Endogenous Intrarenal Adenosine Preserves Renal Blood Flow in One-Kidney, One Clip Rats

RICHARD E. KATHOLI, ROBERT D. CREEK, AND WILLIAM P. McCANN

SUMMARY Intrarenal adenosine concentration is threefold greater in the one-kidney, one clip hypertensive rat compared with normotensive animals. Since exogenously administered adenosine may increase renal blood flow by direct vasodilation, inhibition of renin release, or prejunctional interruption of adrenergic neurotransmission, these studies examined whether endogenous intrarenal adenosine maintains renal blood flow distal to renal arterial stenosis. Administration of theophylline, which blocks the direct vasodilating effect of adenosine and antagonizes the inhibitory effect of adenosine on renin release and sympathetic neurotransmission, resulted in marked renal vasoconstriction in one-kidney, one clip hypertensive animals. This theophylline-induced renal vasoconstriction was markedly attenuated by angiotensin II blockade with saralasin and was unchanged by renal denervation or $\beta_1$-adrenergic blockade with atenolol. These findings indicate that the marked renal vasoconstriction in one-kidney, one clip hypertension during theophylline administration is mainly mediated by angiotensin II, is to a lesser degree due to inhibition of adenosine-induced vasodilation, and is independent of sympathetic influences. These data suggest that endogenous interstitial adenosine preserves renal blood flow in one-kidney, one clip hypertension mainly by inhibiting renin release. (Hypertension 11: 651-656, 1988)

KEY WORDS • renovascular hypertension • renin-angiotensin system • renal nerves • prostaglandins • sympathetic nervous system

ADENOSINE is readily released by renal proximal tubular cells into the interstitial and tubular fluid when renal blood flow is reduced.1-3 Studies from our laboratory indicate that urinary adenosine concentration and excretion are threefold greater in the one-kidney, one clip hypertensive rat compared with normotensive animals.4 Since long-term intrarenal administration of adenosine may increase renal blood flow by direct vasodilation, inhibition of renin release, or prejunctional interruption of adrenergic neurotransmission,5-11 we hypothesized that increased endogenous intrarenal adenosine contributes to the maintenance of renal blood flow distal to renal arterial stenosis in the one-kidney, one clip hypertensive rat. The objectives of these experiments were twofold: 1) to determine whether enhanced endogenous intrarenal concentration of adenosine in the one-kidney, one clip hypertensive rat contributes to the maintenance of renal blood flow, and 2) to determine the mechanisms by which intrarenal adenosine influences renal vascular resistance in this model.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats (weight, 534 ± 8 g; Charles River Breeding Laboratories, Wilmington, MA, USA) 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. Systolic blood pressure of all animals was measured twice weekly with the tail-cuff method without anesthesia (Narco Bio-Systems, Houston, TX, USA). 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 hyperten-
sion characterized by a positive sodium balance and enhanced sympathetic activity. Hypertensive animals and normotensive controls were then instrumented under pentobarbital anesthesia (50 mg/kg i.p., with a maintenance infusion of 0.1 mg/kg/min i.v.) with 0.025-in. (inside diameter) microline catheters in a femoral artery for mean arterial pressure measurement and in femoral veins for drug infusion. 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) was placed in the left renal artery for intrarenal theophylline infusion. 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. A pulsed Doppler flow probe (Pulsed-Doppler flowmeter, University of Iowa, Iowa City, IA, USA) was placed around the proximal left renal artery for measurement of renal blood flow. Zero flow was checked by occlusion at the end of the experiment. Renal vascular resistance was calculated using the following formula: mean arterial pressure (mm Hg)/mean blood flow (kHz). \[ R = \frac{P_{\text{mean}}}{Q_{\text{mean}}} \]

**Protocol**

To examine the effects of endogenous intrarenal adenosine on renal blood flow 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, Richmond, IN, USA) ad libitum. During experimental observations, arterial pressure and renal blood flow were recorded continuously on a Grass polygraph (Model 79D, Quincy, MA, USA). To remove the influence of prostaglandins, which are enhanced in an anesthetized animal and which have a compensatory vasodilating effect on the renal circulation, all animals were pretreated with indomethacin (5 mg/kg i.v.). Such treatment has been shown to effectively reduce renal prostaglandin synthesis in the rat and block vasoactive responses to arachidonic acid. Baseline values were established after at least an hour’s equilibration period. The response to an intrarenal infusion of theophylline (45 μg/kg/min i.v.) on mean arterial pressure, renal blood flow, and renal vascular resistance was initially determined. This dose of theophylline is known to have no influence on cyclic nucleotide phosphodiesterase activity. After learning that administration of theophylline, which blocks the direct vasodilating effect of adenosine and antagonizes the inhibitory effect of adenosine on renin release and sympathetic neurotransmission, resulted in an increase in renal vascular resistance, the mechanisms by which intrarenal adenosine influences renal flow were examined. The effect of angiotensin II on mean arterial pressure and renal vascular resistance was assessed by administering saralasin (3 μg/kg/min i.v.) for at least 30 minutes. This dose of saralasin inhibits the pressor response to a test dose (20 ng/kg) of angiotensin II. To assess sympathetic influences on renin release, a subset of animals underwent β2-adrenergic blockade with atenolol (0.5 mg/kg i.v.). To determine whether the renal vasoconstriction after theophylline was due to increased efferent renal nerve activity, a subset of animals underwent renal denervation. Renal denervation was accomplished by stripping the renal arterial adventitia and painting the renal artery with 20% phenol (wt/vol) in ethanol.

Time controls were run in a subset of hypertensive and normotensive animals. These animals received the vehicle of the substances infused during a time equivalent to that of the experimental groups. Numerical results were expressed as means ± SE. Values used for analysis represented the mean value during the last 5 minutes of each infusion period. Changes in mean arterial pressure, renal blood flow, and renal vascular resistance to these interventions were compared by repeated-measures analysis of variance. Comparisons were also made against a time control. Individual comparisons were made using Duncan’s multiple range test. Changes were reported as significant if the p value was less than 0.05.

**Results**

In six one-kidney, one clip hypertensive animals, theophylline administration resulted in no significant change in mean arterial pressure (from 175 ± 7 to 178 ± 8 mm Hg) while renal blood flow decreased 32% (p < 0.001) and renal vascular resistance increased 40% (p < 0.001). In six normotensive control animals, theophylline administration resulted in no significant change in mean arterial pressure (from 118 ± 5 to 120 ± 5 mm Hg), renal blood flow (4% decrease), or renal vascular resistance (6% increase). In these initial experiments, when saralasin was then administered to hypertensive animals after theophylline-induced renal vasoconstriction, renal blood flow and renal vascular resistance returned to baseline levels.

Subsequently, to examine further the mechanisms by which endogenous intrarenal adenosine influences renal vascular resistance, saralasin was administered to hypertensive and normotensive animals for at least 30 minutes before theophylline administration; it was administered simultaneously with theophylline for at least 30 minutes, and was later discontinued. As shown in Figure 1, Point B, continuous infusion of saralasin resulted in a significant 10% decrease in mean arterial pressure and a significant 8% increase in renal blood flow in hypertensive and normotensive animals. During the saralasin infusion, theophylline administration (Point C) resulted in no change in mean arterial pressure and a significant 10% decrease in renal blood flow in one-kidney, one clip animals. In contrast, no changes were observed in mean arterial pressure or renal blood flow in normotensive animals with the addition of theophylline (Point C). As shown at Point D, discontinuing the saralasin infusion resulted in a further 32% decrease in renal blood flow and a significant 10% increase in mean arterial pressure in
hypertensive animals. Discontinuing the saralasin infusion in normotensive animals resulted in a significant 13% decrease in renal blood flow and a significant 8% increase in mean arterial pressure (comparing Point D with Point C).

As shown in Figure 2, hypertensive and normotensive animals pretreated with atenolol had the same mean arterial pressure and renal blood flow responses to saralasin and theophylline and to discontinuing saralasin compared with respective groups described in Figure 1. As shown in Figure 3, hypertensive and normotensive animals that had undergone prior renal denervation had the same renal vascular response to saralasin and theophylline and to discontinuing saralasin compared with respective groups described in Figure 1. The mean arterial pressure of renal denervated hypertensive animals was significantly lower compared with the hypertensive animals with intact renal nerves described in Figure 1. Finally, in three one-kidney, one clip hypertensive animals and three normotensive animals, time control experiments showed no significant changes in mean arterial pressure or renal blood flow.

Discussion

Our study provides evidence that 1) theophylline administration to one-kidney, one clip hypertensive animals results in a marked decrease in renal blood flow, and 2) this theophylline-induced renal vasoconstriction is mainly mediated by angiotensin II. Taken together, these data suggest that endogenous interstitial adenosine preserves renal blood flow in one-kidney, one clip hypertension mainly by inhibiting renin release.

Previously, we reported another role for intrarenal adenosine in one-kidney, one clip hypertension. Our studies have suggested that increased intrarenal adenosine is a stimulus for afferent renal nerve activity with resultant increased sympathetic nervous system activity, thus producing hypertension in this model. We also previously observed that theophylline, a competitive antagonist of purinergic adenosine receptors, does
not alter the arterial pressure response to intrarenal adenosine infusion. This finding suggested that the afferent renal nerve excitatory action of adenosine is mediated by a different receptor. Consistent with this possibility is the observation by others that theophylline does not significantly alter the excitatory action of adenosine on cat carotid chemoreceptors. Thus, theophylline was used in the present experiments to investigate a role for intrarenal adenosine in the maintenance of renal blood flow distal to renal arterial stenosis without altering intrarenal adenosine's influence on arterial pressure through the afferent renal nerves.

Based on studies in which exogenous adenosine has been chronically infused into the renal artery, we hypothesized that adenosine could increase or maintain renal blood flow distal to arterial stenosis by direct vasodilation, by inhibition of renin release, or by prejunctional interruption of adrenergic neurotransmission. Theophylline has been shown to block these actions of adenosine. Theophylline treatment in one-kidney, one clip hypertensive animals resulted in a marked decrease in renal blood flow that could be markedly attenuated by angiotensin II blockade. These findings suggested that endogenous intrarenal adenosine maintains renal blood flow in this model by inhibiting renin release. Adenosine's inhibition of renin in this model occurred independently of prostaglandin or sympathetic influences since similar responses were observed in indomethacin-treated animals that underwent β-adrenergic blockade and renal denervation. A small inhibitory effect of adenosine on renin was also observed in normotensive animals.

These experiments revealed a small direct renal vasodilating effect of adenosine in hypertensive animals that was not observed in normotensive animals. Renal vasoconstriction was observed during theophylline administration in hypertensive animals when prostaglandin and angiotensin II influences on renal blood flow had been removed (Point C in the figures). The presence of a direct renal vasodilating effect of adenosine in hypertensive animals but not in normotensive animals is consistent with our previous findings demonstrating that intrarenal adenosine is threefold greater in the one-kidney, one clip hypertensive rat compared with normotensive animals. However, we found that the decrease in renal blood flow during theophylline did not appear to be mediated by increased efferent
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Mean arterial pressure and percent change in renal blood flow response to saralasin (Sar⁻¹-Ala⁻⁸-angiotensin II) administration and intrarenal theophylline infusion after renal denervation in indomethacin-pre-treated one-kidney, one clip hypertensive rats (●; top panel) and normotensive animals (○; bottom panel). Asterisk (p<0.05) and dagger (p<0.01) indicate significant difference compared with the preceding intervention. See Figure 1 for explanation of Points A, B, C, and D.

Figure 3.

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renal nerve activity. This latter observation is consistent with reports suggesting that there is an attenuation of efferent sympathetic control of renal vascular resistance in this model of hypertension. Thus, we found support for our hypothesis that intrarenal adenose may also preserve renal blood flow in one-kidney, one clip hypertension by direct vasodilation, but we found no evidence that intrarenal adenose preserved renal blood flow by prejunctional inhibition of sympathetic neurotransmission.

We and others have found that the established one-kidney, one clip hypertensive rat model is characterized by a positive sodium balance and increased sympathetic nervous system activity with "normal" renin-angiotensin system activity. Our present studies suggest that endogenous intrarenal adenose contributes to the negative feedback on renin release that occurs in this model. Recent studies of others suggest that intrarenal adenose also inhibits renin release in two-kidney, one clip hypertension. In the two-kidney, one clip renovascular model, adenose receptor blockade with caffeine resulted in a sevenfold increase in plasma renin activity. Our studies further demonstrate that a major hemodynamic effect of the inhibition of renin release by intrarenal adenose in the one-kidney, one clip hypertensive rat is the preservation of renal blood flow distal to renal arterial stenosis.

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