Intrarenal Adenosine Produces Hypertension Via Renal Nerves in the One-Kidney, One Clip Rat

RICHARD E. KATHOLI, WILLIAM P. MCCANN, AND W. THOMAS WOODS

SUMMARY The afferent renal nerves enhance sympathetic activity in the one-kidney, one-clip hypertensive rat. We have also found adenosine-sensitive nerve endings in the renal pelvis that, when stimulated, increase sympathetic activity producing hypertension. To determine whether adenosine, which is excreted when renal blood flow is reduced, activates the afferent renal nerves in one-kidney, one-clip hypertension, urinary adenosine concentration was lowered by infusing adenosine deaminase into the renal artery. Urinary adenosine concentration was threefold greater in one-kidney, one-clip hypertensive animals compared with normotensive controls. Intrarenal infusion of adenosine deaminase in one-kidney, one-clip rats lowered urinary adenosine to an undetectable level and attenuated the hypertension. Both plasma norepinephrine levels and the fall in mean arterial pressure after ganglionic blockade decreased during intrarenal adenosine deaminase infusion in one-kidney, one-clip animals. Renal denervation in one-kidney, one-clip animals prevented the changes in mean arterial pressure and plasma norepinephrine levels during intrarenal adenosine deaminase infusion. In contrast to findings in hypertensive animals, intrarenal infusion of adenosine deaminase produced no change in arterial pressure in normotensive controls. These data indicate that urinary adenosine concentration is enhanced in one-kidney, one-clip hypertension and suggest that when urinary adenosine concentration is lowered, sympathetic activity and hypertension become attenuated in this model if the renal nerves are intact. (Hypertension 7 [Suppl I]: I-88-I-93, 1985)

KEY WORDS  * adenosine deaminase  * renal denervation  * sympathetic nervous system  * renin-angiotensin system  * afferent renal nerves

STUDIES from our laboratory suggest that the afferent renal nerves contribute to the pathogenesis of one-kidney, one-clip hypertension by enhancing the activity of the sympathetic nervous system.1-3 To test further the hypothesis that increased afferent renal nerve signals from a clipped kidney increase the level of sympathetic nervous system activity in this model, it is necessary to identify the stimulus to afferent renal nerve discharge.

Previous studies from our laboratory and others have identified adenosine as a likely candidate to stimulate chemoreceptive nerve endings in renovascular hypertension. Adenosine is readily released by kidney proximal tubular cells into the tubular fluid when renal blood flow is reduced.4 Intrarenal administration of adenosine enhances afferent renal nerve signals5 with resultant increased sympathetic nervous system activation producing hypertension.5-7 These adenosine-sensitive nerve endings are located within or near the renal pelvis.5

The objectives of these experiments were twofold: (1) to determine whether urinary adenosine concentration and excretion were greater in the one-kidney, one-clip hypertensive rat compared with normotensive control animals, and (2) to determine whether renal artery adenosine deaminase administration in the rat with established one-kidney, one-clip hypertension results in decreased urinary adenosine concentration and excretion in association with decreased sympathetic activity and decreased arterial pressure.

Methods

Animal Preparation

Male Sprague-Dawley rats (521 ± 9 g) obtained from Charles River Breeding Laboratories (Wilmington, MA) were subjected to clipping (0.40-mm silver clip) of the distal left renal artery. One week later a right nephrectomy was performed. Uninephrectomized age-matched and sex-matched animals were used as normotensive controls. Throughout the study,
the animals were housed in a room with constant temperature (24 ± 1°C) and humidity (60 ± 5%) and light from 0600 to 1800 hours. Systolic blood pressure of all animals was measured twice weekly with the tail cuff method without anesthesia (Narco Biosystems, Inc., Houston, TX). Animals were followed after renal artery clipping until stable hypertension was achieved. Previous studies have shown that within 2 to 3 weeks after clipping, these animals have hypertension characterized by a positive sodium balance and enhanced sympathetic activity. Hypertensive animals and normotensive controls were then instrumented under ether anesthesia with 0.025-inch (inside diameter) microline catheters in a femoral artery and vein and a PE-10 catheter (prepared by stretching the tubing so that the dimension at the tip of the catheter is approximately 70 μm outside diameter) in the left renal artery. The renal artery catheter was introduced through the left common carotid artery down the descending aorta into the left renal artery ostium. After the catheter was in the artery, the kidney surface was inspected for signs of ischemia. The renal catheter was held in place by ligatures at the neck. All catheters were brought under the skin to the back of the neck and protected. Animals recovered at least 48 hours before experiments were begun.

Protocol
To examine the effects of intrarenal adenosine deaminase administration on sympathetic activity and mean arterial pressure in the one-kidney, one-clip hypertensive rat, animals were maintained on a purified basal diet (0.141 mEq of sodium and 0.232 mEq of potassium per gram; Ralston Purina Company, Richmond, IN) ad libitum and studied in the conscious, resting state. To compare urinary adenosine concentration and excretion, hypertensive animals and normotensive control animals were kept in metabolic cages as previously described. During experimental observations arterial pressure was recorded continuously (Hewlett-Packard Recorder, Palo Alto, CA) and analyzed continuously (Digital Equipment Corporation LSI-II computer, Maynard, MA). Plasma norepinephrine concentration and mean arterial pressure decrease in response to ganglionic blockade with 30 mg/kg of hexamethonium bromide were used as indices of peripheral sympathetic nervous system activity during baseline and during intrarenal adenosine deaminase administration. All animals were studied at the same time of day under the same environmental conditions to avoid diurnal variation or ambient temperature influences on plasma norepinephrine levels. One-half milliliter of whole blood from a donor rat was infused as a volume replacement after plasma norepinephrine sampling. Two hours later, under the same conditions and after a stable mean arterial pressure was obtained, 30 mg/kg of hexamethonium bromide was infused intravenously and the maximum decrease in mean arterial pressure was recorded. This dose of hexamethonium bromide has been shown to interrupt sympathetic transmission controlling the cardiovascular system in the rat. Arterial pressure response to a 20-minute intravenous infusion of Sar′-Ala′-angiotensin II (2 μg/kg/min) was used as an index of renin-angiotensin system activity. This dose of Sar′-Ala′-angiotensin II inhibits the pressor response to a test dose of 20 ng/kg of angiotensin II.

After establishing baseline values, the effect of intrarenal adenosine deaminase in conscious, resting one-kidney, one-clip animals on arterial pressure and activity of the sympathetic and renin-angiotensin systems was determined. Adenosine deaminase (type III, solution in 50% glycerol and 0.01 M potassium phosphate, pH 6.0. 1000 units/ml; Sigma Co., St. Louis, MO) was infused into the renal artery at increasing doses (0.5, 1.0, and 2.0 units/kg/min) every 30 minutes. One unit of adenosine deaminase deaminates 1.0 μmol of adenosine to inosine per minute at pH 7.5, 25°C. As a control for the potential effect of the vehicle, heat-denatured adenosine deaminase was infused for 30 minutes in each animal. To determine whether the arterial pressure lowering effect of adenosine deaminase was mediated by the afferent renal nerves, nine of the hypertensive animals subsequently underwent renal denervation. Renal denervation was accomplished through a flank incision by stripping the renal artery adventitia and painting the renal artery with 20% phenol (wt/vol) in ethanol.

Subsequently, all animals were anesthetized with α-chloralose (100 mg/kg) and the left ureter cannulated. Urinary adenosine concentration and excretion were measured during baseline, during intrarenal adenosine deaminase, and 30 minutes after discontinuing adenosine deaminase in all animals. Urine samples for adenosine were collected in tubes immersed in ice and then were rapidly frozen and stored at −20°C. At the end of the experiment the kidneys were removed and frozen in liquid nitrogen for subsequent measurement of norepinephrine content.

Biochemical Measurement and Statistics
Plasma norepinephrine was measured using a modification of the radioenzymatic method of Peuler and Johnson. Kidney norepinephrine content was measured by high-performance liquid chromatography. Urine for adenosine analysis was thawed, remixed, and centrifuged at 4°C to remove sediments. One sample (0.6-ml volume) was treated with 0.025 ml of adenosine deaminase in water (25 units/ml) and another with 0.025 ml of water. After being left at 20°C for 30 minutes to destroy adenosine in the enzyme-treated sample, both samples were treated with 0.6 ml of 12% trichloroacetic acid to remove protein and then were centrifuged. One ml of each supernatant was extracted with ether, treated with chloroacetaldehyde in sodium acetate buffer to form the fluorescent ethereal derivative of adenosine, and then extracted with ether again. Water standards of adenosine (2 μg/ml) were treated identically. Adenosine concentration was determined by a high-performance liquid chromatographic method developed for arabinosyladenine modified by the use of 0.025 M sodium phosphate (pH...
7 7) in place of borate buffers. Adenosine concentrations were calculated from the difference in peak heights at the appropriate retention time (approximately 15 minutes) between the enzyme-treated and untreated members of the pair, and from the net peak height of the concomitant standards, and were corrected for dilution factors. The sensitivity of the method in urine is 0.04 μg/ml, and the coefficient of variance is 8.2% at a concentration of 1.3 μg/ml. Recovery was 99% at this concentration. Numerical results were expressed as means ± SE.

Changes in arterial pressure, plasma norepinephrine levels, arterial pressure decreases to hexamethonium, and Sar'-Ala'-angiotensin II in response to intrarenal adenosine deaminase were compared with baseline by analysis of variance. Changes were reported as significant if the p value was less than 0.05.

Results

Urinary Adenosine
Clipping of the renal artery produced a rise in systolic blood pressure from 121 ± 4 to 185 ± 5 mm Hg (p < 0.001) by 2 weeks. As shown in Table 1, urinary adenosine concentration and excretion were threefold greater in conscious one-kidney, one-clip hypertensive animals than in age-matched and sex-matched one-kidney normotensive controls.

Intrarenal Adenosine Deaminase
In conscious, resting normotensive and hypertensive animals, intrarenal infusion of denatured adenosine deaminase for 30 minutes had no effect on mean arterial pressure. As shown in Figure 1, intrarenal infusion of adenosine deaminase (1.32 ± 0.15 units/kg/min) resulted in a significant sustained fall in mean arterial pressure in conscious one-kidney, one-clip hypertensive animals (p < 0.01). Mean arterial pressure remained decreased throughout the 120 minutes of intrarenal adenosine deaminase infusion. After discontinuing intrarenal adenosine deaminase administration (recovery), mean arterial pressure in hypertensive animals returned to baseline levels within 30 minutes. In contrast, intrarenal infusion of adenosine deaminase (2 units/kg/min) in conscious one-kidney normotensive controls did not change mean arterial pressure. Subsequently, it was determined that intrarenal adenosine deaminase administration in one-kidney, one-clip hypertensive animals and in control animals significantly lowered urinary adenosine concentration (p < 0.01).

Within 30 minutes after discontinuing the enzyme infusion (recovery phase) urinary adenosine concentration returned to baseline levels (see Figure 1).

Plasma norepinephrine levels of conscious, resting, unrestrained one-kidney, one-clip hypertensive animals during intrarenal adenosine deaminase infusion decreased (p < 0.01) from 425 ± 38 to 278 ± 22 pg/ml. Plasma norepinephrine levels of normotensive control animals during intrarenal adenosine deaminase infusion did not change (289 ± 25 before and 283 ± 24 pg/ml during infusion of adenosine deaminase). There was no significant difference in plasma norepi-

Table 1: Systolic Blood Pressure and Urinary Adenosine Concentration and Excretion of Conscious One-Kidney, One-Clip Hypertensive and Normotensive Control Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Systolic BP (mm Hg)</th>
<th>Volume/24 hr (ml)</th>
<th>ADO concentration (μg/ml)</th>
<th>ADO excretion (μg/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT (n = 15)</td>
<td>124 ± 2</td>
<td>25 ± 3 ± 0.9</td>
<td>0.26 ± 0.06</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>1K-1C (n = 18)</td>
<td>185 ± 5*</td>
<td>26 ± 2 ± 0.8</td>
<td>0.83 ± 0.16*</td>
<td>0.91 ± 0.14*</td>
</tr>
</tbody>
</table>

Values are means ± SE
BP = blood pressure, ADO = urinary adenosine, NT = normotensive controls, 1K-1C = one-kidney, one-clip hypertensive animals
*p < 0.01 compared with control
nephrine levels between hypertensive and normoten-
sive animals during intrarenal adenosine deaminase
administration.

Table 2 shows mean arterial pressure of hyperten-
sive and control animals before and after administra-
tion of 30 mg/kg of hexamethonium bromide. Re-
sponses to ganglionic blockade were determined in
one-kidney, one-clip hypertensive and normotensive
control animals before and during intrarenal adenosine
deaminase administration. Ganglionic blockade result-
ed in a significant (p < 0.01) decrease in mean arterial
pressure in both groups before and during intrarenal
adenosine deaminase. The absolute (p < 0.01) and the
percent (p < 0.05) decreases in mean arterial pressure
were significantly greater in hypertensive animals dur-
ing baseline period compared with hypertensive ani-
mals during intrarenal adenosine deaminase or control
animals.

In response to Sar¹-Ala²-angiotensin II, the absolute
and percent decreases in mean arterial pressure in one-
kidney, one-clip animals (11 mm Hg; 8%) were sig-
ificantly greater (p < 0.05) than those of control
animals (5 mm Hg; 5%). During intrarenal adenosine
deaminase infusion, the responses to Sar¹-Ala²-angio-
tensin II were unchanged compared with baseline for
each group respectively: one-kidney, one-clip ani-
mals, 10 mm Hg, 8%; and controls, 5 mm Hg, 5%

<table>
<thead>
<tr>
<th>Group</th>
<th>Prehex</th>
<th>Posthex</th>
<th>Absolute decrease</th>
<th>Percent decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1K-IC (n = 18)</td>
<td>143 ± 6</td>
<td>80 ± 5</td>
<td>63 ± 2*</td>
<td>44 ± 4*</td>
</tr>
<tr>
<td>1K-IC. ADA (n = 18)</td>
<td>122 ± 5</td>
<td>79 ± 4</td>
<td>43 ± 2*</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>NT (n = 15)</td>
<td>101 ± 4</td>
<td>65 ± 5</td>
<td>36 ± 2*</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>NT. ADA (n = 15)</td>
<td>105 ± 4</td>
<td>66 ± 4</td>
<td>39 ± 2*</td>
<td>37 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE
MAP = mean arterial pressure. Prehex, Posthex = before and after hexamethonium.
1K-IC = one-kidney, one-clip hypertensive animals, ADA = during intrarenal adenosine deaminase infusion.
NT = normotensive controls
*p < 0.01.  †p < 0.05. compared with control


discussion

Our study provides evidence that (1) urinary aden-
osine concentration and excretion are threefold greater
in one-kidney, one-clip hypertensive rats compared
with normotensive control animals; (2) when urinary
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this model, peripheral sympathetic nervous system ac-
tivity and arterial pressure decrease if the renal nerves
are intact; and (3) the depressor effect of intrarenal
adenosine deaminase administration in hypertensive
animals is similar to that seen with renal denervation.
These experiments strongly support the concept that
intact afferent renal nerves located in the renal pelvis
are important in the maintenance of hypertension in the
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Under control conditions, small but measurable
quantities of adenosine are present in the urine.4 Urin-
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minutes of total occlusion of the renal artery. Both
stop-flow experiments and histochemical studies suggest that the elevated levels of adenosine found in the urine during decreased renal blood flow are formed by proximal tubular cells and released directly into the tubular fluid. Adenosine appears to accumulate in the kidney in an inverse relationship with renal blood flow. Renal blood flow in one-kidney, one-clip hypertension is decreased at least 30% compared with control levels of flow, while renal perfusion pressure distal to a clip is normal. We found a threefold elevation in urinary adenosine concentration and excretion with this reduced level of renal blood flow. The elevated concentration of urinary adenosine, which results from this reduced level of renal blood flow, is well above levels that occur when physiological doses of exogenous adenosine are infused intrarenally. Thus, these adenosine levels within the tubule or renal pelvis may exert a potent physiological effect.

For the renal nerves to play a long-term role in the maintenance of renovascular hypertension, the receptor for afferent renal nerve stimulation is more likely to be a chemoreceptor than a stretch receptor. Stretch receptors characteristically reset their activation thresholds in response to a chronic change in length, while chemoreceptors do not reset. To test the importance of increased urinary adenosine concentration and excretion in the pathogenesis of one-kidney hypertension required development of a method of interrupting adenosine's proposed action. We had previously found that theophylline, a competitive antagonist of purinergic adenosine receptors, does not alter the arterial pressure response to intrarenal adenosine, which suggests that this excitatory action of adenosine is mediated by a different receptor. Consistent with this possibility was the observation by others that theophylline does not significantly alter the excitatory action of adenosine on cat carotid chemoreceptors. Thus, theophylline could not be used to test our hypothesis. Another approach to interrupting adenosine's proposed action was to lower urinary adenosine concentration by infusing adenosine deaminase into the kidney. The molecular mass of adenosine deaminase is less than 40,000 daltons, so this enzyme is small enough to be filtered and exert its effect in the tubular and pelvic fluid. Since adenosine is released from proximal tubular cells into tubular fluid and adenosine sensitive nerve endings appear to be located within or near the renal pelvis, adenosine deaminase might be used to deaminate adenosine to inosine and thus lower urinary pelvic adenosine concentration. We have previously shown that renal artery administration of inosine does not change arterial pressure.

In one-kidney, one-clip hypertensive rats, lowering urinary adenosine concentration was associated with decreased sympathetic activity and attenuation of the hypertension with no change in activity of the renin-angiotensin system. This observation provided strong evidence for an important role for intrarenal adenosine in this model of hypertension. Further support for the hypothesis that intrarenal adenosine contributes to one-kidney, one-clip hypertension was the return of arterial pressure to hypertensive levels in association with recovery of urinary adenosine concentration after intrarenal adenosine deaminase was discontinued.

Lowering urinary adenosine concentration to low levels in normotensive animals did not alter arterial pressure. This finding suggested that intrarenal adenosine does not contribute to the maintenance of arterial pressure under normal conditions. The lack of changes in arterial pressure and sympathetic activity during intrarenal adenosine deaminase infusion in normotensive animals and in hypertensive animals after renal denervation suggested that adenosine deaminase per se does not have nonspecific antihypertensive effects.

We previously have found that renal denervation lowers sympathetic activity and arterial pressure in the one-kidney, one-clip hypertensive rat. We have interpreted this response to renal denervation as being caused by afferent renal nerve interruption. In this study, we found a similar lowering of sympathetic activity and arterial pressure in this model by selectively lowering urinary adenosine concentration. These data are consistent with the hypothesis that increased urinary adenosine concentration stimulates renal pelvic afferent fibers, which enhance sympathetic nervous system activity. In further support of the concept that intrarenal adenosine alters sympathetic activity by the afferent renal nerves are the findings that lowering urinary adenosine concentration lowers sympathetic activity when the renal nerves are intact, but that no response is seen when urinary adenosine concentration is lowered in renal-denervated one-kidney, one-clip hypertensive animals.

Acknowledgments
The authors express their gratitude to Sandra Whiteny for her technical assistance and to Dr. Charles R. Katholi for assistance in statistical analysis of the data. Sar1-Ala4-angiotensin II was supplied by Norwich Eaton Pharmaceuticals, Inc., Norwich, New York.

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