Adenosine and Renal Sodium Handling: Direct Natriuresis and Renal Nerve-Mediated Antinatriuresis¹

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ABSTRACT

Adenosine infusion is associated with natriuresis as well as antinatriuresis. The physiologic significance of these opposite effects is unknown but may have to do with different conditions of ischemia, in which adenosine accumulates. These effects were characterized in the rat. First, intrarenal and systemic infusions within one animal were performed. Intussus 10 μg/min into the left renal artery increased sodium by ~50%; however, the subsequent infusion of 50 μg/min into the thoracic aorta decreased sodium excretion by ~60%, in association with a small reduction of blood pressure. Second, to explore the effect of intrarenal adenosine on tubular sodium handling, free-flow micropuncture experiments were performed. The intrarenal infusion of 10 μg/min again caused sodium excretion, but no change in GFR, volume, and sodium deliveries up to the early distal tubule was found. Apparently, the direct effect of adenosine in the kidney is sodium excretion, by a tubular action beyond the early distal tubule. Third, to further characterize the indirect effect, which apparently is sodium retention, adenosine was infused systematically at low rates, in order to avoid a decrease in blood pressure. A 25 μg/min infusion again caused sodium retention, in the absence of a fall in blood pressure. After acute left renal denervation, the antinatriuretic effect disappeared in the denervated kidney but remained in the right kidney. These data suggest that increased intrarenal adenosine suppresses sodium reabsorption at some distal nephron site, appropriately decreasing the workload of the kidney. On the other hand, systemic adenosine stimulates sodium reabsorption, an effect that is appropriate to improve systemic circulation and depends on the renal nerves.

Key Words: Adenosine, renal physiology, renal sodium handling, renal nerves

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1064-4563/95/05-14911503.00/0
Journal of the American Society of Nephrology
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The effects of adenosine on renal sodium handling are complex. First, the physiologic action of intrarenally released adenosine is probably natriuresis by decreased tubular sodium reabsorption (1,2). However, it is unclear in which tubule segment adenosine accomplishes this effect. Second, systemically administered adenosine has been associated with sodium retention (3). The background of this paradox with intrarenal administration is unclear as well.

With respect to the first question, several studies have used either in vitro or in vivo microperfusion to study the effects of adenosine on the proximal tubule (4), the loop of Henle (5), or the collecting ducts (6,7). The data suggested that adenosine can increase proximal bicarbonate reabsorption (4), suppress sodium reabsorption in Henle's loop (5), and oppose vasopressin-dependent water reabsorption in collecting tubule (6,7). However, in each of these studies, artificial tubule fluid not containing adenosine was used, although natural tubule fluid contains some 0.5 × 10⁻⁶ M adenosine (8). It cannot be excluded that luminal adenosine affects tubular reabsorption (9). Free-flow micropuncture studies, which are not subject to this problem, are not available.

The paradoxical sodium retention caused by systemically infused adenosine could be attributed to the accompanying fall in blood pressure (3). However, this hypotension actually indicates that adenosine was given in supraphysiologic amounts; the infusion of adenosine antagonists is not usually associated with a rise in blood pressure, indicating little contribution of endogenous adenosine to blood pressure. Thus, the sodium retention reported during systemic adenosine infusion may be specific for the model used rather than represent a physiologic action. However, antinatriuresis was also observed during the aortic infusion of a nonhypotensive dose of adenosine (10). This effect of systemically infused adenosine could conceivably be mediated indirectly through the renal nerve, because the intracoronary infusion of adenosine in the dog has been demonstrated to increase renal nerve activity (11). The questions presented above can be resolved by studying whether a nonhypotensive dose of intrarenal and systemic adenosine can cause, respectively, sodium excretion and retention within one animal and whether the latter effect requires an intact renal nerve.

If indeed an increase in extrarenal adenosine may cause antinatriuresis, while intrarenal adenosine causes natriuresis, this has important implications for our understanding of the physiologic role of adenosine. It would suggest that an extrarenal increase in adenosine, such as exists during local ischemia, would lead to volume retention and thus to increased
vascular filling and increased perfusion of ischemic sites. An intrarenal increase of adenosine content, however, would lead to decreased reabsorption and, thus, a decreased oxygen consumption by the kidney, which would prevent ischemic damage, as suggested by the metabolic feedback hypothesis (3).

We have therefore first set out to document both the natriuretic and the antinatriuretic action of adenosine in the same animal. This was done to rule out that any methodologic difference other than the route of administration led to the contrasting responses as reported previously. Next, we investigated the tubular site of action of intrarenal adenosine by free-flow cortical micropuncture. Finally, we investigated the role of the renal nerve in the antinatriuresis induced by the nonhypotensive systemic infusion of adenosine. Rather than using adenosine analogues, attractive because of their possible receptor specificity, we used adenosine itself. Whereas adenosine analogues are resistant to degradation and cellular uptake, the degradation of adenosine itself is so fast that no spillover into the systemic circulation occurs and the obviously confounding effects of systemically administered adenosine are eliminated.

METHODS

Animal Preparation

Male Sprague-Dawley rats (weight range, 200 to 300 g) were maintained on normal rat chow containing ~100 mmol sodium/kg dry wt. At the day of the experiment, rats were anesthetized by an ip injection of 110 mg/kg body wt of 5-sec-butyl-5-ethyl-2-thio-barbituric acid (Inactin, Byk-Gulden, Germany). Rats were placed on a heated animal table; rectal temperature was maintained at 36 to 37°C. They were prepared as for micropuncture as described previously (12). In brief, tracheostomy was performed, two PE50 catheters (Medical Hospital Supplies, Den Bosch, The Netherlands) were placed in the left jugular vein for infusions, and one was placed in the left femoral artery for continuous blood pressure measurement and sampling of arterial blood. Blood pressure was monitored with a Statham P23Db transducer (Ohmeda, Breda, The Netherlands), and a Minimon 7132A recorder (Kontron Instruments, Abcoude, The Netherlands). After a midline incision, the bladder and left ureter were catheterized with PE50 tubing to collect urine from both kidneys separately. Abdominal incisions were made with an electrocauter to minimize blood losses. The left kidney was exposed by a lateral incision, freed from perirenal fat, and placed on a plastic holder. The following variations were made on this standard preparation: for Protocols I and II, a piece of PE10 tubing (Medical Hospital Supplies, Den Bosch, The Netherlands), tapered to give a tip of <0.3 mm outer diameter, was inserted into the left renal artery from the right femoral artery. The correct placement of this catheter was checked visually, and the tip was generally within the first 1 mm of the vessel. In Protocol III, rats were investigated either with intact renal nerves or after acute left kidney denervation. Denervation was carried out according to the method described by Bello-Beuus et al. (13). Briefly, the left renal artery was stripped and subsequently wrapped with a piece of cottonwool soaked in a solution of 10% phenol in ethanol for 1 to 2 min. Denervation was judged to be adequate when an initial increase in urine flow was seen combined with a sustained twofold to threefold increase in sodium excretion compared with the right kidney. Furthermore, rats in Protocol III had a PE10 catheter inserted 2 to 3 cm into the right carotid artery.

After the insertion of the venous catheters, the infusion of 5% bovine serum albumin in 0.9% saline was started at a rate of 1 mL/100 g body wt for 45 min to replace surgical losses. At the same time, a continuous infusion of 10% insulin (polyfructosan. Inutest®1, Laevosan GmbH, Linz, Austria) and 0.5% p-aminohippuric acid (PAH) in 0.9% NaCl was started at 1.2 mL/h. When the bovine serum albumin infusion was stopped, an infusion of saline at 10 μL/min was started through the left renal artery catheter (Protocols I and II) or the carotid artery catheter (Protocol III). After the equilibration period, three different experimental protocols were followed.

Experimental Protocols

Protocol I: Infusion Into the Left Renal Artery or Thoracic Aorta. During the 90-min equilibration period, saline was infused into the left renal artery of five rats. After a 10-min baseline collection, adenosine in saline was infused at two doses (5 and 10 μg/min) at a constant rate (10 μL/min) into the left renal artery. After each change in adenosine concentration, a 15-min equilibration period was observed, after which, urine was collected for 10 min. After the adenosine infusions, saline was again infused into the left renal artery, and a 10-min recovery collection was made. Then, the catheter was carefully retracted into the aorta and moved up into the thoracic aorta. The infusion rate was maintained at 10 μL/min. After a 15-min equilibration period, adenosine was again infused, at 50 μg/min. This infusion was expected to yield an adenosine concentration in both renal arteries comparable to that during the intrarenal adenosine infusions. A 15-min urine collection was made, and saline was then infused into the aorta for a 10-min recovery collection. Blood samples (200 μL) were taken at regular intervals.

Protocol II: Micropuncture During Intrarenal Infusion of Adenosine. During the 90-min equilibration period, late proximal and early distal tubules were identified by injecting a small amount of lissamine green-stained saline into a random proximal tubule with a small (outer diameter <2.5 μm), sharpened micropipet. Rats were then infused either with saline (controls, N = 8) or 10 μg/min adenosine (N = 8) in saline, both at 10 μL/min. After a 15-min equilibration period, a timed urine collection was started. During this period (60 min), three late proximal and three early distal fluid samples were obtained with sharpened micropettes as described previously (12). Tubular fluid samples were immediately stored at ~20°C. A 200-μL blood sample was taken before and after the micropuncture period.

Protocol III: Effect of Denervation on Adenosine-Induced Antinatriuresis. After surgery, the rats were left for at least 2 h to ensure that sodium excretion from the denervated kidneys had stabilized. Infusion through the carotid artery catheter was kept at a constant rate of 10μL/min throughout the experiment. Six groups of rats were investigated (N = 6 for each group): infusion of saline or 10 or 25 μg/min of adenosine, either in rats with intact nerves or in rats after acute left kidney denervation. After the equilibration period, two 15-min baseline collections were made, after which, the carotid artery infusion of saline was either continued or switched to adenosine (10 or 25 μg/min). The highest dose that did not affect blood pressure was 25 μg/min. After a 20-min equilibration period, two 15-min collections were
made during the experimental period. Then, the adenosine infusions were switched to saline, and after 60 min, a 30-min recovery sample was collected. Blood samples (200 μL) were taken at regular intervals.

Analytical techniques

Urine volume was determined gravimetrically. Urine and plasma sodium concentrations were measured by flame photometry (Instrumentation Laboratory, IL 543, IJsselstein, The Netherlands). Inulin concentrations in plasma and urine were determined photometrically with indoleacetic acid after hydrolyzation to fructose (14). Plasma and urine PAH was determined photometrically by a chromogenic aldehyde reaction (15). The volume of tubular fluid samples was determined with constant-bore capillaries (Drummond Micropacs, 1 μL, Ankersmit, Breda, The Netherlands). Tubular fluid inulin concentration was determined according to Vurek and Pegram (16) with modifications described elsewhere (17). Tubular fluid sodium concentration was measured as described elsewhere (18). In brief, a sample of 3 nL was transferred to a graphite microboat, which was then inserted into a rectangular graphite cuvette of a Thermo Jarrell Ash SH12 atomic absorption spectrophotometer (TJA Europe, Breda, The Netherlands) and atomized at 1,900°C.

Calculations

The renal clearances of inulin and PAH were calculated by the use of a standard formula (19) as a measure of GFR and estimated RPF (ERPF), respectively. Fractional delivery to the micropuncture segments was calculated as 1/(T/P)inulin for water and [T/P]urea/[T/P]inulin for sodium, in which [T/P] is the tubular fluid/plasma concentration ratio. Single-nephron GFR was calculated as tubular fluid flow x [T/P]inulin. Results are expressed as mean ± SE.

RESULTS

Protocol 1: Intrarenal Versus Aortic Infusion of Adenosine

The infusion of adenosine into the renal artery caused an increase in urine flow and sodium excretion (Figure 1A and B). No effects were found on GFR or ERPF (Figure 1C and D) or on filtration fraction (not shown). Although urine flow and sodium excretion were slightly higher at 10 compared with 5 μg/min, there was no statistical difference in the response at both levels. Right kidney excretory function was not affected by the infusion of adenosine into the left renal artery. The effects of adenosine were fully reversible, with return to baseline values after switching to saline. When the infusion catheter was subsequently removed from the renal artery and adenosine was infused into the thoracic aorta at 50 μg/min, blood pressure fell from 107 ± 3 to 95 ± 6 mm Hg (P < 0.05) (Figure 2). Urine flow and the renal excretion of sodium fell in both kidneys, as did GFR and ERPF (Figure 1). Again, filtration fraction was not affected. Switching the aortic infusion to saline caused a return to baseline values for blood pressure and renal excretions and hemodynamics.

Figure 1. Urine flow, sodium excretion, GFR, and ERPF during subsequent intrarenal and aortic infusion of adenosine in the same animal. Adenosine was infused at 0, 5, 10, and 0 μg/min into the left renal artery (intrarenal) and at 50 and 0 μg/min into the thoracic aorta. Open bars represent the left kidney; solid bars represent the right kidney. Data are mean ± SE; *P < 0.05 versus previous 0 μg/min infusion by repeated-measures analysis of variance and Tukey's multicomparison test.

Figure 2. Mean arterial pressure (MAP) during the intrarenal and aortic infusion of adenosine. Adenosine infusion and statistics as in Figure 1.

Protocol II: Micropuncture During Intrarenal Infusion of Adenosine

The infusion of 10 μg/min of adenosine into the left renal artery increased both urine flow and sodium excretion, as in Protocol 1. Mean arterial pressure was not affected (106 ± 3 mm Hg in controls; 104 ± 2 mm Hg in the adenosine-infused group; not significant).
TABLE 1. Intrarenal infusion of adenosine: whole-kidney and micropuncture data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (N = 8)</th>
<th>Adenosine (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Flow (µl/min)</td>
<td>4.5 ± 0.3</td>
<td>18.0 ± 2.2b</td>
</tr>
<tr>
<td>Retention (%)</td>
<td>0.21 ± 0.11</td>
<td>4.4 ± 0.29</td>
</tr>
</tbody>
</table>

Both single-nephron GFR (measured from the early distal tubule) and tubular fluid flow at the late proximal and early distal puncture site were similar in the adenosine-infused group compared with controls (Table 1). Fractional deliveries of water and sodium to both puncture sites were also not different, although the
The experimental groups are: innervated time controls (TC Inn), time controls after left kidney denervation (TC-Ldnx), and animals infused with 10 or 25 \( \mu \text{g/min} \) of adenosine during the experimental period with either intact renal nerves (Inn) or after acute left kidney denervation (Ldnx). For details, see Methods. Data were analyzed by the use of two-way analysis of variance and a least significant difference test. *\( P < 0.05 \), experimental period versus baseline period; \( \#P < 0.05 \), left versus right kidney in the same period.

Urine flow increased at 10 \( \mu \text{g/min} \) only in the Ldnx group, but did so in both kidneys (Figure 5). However, urine flow in the denervated left kidney was significantly higher than in the innervated right kidney. At 25 \( \mu \text{g/min} \), urine flow increased in both the innervated and the left kidney–denervated groups. In the Ldnx group, urine flow was again higher in the left as compared with the right kidney. Adenosine infusion at either 10 or 25 \( \mu \text{g/min} \) did not affect GFR, ERPF, or filtration fraction in either innervated or denervated kidneys (not shown).

**DISCUSSION**

This study shows that the intrarenal infusion of adenosine in rats increases sodium excretion, whereas systemic infusion in the same animal does the opposite. The natriuretic effect of intrarenal adenosine occurred in the absence of a change in GFR, suggesting that adenosine suppresses tubular sodium reabsorption. However, we found no effect of intrarenal adenosine infusion on sodium handling proximal to the superficial early distal tubule. The antinatriuretic effect of systemic adenosine infusion was also found in the absence of a significant fall in blood pressure. In the latter experiments, this antinatriuretic effect was markedly attenuated by acute renal denervation.

Previous studies on the renal actions of adenosine have mostly been performed with the systemic administration of adenosine or adenosine agonists. Administered in this way, adenosine was found to decrease sodium excretion and GFR in rats (10), dogs (20), and humans (21). However, more recently, it was found that if adenosine is administered directly into the renal artery, it stimulates sodium excretion (1), an effect that can be blocked by the A1 antagonist DPCPX (2). Whether this discrepancy concerns a methodologic rather than a physiologic difference is unclear. However, this study establishes firmly that the effect of exogenous adenosine on sodium excretion indeed depends on the route of administration, because within the same animal, both natriuresis and antinatriuresis can be observed after, respectively, intrarenal and systemic infusion.

In further experiments, we focused on the renal hemodynamic and tubular effects of a natriuretic intrarenal adenosine infusion. Whereas in our first experiments, the tendency of intrarenal adenosine infusion to increase ERPF was not significant (Figure 1D), a statistically significant increase was found during a similar dosage in the micropuncture experiments (Table 1). This discrepancy is probably the result of the difference in the length of the clearance periods in both protocols, because renal vasodilation during intrarenal adenosine infusion does not occur immediately (22) Filtration fraction decreased, be-
cause the GFR did not change, suggesting predominately efferent vasodilation. A similar response to intrarenal adenosine infusion in rats has been reported previously by others (1,2). Presumably, in the rat, efferent arteriolar (vasodilatory) A2 receptors predominate over afferent arteriolar (vasoconstrictive) A1 receptors (22). Experiments with the intrarenal infusion of adenosine and receptor-specific agonists in the dog led to a similar conclusion for that species (23).

To investigate the direct effects of exogenous adenosine on tubular sodium reabsorption, we performed in vitro micropuncture experiments during the intrarenal infusion of 10 µg/min adenosine. Despite a clear natriuretic effect of the infusion, we observed no significant change in single-nephron filtration and in tubular fluid flow and fractional deliveries in the late proximal and early distal nephron levels (Figure 3). However, Takeda et al. (4) found that specific adenosine A1 antagonists inhibit the basolateral Na⁺-3HCO₃⁻ cotransporter in the in vitro perfused rabbit proximal convoluted tubule. This suggests that endogenous adenosine stimulates proximal reabsorption, which contrasts with the finding here that exogenous adenosine had no measurable effect in this segment. The option should therefore be considered that adenosine has differential effects, depending on its intrarenal concentration. Possibly, physiologic concentrations of adenosine stimulate reabsorption in the proximal nephron, whereas higher concentrations, such as those achieved by intrarenal infusion or ischemia, do not stimulate proximal reabsorption further, but instead inhibit reabsorption at a more distal nephron site.

Two preliminary studies (5,9) have found an inhibiting effect of adenosine on sodium reabsorption in the loop of Henle of the rat. Seney and Seikaly infused adenosine intrarenally and perfused loops of Henle in vivo (5), whereas Bell et al. performed the experiments in isolated perfused thick ascending limbs (9). It is unclear why these results differ from those of our study, in which no effect of adenosine on loop reabsorption was seen. Methodologic differences between the perfused tubule systems and our free-flow micropuncture experiments could be responsible. Obviously, any microanalytical technique, including the method of free-flow micropuncture described here, has its intrinsic limitations. However, with these limitations in mind, our results do not provide evidence for an effect of intrarenally infused adenosine on tubular segments up to the early distal tubule. Thus, our observation would be in line with other findings that adenosine can inhibit the vasopressin-induced reabsorption of water and sodium in the collecting ducts (6,7).

In view of the natriuretic effect of intrarenally infused adenosine, the antinatriuresis after systemic adenosine infusion must be mediated indirectly. The vasodilatory action of systemically infused adenosine often results in a decrease in blood pressure (21), which would reduce renal excretion. However, antinatriuresis after systemic adenosine administration has also been reported in the absence of a decrease in blood pressure (10). Another system that could be activated by adenosine and lead to antinatriuresis is the sympathetic nervous system. Activation could occur through chemoreceptors in, for example, the carotid body (24). Thus, we hypothesized that systemic adenosine infusion stimulates arterial chemoreceptors, resulting in increased renal nerve activity and antinatriuresis. To test this hypothesis, we infused adenosine into the carotid artery in dosages without a significant effect on mean arterial pressure and assessed its effect on innervated and acutely denervated kidneys. It appeared that acute unilateral renal denervation greatly diminished the antinatriuresis after systemic adenosine in the denervated kidney but not in the intact kidney. Previously, Cook and Churchill (25) found no effect of acute denervation on the antinatriuretic response to an intravenous infusion of the A1 agonist cyclohexyladenosine. However, the dosage was such that mean arterial pressure decreased significantly, which may explain the unaltered antinatriuretic response.

Together, our data strongly suggest that the reduction in sodium excretion during adenosine infusion was at least partly the result of increased renal nerve activity. The peripheral activation of arterial chemoreceptors by adenosine, as mentioned above, is a likely option. We cannot exclude a triggering role of baroreceptor activation, following from the adenosine-associated decrease in blood pressure, although the latter was small and not statistically significant. More important, our data may explain why systemically administered adenosine antagonists also cause natriuresis (26,27). At first sight, this is a paradox in view of the natriuretic effect of intrarenal adenosine. However, it is conceivable that the effect of systemically administered adenosine antagonists also reflects interaction with arterial chemoreceptors rather than direct interaction with the kidney. Indeed, the direct intrarenal administration of adenosine A1 antagonists did not affect sodium excretion, even if given at a concentration sufficient to prevent the diuretic effect of simultaneously administered intrarenal adenosine (2,28).

Finally, the differential effects of adenosine on urine flow deserve attention. The systemic infusion of adenosine at rates of 10 and 25 µg/min was associated with increased urine flow (Protocol III), whereas an infusion rate of 50 µg/min was followed by antidiuresis (Protocol I). Most likely, the antidiuresis during the highest infusion rate was due to a nonspecific effect, because it occurred in association with a decrease in GFR and ERPF and antinatriuresis. By contrast, the diuresis found during the low infusion rates was dissociated from sodium excretion, which decreased, and renal hemodynamics, which were not altered. This suggests a specific diuretic mechanism. Indeed, it has been reported that adenosine reduces vasopressin-induced water reabsorption in the inner medul-
lary collecting ducts in vitro (6,7). We therefore propose that the diuretic effect of systemic adenosine administration concerns a direct interaction of adenosine with the kidney. Although a similar mechanism can have contributed to the diuresis found after intrarenal adenosine infusion, this cannot be dissociated from the natriuretic response. Interestingly, the increase in urine flow was stronger in the denervated kidneys in comparison to the innervated kidneys of the same animal (Figure 5). Taken together, our data suggest that systemic adenosine can counteract its own direct diuretic effect through its effects on blood pressure, the renal nerve, and GFR.

In conclusion, it appears that the route of administration determines the renal effect of adenosine. Systemic adenosine leads to a decrease in sodium excretion, which in the absence of a major change in blood pressure, appears to depend on renal nerve activity. The inhibitory action of adenosine on tubular sodium reabsorption is most likely located downstream from the early distal tubule. Diuresis is probably also a local effect of adenosine, which can be counteracted by blood pressure and renal nerve activity. The data presented here stress the contrasting effects of intrarenal and extrarenal adenosine, which is relevant for our understanding of the physiologic role of adenosine. Extrarenal adenosine accumulation, due to local or generalized ischemia, probably stimulates renal nerve activity, in order to conserve sodium and improve the circulation. On the other hand, intrarenal adenosine accumulation, signifying imbalance between workload and aerobic metabolism, leads to diminished sodium reabsorption and, in line with the metabolic feedback hypothesis (2), thus acts to diminish workload in the kidney.

ACKNOWLEDGMENTS

This study was supported by Grant C83.1301 from the Dutch Kidney Foundation. The technical assistance of Remmert de Roos and Fatima el Khalouki is gratefully acknowledged.

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