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Role of renal medullary adenosine in the control of blood flow and sodium excretion

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Zou, Ai-Ping, Kasem Nithipatikom, Pin-Lan Li, and Allen W. Cowley, Jr. Role of renal medullary adenosine in the control of blood flow and sodium excretion. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R790–R798, 1999.—This study determined the levels of adenosine in the renal medullary interstitium using microdialysis and fluorescence HPLC techniques and examined the role of endogenous adenosine in the control of medullary blood flow and sodium excretion by infusing the specific adenosine receptor antagonists or agonists into the renal medulla of anesthetized Sprague-Dawley rats. Renal cortical and medullary blood flows were measured using laser-Doppler flowmetry. Analysis of microdialyzed samples showed that the adenosine concentration in the renal medullary interstitial dialysate averaged 212 ± 5.2 nM, which was significantly higher than 55.6 ± 5.3 nM in the renal cortex ($n = 9$). Renal medullary interstitial infusion of a selective A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX; 300 pmol·kg^{-1}·min^{-1}, $n = 8$), did not alter renal blood flows, but increased urine flow by 37% and sodium excretion by 42%. In contrast, renal medullary infusion of the selective A_2 receptor blocker 3,7-dimethyl-1-propargylxanthine (DMPX; 150 pmol·kg^{-1}·min^{-1}, $n = 9$) decreased outer medullary blood flow (OMBF) by 28%, inner medullary blood flows (IMBF) by 21%, and sodium excretion by 35%. Renal medullary interstitial infusion of adenosine produced a dose-dependent increase in OMBF, IMBF, urine flow, and sodium excretion at doses from 3 to 300 pmol·kg^{-1}·min^{-1} ($n = 7$). These effects of adenosine were markedly attenuated by the pretreatment of DMPX, but unaltered by DPCPX. Infusion of a selective A_3 receptor agonist, N^6-benzyl-5′-(*N*-ethylcarbonxamido)adenosine (300 pmol·kg^{-1}·min^{-1}, $n = 6$) into the renal medulla had no effect on medullary blood flows or renal function. Glomerular filtration rate and arterial pressure were not changed by medullary infusion of any drugs. Our results indicate that endogenous medullary adenosine at physiological concentrations serves to dilate medullary vessels via A_2 receptors, resulting in a natriuretic response that overrides the tubular A_1 receptor-mediated antinatriuretic effects.

renal hemodynamics; renal medulla; laser-Doppler flowmetry

ADENOSINE PLAYS an important role in cardiovascular homeostasis (6). Studies have indicated that adenosine is produced in response to tissue hypoxia and may serve as a negative feedback regulator to dilate vessels and to increase tissue blood perfusion, thereby returning the oxygen supply-to-demand ratio toward normal (6, 34). This adenosine feedback mechanism was first demonstrated to participate in the local regulation of coronary blood flow (5) and was extended to explain various types of active hyperemia in other tissues, including exercising skeletal muscle and the postprandial gastrointestinal circulation (14). In the kidney, however, adenosine-mediated metabolic regulation is more complex because of the marked regional heterogeneity of renal structure and function and the different effects of adenosine on pre- and postglomerular vessels (10, 21, 34). There is evidence, however, that this adenosine feedback regulatory process in the kidney may play an important role in the control of renal vascular tone and tubular transport. It has been proposed that adenosine may be produced by transporting epithelium and increase in response to metabolic demand in the kidney (8, 10, 11). Adenosine serves as a paracrine to decrease metabolic demand of the tubular cells through the contraction of cortical preglomerular arterioles, resulting in reduction of glomerular filtration rate (GFR), and to increase peritubular blood oxygen supply by dilating postglomerular vessels. These hemodynamic effects of adenosine appear to work together with its inhibitory action on tubular transport to adjust the metabolic supply and demand toward a level of transport activity appropriate for the oxygen and substrate availability of the tissue (34).

Recently, adenosine receptors have been cloned and designated A_1, A_{2a}, A_{2b}, and A_3 receptors (25). With the use of ligand binding, autoradiography, and measurement of adenylyl cyclase activity, two classical adenosine receptors, A_1 and A_2, have been shown in the renal cortex, outer medulla, and inner medulla (2, 36). These two subtypes of adenosine receptors have also been functionally localized to specific nephron segments, including glomeruli, thick ascending limb, and papillary collecting duct (2, 13). Recent studies using RT-PCR have detected the mRNA of these two subtypes of adenosine receptors in most segments along the nephron and in outer medullary descending vasa recta (15, 35). It is generally concluded that A_1 and A_{2a} receptors are widely distributed throughout the nephron and renal vasculature (21). It was demonstrated that stimulation of A_1 receptors produced preglomerular vasoconstriction, activation of tubuloglomerular feedback response, and consequently reduction of GFR and sodium excretion (26, 34). Moreover, tubular A_1 receptor activation may increase cortical and medullary tubular sodium reabsorption (18). In contrast, activation of A_2 receptors, including A_{2a} and A_{2b}, dilates pre- and postglomerular vessels and inhibits tubular sodium reabsorption (18, 34). Therefore, A_1 receptors are considered antidiuretic and antinatriuretic adenosine receptors, and A_2 receptors are seen as diuretic and natriuretic adenosine receptors.

A_3 receptors have been identified in different animal tissues, including rat and pig kidneys (7, 17). This novel adenosine receptor subtype is involved in the release of autocoids or paracrines, such as histamine, cytokines, leukotrienes, thromboxanes, and proteases, from mast cells and other interstitial cells in response to inflammatory or noninflammatory stimulations. Activation of A_3 receptors was reported to contribute to the development of asthma, ischemic preconditioning (17), and adenosine-mediated vasoconstriction (30). It remains to be determined whether A_3 receptors contribute to the control of medullary blood flow and tubular function.

The blood supply of the renal medulla is derived from the cortical efferent arterioles of the juxtamedullary glomeruli (16). Intrarenal administration of exogenous adenosine or adenosine A_2 receptor agonists produced a marked increase in medullary blood flow and oxygenation (1, 10). However, the physiological role of endogenous medullary adenosine in the control of medullary circulation remains unknown. It is well known that there is a relatively hypoxic milieu in the outer medulla due to active transport of medullary thick ascending limb of Henle (TALH) and limited oxygen supply resulting from shunting of O_2 in the outer medulla. On the presumption that adenosine feedback mechanism also occurs in the renal medulla, adenosine level in this region should be higher relative to the renal cortex and it may play an essential role in the control of medullary blood flow. Moreover, studies have indicated that medullary blood flow and renal interstitial hydrostatic pressure participate in the control of sodium and water excretion. Increases in medullary blood flow inhibit tubular transport and result in diuresis and natriuresis (9). The extent to which endogenous medullary adenosine contributes to the control of renal sodium and water excretion through its medullary hemodynamic effect is unknown.

The present studies were designed to determine and compare medullary and cortical interstitial adenosine concentrations using microdialysis and fluorescence HPLC techniques and to examine the role of endogenous medullary adenosine in the control of medullary blood flow and renal sodium and water excretion. A renal interstitial catheter was implanted to deliver adenosine and its receptor agonists or antagonists into the renal medullary interstitial space, and changes in regional blood flows were determined by laser-Doppler flowmetry.

MATERIALS AND METHODS

Surgical Preparation

Experiments were performed on male Sprague-Dawley rats weighing between 250 and 300 g. The rats were fed a normal salt diet (1% NaCl). Before experiments, rats was fasted overnight but allowed free access to water. They were anesthetized with ketamine (30 mg/kg body wt im) and Inactin (50 mg/kg body wt ip) and placed on a thermostatically controlled warming table to maintain body temperature at 37°C. After tracheotomy, cannulas were placed in the right femoral vein and artery for intravenous infusions and measurement of arterial pressure. An abdominal incision was made, and the left kidney was placed in a stainless steel cup for implantation of optical fibers to measure cortical and medullary blood flows or for implantation of microdialysis probes to dialyze adenosine from the renal interstitium. An interstitial catheter was implanted into the renal medulla for renal medullary interstitial infusion of drugs. After implantation, a 0.9% solution of sodium chloride was continuously infused at a rate of 0.5 ml/h to maintain the patency of the catheter (19). Ureters were cannulated for collection of urine. The animals received an intravenous infusion of 2% bovine serum albumin in a 0.9% sodium chloride solution, at a rate of 3 ml/h throughout the experiment to replace fluid losses and maintain a stable hematocrit of ~43 ± 3%.

Experimental Protocols

Protocol 1: Microdialysis analysis of adenosine concentration in the renal cortex and medulla. In vivo microdialysis study was performed according to the technique described by Baranowski and Westenfelder (3). Briefly, the rats ($n = 9$) were anesthetized and surgically prepared as described above. The left kidney was exposed and immobilized by placing it dorsal side up in a kidney cup. A microdialysis probe (Bioanalytical Systems, West Lafayette, IN) with 0.5-mm tip diameter, 1-mm dialysis length, and a 20-kDa transmembrane diffusion cutoff was inserted into the renal cortex (1.5 mm in depth) horizontally from kidney pole and another was inserted into the renal medulla (5 mm in depth) from dorsal surface. The cortical probe was perfused at 2 µl/min with PBS (in mM: 80 NaCl, 40.5 Na_2HPO_4, and 9.5 NaH_2PO_4, pH 7.4 and osmolarity 300 mosM), and the medullary probe was perfused with PBS containing (in mM) 205 NaCl, 40.5 Na_2HPO_4, and 9.5 NaH_2PO_4 (pH 7.4 and osmolarity 550 mosM). The dialysate was collected for 30 min and then either reacted with fluorescence dye (chloroacetaldehyde) immediately or stored at −80°C until HPLC analysis.

Protocol 2: Effects of renal medullary interstitial infusion of A_1 and A_2 receptor antagonists on medullary blood flow and renal function. These experiments were performed in 22 rats to evaluate the role of endogenous adenosine in the control of renal medullary blood flow. The rats were anesthetized and surgically prepared as described above. The left kidney was exposed and immobilized by placing it dorsal side up in a kidney cup. Laser-Doppler flowmeters (model Pf3, PERIMED KB) were used to simultaneously determine the changes in three regions of the left kidney with one optical fiber (diameter of 500 µm) implanted in the renal cortex, another in the outer medulla, and a third in the inner medulla as described previously (19). The implanted fibers were optically connected to an external probe specifically designed for such applications (model Pf318A, PERIMED KB) using fused silica matching liquid (#50350, Cargille Laboratories, Cedar Grove, NJ) to minimize loss of light at the connection. The laser-Doppler signal, which is the product of the number of moving particles and their velocity, is thereby used as an index of changes of blood flow in the different portions of the kidney (19). After surgery and a 60-min equilibration period, continuous measurements of mean arterial pressure (MAP) and cortical, outer, and inner medullary blood flows were obtained throughout the experiment. Saline was infused into the renal medullary interstitium for two 20-min control periods. At the end of the second control period, either a selective A_1 antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) ($n = 8$, 300 $pmol \cdot kg^{-1} \cdot min^{-1}$), or A_2 antagonist, 3,7-dimethyl-1-propargylxanthine (DMPX) ($n = 9$, 150 $pmol \cdot kg^{-1} \cdot min^{-1}$), was infused into the renal medullary interstitium for 30 min. Doses of DPCPX and DMPX for renal medullary interstitial infusion were chosen on the basis of those used in previous in

vivo studies, at which DPCPX and DMPX specifically blocked A_1 and A_2 receptors, respectively (23, 27).

To determine whether endogenous medullary adenosine contributes to the control of sodium and water excretion, we also examined the effects of A_1 and A_2 receptor antagonists on renal function. Seventeen rats were surgically prepared as described above. The animal received an intravenous infusion of 2% bovine serum albumin in 0.9% sodium chloride, and [^{3}H]inulin (1 μCi/ml) was added to the infusion solution for measurement of GFR. After surgery and a 1-h equilibration period, urine flow, sodium excretion, and GFR were measured in both left and right kidneys during a 30-min control period. DPCPX was then infused through the renal interstitial catheter into the renal medulla at a rate of 0.5 ml/h to deliver a dose of 300 pmol·kg^{-1}·min^{-1} ($n = 6$), or DMPX was infused at a dose of 150 pmol·kg^{-1}·min^{-1} ($n = 11$). Fifteen minutes after drug infusions were started, urine and plasma samples were again collected over a 30-min period from the left and right kidneys.

Protocol 3: Effects of renal medullary interstitial infusion of adenosine on medullary blood flow and renal function. Nineteen rats were anesthetized and surgically prepared as described above to determine the effects of renal medullary interstitial infusion of adenosine on medullary blood flow. The left kidney was exposed and immobilized by placing it dorsal side up in a kidney cup. After surgery and a 60-min equilibration period, continuous measurements of MAP and laser-Doppler measurements of cortical, outer, and inner medullary blood flow were obtained throughout the experiment as described in *protocol 2*. Saline was infused into the renal medullary interstitium for two 20-min control periods. At the end of the second control period, adenosine was infused into the renal medullary interstitium for 20 min at doses of 3, 30, and 300 pmol·kg^{-1}·min^{-1} ($n = 7$). Finally, the interstitial infusion was switched back to saline for 30 min. To define the receptor type responding to exogenous adenosine on medullary vessels, either DPCPX ($n = 6$, 300 pmol·kg^{-1}·min^{-1}) or DMPX ($n = 6$, 150 pmol·kg^{-1}·min^{-1}) was infused into the renal medullary interstitial space before and during adenosine infusion. Simultaneously, plasma and urine samples from the left and right kidneys were collected to measure sodium and water excretion during control and experimental periods as described above. In time control experiments ($n = 6$), saline vehicle alone was infused into the renal medullary interstitium during control and experimental periods.

Protocol 4: Effects of renal medullary interstitial infusion of A_3 receptor agonist on medullary blood flow and renal function. Because a specific A_3 receptor antagonist is not yet available, we examined the effect of a selective A_3 receptor agonist, N^6-benzyl-5′-(N-ethylcarbonxamido)adenosine (N^6-benzyl-NECA) on renal medullary blood flow. Six rats were anesthetized and surgically prepared as described above for continuous measurements of MAP and laser-Doppler measurements of cortical, outer, and inner medullary blood flows. Saline was infused into the renal medullary interstitium for two 20-min control periods. At the end of the second control period, N^6-benzyl-NECA was infused into the renal medullary interstitium for 40 min at a dose of 300 pmol·kg^{-1}·min^{-1} ($n = 6$) followed by a 30-min interstitial infusion of saline. Plasma and urine samples from the left and right kidneys were collected to measure sodium and water excretion during control and experimental periods as described above. The dose of N^6-benzyl-NECA was chosen on the basis of its A_3 affinity analysis ($K_i = 6.1$ nM) (31) and previous in vivo study (28).

HPLC Assay of Adenosine

HPLC assay of adenosine was performed as we described previously (24). The cortical and medullary dialysates (50 μl) were transferred into autosample vials and mixed with 4 μl of 1 M acetate buffer (pH 7.5) and 2 μl of 50% chloroacetaldehyde. The vials were capped and incubated at 60°C for 4 h. Reaction mixtures (5 μl) were injected and chromatographed on a 1090 Series II liquid chromatograph (Hewlett-Packard, Palo Alto, CA) using an autosampler and a column-switching valve. A shielded hydrophobic phase column, HiSep, 250 × 2.1 mm (Supelco, Bellefonte, PA) and an ODS-S C18, 250 × 2.0 mm (Metachem Technologies, Torrance, CA) with an isochratic mobile phase of 10% acetonitrile and 90% of 0.1 M sodium acetate and 0.002 M 1-octanesulfonic acid were used for separation. The flow rate was 0.2 ml/min. The eluate from the HiSep column was bypassed to waste 6 s after injection. After 5 min, the effluent was switched back to the C18 column for further separation and passed through the detector. The fluorescence was detected by an FS 970 LC Fluorometer (Kratos Analytical Instruments, Ramsey, NJ) with an excitation wavelength of 274 nm and a 370-nm loop-pass filter for emission. The chromatograph was recorded, and the peaks were integrated on a 3392 integrator (Hewlett-Packard). The run time was 20 min with 5 min post run (24). To determine derivatization reaction recovery, a known concentration of adenosine was reacted with chloroacetaldehyde as described above, and 1,N^6-ethenoadenosine standard was serially diluted to construct the standard curve.

Analytical Techniques

[^{3}H]inulin concentrations of urine and plasma samples were determined using a liquid-scintillation counter (model 2450, Packard Instrument, Downers Grove, IL). Urine flow rate was determined gravimetrically. Sodium and potassium concentrations of urine and plasma samples were measured using a flame photometer. GFR was calculated as the product of urine flow and the urine-to-plasma inulin concentration ratio. Urinary excretion data, renal blood flow, and GFR were all factored per gram kidney weight.

Statistics

Data are presented as means ± SE. The significance of differences within and between groups was evaluated using an ANOVA for repeated measures followed by a Duncan's multiple-range test and using a paired t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Protocol 1: Renal Interstitial Concentration of Adenosine in the Renal Medulla and Cortex

A typical reverse phase-HPLC chromatogram depicting the profile of the fluorescent derivatives of adenosine is presented in Fig. 1*A*. Adenosine (1 μM) was reacted with chloroacetaldehyde to form a fluorescence product, 1,N^6-ethenoadenosine. This fluorescence product coeluted with synthetic 1,N^6-ethenoadenosine standard with a retention time of 9 min. When the sample containing adenosine was incubated with adenosine deaminase (1 U), the 9-min peak disappeared (Fig. 1*B*). Adenosine concentrations of renal cortical and medullary dialysates are presented in Fig. 1*C*. Adenosine concentration in renal medullary interstitial dialysate

averaged 212 ± 5.2 nM, which was significantly higher than 55.6 ± 5.3 nM in cortical dialysate.

Protocol 2: Effects of Renal Medullary Interstitial Infusion of A_1 and A_2 Receptor Antagonists on Medullary Blood Flow and Renal Function

The effects of renal medullary interstitial infusion of DPCPX and DMPX on cortical and medullary blood flows are presented in Fig. 2. In control periods, the laser-Doppler signals from the fibers implanted in the renal cortex and outer and inner medulla averaged 1.56 ± 0.2, 0.80 ± 0.12, and 0.51 ± 0.1 V, respectively. The cortical flow signal was not altered from control values during the renal medullary interstitial infusion of either the A_1 or the A_2 antagonist. Renal medullary interstitial infusion of the A_2 antagonist DMPX significantly decreased outer and inner medullary flow signals by 28.5 and 27.5% compared with control flow signal, respectively. In contrast, infusion of the A_1 antagonist DPCPX had no effect on flow signals in either of the medullary regions. MAP was not altered from the control level (115 ± 3 mmHg) during renal medullary interstitial infusion of the A_1 and A_2 antagonists.

The effects of renal medullary interstitial infusion of the A_1 and A_2 antagonists on renal function are summarized in Table 1. GFR, urine flow rate, and sodium and potassium excretion all were similar in the infused and contralateral kidney during the control period, indicating that implantation of an infusion catheter in the renal medulla and renal medullary interstitial infusion of saline had no significant effect on renal tubular or vascular function. Renal medullary interstitial infu-

Fig. 2. Effect of renal medullary interstitial infusion of 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), A_1 receptor antagonist ($n = 9$), and 3,7-dimethyl-1-propargylxanthine (DMPX), A_2 receptor antagonist ($n = 8$) on cortical and medullary blood flows. C, control period; CBF, cortical blood flow; OMBF, outer medullary blood flow; IMBF, inner medullary blood flow. *Significant difference from control periods.
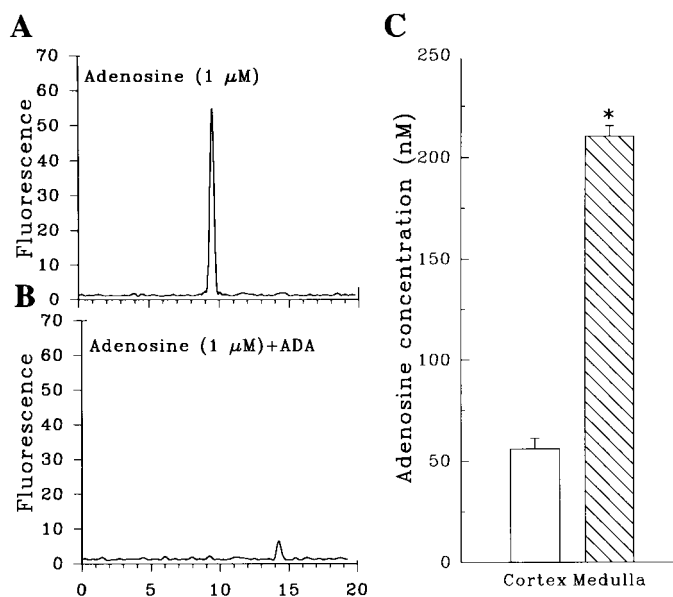

Fig. 1. Adenosine concentrations in renal cortical and medullary interstitial dialysates. *A*: representative HPLC chromatogram of adenosine fluorescent derivatives. *B*: HPLC chromatogram of adenosine fluorescent derivatives after 1 μM adenosine standard solution was incubated with 1 IU adenosine deaminase (ADA). *C*: summary of adenosine concentration in cortical and medullary interstitial dialysates ($n = 9$). *Significant difference from cortical concentration.
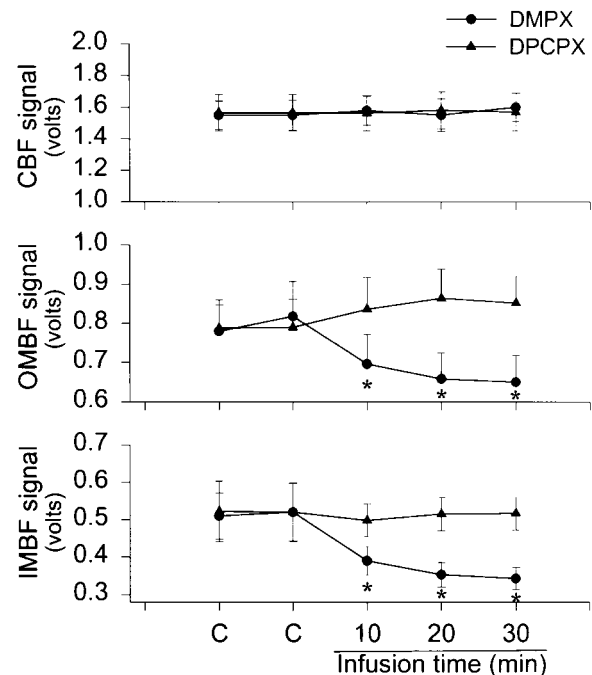
sion of the A_1 antagonist DPCPX produced an increase in urine flow rate and sodium excretion of 28.3 and 29.7%, respectively, in the absence of changes in GFR. Urine flow rate, sodium excretion, and GFR in the contralateral control kidney were not significantly altered during these experiments, indicating that medullary interstitial infusion of DPCPX was localized in the infused kidney. In contrast, renal medullary interstitial infusion of the A_2 receptor antagonist DMPX reduced urine flow rate and sodium excretion by 24.3 and 25.7%, respectively, without changes in GFR. In the contralateral control kidney, urine flow rate, sodium excretion, and GFR were not significantly altered during these experiments. MAP remained unchanged throughout all of the above studies.

Protocol 3: Effects of Renal Medullary Interstitial Infusion of Adenosine on Medullary Blood Flow and Renal Function

Renal medullary interstitial infusion of adenosine produced a concentration-dependent increase in medullary blood flows. As shown in Fig. 3, adenosine infusion at a dose of 300 pmol·kg^{-1}·min^{-1} had no effect on cortical flow signal but significantly increased outer and inner medullary flow signals by 30 and 25%, respectively. Both outer and inner medullary blood flows returned to levels not significantly different from control during a 20-min postcontrol period. Vehicle infusion had no effect on cortical and medullary blood flows.

Table 1. *Effects of renal interstitial infusion of A_1 and A_2 receptor antagonists on renal function*

	Infused Kidney		Contralateral Kidney	
	Control	Antagonist	Control	Antagonist
A_1 antagonist DPCPX (300 pmol/kg/min, n = 6)				
GFR, ml·min^{-1}·g kidney wt^{-1}	0.88 ± 0.13	0.90 ± 0.04	0.81 ± 0.01	0.74 ± 0.06
Urine flow rate, µl·min^{-1}·g kidney wt^{-1}	11.6 ± 1.98	$19.3 \pm 3.21^*$	9.1 ± 1.50	11.8 ± 3.04
Sodium excretion, µmol·min^{-1}·g kidney wt^{-1}	1.96 ± 0.43	$3.15 \pm 0.60^*$	1.59 ± 0.31	1.99 ± 0.39
Potassium excretion, µmol·min^{-1}·g kidney wt^{-1}	0.95 ± 0.12	1.24 ± 0.13	0.91 ± 0.06	1.06 ± 0.16
A_2 antagonist DMPX (150 pmol/kg/min, n = 11)				
GFR, ml·min^{-1}·g kidney wt^{-1}	0.83 ± 0.09	0.91 ± 0.13	0.74 ± 0.10	0.86 ± 0.10
Urine flow rate, µl·min^{-1}·g kidney wt^{-1}	12.0 ± 1.94	$8.79 \pm 1.92^*$	10.7 ± 2.19	10.8 ± 3.27
Sodium excretion, µmol·min^{-1}·g kidney wt^{-1}	2.24 ± 0.43	$1.35 \pm 0.21^*$	2.16 ± 0.67	2.04 ± 0.46
Potassium excretion, µmol·min^{-1}·g kidney wt^{-1}	1.11 ± 0.19	0.90 ± 0.12	1.16 ± 0.38	1.02 ± 0.24

Values are means $\pm$ SE. DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMPX, 3,7-dimethyl-1-propargylxanthine; GFR, glomerular filtration rate. $^*P < 0.05$ indicates significant difference from control value.

Figure 4 presents the effects of renal medullary interstitial infusion of adenosine on sodium and water excretion. A concentration-dependent increase in urine flow rate and sodium excretion was observed during renal medullary interstitial infusion of adenosine. Infusion of adenosine even at the highest doses studied (300 pmol·kg^{-1}·min^{-1}) did not alter sodium and water excretion in the contralateral kidney. During the postcontrol period, urine flow rate and sodium excretion returned to control levels.

The effects of A_1 and A_2 receptor blockade on adenosine-induced changes in renal regional blood flows and sodium excretion are depicted in Fig. 5. Renal medullary interstitial infusion of adenosine at a dose of 300 pmol·kg^{-1}·min^{-1} increased outer medullary flow signal by 0.23 V (29%) and inner medullary flow signal by 0.13 V (25%) and had no effect on the cortical flow signal. Adenosine infusion also significantly increased urine flow rate and sodium excretion. Blockade of A_1 receptor by DPCPX had no effect on baseline renal blood flows, but increased water excretion from 12.8 ± 1.9 to 21.3 ± 2.8 µl·min^{-1}·g kidney wt^{-1} and sodium excretion from 1.43 ± 0.28 to 2.73 ± 0.36 µmol·min^{-1}·g kidney wt^{-1}. However, DPCPX did not alter adenosine-induced increase in outer and inner medullary blood flows and in sodium and water excretion. In contrast,
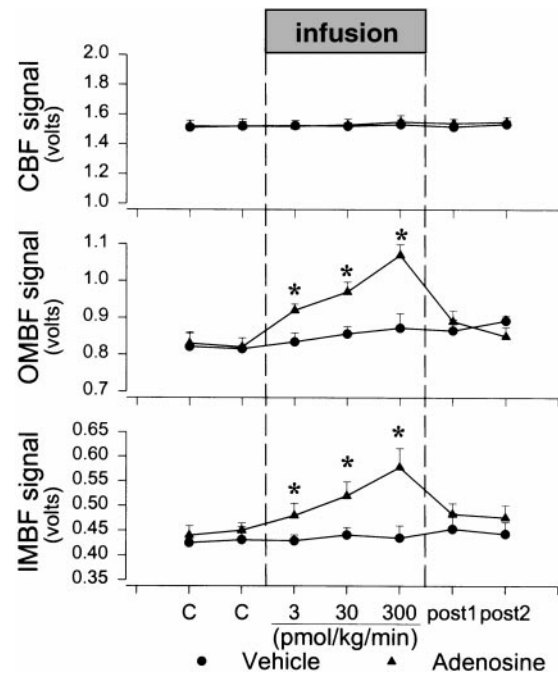

Fig. 3. Effect of renal medullary interstitial infusion of adenosine on cortical and medullary blood flows ($n = 7$). Post, postcontrol period. *Significant difference from control periods.

blockade of A_2 receptor by DMPX not only decreased baseline medullary blood flows (outer from 0.82 ± 0.09 to 0.65 ± 0.07 V and inner from 0.45 ± 0.04 to 0.34 ± 0.03 V) but also abolished the increase in medullary blood flows during medullary interstitial infusion of adenosine. DMPX also significantly attenuated the adenosine-induced increase in water and sodium excretion. MAP remained unchanged throughout all of the above studies.
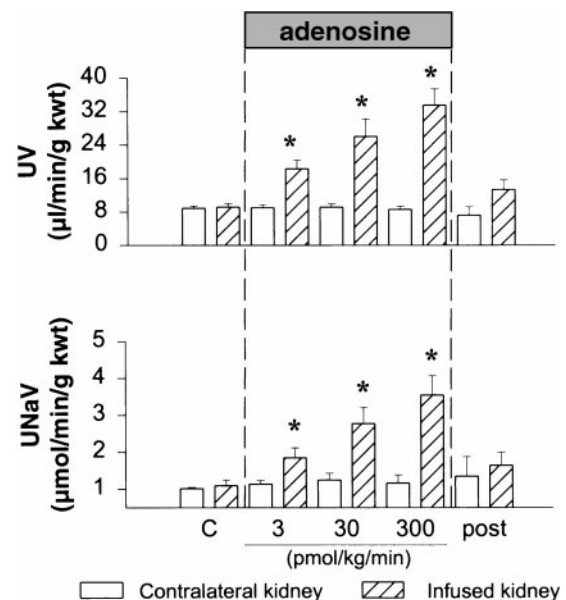

Fig. 4. Effect of renal medullary interstitial infusion of adenosine on sodium (UNaV) and water (UV) excretion ($n = 7$). *Significant difference from control periods.
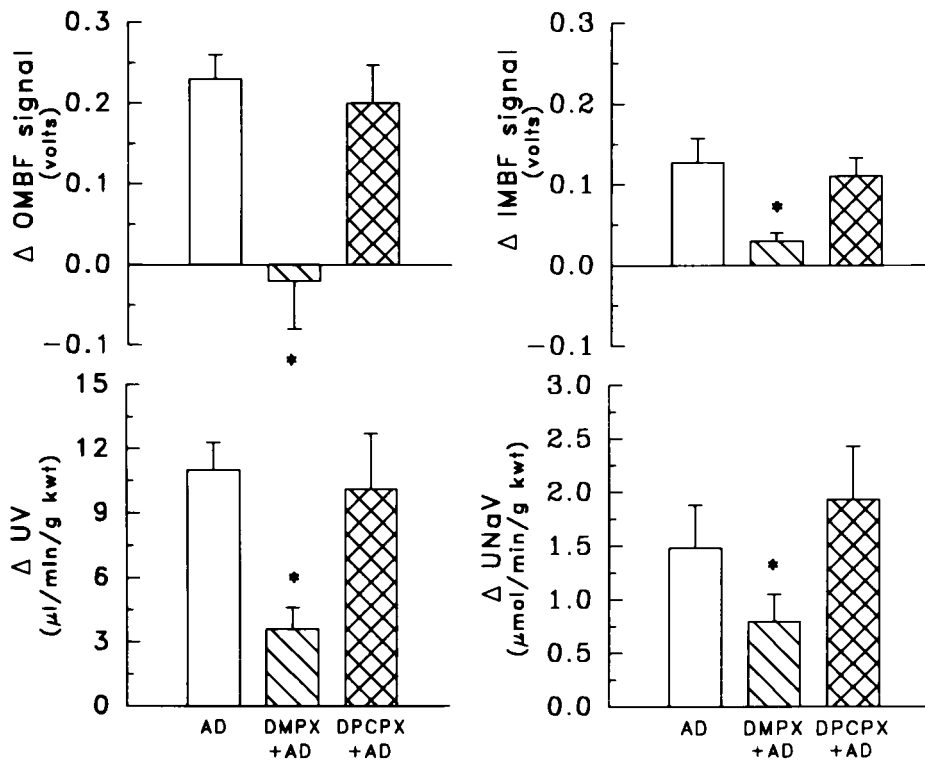
Fig. 5. Effect of DPCPX, A_1 receptor antagonist ($n = 6$), and DMPX, A_2 receptor antagonist ($n = 6$), on adenosine-induced alterations of medullary blood flows and renal function. AD, adenosine. *Significant difference from values obtained when adenosine alone was infused into the renal medullary interstitium.

Protocol 4: Effects of Renal Medullary Interstitial Infusion of A_3 Receptor Agonist on Medullary Blood Flow and Renal Function

A_3 receptor agonist N^6-benzyl-NECA produced significant decrease in MAP when infused intravenously at a dose of 300 pmol·kg^{-1}·min^{-1} (data not shown). Renal medullary interstitial infusion of N^6-benzyl-NECA at the same dose had no effect on cortical and medullary flow signals, renal function, or MAP (Table 2).

DISCUSSION

Recent studies have indicated that the effects of adenosine on renal function depend on its concentration in the extracellular space (2, 26). In the present studies, we evaluated adenosine concentrations in renal medullary interstitial dialysate by microdialysis

Table 2. *Effects of renal medullary interstitial infusion of N^6-benzyl-NECA on mean arterial pressure, regional renal blood flow and sodium and water excretion*

	Control	N^6-Benzyl-NECA
Mean arterial pressure, mmHg	107 ± 2.60	109 ± 4.26
Cortical flow signal, V	1.58 ± 0.13	1.67 ± 0.15
Outer medullary flow signal, V	0.67 ± 0.09	0.65 ± 0.10
Urine flow rate, μl·min^{-1}·g kidney wt^{-1}	10.1 ± 2.56	10.1 ± 1.85
Sodium excretion, μmol·min^{-1}·g kidney wt^{-1}	2.39 ± 0.31	2.30 ± 0.36

Values are means $\pm$ SE ($n=6$). N^6-benzyl-NECA, N^6-benzyl-5′-(N-ethylcarbonxamido)adenosine.

and fluorescence HPLC analysis. We have found that adenosine concentrations in interstitial dialysate were significantly higher in the renal medulla than in the cortex. Our results are consistent with those by Siragy and Linden (33), who reported that adenosine concentration in renal medullary dialysate from rats on normal salt diet (0.28% NaCl) was 157 ± 6 nM, which is significantly higher than 63 ± 6 nM in renal cortical dialysate. However, cortical adenosine concentration detected in the present study is much lower compared with the value (199 ± 53 nM) reported by Baranowski and Westenfelder (3). The reason for this discrepancy may be due to the different length of dialysis tubing in our probes (1 mm in length) from theirs (2 mm). The probe with a long dialysis tubing may dialyze adenosine from the deeper cortical area close to renal medulla if inserted from the dorsal surface as carried out by Baranowski and Westenfelder (3). For dialysis of cortical adenosine, we inserted the probe from the kidney pole into the renal cortex ~1.5 mm from the outer renal surface, which was similar to the method used by Siragy and Linden (33). Therefore, cortical adenosine concentration detected in the present study represents a concentration in the outer renal cortex.

Although the present study did not examine the mechanism leading to high concentration of renal medullary interstitial adenosine, a high metabolic activity and limited oxygen supply in the renal outer medulla may increase the production of adenosine in this region. Recent studies have demonstrated that the medullary TALH releases adenosine in response to hypoxia (11). Under control conditions, P_{O_2} in the renal medulla has been found to be <20 mmHg (10, 11, 20). Therefore, a low intracellular P_{O_2} and high transport

activity in the medullary TALH may result in enhanced production and release of adenosine in this kidney region (8, 11).

The role of adenosine in the control of the medullary oxygenation and the protection of the cells of medullary TALH from hypoxic damage has been extensively examined (11, 12). In the isolated perfused kidney, addition of adenosine deaminase or an adenosine efflux inhibitor 6-nitrobenzylthionosine to the perfusate resulted in exaggerated hypoxic damage to the cells of medullary thick ascending limb (12). This suggests that endogenous interstitial adenosine may play an important role in protecting medullary thick ascending limb from hypoxic injury. In the same preparation, an adenosine analog, $R(-)$-phenylisopropyladenosine, markedly reduced hypoxic injury to these cells when added to the perfusate (12). The protecting effect of adenosine has been proposed to be associated with the medullary oxygenation because renal interstitial infusion of adenosine significantly increased medullary P_{O_2} (10) and medullary blood flow (1), indicating that increase in medullary adenosine is of substantial importance in protecting medullary tubular cells from hypoxic injury through an increase in medullary blood flow and medullary oxygen supply.

Despite these observations, there has been no direct evidence suggesting that endogenously produced adenosine in the renal medulla participates in the regulation of medullary blood flow and hence medullary oxygenation under physiological conditions. In the present studies, we examined the role of endogenous medullary adenosine in the control of medullary blood flow by infusing specific adenosine receptor antagonists into the renal medullary interstitial space while measuring changes in blood flow to this region. Infusion of the A_2 receptor antagonist DMPX into the renal medullary interstitial space markedly decreased blood flow in both the outer and inner medulla of the anesthetized rats. In contrast, infusion of the A_1 receptor antagonist DPCPX had no effect on medullary blood flows. These results indicate that endogenous medullary adenosine contributes importantly to medullary vascular tone via A_2 receptors. Conversely, elevations of medullary adenosine concentrations by administration of adenosine into medullary interstitial space increased medullary blood flow. These findings demonstrate that medullary adenosine exerts vasodilator effect under normal conditions and during states of elevated adenosine.

However, Silldorf et al. (32) observed that adenosine at 10^{-12}–10^{-7} M produced vasoconstriction of isolated perfused outer medullary descending vasa recta, but by A_1 receptor activation. In the present study, we did not observe A_1 receptor-mediated vasoconstrictor effects on medullary vessels in the intact blood-perfused kidney, although the A_1 receptor blocker DPCPX was infused into the renal medullary interstitial space at a dose sufficient to alter tubular function. Our data are consistent with previous reports suggesting that interstitial infusion of A_1 receptor agonists had no effect on medullary blood flow and oxygenation (1). The effects of A_1 receptor blockade or activation on medullary vessels in

vivo may be related to a high basal concentration of adenosine in the renal medullary interstitium, which could mask vasoconstrictor actions of A_1 receptors by predominately stimulating A_2 receptors. Indeed, a vasodilator effect with high concentration of adenosine (10^{-6}–10^{-5} M) was also observed in the in vitro isolated and perfused vasa recta (32).

Recent studies in our laboratory and others showed that medullary blood flow plays an important role in the control of renal sodium and water excretion (13). It has been also reported that infusion of adenosine into the renal artery, at doses that did not activate systemic receptor sites, produced marked natriuresis and diuresis in rats (22, 37). The mechanism by which intrarenal adenosine produced natriuresis remains unknown. In the present study, we determined whether elevation of medullary adenosine contributed to the control of renal sodium and water excretion by increasing medullary blood flow. We found that renal medullary interstitial infusion of the selective A_2 receptor antagonist that reduced medullary blood flow significantly decreased sodium and water excretion in anesthetized rats. These observations indicate that adenosine contributes to the normal maintenance of sodium and water excretion via A_2 receptor stimulation under physiological conditions. In contrast, renal medullary infusion of specific A_1 receptor blocker increased sodium and water excretion. Because A_1 receptor blockade had no effect on medullary blood flow, we propose that this A_1 receptor-mediated antinatriuretic and antidiuretic effect may be due to a direct effect on medullary tubular transport. Taken together, the results suggest that medullary adenosine could contribute importantly to the control of sodium and water excretion largely through the vascular effects.

Our findings are not consistent with conclusions reached by others with exogenously infused adenosine, which reduced chloride reabsorption in the loop of Henle and sodium reabsorption in the inner medullary collecting duct (4, 29). The reason for this discrepancy is not apparent, but it is possible that adenosine at physiological concentrations has direct stimulatory effects on tubular transport mediated by A_1 receptors. A recent study by Ling et al. (18) using A6 distal nephron cells and patch-clamp technique supports this view. They found that adenosine at physiological concentrations (nanomolar range) increased the activity of amiloride-sensitive Na^+ channels on the apical membrane, and increases in adenosine concentration to micromolar range inhibited the activity of these Na^+ channels. The A_1 receptor antagonist DPCPX blocked the stimulatory effect of low concentrations of adenosine on the Na^+ channel activity, and the A_2 receptor antagonist DMPX blocked the inhibitory effects of high concentrations of adenosine on the Na^+ channel activity.

Recently, the molecular and pharmacological characteristics of a novel adenosine receptor subtype designated A_3 were described (38). It was shown that activation of A_3 receptors produced hypotension, mast cell degranulation, and vasoconstriction (17, 30). A_3 receptor-mediated adenosine effects on renal medullary blood

flow and renal function have not been previously studied. In the present study, we found that renal medullary infusion of benzyl-NECA, a specific A_3 receptor agonist, had no effect on either medullary blood flow or sodium and water excretion, even at a dose that decreased arterial blood pressure when infused intravenously. Therefore, it appears that A_3 receptors do not participate in the control of medullary blood flow and sodium and water excretion.

In summary, the present data provide evidence that high concentrations of adenosine are present in the renal medullary interstitial dialysate. Endogenous adenosine dilates medullary vessels and increases medullary blood flow via A_2 receptors, resulting in a natriuretic response. This A_2 receptor-mediated effect of adenosine on renal medullary hemodynamics overrides A_1 receptor-mediated tubular antinatriuretic effects and hence plays an important role in the control of renal sodium and water excretion. A_3 receptors do not participate in the effect of adenosine on medullary circulation and sodium and water excretion.

Perspectives

Our previous studies have demonstrated that medullary blood flow was poorly autoregulated and rise of renal perfusion pressure increases medullary blood flow, which provides a signal that transmits increases in arterial pressure to the tubules to enhance sodium and water excretion. It will be intriguing to determine whether a high concentration of medullary adenosine and its vasodilator effect shown in the present study contribute to the poor autoregulation of medullary blood flow and pressure natriuretic response. Moreover, because medullary blood flow participates in the long-term control of arterial blood pressure (9), adenosine as an endogenous vasodilator in the renal medullary circulation would be expected to be a medullary antihypertensive factor.

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