Renal Arteriolar Angiotensin Responses During Varied Adenosine Receptor Activation

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Abstract We performed experiments to test the hypothesis that endogenous adenosine acts as an essential cofactor required for eliciting angiotensin II (Ang II)—induced afferent and/or efferent arteriolar vasoconstriction. Enalaprilat (2 mg IV) was administered to anesthetized rats to reduce endogenous Ang II levels. Kidneys and blood were harvested from these animals and used for study of renal microvascular function using the in vitro blood-perfused juxtamedullary nephron technique. Arteriolar inside diameter was monitored videomicroscopically in (1) normal kidneys, (2) kidneys subjected to adenosine receptor blockade (100 μmol/L 1,3-dipropyl-8-p-sulfophenylxanthine), and (3) kidneys continuously exposed to 1 μmol/L adenosine. Under resting conditions, arteriolar diameters were similar in all three groups of kidneys, averaging 24.8±1.0 μm (n=23) in afferent arterioles and 24.0±0.9 μm (n=16) in efferent arterioles. In normal kidneys, adenosine (10 μmol/L) decreased both afferent (10.2±2.0%) and efferent (6.5±0.8%) diameters, an effect that was absent in kidneys subjected to adenosine receptor blockade. Ang II (10 pmol/L to 100 nmol/L) elicited dose-dependent vasoconstriction of both vascular segments in normal kidneys. At a concentration of 100 nmol/L, Ang II decreased afferent diameter by 36.8±8.5% and efferent diameter by 30.8±9.6%. Neither afferent nor efferent arteriolar Ang II dose-response relations were significantly different in kidneys treated with low-dose adenosine or the adenosine receptor blocker. These observations refute the hypothesis that a receptor-mediated action of adenosine is required for Ang II—induced constriction of juxtamedullary afferent or efferent arterioles. Furthermore, subconstrictor adenosine levels do not potently modulate renal arteriolar vasoconstrictor responses to Ang II.

Key Words • arterioles • renal circulation • angiotensin II • receptors, purinergic • adenosine

Within the renal microvasculature, angiotensin II (Ang II) is capable of evoking vasoconstriction at both preglomerular and postglomerular sites; however, under some experimental conditions, the peptide seems to exert a predominantly efferent vasoconstrictor effect. Such observations have led to the suggestion that the effects of Ang II on afferent and/or efferent arterioles might be modulated by the action of an endogenously produced substance. The substances proposed to modulate renal microvascular Ang II responsiveness include vasodilator prostaglandins, endothelium-derived relaxing factor, and adenosine. The proposed role of adenosine is unique among these substances in that local production of this compound is postulated to accentuate rather than attenuate vasoconstrictor responses to Ang II. For example, Hall and Granger reported that intrarenal adenosine infusion alters the influence of Ang II on glomerular function in the dog in a manner consistent with an unmasking of a preglomerular vasoconstrictor effect of Ang II. These investigators proposed that prevailing interstitial adenosine levels may be an important determinant of afferent arteriolar Ang II responsiveness, whereas efferent responses to Ang II appear to be adenosine independent. Reports that both adenosine and its receptor agonists exert differing influences on afferent and efferent arteriolar resistances lend credence to the postulate that adenosine may selectively modulate afferent arteriolar Ang II responsiveness. Although recent micropuncture studies suggest that coactivation of Ang II and adenosine receptors may be required for either agonist to act as a potent preglomerular vasoconstrictor, studies using the hydronephrotic rat kidney indicate that Ang II can induce preglomerular arteriolar constriction through a mechanism that is independent of adenosine. Thus, conflicting data exist concerning the ability of adenosine to selectively modulate afferent arteriolar Ang II responsiveness.

The primary evidence against a modulatory role of adenosine in determining the renal microvascular response to Ang II was derived from studies using hydro nephrotic kidneys, which lack intact structural or functional tubulovascular interrelationships. Because renal tissue adenosine levels are determined primarily by the metabolic activity required for tubular transport processes in the normal kidney, tissue adenosine concentration and its effect on Ang II responsiveness may be underestimated in the nonfiltering hydronephrotic kidney. No direct data are available detailing the role of endogenous adenosine in modulating arteriolar Ang II responses of filtering and transporting nephrovascular units. Therefore, we performed the present studies to test the hypothesis that a receptor-mediated action of endogenous adenosine modulates afferent and/efferent arteriolar responses to Ang II in intact nephrovascular units.

Methods All experiments used the in vitro blood-perfused juxtamedullary nephron technique to provide direct access to afferent and efferent arterioles of the rat kidney and were performed in accordance with the guidelines of the Advisory Committee.
for Animal Resources of Tulane University School of Medicine. Male Sprague-Dawley rats weighing 350 to 410 g were anesthetized with pentobarbital sodium (40 mg/kg IP). Thirty minutes after enalaprilat (2 mg IV) administration, tissue was harvested for use in vitro experiments. Kidney Ang II levels measured by radioimmunoassay are reduced by 60% and Ang II levels in the perfusate are undetectable in tissue pretreated with enalaprilat (unpublished observations).

Kidney donor rats were subjected to the following procedure: After enalaprilat administration, a cannula was introduced into the aorta, after which the right renal artery, thus initiating perfusion of the right kidney with Tyrode’s solution (pH 7.40) containing 773 μmol/L (51 g/L) bovine serum albumin. 1 The [Ca2+] of this perfusate solution was measured (model 634 Ca2+/pH Analyzer, Ciba-Corning Diagnostics, Pensacola, Fla) and adjusted to 1.0 to 1.2 mmol/L. While the perfusion was maintained, the kidney was removed from the rat, and the dorsal third of the organ was removed, retaining the papilla intact. The tip of the papilla was reflected away from the pelvic region and maintained in that position by insect pins. The pelvic mucosa and underlying adipose tissue were carefully removed, thus exposing the vascular and tubular structures of the inside cortical surface. Large venous structures were removed, and tight ligatures were placed around the distal aspects of the accessible arterial supply. This procedure restricted perfusion to a small percentage of the total nephron population, including the juxtamedullary nephrons that comprise the inside cortical surface.

The Tyrode’s perfusate was replaced with a perfusate derived from homologous blood obtained by exanguination of enalaprilat-treated, acutely nephrectomized rats. The blood was collected in a syringe containing 300 U heparin, centrifuged at 4°C, and plasma and erythrocytes were collected separately. Plasma [Ca2+] was measured and adjusted to 1.0 to 1.2 mmol/L. The plasma was passed sequentially through 5- and 0.22-μm filters and mixed with erythrocytes to achieve a hematocrit of 33%. The reconstituted blood was placed in a perfusate reservoir and pressurized under a pressure (measured at the tip of the perfusion cannula) of 110 mm Hg. The inner cortical surface of the kidney was continuously superfused with warmed (37°C) Tyrode’s solution containing 152 μmol/L (10 g/L) bovine serum albumin. Afferent arteriolar diameter was measured at 6-second intervals for the duration of the experiment. The average diameter during that were reproducible to within 1 μm. Inside diameter was measured from a single arteriolar site at 6-second intervals for the duration of the experiment. PSPX was purchased from Research Biochemicals, Natick, Mass. Enalaprilat was provided by Merck Sharp & Dohme Research Laboratories, Rahway, NJ. All other chemicals were purchased from Sigma Chemical Co, St Louis, Mo.

Perfusate and bathing solutions were prepared fresh daily. Stock solutions of Ang II (100 μmol/L) and adenosine (1 mmol/L) were stored in aliquots at −70°C and diluted, as necessary, with standard Tyrode’s superfusate solution on the day of the experiment. PSPX was purchased from Research Biochemicals, Natick, Mass. Enalaprilat was provided by Merck Sharp & Dohme Research Laboratories, Rahway, NJ. All other chemicals were purchased from Sigma Chemical Co, St Louis, Mo.

Microvessel inside diameter was measured from videotaped images with a digital image-sensing monitor. The measurement system was calibrated using a stage micrometer (smallest division, 2 μm) and yielded arteriolar diameter measurements that were reproducible to within 1 μm. Inside diameter was measured from a single arteriolar site at 6-second intervals for the duration of the experiment. The average diameter during that were reproducible to within 1 μm. Inside diameter was measured from a single arteriolar site at 6-second intervals for the duration of the experiment. The average diameter during the final 2 minutes of each 5-minute treatment period was used for statistical analysis. Statistical differences were evaluated by analysis of variance for repeated measures and least significant difference comparison using the PC ANOVA statistical package (Human Systems Dynamics, Northridge, Calif). Comparisons between groups used the unpaired t test. A value of P<.05 was considered significant. Data are presented as mean±SEM.

Results
In normal kidneys, inside diameters of juxtamedullary afferent and efferent arterioles averaged 27.6±2.0 (n=6) and 24.8±1.2 (n=5) μm, respectively. Adenosine (10 μmol/L) reduced afferent arteriolar diameter to 24.8±2.0 μm, and efferent diameter decreased to 23.2±1.2 μm (Fig 1). The vasoconstrictor effect of adenosine was statistically significant in each arteriolar type and did not differ significantly between afferent and efferent arterioles. Removal of adenosine from the superfusate bathing solution allowed complete recovery of afferent diameter to 27.5±2.0 μm, whereas efferent diameter was restored to 24.7±1.2 μm. Thus, 10 μmol/L adenosine evoked a reversible vasoconstriction of both afferent and efferent arterioles of juxtamedullary nephrons in normal kidneys.

In PSPX-treated kidneys, afferent arteriolar diameter averaged 23.3±1.5 μm (n=8) and efferent diameter was 25.4±1.8 μm (n=5). These values did not differ significantly from arteriolar diameters in normal kidneys (ie, in the absence of PSPX). In contrast with the behavior of normal kidneys, adenosine failed to alter arteriolar diameters significantly in PSPX-treated kidneys (Fig 1). Afferent arteriolar diameter in PSPX-treated kidneys was 23.3±1.5 μm during exposure to 10 μmol/L adenosine, whereas efferent diameter averaged 25.3±1.8 μm. Thus, treatment of the in vitro blood-perfused juxtamedullary arteriole microvasculature with 100
In kidneys subjected to continuous adenosine exposure, afferent and efferent arteriolar diameters averaged 25.0±1.5 (n=9) and 21.5±1.5 (n=6) μm, respectively, before adenosine exposure. After addition of 1 μmol/L adenosine to the bathing solution, afferent diameter was 24.3±1.5 μm and efferent diameter was 22.2±1.5 μm. These values did not differ significantly from arteriolar diameters before exposure to adenosine, in accord with our previous observation that 10 μmol/L adenosine is required to evoke a significant change in afferent arteriolar diameter.17 Furthermore, arteriolar diameters in tissue exposed to 1 μmol/L adenosine did not differ from PSPX-treated vessels. Thus, baseline arteriolar diameters were similar in all three treatment groups.

Responses of afferent arterioles to Ang II are depicted in Fig 2 (top). In normal kidneys, Ang II evoked dose-related decreases in afferent arteriolar inside diameter from 27.5±2.0 μm to values averaging 27.4±2.0 (10 pmol/L), 26.5±2.0 (100 pmol/L), 25.2±2.0 (1 nmol/L), 22.3±2.4 (10 nmol/L), and 17.1±2.5 (100 nmol/L) μm. In the adenosine receptor blockade group, Ang II decreased afferent diameter from 23.3±1.5 μm to 22.7±1.4 (10 pmol/L), 22.1±1.3 (100 pmol/L), 21.5±1.3 (1 nmol/L), 18.5±1.5 (10 nmol/L), and 14.3±2.1 (100 nmol/L) μm. Thus, adenosine receptor blockade with 100 μmol/L PSPX failed to elicit any discernible shift in the concentration-related afferent arteriolar vasoconstrictor response to Ang II. In kidneys exposed continuously to 1 μmol/L adenosine, Ang II caused similar concentration-related decreases in afferent arteriolar diameter, from 24.3±1.5 μm to values averaging 23.5±1.4 (10 pmol/L), 22.7±1.6 (100 pmol/L), 21.6±1.8 (1 nmol/L), 19.6±1.8 (10 nmol/L), and 18.4±1.7 (100 nmol/L) μm. None of these values differed significantly from those observed in normal or PSPX-treated afferent arterioles at any given Ang II concentration.

In Fig 2 (bottom) illustrates the effects of Ang II on efferent arterioles. In normal kidneys, Ang II induced concentration-related efferent arteriolar vasoconstriction that was quantitatively similar to that observed in afferent arterioles. Ang II reduced efferent arteriolar inside diameter from 24.7±1.2 μm to 24.1±1.2 (10 pmol/L), 23.3±1.2 (100 pmol/L), 22.8±1.3 (1 nmol/L), 20.0±2.1 (10 nmol/L), and 17.3±2.8 (100 nmol/L) μm. In PSPX-treated kidneys, angiotensin evoked similar concentration-related efferent vasoconstriction. Efferent diameter was decreased from 25.3±1.7 μm to 24.6±1.7 (10 pmol/L), 24.7±1.7 (100 pmol/L), 23.9±1.8 (1 nmol/L), 20.8±1.8 (10 nmol/L), and 19.9±2.0 (100 nmol/L) μm in PSPX-treated kidneys. Thus, there was no statistically significant effect of PSPX treatment on Ang II-induced efferent arteriolar vasoconstriction. In kidneys exposed to 1 μmol/L adenosine, Ang II reduced efferent diameter from 22.2±1.5 μm to 22.0±1.4 (10 pmol/L), 21.6±1.4 (100 pmol/L), 21.1±1.3 (1 nmol/L), 19.4±1.5 (10 nmol/L), and 18.1±1.3 (100 nmol/L) μm. These efferent arteriolar Ang II responses in adenosine-supplemented tissue did not differ significantly from those observed in normal or PSPX-treated kidneys at any given Ang II concentration.

Discussion

The results of the present study reveal a vasoconstrictor influence of adenosine on both afferent and efferent arterioles of juxtamedullary nephrons. This vasoconstriction was abolished in the presence of the adenosine receptor antagonist PSPX; however, Ang II concentra-
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Decision-response profiles were not influenced by adenosine receptor blockade or adenosine supplementation. These observations have numerous implications regarding the renal microvascular responses to adenosine and Ang II.

Adenosine interacts with $A_1$ receptors to elicit vasodilation of most vascular beds; however, the kidney and skin are distinct in that the vasculature is also endowed with $A_2$ receptors known to evoke vasoconstriction. In the renal vasculature, afferent arterioles are known to be endowed with $A_2$ receptors, whereas $A_3$ receptors are thought to mediate vasodilation in effenter arterioles alone or both afferent and effenter arterioles. Reports are available detailing the effects of adenosine and adenosine agonists on the renal microvasculature of split nephros, renal implants into hamster cheek pouch, and isolated afferent arterioles; however, all three of these experimental models provide access to renal microvascular structures in settings that preclude the glomerular filtration and tubular transport processes of the nephron.

In contrast, studies in the kidney have suggested that adenosine altered the glomerular filtration rate response to Ang II by allowing a marked postglomerular vasodilation. In a recent report, the vasodilator component lagged 70 seconds behind the vasoconstriction. In the present study, the time courses of afferent and effenter vasoconstrictor responses to adenosine were similar (data not shown). Responses of both vessel segments displayed a sustained decrease in diameter, with no tendency for recovery to control values during the 5-minute treatment period. Differences in these observations likely arise from the mode of adenosine preparation (functional versus adventitial). A transendothelial diffusion barier to adenosine and/or an endothelium-dependent component of the response could underlie a more prominent vasodilator influence of exogenous adenosine when administered intravascularly. The results of the present study, which used topically applied adenosine, may better indicate the influence of adenosine produced endogenously by the transporting epithelium and its subsequent approach to the vasculature via the interstitial compartment.

In addition to the direct vasoactive influence of adenosine, this substance may interact with Ang II to determine vascular tone. Numerous reports indicate that Ang II levels are important modulators of the renal hemodynamic response to adenosine. Although some data fail to support this contention, the ability of adenosine to modulate Ang II-induced vasoconstriction is less well defined. In the mesenteric vasculature, for example, the action of exogenous adenosine can attenuate Ang II-induced vasoconstriction. In contrast, studies in the kidney have suggested that adenosine may act through $A_1$ receptors to enhance Ang II-induced vasoconstriction. This postulate was first raised with regard to the mechanism of postocclusive renal vasoconstriction. Hall and Granger more fully addressed the hypothesis through comparison of the effects of Ang II on glomerular filtration rate in the presence and absence of an intrarenal adenosine infusion. During adenosine infusion, Ang II evoked a reduction in glomerular filtration rate that was not observed in dogs not receiving adenosine. Estimates of preglomerular and postglomerular resistance responses suggested that adenosine altered the glomerular filtration rate response to Ang II by allowing a marked preglomerular vasoconstrictor response to the peptide. The effects of Ang II on postglomerular resistance were not influenced by adenosine infusion. Thus, tissue adenosine levels were proposed to exert important functional consequences by determining whether Ang II evokes both preglomerular and postglomerular vasoconstriction or a more prominent afferent arteriole action. Based on their observations, Hall and Granger suggested that high adenosine levels that might accompany malignant hypertension, acute renal failure, or ischemia
could severely reduce glomerular filtration rate by causing Ang II to constrict preglomerular vessels. More recently, Schnermann and coworkers reported data which indicated that endogenously formed adenosine and Ang II act in a synergistic manner to elicit preglomerular vasoconstriction in the anesthetized rat. This observation led to the hypothesis that coactivation of Ang II and adenosine receptors is required for either agonist to act as a potent afferent arteriolar vasoconstrictor. However, Dietrich and colleagues were unable to document an adenosine-dependent component of Ang II-induced vasoconstriction in the hydropnephrotic rat kidney, a fact that might reflect the uncertain state of endogenous adenosine production in that experimental setting.

The present studies were performed to address this issue in a setting that provides access to the renal microvasculature while maintaining glomerular and tubular function, thus more closely reflecting the ability of endogenous adenosine to modulate Ang II responsiveness. The in vitro blood-perfused juxtamedullary nephron technique is particularly useful for these studies because both the afferent and efferent arterioles are responsive to exogenous Ang II. Thus, the strategy in determining the role of endogenous adenosine in modulating Ang II responsiveness was to compare Ang II concentration-response profiles in normal tissue and in tissue subjected to pharmacologic blockade of adenosine receptors or supplementation of adenosine levels. If endogenous adenosine acts as a cofactor that is required to elicit Ang II-induced afferent and/or efferent arteriolar vasoconstriction, Ang II–dependent vasoconstriction should be attenuated or abolished during adenosine receptor blockade. In agreement with the data of Dietrich et al from the hydropnephrotic kidney, the results of the present study indicate that the concentration-dependent Ang II–induced vasoconstriction of both afferent and efferent arterioles is sustained during documented PSPX-induced blockade of adenosine receptors. Because Ang II responses were not influenced by adenosine receptor blockade, these data refute the hypothesis that a receptor-mediated action of endogenous adenosine is required for expression of Ang II–induced constriction of juxtamedullary arterioles. Furthermore, supplementation of endogenous adenosine with a constrictor concentration of exogenous adenosine failed to enhance Ang II responsiveness of either afferent or efferent arterioles. Thus, local adenosine concentrations do not appear to be a potent determinant of vascular responsiveness to Ang II in the juxtamedullary nephron population, although we cannot rule out the possibility that adenosine levels high enough to exert a direct vasoconstrictor influence might interact with Ang II in a more than additive manner.

Discrepancies between these observations and previous reports may reflect functional differences between superficial and juxtamedullary nephrons or a heightened influence of endothelium-dependent factors in previous studies that used intravascular administration of adenosine or adenosine analogues.

In summary, the vasoconstrictor influence of exogenous adenosine on afferent and efferent arterioles and the blockade of these responses by PSPX suggest that adenosine A1 receptors are present at both preglomerular and postglomerular sites in rat juxtamedullary nephrons. In accord with our previous observations, resting arteriolar diameters did not differ between normal and PSPX-treated kidneys, indicating that endogenous adenosine levels do not exert a substantial tonic vasoconstrictor influence on the renal microvasculature when studied using the in vitro blood-perfused juxtamedullary nephron technique. Both afferent and efferent arteriolar responses to Ang II were sustained during PSPX blockade of adenosine receptors, thus refuting the hypothesis that a receptor-mediated action of adenosine is required for expression of Ang II–induced constriction of juxtamedullary afferent arterioles. Furthermore, neither arteriolar segment exhibited enhanced Ang II responsiveness in the presence of subconstrictor concentrations of exogenous adenosine. We conclude that the Ang II responsiveness of juxtamedullary afferent and efferent arterioles does not require a receptor-mediated action of adenosine and is not potentially modulated by tissue adenosine concentration.

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