Hemodynamic Effects of Adenosine in Conscious Hypertensive and Normotensive Rats

AKIHIRO OHNISHI, ITALO BIAGGIONI, GILBERT DERAY, ROBERT A. BRANCH, AND EDWIN K. JACKSON

SUMMARY Mean arterial pressure and heart rate were measured during intra-aortic arch (i.a.a.), intravenous, and suprarenal artery (s.r.a.) infusions of adenosine in conscious, unrestrained normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) in the absence and presence of ganglionic blockade. In both groups, i.a.a. and i.v. infusions of adenosine induced comparatively larger dose-dependent reductions in mean arterial pressure than did s.r.a. infusions. In WKY, i.a.a. and i.v. infusions of adenosine were equipotent in reducing mean arterial pressure. In contrast, i.a.a. infusion of adenosine was approximately twice as potent as i.v. infusion in SHR. Also, SHR were approximately 6.5 and 2.6 times more sensitive to i.a.a. and i.v. infusions of adenosine, respectively, than were WKY. Further, i.a.a. and s.r.a. infusions of adenosine caused tachycardia in WKY, while i.v. infusions did not alter heart rate. In SHR, neither i.a.a. nor s.r.a. infusion of adenosine altered heart rate, but i.v. infusion induced a profound bradycardia. In ganglionic-blocked WKY that received a norepinephrine infusion to restore blood pressure and heart rate to pre-ganglionic blockade levels, depressor responses to i.a.a. infusion of adenosine were unchanged while the increase in heart rate was abolished. In SHR, ganglionic blockade markedly decreased the depressor response to i.a.a. and i.v. infusions of adenosine and abolished the bradycardic response to i.v. infusion. These results suggest that adenosine is an effective hypotensive agent in both WKY and SHR; however, marked between-strain differences exist in the cardiovascular response to adenosine. These differences most likely are due to changes in adenosine-pulmonary interactions and increases in the importance of adenosine-autonomic interactions in SHR. (Hypertension 8: 391-398, 1986)

KEY WORDS • adrenergic neurotransmission • autonomic nervous system • ganglionic blockade • antihypertensive agents • pulmonary endothelium

ADENOSINE is a ubiquitously occurring natural substance with profound effects on the cardiovascular system. Given parenterally in pharmacological doses, it is a potent vasodilator and produces a marked reduction in blood pressure.1-6 The hemodynamic effects of adenosine may be the result of one or more of its many actions. Adenosine has a direct relaxing action on vascular smooth muscle,7-8 which causes vasodilatation in most vascular beds. Adenosine has been shown to inhibit neurotransmitter release from noradrenergic nerve terminals in isolated organs9-12 and to oppose the actions of catecholamines in the heart.13 Adenosine also inhibits renin release from the kidneys.14-16 Although most of adenosine’s actions are hypotensive, when infused intrarenally in some animal models it will produce hypertension secondary to activation of renal afferent nerves.17,18

Most of the studies dealing with the hemodynamic effects of adenosine have been performed in normotensive, anesthetized animals. Both the state of anesthesia and the presence or absence of arterial hypertension might be expected to modify cardiovascular responses to adenosine. Since adenosine analogues may have utility as antihypertensive drugs, it is important to determine the cardiovascular actions of adenosine in the conscious, hypertensive state. Therefore, the present study investigated the cardiovascular actions of adenosine infusions in conscious, unrestrained, normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR).

In the investigation of the cardiovascular effects of adenosine, an important methodological consideration is the site of infusion. As adenosine is rapidly taken up...
by cells and metabolized intracellularly, it has a very short half-life. Consequently, the highest concentration of adenosine will be achieved in those tissues most proximal to the catheter tip. Therefore, the qualitative or quantitative response to adenosine may vary according to the site of infusion. Rather than arbitrarily select a single site of infusion, we chose to infuse adenosine at three separate sites: the aortic arch, the vena cava, and the abdominal aorta just proximal to the renal arteries.

A second objective of this investigation was to determine the relative contribution of the autonomic nervous system to the overall hemodynamic response to adenosine. Adenosine is a potent and effective inhibitor of noradrenergic neurotransmission. Consequently, if basal sympathetic tone is high, a substantial portion of the hypertensive or bradycardic response to adenosine might be mediated by inhibition of sympathetic neurotransmission. To test this hypothesis, the hemodynamic responses to adenosine in SHR and normotensive rats were compared before and after ganglionic blockade with chlorisondamine. To avoid interpretational problems due to baseline shifts, hemodynamic parameters were restored to normal in chlorisondamine-treated animals by a continuous infusion of norepinephrine.

Materials and Methods

Studies were performed on 18 male SHR (318 ± 4 [SD] g) and 13 male WKY (351 ± 10 g) obtained from Taconic Farms (Germantown, NY, USA). Rats were maintained on a diet containing Na+, 170 mEq/kg, and K+, 246 mEq/kg (Allied Mills, Chicago, IL, USA) and were given tap water ad libitum. Animals were anesthetized with pentobarbital (50 mg/kg i.p.), and a polyethylene 50 catheter (inside diameter, 0.1 mm; outside diameter, 0.3 mm) was implanted in the aortic arch through the right carotid artery for intra-aortic arch (i.a.a.) infusions, in the femoral vein for intravenous infusions, and in the abdominal aorta just above the renal arteries through the femoral artery for suprarenal aortic (s.r.a.) infusions. In some experiments both femoral veins were cannulated. The positions of the catheters were confirmed at autopsy. The catheters were brought subcutaneously to the back of the neck and exteriorized. They were protected with a metal coil attached to the animal with a jacket and connected to a miniature channel swivel (Alice King Chatham Medical Arts, Los Angeles, CA, USA). The rats could move freely, eat, and drink with this system in place. After the implantation procedure, the rats were allowed to recover for at least 24 hours.

Mean arterial pressure (MAP) and heart rate (HR) were monitored through one of the arterial catheters connected to a Hewlett-Packard pressure transducer interfaced to a Hewlett-Packard physiograph (Andover, MA, USA). All adenosine infusions were performed at a fixed rate of 0.04 ml/min using a Harvard syringe pump (South Natick, MA, USA).

Dose-response relationships to adenosine were obtained at three different sites of adenosine infusion in six SHR and seven WKY. Adenosine (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in normal saline in concentrations of up to 10 mg/ml. Appropriate dilutions in normal saline were made for the individual doses. After a baseline infusion of saline, six doses of adenosine were infused at a given site in a cumulative fashion: 0.01, 0.033, 0.066, 0.1, 0.33, 0.66, and 1.0 mg/kg/min; each dose was infused for 15 minutes. After a 15-minute washout, the site of infusion was changed and the procedure was repeated. The order of i.v., i.a.a., and s.r.a. infusions was randomized. Both MAP and HR were monitored throughout the study.

In a separate series of experiments, the relative contribution of sympathetic tone to the inhibitory effect of adenosine on blood pressure was studied in six SHR and six WKY. Cumulative dose responses to adenosine infused into the aortic arch were studied before and during ganglionic blockade with chlorisondamine (Ciba-Geigy, Summit, NJ, USA), 10 mg/kg s.c. Cumulative dose responses to i.v. infusions of adenosine also were measured in six additional SHR before and after ganglionic blockade. In each experiment using ganglionic blockade, blood pressure and HR were restored to baseline values by an i.v. infusion of norepinephrine (Sigma).

The relationships between MAP or HR and dose of adenosine at different sites of infusion were compared using a Model I two-factor analysis of variance with replication. In this analysis, Factor A (fixed) was the site of infusion (3 levels) and Factor B (fixed) was the dose of adenosine (8 levels) and Factor B (fixed) was the site of infusion (3 levels). If this analysis indicated a significant difference among the three relationships, the residual mean square from the analysis of variance was applied in a Student Newman-Keuls test to determine which curves were different. A similar analysis was used to compare MAP or HR rate with adenosine dose relationships before and after ganglionic blockade, except that multiple range testing was unnecessary, since Factor B consisted of only two levels. Within a particular adenosine dose versus HR or blood pressure curve, the HR or MAP during saline infusion was compared with the HR or MAP obtained during adenosine infusions using a Model III two-factor analysis of variance without replication. In this analysis, Factor A (random) was the animal (6 or 7 levels) and Factor B (fixed) was the dose of adenosine (8 levels). If this analysis revealed a significant difference among the levels of Factor B, the residual mean square from the analysis of variance was applied in a Dunnett's test to determine which doses of adenosine produced significant changes in HR or MAP compared with control. Statistical analyses were performed as described by Zar. All null hypotheses were two-tailed, and the criterion of significance was a p level less than 0.05. Computations were performed on the Vanderbilt Digital Electronics Corporation 1099 computer (Maynard, MA, USA) using the Statistical Package for the Social Sciences.
Results

Baseline values for MAP and HR just before each infusion in WKY and SHR are given in Table 1. In normotensive WKY, i.a.a. infusions of adenosine induced dose-dependent reductions in MAP (Figure 1); an infusion rate of 0.52 ± 0.11 mg/kg/min was required to reduce MAP by 25% (Table 2). The i.v. infusions of adenosine in WKY also caused dose-dependent reductions in MAP that were nearly identical to the i.a.a. dose responses (see Figure 1 and Table 2). Although s.r.a. infusions produced dose-dependent reductions in MAP, the responses to s.r.a. infusions of adenosine were comparatively less for any given dose than those induced by i.a.a. and i.v. infusions.

In SHR, adenosine infusion also produced a dose-dependent decrease in MAP (see Figure 1). However, i.a.a. infusions at a rate of 0.08 ± 0.02 mg/kg/min induced a 25% reduction in MAP, a value significantly different from that observed in WKY (p < 0.01, Student's t test; see Table 2). Both i.a.a. and i.v. infusions of adenosine in SHR produced significantly different responses from those seen in WKY. The dose-response curve shifted to the right following i.v. infusion compared with the response after i.a.a. infusion, and a twofold increase in the dose was required to achieve a 25% reduction in MAP in the same rat (p < 0.01). The s.r.a. infusions of adenosine in SHR induced lesser hypotensive responses than did i.a.a. or i.v. infusions (see Figure 1).

The HR response to adenosine infused at different sites varied between WKY and SHR (Figure 2). The i.a.a. and s.r.a. infusions in WKY induced small but significant increases in HR. In contrast, i.v. infusions at concentrations that induced reductions in MAP

<table>
<thead>
<tr>
<th>Adenosine (mg/kg/min)</th>
<th>Infusion</th>
<th>Control</th>
<th>Pre-GB</th>
<th>GB</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.a.a.</td>
<td>SHR (n = 6)</td>
<td>0.08 ± 0.02</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>WKY (n = 7)</td>
<td>0.52 ± 0.11†</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>i.v.</td>
<td>SHR (n = 6)</td>
<td>0.19 ± 0.06*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>WKY (n = 7)</td>
<td>0.50 ± 0.141</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>i.a.a.</td>
<td>SHR (n = 6)</td>
<td>—</td>
<td>0.10 ± 0.02</td>
<td>0.41 ± 0.08§</td>
</tr>
<tr>
<td></td>
<td>WKY (n = 6)</td>
<td>—</td>
<td>0.43 ± 0.09</td>
<td></td>
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</tbody>
</table>

Values are means ± SEM. Dash indicates that the category is not applicable.

GB = ganglionic blockade; i.a.a. = intra-aortic arch.

*p < 0.05, compared with i.a.a. infusion values.
†p < 0.01, compared with pre-GB values.
§p < 0.05, compared with values in SHR.
||p < 0.01, compared with pre-GB values.

Values are means ± SEM. MAP = mean arterial pressure; HR = heart rate; i.a.a. = intra-aortic arch; s.r.a. = suprarenal artery.
equivalent to those produced by i.a.a. infusions did not affect HR in WKY. In SHR, neither i.a.a. nor s.r.a. infusions of adenosine altered HR, despite the fact that i.a.a. infusion of adenosine caused substantial reductions in MAP. In SHR, the i.v. route of administration was associated with a significant dose-dependent reduction in HR (see Figure 2).

The MAP and HR values before and after ganglionic blockade and after restoration of MAP and HR with norepinephrine are shown in Table 3. The norepinephrine dose needed to restore MAP and HR to baseline values was the same in SHR and WKY. Dose-response curves for i.a.a. infusions of adenosine before and after ganglionic blockade are shown in Figures 3 and 4. Ganglionic blockade produced a parallel shift to the right of the MAP dose-response curve in SHR with a fourfold increase in the dose required to achieve a 25% reduction in MAP (see Table 2). In contrast, ganglionic blockade in WKY did not alter the sensitivity to i.a.a. infusion of adenosine (see Figure 3 and Table 2).

Ganglionic blockade blunted the tachycardic effect of i.a.a. infusion of adenosine in the WKY but did not influence HR either before or during ganglionic blockade in the SHR (see Figure 4). As shown in Figure 5, ganglionic blockade in SHR resulted in a shift to the right of the MAP dose-response curve to i.v. infusion of adenosine and abolished the bradycardic response to adenosine.

Discussion

Adenosine, infused either intravenously or into the aortic arch, produced a substantial hypotensive effect in conscious, unrestrained SHR and normotensive WKY. These data clearly indicate that, under normal physiological conditions, adenosine is a potent and efficacious hypotensive agent in both normotensive and hypertensive animals. Even so, the cardiovascular response to adenosine differs in several important as-

<table>
<thead>
<tr>
<th>Infusion</th>
<th>MAP (mm Hg)</th>
<th>HR (beats/min)</th>
<th>NE (μg/kg/hr)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-GB</td>
<td>Post-GB</td>
<td>After NE</td>
</tr>
<tr>
<td>i.a.a.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>186±9</td>
<td>110±6</td>
<td>182±9</td>
</tr>
<tr>
<td>WKY (n = 6)</td>
<td>130±3</td>
<td>79±2</td>
<td>131±4</td>
</tr>
<tr>
<td>i.v.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR (n = 6)</td>
<td>170±8</td>
<td>103±5</td>
<td>174±7</td>
</tr>
</tbody>
</table>

All data are means ± SEM. MAP = mean arterial pressure; HR = heart rate; GB = ganglionic blockade; NE = norepinephrine; i.a.a. = intra-aortic arch.

*Amount needed to restore MAP and HR to baseline values.
One important difference between SHR and WKY is their sensitivity to adenosine. When infused into the aortic arch, a given dose of adenosine caused a greater hypotensive response in SHR than in WKY (compare i.a.a. infusion curves in left and right panels of Figure 1 and doses of adenosine required to achieve a 25% reduction in MAP in Table 2). This difference persisted regardless of whether the reduction in blood pressure was expressed as percentage change or absolute change. A second important distinction between SHR and WKY, and one that could explain the difference in sensitivity to adenosine, is the role that adenosine-autonomic interactions play in the overall depressor response. In SHR, ganglionic blockade with chlorisondamine markedly reduced the depressor response to i.a.a. infusion of adenosine, whereas ganglionic blockade in WKY did not influence the hypotensive response to i.a.a. infusion of adenosine. Attenuation of depressor responses to adenosine by ganglionic blockade in SHR could not have been due to a reduction in baseline blood pressure, since blood pressure was restored to pre-ganglionic blockade levels with norepinephrine. Rather, these data demonstrate that adenosine-autonomic interactions contribute...
substantially to the net depressor response to adenosine in SHR, but not in WKY.

Adenosine is a potent inhibitor of neurotransmitter release from sympathetic nerve terminals.\textsuperscript{9-11} Further, both sympathetic tone\textsuperscript{20-22} and the release of norepinephrine from noradrenergic nerve terminals per impulse\textsuperscript{23,24} are elevated in SHR. Therefore, the sympathetic nervous system contributes comparatively more to basal blood pressure levels in SHR than in WKY. This information suggests that adenosine-mediated attenuation of noradrenergic neurotransmission should contribute more to the hypotensive response in SHR than in WKY. This hypothesis is consistent with our observations that adenosine lowered blood pressure more effectively in SHR than in WKY and that ganglionic blockade reduced the depressor response to adenosine in SHR but not in WKY.

We are not suggesting that prejunctional inhibition of neurotransmission by adenosine is unimportant in the WKY. Adenosine can enhance sympathetic discharge in at least two ways. First, by lowering blood pressure adenosine unloads high pressure baroreceptors, which would reflexly increase sympathetic tone. Second, adenosine stimulates chemoreceptors in the renal pelvis, which would also increase sympathetic tone.\textsuperscript{25} Consequently, reflex activation of the sympathetic nervous system by adenosine should attenuate the depressor response to adenosine. However, the observation that ganglionic blockade does not increase the depressor response to adenosine in WKY is inconsistent with this prediction. This incongruity can be reconciled by postulating that adenosine-mediated prejuncional inhibition of norepinephrine release counteracts any increases in sympathetic tone so that the net contribution of adenosine-sympathetic interactions to the depressor response is trivial. Thus, our observations suggest that adenosine-mediated inhibition of noradrenergic neurotransmission plays an important role in the depressor response to adenosine during both high and low basal sympathetic tone. In the former situation, prejuncional inhibition contributes to the actual depressor response, and in both instances prejuncional inhibition prevents the attenuation of the depressor response by reflex sympathetic activation.

A third intriguing distinction between SHR and WKY with respect to adenosine responsiveness is the difference between depressor-response curves when adenosine is administered into the aortic arch rather than intravenously. In SHR, the depressor potency of adenosine is two times greater when infused into the aortic arch than when infused into the vena cava. In contrast, the i.a.a. and i.v. dose–depressor response curves for adenosine in WKY are nearly identical. A possible explanation for these data is that in SHR the lung extracts approximately 50% of the presented adenosine, whereas pulmonary extraction is essentially nil in WKY. However, other mechanisms besides differences in pulmonary extraction of adenosine could explain our observations. Whether pulmonary extraction of adenosine differs in SHR and WKY and, if it does, what impact this putative difference in pulmonary adenosine extraction has on circulating adenosine levels in SHR and WKY are currently under investigation.

A fourth difference between SHR and WKY is the manner in which adenosine influences HR. In SHR, i.a.a. and s.r.a. infusions of adenosine did not change HR, whereas HR in WKY was accelerated by i.a.a. or s.r.a. infusion of adenosine. In addition, i.v. infusion of adenosine caused a profound bradycardia in SHR but did not affect HR in WKY. The actions of adenosine on HR are complex; the net effect is a function of several component actions. By lowering blood pressure, adenosine would unload high pressure arterial baroreceptors and, thereby, tend to reflexly increase sympathetic tone to the heart. Also, by stimulating renal chemoreceptors, adenosine may increase renal
afferent tone, which also may increase sympathetic tone to the heart. On the other hand, adenosine-mediated inhibition of noradrenergic neurotransmission at the sinoatrial (SA) node and its direct negative chronotropic effect would oppose baroreceptor-mediated and chemoreceptor-mediated increases in cardiac sympathetic tone. Therefore, the net influence of adenosine on HR may depend on the concentration of adenosine achieved at the SA node and on basal sympathetic tone.

Because of adenosine’s extremely short half-life, when infused into the arterial circulation the amount of adenosine presented to the SA node would be low. Consequently, inhibition of noradrenergic neurotransmission at the SA node would be insignificant, so that adenosine-induced activation of sympathetic tone would increase HR. This prediction corresponds to our observation that i.a.a. infusion of adenosine increased HR in WKY, an increase that was abolished by ganglionic blockade. On the other hand, when infused intravenously, adenosine concentrations at the SA node would be high due to delivery of adenosine through the coronary arteries and, perhaps, due to direct diffusion of adenosine from the right atrium into the SA node. High levels of adenosine at the SA node would attenuate the consequences of increased sympathetic drive.

This response could explain the lack of a net effect of i.v. infusion of adenosine on HR in WKY. If adenosine is administered intravenously to animals with high basal sympathetic tone, a reduction in HR would be anticipated due to inhibition of noradrenergic neurotransmission. Accordingly, i.v. infusion of adenosine produced a marked bradycardia in SHR, an effect that was abolished by ganglionic blockade. An important implication of the latter observation is that a direct negative chronotropic effect of adenosine is not important in mediating adenosine-induced bradycardia in SHR.

As mentioned previously, evidence indicates that the renal pelvis is equipped with atypical adenosine receptors that mediate an increase in renal afferent discharge and, consequently, cause a reflex activation of the sympathetic nervous system. We found that when adenosine was infused above the renal arteries in WKY, a tachycardia resulted that was out of proportion to the reduction in blood pressure. For instance, the increases in HR induced by s.r.a. infusions of adenosine were equal to those induced by i.a.a. infusions, despite the fact that the reduction in blood pressure induced by s.r.a. infusions of adenosine was much less than that induced by i.a.a. infusions. This observation supports the concept that adenosine reflexly activates the sympathetic nervous system by stimulating renal adenosine receptors. Also in support of this concept is the observation that s.r.a. infusion of adenosine was much less efficacious than i.v. or i.a.a. infusion in lowering MAP.

In conclusion, our data indicate that adenosine is an effective hypotensive agent in conscious, unrestrained normotensive WKY and SHR. However, important between-strain differences exist in the cardiovascular response to adenosine. These differences most likely are due to changes in adenosine-pulmonary interactions and increases in the importance of adenosine-autonomic interactions in SHR.

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_Hypertension_. 1986;8:391-398
doi: 10.1161/01.HYP.8.5.391

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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