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J Clin Invest. 1996;98(1):18-23. https://doi.org/10.1172/JCI118764.

Research Article

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Adenosine Modulates Vasomotor Tone in Outer Medullary Descending Vasa Recta of the Rat

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Abstract

Adenosine is generated within the renal medulla under hypoxic conditions and is known to induce net vasoconstriction within the renal cortex while increasing medullary blood flow and oxygenation. To test the hypothesis that vasoconstriction of outer medullary descending vasa recta (OMDVR) is modulated by adenosine, we examined the effects of adenosine and adenosine A_1 and A_2 receptor subtype agonists on in vitro perfused control and preconstricted rat OMDVR. Constriction with angiotensin II (ANG II, 10^{-9} M) was attenuated by adenosine in a concentration-dependent manner ($EC_{50} = 2.0 \times 10^{-7}$ M, $P < 0.05$). Similarly, an adenosine A_2 agonist (CGS-21680, 10^{-7} M), but not an adenosine A_1 agonist (cyclohexyladenosine, 10^{-6} M), attenuated ANG II–induced vasoconstriction. Under control conditions, ablumenal application of adenosine (10^{-12} to 10^{-5} M) elicited a biphasic response. Additionally, cyclohexyladenosine (10^{-6} M) caused vasoconstriction and CGS-21680 (10^{-6} M) had no effect on untreated vessels. Finally, an influence of ANG II receptor stimulation on adenosine A_1 receptor–mediated vasoconstriction could not be shown. These data suggest that OMDVR possess both A_1 and A_2 adenosine receptors and that they mediate constriction and dilatation, respectively. We conclude that adenosine is a potent modulator of OMDVR vasomotor tone and that its net effect is dependent upon local concentrations. (*J. Clin. Invest.* 1996. 98:18–23.) Key words: microcirculation • microperfusion • videomicroscopy • vasoconstriction • vasodilatation

Introduction

The renal medullary microvasculature must satisfy the conflicting requirements of preserving corticomedullary gradients of NaCl and urea while maintaining adequate oxygen and nutrient delivery to the medulla. To accomplish the former, descending and ascending vasa recta (AVR)[1] are arranged in a countercurrent distribution. However, it has been proposed that this countercurrent relationship interferes with overall medullary oxygenation by allowing diffusive efflux of O_2 from the descending to the ascending limb of the microvascular exchanger. It seems likely that diffusive loss of oxygen, coupled with presence of the highly metabolic medullary thick ascending limb of Henle (MTAL) accounts for the low oxygen tensions that have been observed in this region by a number of investigators (1–4).

Adenosine appears to have a unique capability to modulate regional blood flow within the kidney (1, 5). In contrast to its net vasodilatory effect on most microvascular beds, adenosine reduces net renal blood flow primarily by constriction of afferent arterioles in the renal cortex (6). However, within the renal medulla, evidence favors a role for adenosine to act as a vasodilator (7, 8). Beach et al. have shown that the MTAL releases adenosine under hypoxic conditions (9). It has also been shown that interstitially administered adenosine improves medullary oxygenation (10) subsequent to increases in medullary blood flow (8). A role for adenosine to function as a defender of the medulla from hypoxic insult is readily inferred from those studies.

The sites at which adenosine exerts its actions in the medulla have not been determined completely. Locally produced adenosine might modulate medullary blood flow through its actions on the arterioles of juxtamedullary glomeruli (11–13). However, in addition to this, all blood flow to the renal medulla must pass through outer medullary descending vasa recta (OMDVR) that lie in vascular bundles of the inner stripe (14) so that OMDVR might also be an important site of action for adenosine. To enable a direct test of the hypothesis that adenosine modulates vasomotor tone in OMDVR, we dissected these vessels and perfused them in vitro. Adenosine and adenosine A_1 and A_2 receptor subtype agonists were applied to the ablumenal surface of microperfused OMDVR in the presence and absence of angiotensin II (ANG II). Videomicroscopic observation verifies the ability of adenosine to modulate OMDVR vasomotor tone in a manner that is dependent upon local adenosine concentration.

Methods

In vitro microperfusion

Details of the methods used to perfuse OMDVR (15) and document their contractility (16, 17) have been published. The techniques are a

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Received for publication 5 September 1995 and accepted in revised form 30 April 1996.

J. Clin. Invest.

0021-9738/96/07/18/06 $2.00
Volume 98, Number 1, July 1996, 18–23

1. *Abbreviations used in this paper:* ANG II, angiotensin II; AVR, ascending vasa recta; CHA, cyclohexyladenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; MTAL, medullary thick ascending limb of Henle; OMDVR, outer medullary descending vasa recta.

minor variation of those originally described by Burg (18). In brief, young female Sprague Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) were anesthetized by intraperitoneal injection of thiopental (50 mg/kg) after which the kidneys were harvested, sliced, and placed into cold (4°C) Hepes buffer (Hepes 5 mM, NaCl 140 mM, Na acetate 10 mM, KCl 5 mM, $MgCl_2$ 1.2 mM, Na_2HPO_4 1.71 mM, NaH_2HPO_4 0.29 mM, $CaCl_2$ 1 mM, alanine 5 mM, glucose 5 mM, and albumin 0.5 gram/dl, adjusted to pH 7.4 and bubbled with 100% O_2). The methods used to dissect OMDVR and construct pipettes for microperfusion have been described in detail (15–17).

Harvested vessels were transferred to the stage of an inverted microscope (Nikon diaphot) equipped with Differential Interference Contrast (DIC, Nomarski) optics, cannulated, and perfused at 37°C with the same Hepes buffer used for dissection. The bath was identical to the perfusate except for addition of hormones. The bath flow rate was 300 μl/min. The addition of hormones during experiments was achieved by three rapid exchanges of the bath from a prewarmed syringe. Micromanipulators, perfusion and collection apparatus, and perfusion chamber were purchased from Instruments Technology and Machinery (San Antonio, TX). Temperature of the perfusion chamber was maintained at 37°C with a feedback system using a CN9111A controller (Omega Engineering, Inc., Stamford, CT).

After a 30-min equilibration period, perfusions were standardized by the adjustment of collection rate to the desired value (5 nl/min in most experiments). Timed collections of vessel effluent were obtained with volumetric constriction pipettes after which collection rate was adjusted by changing the pressure in the perfusion pipette. Driving pressure, once adjusted, was maintained constant throughout the remainder of each experiment. Continuous measurement of lumenal pressure in the microperfused vessel has not been feasible; however, we have performed separate experiments that show lumenal pressures to be < 15 mmHg (16).

Videomicroscopy and measurement of vessel diameters

To evaluate the effects of vasoactive agents on OMDVR diameters, microperfusion experiments were recorded on video tape. The inverted microscope was equipped with a 20/80% beam splitter and a side port for attachment of a video camera (Dage-MTI, CCD model 72). During experimentation, OMDVR were observed with a ×40 objective to yield a final magnification of 1,300 on the video screen. Experiments were recorded on a VCR (model AG 1960; Panasonic) with a microphone for audio recording of experimental events. During playback, vessel diameters were measured by video edge detection (model VED 103; Crescent Electronics) at the point of greatest constriction or dilation. Changes in vessel diameter are expressed as percent constriction, defined in terms of the basal diameter in the absence of hormones (Do) and the experimental diameter (D) by the expression, %Constriction = $(1 - D/Do) \times 100$.

Reagents

ANG II, saralasin ([Sar^1, Ala^8] ANG II), adenosine, and CHA (cyclohexyladenosine) were purchased from Sigma Immunochemicals (St. Louis, MO). DPCPX (8-cyclopentyl-1,3-dipropylxanthine) and CGS-21680 (2-*p*-(2-carboxyethyl)phenethylamino-5′-*N*-ethylcarboxamido adenosine hydrochloride) were purchased from Research Biochemicals International (Natick, MA). CHA and DPCPX were dissolved in ethanol while all other agents were dissolved in deionized water. After solubilization, all pharmacological agents were stored at −20°C in small aliquots of 10^{-2} to 10^{-5} M. Aliquots were thawed and diluted at least 1,000-fold on the day of the experiment. Unused portions were discarded. Similar dilutions of vehicle were not vasoactive.

Experimental protocol

Time course of ANG II–induced vasoconstriction and vasodilatation by adenosine of OMDVR. In one series of experiments, after equilibration, ANG II (10^{-9} M) was added to the bath. Vessel diameters were measured every minute for the first 10 min and every 5 min thereafter for an additional 20 min. A separate set of vessels was treated in a similar fashion except that adenosine (10^{-6} M) was added to the bath after 5 min of exposure to ANG II. Vessel diameters were measured at the same intervals.

Concentration-dependent effects of ANG II and adenosine on ANG II–induced vasoconstriction in OMDVR. After cannulation, OMDVR were perfused for 30 min for equilibration. Subsequently ANG II was added at 10-fold increments (10^{-11} to 10^{-8} M) to the bath and diameters were measured after 5 min at each concentration. In a previous publication (17) we measured lumenal diameters directly from the calibrated video screen with calipers. To further limit opportunity for investigator bias, we now use video edge detection. For comparison, vessel diameters were measured directly from the video screen with calipers (inner and outer diameters) and with video edge detection (Fig. 1, $n = 5$). Video edge detection was otherwise used exclusively throughout these studies.

In separate experiments, vessels were preconstricted for 5 min with ANG II (10^{-9} M) after which adenosine (10^{-9} to 10^{-5} M) was added to the bath along with ANG II (10^{-9} M) or sham exchange with ANG II (10^{-9} M) was performed. Vessel diameters were measured 5 min after each bath exchange.

Selective stimulation of the A_1 and A_2 receptor subtypes during ANG II–induced constriction. To determine the effect of A_1 receptor stimulation on the response to ANG II we used the specific A_1 agonist, CHA. After control measurements, ANG II (10^{-9} M) was added to the bath for 5 min after which, CHA (10^{-6} M) was added along with ANG II. Finally, CHA was removed from the bath leaving ANG II (10^{-9} M) alone.

Similar experiments were conducted to determine the effect of A_2 receptor stimulation on the vasoconstrictor response of ANG II (10^{-9} M). In this experiment CGS-21680 (10^{-7} M) was added to the bath after a 5-min pretreatment with the A_1 blocker DPCPX (10^{-8} M). DPCPX was added in this protocol to assure selective stimulation of A_2 receptors. Again, recovery of constriction in the presence of ANG II was determined after removal of CGS-21680 and DPCPX from the bath. For comparison, adenosine (10^{-6} M) was examined in a similar protocol.

Direct effects of adenosine, A_1, and A_2 receptor stimulation. After control diameter measurements, CHA (10^{-6} M) or CGS-21680 (10^{-6} M) was added to the bath. Vessel diameters were measured after 5 min. The bath was then repeatedly exchanged with control buffer and recovery was reexamined after 5 min. Pilot experiments showed that

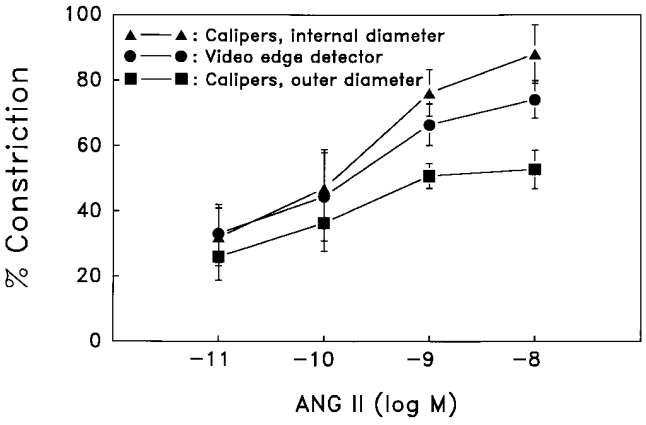

Figure 1. Concentration-dependent vasoconstriction induced by ANG II (10^{-11} to 10^{-8} M, $n = 5$) added to the bath. Comparison of caliper and video edge detector measurements of vessel diameters is displayed as percent constriction from baseline versus log molar ANG II concentration.

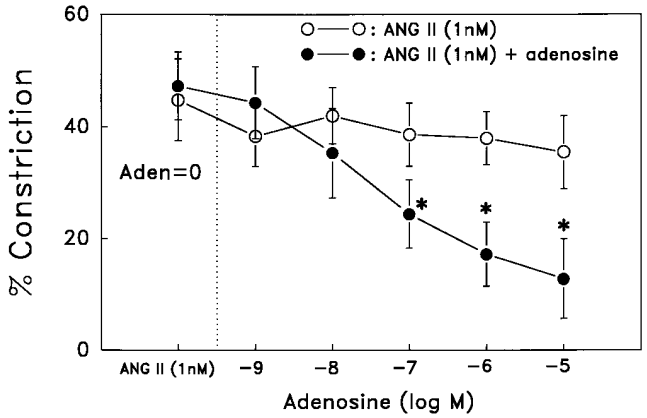

Figure 2. Concentration dependence of vasodilation of OMDVR by adenosine after ANG II (10^{-9} M) preconstriction. Percent constriction during ANG II administration is shown in the presence (*closed circles*, $n = 9$) and absence (*open circles*, $n = 7$) of log molar increments in adenosine concentration. *Significant reversal of constriction after adenosine administration ($P < 0.05$).

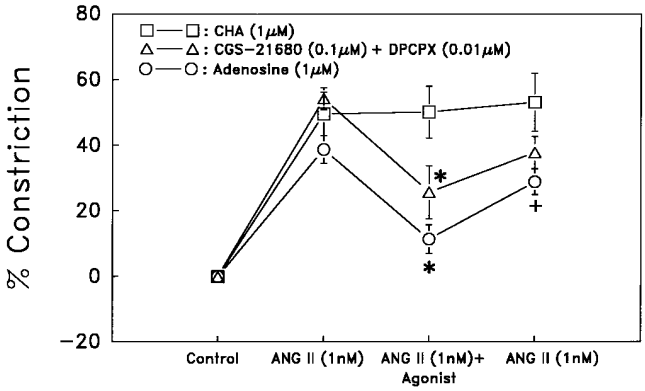

Figure 3. Effects of adenosine receptor agonists on ANG II–induced constriction. Effect of CHA, an adenosine A_1 receptor agonist (10^{-6} M, *squares*, $n = 7$), CGS-21680, an A_2 receptor agonist (10^{-7} M, *triangles*, $n = 8$), or adenosine (10^{-6} M, *circles*, $n = 7$) on OMDVR preconstricted with ANG II (10^{-9} M) is shown. Significant reversal of constriction was produced by adenosine and CGS-21680 (*$P < 0.05$). Recovery of constriction achieved significance after removal of adenosine ($^{+}P < 0.05$) but not CGS-21680.

vessels treated with CGS-21680 (10^{-6} M) showed no change in diameter so that this maneuver was followed by treatment with ANG II (10^{-9} M) to demonstrate intact vasoactivity of the vessels. Additionally, concentration-dependent effects of adenosine (10^{-12} to 10^{-5} M in log molar increments) were examined. Vessel diameters were measured 5 min after each concentration change.

Effects of ANG II receptor blockade on CHA- or adenosine-induced constriction. To examine the proposed interaction of adenosine and ANG II in the renal microcirculation we tested the ability of an adenosine A_1 receptor antagonist to block the effects of ANG II and the effects of ANG II receptor blockade to inhibit vasoconstriction induced by A_1 receptor stimulation. Furthermore, the ability of ANG II to modulate adenosine-induced constriction was also examined.

In one set of experiments, vessels were pretreated with DPCPX (10^{-5} or 10^{-8} M) after which ANG II (10^{-9} M) was added in the presence of DPCPX. Recovery after removal of DPCPX was observed. DPCPX (10^{-8} M) was tested against CHA (10^{-6} M) in a similar protocol. In an analogous experiment, the ability of saralasin (10^{-7} M) to inhibit ANG II (10^{-9} M) or CHA (10^{-6} M) was examined. In all cases antagonists were added to the bath for 5 min before addition of agonist. Finally, the ability of ANG II pretreatment to modulate adenosine induced constriction (10^{-12} to 10^{-8} M) was examined. Adenosine was added to the bath in log molar increments in the presence of a threshold (10^{-14} M) or suprathreshold (10^{-11} M) pressor concentration of ANG II after a 5-min pretreatment with ANG II alone.

Statistical analysis

Experimental results are reported as mean±SE. Statistical comparisons use a paired *t* test or repeated measures ANOVA as appropriate. For ANOVA, significance was determined by the Student-Newman-Keuls test. *P* values < 0.05 are considered significant.

Results

Stability of ANG II–induced vasoconstriction and adenosine-induced relaxation. OMDVR exposed to ablumenal application of ANG II (10^{-9} M, $n = 7$) constricted from a mean diameter of 10.8±1.0 μm to reach a minimum of 5.5±0.9 μm in ~2 min (data not shown). The constriction waned slightly after peaking and then remained stable for the duration of exposure to ANG II (30 min). The addition of adenosine (10^{-6} M) after 5 min of exposure to ANG II resulted in attenuation of constriction which was apparent almost immediately but which reached significance only after 10 min.

Concentration dependence of ANG II–induced vasoconstriction and attenuation by adenosine. Vasoconstriction with ANG II was concentration dependent ($P < 0.05$, $n = 5$, Fig. 1). Vessels typically demonstrated localized foci of constriction as shown in prior photomicrographs (17). OMDVR (10.2±0.8 μm) preconstricted with ANG II (10^{-9} M, 5.4±0.6 μm) were significantly dilated by adenosine (10^{-7} to 10^{-5} M, 9.1±1.3 μm, $n = 9$, Fig. 2) in a concentration-dependent manner. Sham exchange with vehicle alone (ANG II, 10^{-9} M) produced stable vasoconstriction ($n = 7$, Fig. 2).

Effect of A_1 and A_2 receptor stimulation on ANG II–induced vasoconstriction. The A_1 receptor agonist CHA (10^{-6} M) had no effect on the response to ANG II (10^{-9} M, $n = 7$, Fig. 3). The A_2 receptor agonist CGS-21680 (10^{-7} M) in the presence of A_1 receptor blockade with DPCPX (10^{-8} M) significantly dilated (5.9±0.5 to 9.7±1.2 μm) ANG II–constricted vessels ($n = 8$, Fig. 3). Recovery of constriction after removal of CGS-21680 did not achieve significance after 5 min (8.0±0.7 μm). Adenosine (10^{-6} M) also significantly dilated ANG II–constricted vessels (5.9±0.6 to 8.5±0.7 μm), however, recovery of constriction after removal of adenosine was significant (6.8±0.5 μm, $n = 7$, Fig. 3). In a separate series of experiments it was demonstrated that DPCPX had no effect on basal diameters (see Fig. 6, *top*).

Direct effects of adenosine, A_1, and A_2 receptor stimulation. CGS-21680 (10^{-6} M, $n = 7$) had no effect on basal OMDVR diameters. The adenosine A_1 receptor agonist CHA, however, constricted vessels from 10.5±1.0 to 4.5±1.1 μm ($P < 0.05$, $n = 8$, Fig. 4). Adenosine itself had a biphasic response, eliciting significant constriction between 10^{-11} M and 10^{-7} M (10.5±0.9 to 8.3±0.8 μm). At concentrations $> 10^{-7}$ M, vasoconstriction reversed and vessel diameter returned toward baseline (10.6±1.0 μm, Fig. 5). When administered to untreated vessels at a concentration of 10^{-6} M, adenosine had no effect on vessel diameters (data not shown).

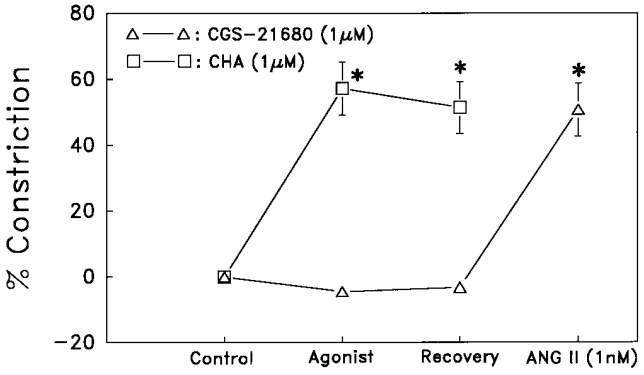

Figure 4. Direct effects of adenosine receptor agonists on basal diameter of microperfused OMDVR. Percent constriction caused by CHA (10^{-6} M, *squares*, $n = 7$, $^*P < 0.05$) or CGS-21680 (10^{-7} M, *triangles*, $n = 8$) is shown. Vessels failed to respond to CGS-21680. To assure the reactivity of those OMDVR they were subsequently treated with ANG II (10^{-9} M) to demonstrate intact vasoconstriction ($^*P < 0.05$).

Interaction of adenosine and ANG II. The A_1 receptor antagonist, DPCPX (10^{-5} or 10^{-8} M, $n = 4$ and 5, respectively), had no effect on basal diameters or ANG II–induced vasoconstriction (10^{-9} M, Fig. 6, *top*). However, DPCPX (10^{-8} M) was highly effective in antagonizing the response to CHA (10^{-6} M, 4.5±1.1 vs. 9.2±1.5 μm, $n = 6$, Fig. 6, *bottom*). Blockade of ANG II receptors with saralasin (10^{-7} M) inhibited constriction with ANG II (10^{-9} M, 7.4±1.4 vs. 13.1±1.1 μm, $n = 6$, Fig. 7, *top*) but failed to abrogate constriction by CHA (10^{-6} M, $n = 8$, Fig. 7, *bottom*). ANG II at concentrations above and below that which induces significant OMDVR vasoconstriction (10^{-11} and 10^{-14} M) also did not modify constriction by adenosine (10^{-12} to 10^{-8} M, data not shown).

Discussion

In most microvascular beds, adenosine is produced locally during periods of increased oxygen demand or decreased oxygen supply to enhance blood flow by inducing local vasodilatation. Within the renal medulla, adenosine dilates glomerular efferent arterioles (19, 20) and constricts glomerular afferent arterioles (11–13, 20) resulting in increased blood flow and diminished glomerular filtration. Presumably, these actions serve to enhance oxygen delivery while decreasing solute load to the nephron, minimizing the need for sodium transport and secondarily reducing local oxygen demand. In this context, the ability of adenosine to enhance medullary blood flow and improve oxygenation seems well established by prior studies (7, 8, 10).

The role of adenosine to modulate vasomotor tone in OMDVR had not been explored previously. To demonstrate the direct effects of adenosine and adenosine receptor subtype agonists on the OMDVR we microperfused these vessels in vitro. Although the binding characteristics of available adenosine receptor agonists have been characterized in nonrenal tissue, our results clearly show that adenosine and specific adenosine A_2 receptor stimulation with the agonist, CGS-21680, abrogates vasoconstriction by ANG II. In contrast, adenosine A_1 receptor stimulation vasoconstricts OMDVR in a manner independent of ANG II receptor occupancy in this preparation.

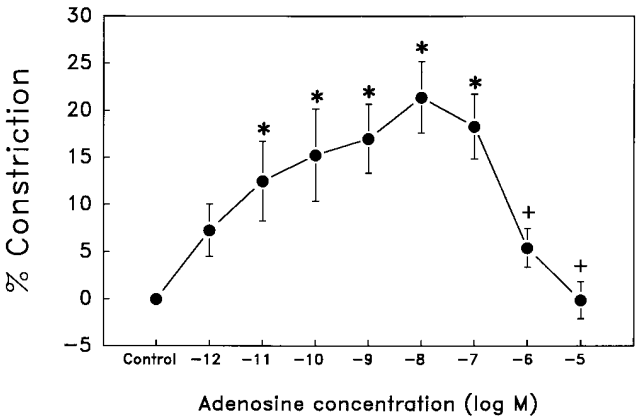

Figure 5. Concentration-dependent effects of adenosine on untreated microperfused OMDVR. Percent constriction caused by adenosine (10^{-12} to 10^{-5} M, $n = 8$) is shown as a function of log molar adenosine concentration ($^*P < 0.05$). Significant reversal of constriction occurred above 10^{-7} M adenosine ($^+P < 0.05$).

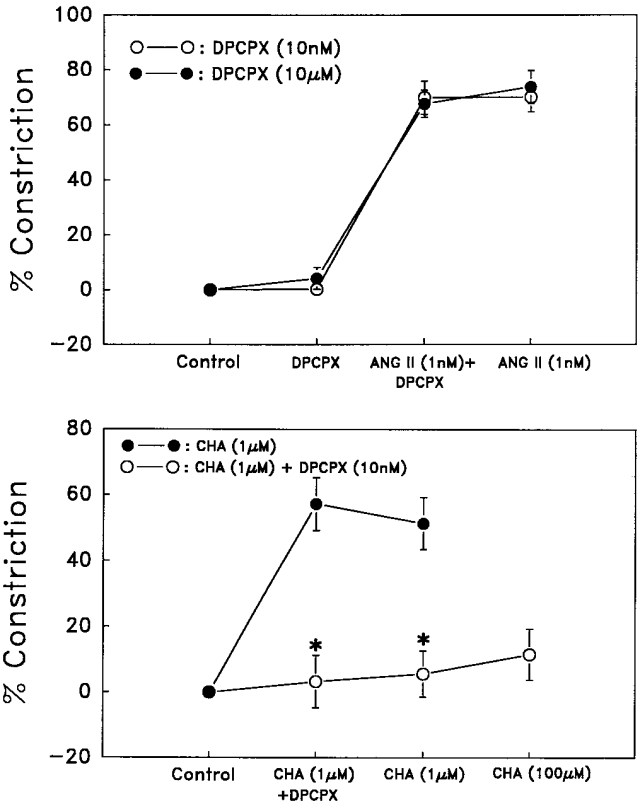

Figure 6. (*Top*) Effect of A_1 receptor blockade on ANG II–induced vasoconstriction. ANG II (10^{-9} M) was added to the bath in the presence of DPCPX, an adenosine A_1 receptor antagonist, at 10^{-6} or 10^{-8} M ($n = 5$ and 4, respectively). No effect on vasoconstriction was observed. (*Bottom*) Effect of DPCPX on CHA-induced vasoconstriction. Antagonism of CHA (10^{-6} M)-mediated constriction by DPCPX (10^{-8} M, *open circles*, $n = 6$, $^*P < 0.05$) is shown along with time controls (*closed circles*, $n = 7$). Constriction by CHA (10^{-6} and 10^{-4} M) did not follow removal of DPCPX even when CHA concentration was increased 100-fold.

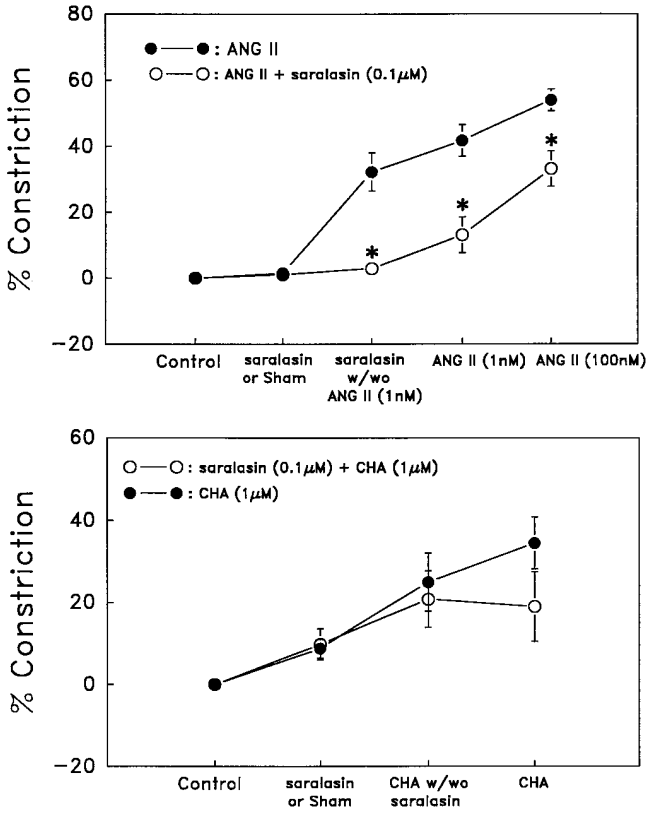

Figure 7. (*Top*) Effect of ANG II receptor blockade by saralasin on ANG II receptor stimulation. The ability of saralasin (10^{-7} M, *open circles*, $n = 6$) to attenuate the response to ANG II (10^{-9} M) and recovery after removal of saralasin is shown along with time controls (*closed circles*, $n = 4$). Asterisks imply significant differences between saralasin-treated and untreated vessels ($P < 0.05$). (*Bottom*) Effect of ANG II receptor blockade by saralasin on CHA-mediated vasoconstriction. The effect of saralasin (10^{-7} M) on CHA (10^{-6} M, *open circles*, $n = 8$)-induced constriction is shown along with time controls (*closed circles*, $n = 8$). No differences were observed.

OMDVR are derived exclusively from the efferent arterioles of juxtamedullary glomeruli in the outer stripe of the outer medulla upon which they coalesce within vascular bundles in the inner stripe. All blood flow to the medulla of the kidney passes through these vessels (14). OMDVR occupy positions in vascular bundles with a specific radial distribution. OMDVR in the center of vascular bundles cross the inner-outer medullary junction to perfuse the inner medulla while OMDVR on the bundle periphery peel off at various levels to supply the interbundle capillary plexus where the MTAL and other outer medullary nephron segments reside (14, 21). It has been speculated by some authors that a radial distribution of action of vasoconstrictors or dilators within the bundles might provide a mechanism to modulate regional perfusion of the medulla (21). Vascular bundles in the inner stripe also contain AVR returning from the inner medulla. It has been suggested that diffusional loss of oxygen from the descending vasa recta to AVR could be responsible for the low oxygen tension found in the renal medulla (3, 4). The MTAL is especially vulnerable to hypoxic damage (22, 23) and has been shown to generate adenosine in response (9). The close proximity of the site of adenosine production to OMDVR on the vascular bundle periphery might provide a mechanism for the MTAL to enhance its own perfusion and oxygenation through dilation of these vessels.

Recently Dinour et al. showed that adenosine infusion into the renal medullary interstitium resulted in an increase in medullary oxygen tension (10). Using laser Doppler measurements, Agmon et al. (8) demonstrated that medullary blood flow increased in response to interstitial adenosine infusion and decreased in response to specific adenosine A_1 receptor stimulation. Localization of these effects to the glomerular arterioles and/or the vasa recta cannot be inferred from their data. Most investigations into the mechanism by which adenosine increases overall renal vascular resistance support dominance of afferent vasoconstriction over efferent vasodilatation (13, 24). We speculate that OMDVR vasodilatation could play a role to modulate vascular resistance downstream of juxtamedullary glomeruli possibly influencing regional perfusion of the medulla.

The physiological effects of adenosine in the kidney are mediated by either the A_1 or A_2 (A_{2a} or A_{2b}) receptor subtypes. Those receptor subtypes mediate selective effects on the renal vasculature. Stimulation of the A_1 receptor produces afferent arteriolar vasoconstriction (11, 12, 24) and a reduction in glomerular filtration rate (19) while A_2 receptor stimulation yields efferent and afferent arteriolar vasodilatation (24, 25). In our hands, stimulation by adenosine or CGS-21680, an A_2 receptor agonist, consistently vasodilates ANG II–preconstricted OMDVR (Figs. 2 and 3) while CHA, an A_1 receptor agonist, was vasoconstrictive in untreated vessels. Furthermore, in untreated vessels, adenosine exhibited vasoconstriction at low concentrations (10^{-12} to 10^{-7} M) and vasodilatation at higher concentrations (10^{-6} to 10^{-5} M, Fig. 5). These results favor the notion that adenosine vasodilates preconstricted OMDVR via the A_2 receptor and vasoconstricts via the A_1 receptor. Prior studies have shown that adenosine is present in vivo at concentrations which would generally vasodilate in vitro perfused OMDVR (0.2 μM, Figs. 2 and 5) (26). Our studies also support a role for the higher affinity A_1 receptors on OMDVR, thereby suggesting potential for regulation of OMDVR tone in a manner that would be dependent upon local tissue adenosine concentration. In this context, we note that several investigators have provided evidence to favor exacerbation of some forms of acute renal failure by A_1 receptor stimulation (27, 28).

Adenosine-induced vasoconstriction via the A_1 receptor has been shown in some experimental preparations to be dependent upon interaction with the renin-angiotensin system. Several in vivo studies have shown that ANG II receptor antagonists (29) or a converting enzyme inhibitor (30) attenuates the vasoconstrictive effects of adenosine. Conversely, Rossi et al. (31), Joyner et al. (13), and Barrett and Droppleman (32) were unable to demonstrate the dependence of A_1 receptor–mediated constriction of renal vessels upon ANG II receptor occupancy. In our preparation, saralasin pretreatment had no effect on CHA-induced constriction. Additionally, the presence of ANG II (10^{-11} or 10^{-14} M) did not affect adenosine-induced constriction, suggesting a lack of interaction between these agents in this preparation. Although we are unable to exclude the possibility of such an interaction in OMDVR, these results support those of Dietrich and Steinhausen who found cortical but not juxtamedullary dependence of A_1 receptor stimulation on the renin-angiotensin system in glomerular arterioles (25).

In summary, our data indicate an ability for adenosine to modulate OMDVR vascular tone through both A_1 and A_2 receptors. Vasodilatory action appears to be mediated by the A_2 receptor subtype while A_1 receptor stimulation results in constriction. Differences in adenosine A_1 and A_2 receptor affinity (1) suggest concentration-dependent effects.

Acknowledgments

This work was supported by National Institutes of Diabetes and Digestive and Kidney Diseases grant DK42495 and a Grant-in-Aid from the American Heart Association. These studies were performed during the tenure of an Established Investigatorship of the American Heart Association (T.L. Pallone).

References

1. Spielman, W.S., and L.J. Arend. 1991. Adenosine receptors and signaling in the kidney. *Hypertension (Dallas).* 17:117–130.

2. Brezis, M., and S. Rosen. 1995. Hypoxia of the renal medulla: its implications for disease. *N. Engl. J. Med.* 332:647–655.

3. Brezis, M., S. Rosen, and F.H. Epstein. 1989. The pathophysiological implications of medullary hypoxia. *Am. J. Kidney Dis.* 13:253–258.

4. Brezis, M., S. Rosen, P. Silva, and F.H. Epstein. 1984. Renal ischemia: a new perspective. *Kidney Int.* 26:375–383.

5. McCoy, D.E., S. Bhattacharya, B.A. Olson, D.G. Levier, L.J. Arend, and W.S. Spielman. 1993. The renal adenosine system: structure, function, and regulation. *Semin. Nephrol.* 13:31–40.

6. Spielman, W.S., and C.I. Thompson. 1982. A proposed role for adenosine in the regulation of renal hemodynamics and renin release. *Am. J. Physiol.* 242: F423–F435.

7. Miyamoto, M., Y. Yagil, T. Larson, C. Robertson, and R.L. Jamison. 1988. Effects of intrarenal adenosine on renal function and medullary blood flow in the rat. *Am. J. Physiol.* 255:F1230–F1234.

8. Agmon, Y., D. Dinour, and M. Brezis. 1993. Disparate effects of adenosine A_1- and A_2-receptor agonists on intrarenal blood flow. *Am. J. Physiol.* 265: F802–F806.

9. Beach, R.E., B.A. Watts, III, D.W. Good, C.R. Benedict, and T.D. DuBose, Jr. 1991. Effects of graded oxygen tension on adenosine release by renal medullary and thick ascending limb suspensions. *Kidney Int.* 39:836–842.

10. Dinour, D., and M. Brezis. 1991. Effects of adenosine on intrarenal oxygenation. *Am. J. Physiol.* 261:F787–F791.

11. Weihprecht, H., J.N. Lorenz, J.P. Briggs, and J. Schnermann. 1994. Synergistic effects of angiotensin and adenosine in the renal microvasculature. *Am. J. Physiol.* 266:F227–F239.

12. Dietrich, M.S., K. Endlich, N. Parekh, and M. Steinhausen. 1991. Interaction between adenosine and angiotensin II in renal microcirculation. *Microvasc. Res.* 41:275–288.

13. Joyner, W.L., R.E. Mohama, T.O. Myers, and J.P. Gilmore. 1988. The selective response to adenosine of renal microvessels from hamster explants. *Microvasc. Res.* 35:122–131.

14. Lemley, K.V., and W. Kriz. 1987. Cycles and separations: the histotopography of the urinary concentrating process. *Kidney Int.* 31:538–548.

15. Pallone, T.L., J. Work, R.L. Myers, and R.L. Jamison. 1994. Transport of sodium and urea in outer medullary descending vasa recta. *J. Clin. Invest.* 93: 212–222.

16. Pallone, T.L. 1994. Vasoconstriction of outer medullary vasa recta by angiotensin II is modulated by prostaglandin E_2. *Am. J. Physiol.* 266:F850–F857.

17. Silldorff, E.P., S. Yang, and T.L. Pallone. 1995. Prostaglandin E_2 abrogates endothelin-induced vasoconstriction in renal outer medullary descending vasa recta of the rat. *J. Clin. Invest.* 95:2734–2740.

18. Burg, M.B. 1972. Perfusion of isolated renal tubules. *Yale J. Biol. Med.* 45:321–326.

19. Osswald, H., W.S. Spielman, and F.G. Knox. 1978. Mechanism of adenosine-mediated decreases in glomerular filtration rate in dogs. *Circ. Res.* 43: 465–469.

20. Thompson, C.I., and W.S. Spielman. 1992. Renal hemodynamic effects of exogenously administered adenosine and polyadenylic acid. *Am. J. Physiol.* 263:F816–F823.

21. Pallone, T.L., C.R. Robertson, and R.L. Jamison. 1990. Renal medullary microcirculation. *Physiol. Rev.* 70:885–920.

22. Brezis, M., S. Rosen, and P. Silva. 1984. Selective vulnerability of the medullary thick ascending limb to anoxia in the isolated perfused rat kidney. *J. Clin. Invest.* 73:182–190.

23. Heyman, S.N., M. Brezis, C.A. Rubinoff, Z. Greenfeld, C. Lechene, F.H. Epstein, and S. Rosen. 1988. Acute renal failure with selective medullary injury in the rat. *J. Clin. Invest.* 82:401–412.

24. Holz, F.G., and M. Steinhausen. 1987. Renovascular effects of adenosine receptor agonists. *Renal Physiol.* 10:272–282.

25. Dietrich, M.S., and M. Steinhausen. 1991. Differential reactivity of cortical and juxtamedullary glomeruli to adenosine-1 and adenosine-2 receptor stimulation and angiotensin-converting enzyme inhibition. *Microvasc. Res.* 45: 122–133.

26. Baranowski, R.L., and C. Westenfelder. 1994. Estimation of renal interstitial adenosine and purine metabolites by microdialysis. *Am. J. Physiol.* 267: F174–F182.

27. Yao, K., H. Kusaka, J. Sano, K. Sato, and A. Karasawa. 1994. Diuretic effects of KW-3902, a novel adenosine A_1-receptor antagonist, in various models of acute renal failure in rats. *Jpn. J. Pharmacol.* 63:281–288.

28. Suzuki, F., J. Shimada, H. Mizumoto, A. Karasawa, K. Kubo, H. Nonaka, A. Ishii, and T. Kawakita. 1992. Adenosine A_1 antagonists. II. Structure-activity relationships on diuretic activities and protective effects against acute renal failure. *J. Med. Chem.* 35:3066–3075.

29. Speilman, W.S., and H. Osswald. 1979. Blockade of postocclusive renal vasoconstriction by an angiotensin II antagonist: evidence for an angiotensin-adenosine interaction. *Am. J. Physiol.* 237:F463–F467.

30. Hall, J.E., J.P. Granger, and R.L. Hester. 1985. Interactions between adenosine and angiotensin II in controlling glomerular filtration. *Am. J. Physiol.* 248:F340–F346.

31. Rossi, N.F., P.C. Churchill, K.A. Jacobson, and A.E. Leahy. 1986. Further characterization of the renovascular effects of N^6-cyclohexyladenosine in the isolated perfused kidney. *J. Pharmacol. Exp. Ther.* 240:911–915.

32. Barrett, R.J., and D.A. Droppleman. 1993. Interactions of adenosine A_1 receptor-mediated renal vasoconstriction with endogenous nitric oxide and ANG II. *Am. J. Physiol.* 265:F651–F659.