Adenosine in Renin-Dependent Renovascular Hypertension

AKIHIRO OHNISHI, PING LI, ROBERT A. BRANCH, ITALO O. BIAGGIONI, AND EDWIN K. JACKSON

SUMMARY Our previous studies support the hypothesis that activation of the renin-angiotensin system by renal ischemia elevates adenosine levels and that adenosine acts in a negative feedback loop to limit renin release and to mitigate some of the hypertension-producing effects of angiotensin II. To further test this hypothesis, we compared the time course of caffeine-induced increases in plasma renin activity with the time course of changes in plasma levels of adenosine in two models of renin-dependent renovascular hypertension. Also, we compared the effects of caffeine on plasma renin activity and arterial blood pressure in renin-dependent versus renin-independent renovascular hypertension. In comparison to sham-operated rats, plasma levels of adenosine in the left and right renal veins and aorta were elevated severalfold in two-kidney, one clip rats (2K1C) 1 week after left renal artery clipping. However, adenosine levels declined during the second and third weeks after clipping. In 2K1C rats treated chronically with caffeine, plasma renin activity was markedly elevated during the first week after operation as compared to non-caffeine-treated 2K1C rats. However, during the second and third weeks after clipping, caffeine had lesser effects on plasma renin activity. A temporal relationship between plasma adenosine levels and caffeine-induced hyperreninemia was also observed in rats with aortic ligation. Caffeine accelerated hypertension in 2K1C rats and rats with aortic ligation (renin-dependent renovascular hypertension), but it had no effect on plasma renin activity or blood pressure in one-kidney, one clip rats (renin-independent renovascular hypertension). These results lend further support to the hypothesis that adenosine functions to mitigate the renin-angiotensin system in renin-dependent renovascular hypertension.

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KEY WORDS • caffeine • renovascular hypertension • adenosine • renin • angiotensin

The nucleoside adenosine may be an endogenous physiological antagonist of the renin-angiotensin system. Not only is adenosine a potent inhibitor of renin release from the renal juxtaglomerular cell; adenosine also exerts many actions that are diametrically opposed to the effects produced by angiotensin II (Ang II). For instance, adenosine acts on most blood vessels to cause vasodilation, whereas Ang II causes vasoconstriction. In the central nervous system, adenosine inhibits sympathetic tone when injected into discrete nuclei associated with the regulation of blood pressure. In contrast, Ang II increases sympathetic tone through a central action. At the sympathetic neurotransmitter junction, adenosine attenuates the release of norepinephrine from nerve varicosities and, in some tissues, reduces the vascular response to norepinephrine. To the contrary, Ang II facilitates both the release of and vascular response to norepinephrine. Although controversial, Ang II may increase glomerular capillary pressure by preferentially constricting the glomerular efferent arteriole, while adenosine may decrease glomerular capillary pressure by constricting the afferent arteriole. The antithetical relationship between adenosine and the renin-angiotensin system suggests that a major physiological role of adenosine may be to protect the cardiovascular system from chronic overactivity of the renin-angiotensin system. One
pathophysiological state associated with chronic overactivity of the renin-angiotensin system is renin-dependent renovascular hypertension. Therefore, adenosine may function to prevent severe hypertension from developing in response to renin-dependent renovascular hypertension.

As an initial test of this idea, we recently examined the effects of chronic blockade of adenosine receptors with caffeine in rats with two-renal, one-clip (2K1C) renovascular hypertension.12 The 2K1C rat is an experimental model of renovascular hypertension in which the renin-angiotensin system is chronically activated and mediates the development of hypertension.13, 14 Consistent with our hypothesis, we found that 1) chronic caffeine administration markedly exacerbates the hypertension in 2K1C rats; 2) chronic treatment with caffeine elevates plasma renin activity (PRA) sevenfold 6 weeks after induction of renal ischemia, 3) inhibition of converting enzyme with enalapril prevents caffeine from worsening hypertension in 2K1C rats; and 4) caffeine does not affect PRA or blood pressure in either normotensive or genetically hypertensive rats that do not have an activated renin-angiotensin system. In a subsequent study, we also discovered that caffeine enhances the slow-pressure response to chronic low dose infusions of Ang II.15 Finally, we obtained preliminary data suggesting that plasma levels of adenosine are elevated in 2K1C rats but are normal in rats with genetic hypertension that have normal PRA.8 Taken together, our data support the following hypothesis: Chronic activation of the renin-angiotensin system by renal ischemia in some manner elevates adenosine levels, and adenosine acts in a negative feedback loop to limit renin release and to attenuate the hypertension-producing effects of Ang II.

In the present study, we tested our hypothesis further by addressing two specific aims. First, we compared the time course of caffeine-induced increases in PRA with the time course of changes in plasma levels of adenosine in two models of renin-dependent hypertension. If our hypothesis is correct, then the time course of caffeine-induced changes in renin release should at least roughly coincide with the time course of changes in adenosine production in response to renin-dependent hypertension. Second, we examined the effects of caffeine on PRA and arterial blood pressure in two models of renin-dependent and one model of renin-independent renovascular hypertension. If our hypothesis is valid, caffeine should increase PRA and arterial blood pressure in renin-dependent, but not in renin-independent, renovascular hypertension.

Materials and Methods

Animal Models

Male Sprague-Dawley rats were obtained from Sasco (Omaha, NE, USA) and acclimated for at least 1 week to our animal care facility. Animals were housed four to a cage and were provided a constant temperature and humidity and light-dark cycle of 12 hours (lights on 0600–1800). All rats were fed a diet of Wayne Lab-Blox (Allied Mills, Memphis, TN, USA) containing 170 mEq Na+/kg and 246 mEq K+/kg, and were given tap water ad libitum. Animals were anesthetized with an intraperitoneal injection of pentobarbital (50 mg/kg), a midline abdominal incision was made, and the cecum and small intestines were exteriorized. In one group of animals, a silver clip with a 0.25-mm gap was placed on the left renal artery and the right kidney remained undisturbed (2K1C rats). In a second group of animals, a silver clip with a gap of 0.20 mm was placed on the left renal artery and the right kidney was removed (one-kidney, one clip [1K1C] rats). In a third group of rats, the aorta was ligated between the origin of the renal arteries as previously described (aortic-ligated [AL] rats).17 After the intestines were restored to their normal position, the abdominal wall was sutured (4-0 silk) and the skin incision was closed with wound clips (9 mm). High-renin renovascular hypertension develops in 2K1C rats13, 14 and AL rats,17 whereas normal-renin renovascular hypertension develops in 1K1C rats.18, 19 2K1C and 1K1C rats were approximately 11 weeks old at the time of operation, and AL rats were approximately 15 weeks old. Sham-operated 2K1C and AL rats had their renal artery or aorta loosely clipped or ligated, but the clip or ligation was removed.

Blood Pressure Measurements

In the 2K1C and 1K1C rats, systolic blood pressure (SBP) was measured with an IITC (Woodland Hills, CA, USA) photoelectric tail-cuff system (Model 20-NW cuff pump, Model 59 pulse amplifier, and Model 65-24 manual scanner) at an ambient temperature of 27 °C. As previously described,20 this technique allowed accurate measurement of SBP without heat-stressing the animals. Each animal was trained for 1 week (three separate training sessions) before SBP was actually recorded. This protocol acclimated the animals to the tail-cuff instrument and thereby reduced variability. For each animal, the SBP was recorded on 2 separate days during a particular week and SBP was measured three times during each session. The average of these six weekly measurements was reported as the SBP for that week.

The tail-cuff method could not be used to measure SBP in rats with aortic ligation because of the aortic ligature. Therefore, 24 hours before arterial blood pressure was to be measured, these rats were lightly anesthetized with ether and the left carotid artery was cannulated with PE-50 tubing. This cannula was tunneled subcutaneously to the back of the neck and exteriorized. The next day, the implanted catheter was connected to a Hewlett-Packard pressure transducer (Model 1280, Palo Alto, CA, USA) and arterial blood pressure was...
recorded on a Hewlett-Packard physiograph (Model 7758A). Heart rate was determined from the arterial tracing.

Protocol 1: Renin Study in 2K1C Rats

Rats were randomized to receive either 0.1% caffeine in drinking water or normal drinking water beginning 1 week before operation (i.e., clipping of left renal artery). Previous studies by von Borstel et al.\textsuperscript{21} and ourselves\textsuperscript{22} indicate that this dose of caffeine provides a pharmacologically relevant plasma level of caffeine (approximately 5–10 µg/ml) that is sufficient to block adenosine receptors. One week before and 1, 2, 3, 4, and 6 weeks after clipping, seven to nine animals were randomly selected from each treatment group for blood pressure, biochemical, and metabolic studies. In all six groups, SBP was measured during the indicated study week as described in the preceding section. Also, during the last 72 hours of the study week, each animal was housed in a metabolic cage (Nalge, Rochester, NY, USA). During the last 24 hours of this 72-hour period, food intake, water intake, and urine output were determined. Urine samples were analyzed for sodium and creatinine levels. Finally, animals were rapidly decapitated, and during the first 3 seconds following decapitation, blood was collected for determination of PRA.\textsuperscript{22} Additional blood was also collected for measurement of plasma creatinine level, plasma sodium concentration, and in some rats, plasma aldosterone level.

Protocol 2: Adenosine Study in 2K1C Rats

2K1C rats and sham-operated rats were randomized to be studied 1, 2, or 3 weeks after operation. At the indicated times, rats were anesthetized with pentobarbital (50 mg/kg i.p.) and a midline incision was made in the abdomen. The intestines were exteriorized to enable access to the aorta or left and right renal veins. In some animals, a blood sample for plasma adenosine determination was withdrawn from the abdominal aorta. In other animals, a blood sample was removed from both the left and right renal veins. Blood samples were obtained by direct puncture and processed as described in the Sample Analysis section.

Protocol 3: Aortic-Ligated Rats

Rats were randomized to receive either 0.1% caffeine in drinking water or normal drinking water. One week later, animals were subjected to either ligation of the aorta or to a sham operation as described in the Animal Models section. Rats were randomized to receive either 0.1% caffeine in drinking water or normal drinking water beginning 1 week before operation. Baseline SBP was measured in both groups, then the animals were subjected to renal artery clipping and contralateral nephrectomy as described in the Animal Models section. SBP was monitored weekly for 4 weeks. In the fourth week after operation, biochemical and metabolic studies were conducted as described for the 2K1C rats in Protocol 1.

Sample Analysis

Plasma adenosine was determined as recently described.\textsuperscript{16} Briefly, blood samples (200 µl) were rapidly aspirated into a prechilled 1-ml plastic syringe containing 200 µl of stopping solution (dipyridamole, erythro-9-(2-hydroxy-3-nonyl)adenine, and heparin; final concentrations of 200 µM, 10 µM, and 2 U/ml, respectively). After centrifugation at 13,000 g for 1 minute, 200 µl of supernatant fluid was withdrawn, and 100 ng of N,N-dimethylguanosine (internal standard) was added. The sample was deproteinized with 60 µl of 15% trichloroacetic acid and centrifuged, and the supernatant fluid was loaded onto an activated (2 ml of methanol followed by 2 ml of water) C\textsubscript{18} Sep-Pak cartridge (Waters Associates, Milford, MA, USA). The Sep-Pak cartridge was washed with 2 ml of water and 2 ml of methanol/water (5:95), and adenosine was eluted with 2 ml of methanol/water (50:50). The eluent was filtered and taken to dryness in a sample concentrator (Savant, Columbus, OH, USA). Samples were reconstituted in 4 µl of mobile phase (0.05 M KH\textsubscript{2}PO\textsubscript{4} buffer, pH 5.5, acetonitrile; 95:5), and 1 µl was injected into a microbore high performance liquid chromatograph (HPLC; Isco, Lincoln, NE, USA) equipped with an Isco 10 cm × 1 mm C\textsubscript{18} reverse-phase column (particle size = 3 µm). The mobile phase was delivered at a flow rate of 20 µl/min, and the absorbance was monitored at 260 nm. The peak height ratio of adenosine to N,N-dimethylguanosine was compared with a standard curve prepared in mobile phase.

Plasma caffeine levels were determined as previously described.\textsuperscript{12} Briefly, 1 mg of β-hydroxyethylxanthines (internal standard) was added to 100 µl of plasma, and this solution was loaded onto an activated (2 ml of methanol followed by 2 ml of water) 1-ml C\textsubscript{18} column (Bond Elut; Analytichem International, Harbor City, CA, USA). Next, the column was washed with 2 ml of water, and methylxanthines were eluted with 400 µl of acetone. The acetone was evaporated under nitrogen and reconstituted in 100 µl of mobile phase, and 10 µl was analyzed by an HPLC system consisting of a pump (Model M45, Waters), an injector (Rheodyne, Cotati,
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CA, USA), a C18 reverse-phase column (Biophase OD5; Bioanalytical Systems, West Lafayette, IN, USA), and an ultraviolet detector (Model 160; Beckman Instruments, Fullerton, CA, USA) set at a wavelength of 280 nm. The mobile phase was 2% acetic acid and 6% acetonitrile and was delivered at 1.5 ml/min. The peak height ratio of caffeine to β-hydroxyethyltheophylline was compared with a standard curve prepared in methylxanthine-free plasma.

Blood samples for PRA were collected in pre-chilled tubes containing 50 μl of 10% EDTA and placed immediately on ice. Samples were centrifuged at 4 °C, and the plasma was frozen (−20 °C) until assayed for PRA as previously described. Plasma and urine were analyzed for creatinine using a creatinine autoanalyzer (Beckman) and for sodium using a flame photometer (Instrumentation Laboratory, Lexington, MA, USA).

Blood samples for measurement of plasma aldosterone were collected in 10% EDTA (30 μl/2 ml of blood). Plasma was obtained by centrifugation and stored at −20 °C. Plasma was adjusted to 2 ml with water and alkalinized with 0.1 ml of 1.0 N NaOH. Samples were then extracted with 20 ml of dichloromethane, and the organic layer was washed with 2.0 ml of 1% acetic acid and then with 2.0 ml of water. Dichloromethane was evaporated under air flow at 37 °C, and samples were reconstituted in 50 μl of ethanol and 250 μl of a 0.05 M borate buffer (pH 8.0) containing 1% bovine gamma globulin. Reconstituted samples (100 μl) were incubated at 4 °C for 24 hours with 100 μl borate buffer, 50 μl antibody (1:4400 final dilution), and 50 μl [3H]aldosterone (4000 dpm). Free and bound [3H]aldosterone were separated with dextrancoated charcoal, and bound radioactivity was determined. Aldosterone levels were quantitated from a standard curve plotted as log aldosterone level versus log bound tracer in the presence and absence of aldosterone. Standards were prepared in an artificial plasma and taken through the same extraction procedure as described for the plasma samples.

Statistical Analysis

All data are presented as means ± SEM. Caffeine-treated animals were compared with control animals with an unpaired two-tailed Student's t test. The criterion of significance was a p level below 0.05.

Results

Protocol 1: Renin Study in 2K1C Rats

As shown in Figure 1, chronic administration of caffeine markedly exacerbated the hypertension-producing response to renal artery clipping. The SBP in caffeine-treated 2K1C rats was significantly elevated compared with that in non-caffeine-treated rats 2, 3, 4, 5, and 6 weeks after clipping, whereas SBP was similar in caffeine-treated control rats and non-caffeine-treated control rats. This independent sampling study involving 85 animals (Table 1 shows group size at each time point) confirms the results of a separate repeated-measures study published previously in which we also found that caffeine worsened renovascular hypertension in the 2K1C rat.

Figure 2 illustrates that chronic caffeine administration greatly enhanced the increase in PRA in response to renal artery clipping. In normal control rats, caffeine administration did not influence PRA. In contrast, caffeine significantly increased PRA in 2K1C rats at most times after renal artery clipping. However, the effect of caffeine on PRA in 2K1C rats appeared to be biphasic. In non-caffeine-treated 2K1C rats, the mean PRA remained between 1.9 ± 0.3 and 4.2 ± 0.5 ng angiotensin I (Ang I)/ml/hr throughout the entire observation period. During the first week after clipping, PRA in the caffeine-treated rats was 19.1 ± 3.5 ng Ang I/ml/hr. However, this value decreased to 10.5 ± 4.7 and 9.6 ± 3.8 ng Ang I/ml/hr (p < 0.01) during the second and third weeks after clipping, respectively. Subsequently, PRA began to rise over the next 3 weeks, reaching a value of 32 ± 8 ng Ang I/ml/hr by the sixth week. Consistent with the effects of caffeine on PRA in 2K1C rats, aldosterone levels were also elevated in caffeine-treated 2K1C rats compared with non-caffeine-treated 2K1C rats 4 weeks after clipping (see Table 1).

Another interesting and potentially important effect of caffeine in rats with 2K1C renovascular hypertension is illustrated in Figure 3. In normal control rats, caffeine did not influence creatinine clearance; however, 3, 4, and 6 weeks after renal artery clipping, creatinine clearance was significantly lower in caffeine-treated 2K1C rats than in non-caffeine-treated 2K1C. During the sixth week, caffeine reduced creatinine clearance by approxi-
hypertension
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TABLE 1. Metabolic Studies in 2K1C Renovascular Hypertensive Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n = 7)</th>
<th>1st week</th>
<th>2nd week</th>
<th>3rd week</th>
<th>4th week</th>
<th>6th week</th>
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<td>Caf (n = 7)</td>
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<td>Caf (n = 7)</td>
<td>No caf</td>
<td>Caf (n = 7)</td>
<td>No caf</td>
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<tr>
<td>Urine volume (ml/day)</td>
<td>15 ± 1</td>
<td>18 ± 1</td>
<td>35 ± 1</td>
<td>15 ± 1</td>
<td>22 ± 1</td>
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<td>Water intake (ml/day)</td>
<td>± 1 ± 1</td>
<td>± 3 *</td>
<td>± 1 ± 1</td>
<td>± 3 *</td>
<td>± 5 ± 2</td>
<td>± 2 ± 3</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
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<td>± 3 ± 1</td>
<td>± 1 ± 1</td>
<td>± 3 ± 1</td>
<td>± 2 ± 2</td>
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<td>Urinary sodium excretion</td>
<td>1590 ± 68</td>
<td>1503 ± 70</td>
<td>1678 ± 213</td>
<td>1461 ± 213</td>
<td>1533 ± 512</td>
<td>1502 ± 70</td>
</tr>
<tr>
<td>(pg/ml)</td>
<td>± 96 ± 2</td>
<td>± 70 ± 2</td>
<td>± 38 ± 213</td>
<td>± 213 ± 213</td>
<td>± 92 ± 142</td>
<td>± 142 ± 83</td>
</tr>
<tr>
<td>Plasma aldosterone (pg/ml)</td>
<td>140 ± 7</td>
<td>134 ± 12</td>
<td>132 ± 12</td>
<td>132 ± 12</td>
<td>135 ± 13</td>
<td>137 ± 12</td>
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</table>

Data are means ± SEM. Caf = caffeine.
*p < 0.05, compared with no caffeine value.

FIGURE 2. PRA in 2K1C renovascular hypertensive rats given either normal drinking water or drinking water containing 0.1% caffeine. Control values are from normal rats. The p values (by unpaired Student's t test) refer to comparisons between caffeine-treated and non-caffeine-treated rats at the indicated time after operation. Each time point represents a separate group of rats. See Table 1 for number of animals at each time point.

FIGURE 3. Creatinine clearance in 2K1C rats given either normal drinking water or drinking water containing 0.1% caffeine. Control values are from normal rats. The p value (by unpaired Student's t test) refers to comparison between caffeine-treated and non-caffeine-treated rats at the indicated time after operation. Each time point represents a separate group of rats. See Table 1 for number of animals at each time point.

These data suggest that caffeine altered the ability of the kidney to maintain a normal glomerular filtration rate following unilateral renal artery clipping.

Table 1 summarizes the metabolic studies in Protocol 1. In control animals caffeine treatment did not affect urine volume, water intake, urinary sodium excretion, or plasma sodium concentration. Similarly, in 2K1C rats caffeine did not influence food intake, urinary sodium excretion, or plasma sodium concentration. However, in 2K1C rats caffeine tended to increase water intake and urine volume at all time points, although this trend achieved statistical significance only during the first week (urine volume) and the fourth week (water intake) after clipping. This effect is consistent with the well-known dipsogenic action of blood-borne Ang II.24 We did not measure plasma levels of caffeine in control rats or 2K1C rats treated with 0.1% caffeine because we have done so previously.12

Protocol 2: Adenosine Study in 2K1C Rats

In one study, we measured the concentration of adenosine in renal venous plasma in 2K1C rats and in sham-operated rats 1, 2, and 3 weeks after operation. As illustrated in Figure 4, one week after operation, plasma levels of adenosine in the right and left renal veins were elevated approximately fivefold to sixfold in 2K1C rats compared with sham-operated controls. However, as the time after
operation increased, there was a linear decrease in renal venous plasma levels of adenosine such that by 3 weeks after operation plasma adenosine levels were no different in 2K1C rats than in control animals.

Figure 5 summarizes the effects of renal artery clipping on plasma levels of adenosine taken from the abdominal aorta. Adenosine levels were elevated at all time points after operation as compared with sham-operated rats. However, reminiscent of the time-course of caffeine-induced increases in PRA, arterial adenosine levels were highest at one week after operation and declined over the next 2 weeks.

Protocol 3: Aortic-Ligated Rats

In Protocol 3, we investigated the effects of caffeine on arterial blood pressure, PRA, and plasma adenosine levels in another model of renovascular hypertension (i.e., the AL rat). Like the 2K1C rat, hypertension in the AL rat is renin-dependent; however, in this model, arterial blood pressure exhibits a rapid and severe increase that resembles malignant hypertension, and by 1 week after ligation both arterial blood pressure and PRA are approaching a physiological maximum.

Figures 6 and 7 depict the time course of aortic ligation-induced increases in mean arterial blood pressure and PRA, respectively. As shown, caffeine treatment accelerated the already rapid rise in arterial blood pressure and PRA that characterizes this model of severe hypertension. However, by the fifth day after aortic ligation, mean arterial blood pressure and PRA were similar in caffeine-treated and non-caffeine-treated rats with aortic ligation.

The effects of aortic ligation on arterial plasma levels of adenosine were also time-dependent in the AL rats. Although adenosine levels were significantly elevated both 3 and 5 days after operation, the effect of aortic ligation on plasma adenosine levels was much greater on Day 3 than on Day 5 (Figure 8).

Finally, as shown in Table 2, caffeine did not influence urine volume, water intake, food intake, urinary sodium excretion, or plasma sodium concentration. However, caffeine did accelerate the rapid decline in creatinine clearance that accompanies aortic ligation-induced hypertension. The plasma levels of caffeine were similar in sham-operated rats and AL rats (see Table 2) and were within the range of caffeine levels that are associated with moderate coffee consumption by humans.
Protocol 4: 1K1C Rats

In Protocol 4, we examined the effects of caffeine in a third model of renovascular hypertension (i.e., the 1K1C rat). Unlike the 2K1C rat, development of hypertension in the 1K1C rat occurs, for the most part, independently of the renin-angiotensin system. As illustrated in Figure 9, chronic administration of caffeine did not alter the increase in SBP in response to renal artery clipping plus contralateral nephrectomy. Likewise, caffeine did not increase PRA in this normal renin model of hypertension (Table 3). As shown in Table 3, caffeine did not affect any other measured metabolic or biochemical parameters, despite the fact that the plasma level of caffeine achieved in the 1K1C rats was similar to that achieved in the AL rats.

Discussion

Over the past several years, we have been testing the hypothesis that adenosine functions as an endogenous physiological antagonist of the renin-angiotensin system. Specifically, we postulate that activation of the renin-angiotensin system results in elevated adenosine levels and that adenosine serves both to inhibit renin release and to attenuate some of the biological effects of Ang II. The conception of this hypothesis was based on the well-known facts that adenosine inhibits renin release and exerts a number of biological actions that would counteract those effects produced by Ang II (see the Introduction for details).

As a starting point, we began testing our hypothesis in the 2K1C renovascular hypertensive rat. This animal model was selected for study because it is a well-characterized paradigm of renovascular hypertension in which the renin-angiotensin system has an unequivocal and major effect on the cardiovascular system. In our previously published study, we reported that chronic administration of the adenosine-receptor blocker caffeine markedly accelerated the hypertensive process in 2K1C rats. Importantly, this effect was prevented by inhibition of angiotensin converting enzyme and was not evident in animals in a normal renin state, regardless of whether they were normotensive or genetically hypertensive. Further, we measured PRA at a
single time point and noted a sevenfold elevation in PRA in 2K1C rats treated with caffeine compared with untreated 2K1C rats. Caffeine did not affect PRA in normotensive or genetically hypertensive rats. In a subsequent study,15 we also reported that blockade of adenosine receptors with caffeine potentiated the slow-pressor, but not the rapid-pressor, response to exogenous Ang II. These experiments supported our hypothesis and prompted the further investigations described in the present report.

In several respects, the results of the experiments described here lend support to our hypothesis. First, we found that in two quite distinct models of renin-dependent renovascular hypertension (i.e., 2K1C rats and AL rats), caffeine exacerbated the development of hypertension. In contrast, caffeine did not affect blood pressure in the 1K1C rat, which is, for the most part, a renin-independent model of renovascular hypertension.18-19 These observations are consistent with the idea that adenosine serves as a physiological antagonist of the renin-angiotensin system, since our hypothesis predicts that blockade of adenosine receptors would worsen only renin-dependent renovascular hypertension.

Also consistent with our hypothesis is the observation that adenosine levels increased, at least temporarily, in 2K1C rats treated with caffeine compared with untreated 2K1C rats. Caffeine did not affect PRA in normotensive or genetically hypertensive rats. Interestingly, the time course of the change in adenosine levels roughly coincided with the magnitude of caffeine-induced changes in PRA. For instance, in the 2K1C rat during the first week, arterial and renal venous levels of adenosine were highest and caffeine increased PRA about 17 ng Ang I/ml/hr. During the subsequent 2 weeks, adenosine levels declined and the effects of caffeine on PRA also decreased. Unfortunately, we did not measure adenosine levels in rats with 2K1C hypertension of greater than 3 weeks' duration, so we cannot speculate as to whether or not there was a secondary rise in adenosine levels that coincided with the secondary increase in PRA. However, since renal function began to deteriorate in caffeine-treated 2K1C rats after several weeks, it is possible that the secondary rise in PRA was mediated solely through renal damage.

The effect of caffeine on PRA in the AL rat also roughly correlated with plasma levels of adenosine. In this model, caffeine increased PRA by 24 ng Ang...

**Table 2. Metabolic Studies in Aortic-Ligated Renovascular Hypertensive Rats**

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<thead>
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<th>Parameter</th>
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<td>Caffeine (n = 7)</td>
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<td>Urine volume (ml/day)</td>
<td>16±1</td>
<td>17±1</td>
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<td>Water intake (ml/day)</td>
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<td>Urinary sodium excretion (µEq/day)</td>
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<td>Plasma sodium concentration (mEq/L)</td>
<td>138±3</td>
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<td>Creatinine clearance (ml/min)</td>
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<td>Plasma caffeine (µg/ml)</td>
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<td>5.6±0.6</td>
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Data are means ± SEM.  
*p < 0.05; compared with no caffeine value.

**Table 3. Metabolic Studies in 1K1C Renovascular Hypertensive Rats**

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<td>Food intake (g/day)</td>
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<td>Plasma sodium concentration (mEq/L)</td>
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<tr>
<td>Creatinine clearance (ml/min)</td>
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<td>Plasma caffeine (µg/ml)</td>
<td>4.8±0.8</td>
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<tr>
<td>Plasma renin activity (ng Ang I/ml/hr)</td>
<td>2.14±0.20</td>
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Data are means ± SEM.
I/ml/hr 3 days after ligation of the aorta and adenosine levels were greatly elevated at this time. However, 5 days after aortic ligation, PRA levels were similar in AL rats with and without caffeine treatment and arterial adenosine levels, although still elevated, had returned toward normal.

The elevation of adenosine in renin-dependent renovascular hypertension is an interesting phenomenon. Teleologically, this response may serve to protect the organism from overactivity of the renin-angiotensin system until other, more long-lasting biochemical adjustments, such as receptor down-regulation, can be made. Presently, we do not know what stimulus releases adenosine in 2K1C rats and AL rats. Further, we cannot deduce from the limited data in this report the anatomical sites from which adenosine is released. However, since Ang II levels are elevated in 2K1C rats and AL rats, one possibility is that Ang II stimulates adenosine release. Also, since arterial levels of adenosine are elevated in renin-dependent hypertension, it is conceivable that Ang II releases adenosine from the lungs. Indeed, in preliminary studies, we have found that Ang II is a potent stimulant of adenosine release from the in situ rat lung (data not shown).

Another interesting result of the present investigation is that caffeine caused a gradual deterioration of renal function in 2K1C rats and accelerated the deterioration of renal function in AL rats. A simple explanation of this phenomenon is that deterioration of renal function is merely secondary to the increase in arterial blood pressure. However, it is possible that the events leading to caffeine-induced deterioration of renal function also involve the blockade of adenosine receptors. Adenosine is thought to constrict the afferent arteriole,21,27 an effect that would decrease glomerular capillary hydrostatic pressure. On the other hand, Ang II may selectively constrict the efferent arteriole,9, 10 leading to an increase in glomerular capillary hydrostatic pressure. Therefore, with respect to glomerular capillary hydrostatic pressure, adenosine and Ang II may be antagonistic to one another. A logical inference from this information is that in renin-dependent hypertension, adenosine may serve to prevent Ang II from increasing glomerular capillary hydrostatic pressure. Therefore, blockade of adenosine receptors would lead to an increase in Ang II-induced glomerular hypertension. Since glomerular hypertension is linked to glomerulosclerosis,28, 29 this scenario, although entirely speculative at this time, could explain the rather striking deterioration of renal function induced by caffeine in 2K1C rats.

In summary, the data described here lend additional support to the hypothesis that endogenous adenosine serve to attenuate the activation and expression of the renin-angiotensin system in renin-dependent renovascular hypertension. A closer examination of this hypothesis seems warranted.

References


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