A1 or A2 receptor mechanisms. If such receptors were involved, we might have expected the nonspecific adenosine receptor antagonists theophylline and SP-T to decrease the antigen-induced histamine release. Such a decrease was not evident from our data (Figure 3B). However, because of the wide variability in the antigen-induced histamine release of the SP-T-treated preparations, we are hesitant to draw specific conclusions from these data. It may also be
that it was only in the presence of EHNA or exogenous adenosine that the tissue levels of adenosine before antigen challenge were high enough to influence histamine release. Studies of theophylline antagonism of adenosine's effects on histamine release in other models are not consistent. Results of earlier studies indicated that theophylline attenuated adenosine modulation of histamine release from isolated rat mast cells, human basophils, and human mast cells. However, Church et al recently reported that 8-phenyl-theophylline did not influence adenosine potentiation of immunologically induced mediator release from rat mast cells. In addition, Lohse et al recently found that methylxanthines did not block the adenosine potentiation of calcium ionophore- or concanavalin A-induced histamine release from rat mast cells. Our data support the conclusions of these most recent studies that adenosine's effect on histamine release is not mediated through A1 or A2 receptors but rather may be dependent upon action at an intracellular site.

Assumptions

The conclusions of our study are based upon the assumption that adenosine levels in the coronary effluent reflect, by some constant factor, the interstitial levels of adenosine in the vicinity of the various effector cells (e.g., vascular smooth muscle, atrioventricular nodal tissue, pacemaker cells, myocardial cells, and mast cells). We concede that this assumption may not be valid. The vascular endothelium under control conditions represents a considerable metabolic barrier for adenosine. It is likely that, under steady-state conditions, venous adenosine concentration significantly underestimates the interstitial adenosine concentration produced by endogenous processes and overestimates that concentration produced by adenosine infusion. The fact that in the present study we found perfusate adenosine concentration to decrease from 10 μM to 5.5 μM as it passed through the coronary bed indicates that significant uptake and/or degradation processes are present in this preparation. It should also be pointed out that during an anaphylactic reaction, significant changes in the metabolic and physical characteristics of the vascular endothelial barrier may occur. If so, variations in adenosine release may be misleading. However, in the absence of any specific information about anaphylaxis-induced alteration in the endothelial barrier for adenosine, we have assumed that changes in adenosine release are proportional to changes in tissue levels of adenosine.

Adenosine release from these hearts under control conditions before antigen challenge was somewhat higher than that reported for guinea pig hearts perfused at constant pressure. This discrepancy may result from several differences in experimental conditions (e.g., 32°C versus 37°C, perfusate contents, and ventricular loading conditions) as well as from the lack of ability of the hearts in the present study to autoregulate flow to meet metabolic needs. The observations that both theophylline and SP-T additions increased the coronary vascular resistance before antigen challenge (Figure 4) suggests that under initial conditions, endogenous adenosine levels were high enough to have vasodilatory effects.

Release of adenosine after antigen challenge under control conditions is substantial and most likely reflects the severity of the mismatch between oxygen supply and demand. Although the absolute peak magnitude of adenosine release after antigen challenge (4.50 ± 0.48 nmol/min/g) is significantly greater than that reported to occur with challenges such as norepinephrine, severe hypoxia, or isoproterenol (1.5, 1.6, and 1.9 nmol/min/g, respectively), the relative increase in adenosine release (15-fold) was similar to those reported in these studies (18-fold, 30-fold, and 14-fold, respectively). The enhancement of the antigen-induced increase in release of adenosine in the presence of exogenous adenosine (Figure 2C) is not clearly understood. It is not likely that this enhancement reflects saturation of an adenosine deaminase system since our data also show that the antigen-induced change in inosine release in the presence of exogenous adenosine is also greatly enhanced (Figure 2C).

A second assumption that we have used is that the effects we are noting are primarily due to adenosine. However, it has been reported that, at high concentrations, exogenous inosine may act as a coronary vasodilator and, furthermore, may influence histamine release from rat mast cells. The source of the inosine in the venous effluent of the hearts in the present study is not fully understood. The decrease in inosine concentration in the presence of EHNA is consistent with the suggestion that at least part of the inosine is a result of deamination of adenosine. However, inosine may also arise from activity of a cytoplasmic 5' nucleotidase acting preferentially upon inosine monophosphate. Given the high levels of inosine in the coronary effluent under initial conditions and especially after antigen challenge, it is indeed possible that this substance may also have significant effects.

Acknowledgments

We appreciate the expert advice of Dr. Ronald R. Regal of the Statistical Center of the University of Minnesota, Duluth. We also thank Richard A. Nelson for his excellent technical assistance with this study and Carol Peterman and Nancy LaVoy for their careful attention to the preparation of the manuscript.

References

42. Schrader J, Haddy FJ, Gerlach E: Release of adenosine, inosine and hypoxanthine from the isolated guinea pig heart during hypoxia, flow-autoregulation and reactive hyperemia. Pflogers Arch 1977;369:1–6

KEY WORDS • adenosine • arrhythmia • atrioventricular conduction • histamine • anaphylaxis • immediate hypersensitivity • theophylline • coronary vascular resistance • cardiac muscle
Effect of adenosine on histamine release and atrioventricular conduction during guinea pig cardiac anaphylaxis.
L J Heller and J F Regal

doi: 10.1161/01.RES.62.6.1147

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/62/6/1147