Renal Effects of Adrenomedullin in the Rat (43959)

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Abstract. **We investigated the effects of a constant infusion of adrenomedullin (ADM) on renal hemodynamics and fluid electrolyte excretion in the rat. Following baseline measurements, eight rats received an intravenous infusion of 5 μg of rat ADM (167 ng/min) for 30 min at 10 μl/min. Eight additional rats received 0.9% saline at 10 μl/min instead of ADM. Renal function was measured during this period and for two consecutive 20-min periods following termination of the ADM or vehicle infusion. Mean arterial pressure decreased from a baseline of 113 ± 3 to 102 ± 1 mm Hg at 25 min of ADM infusion and returned towards control after the ADM infusion was terminated. This modest hypotensive effect was associated with an increase in heart rate from 366 ± 10 to 384 ± 9 bpm, which continued to remain elevated after the ADM infusion was stopped. Urinary sodium excretion increased from 348 ± 57 to 813 ± 172 nEq/min during ADM infusion and continued to increase to 1141 ± 347 nEq/min after the infusion of ADM was terminated. Urinary potassium excretion increased from 1.94 ± 0.22 to 2.75 ± 0.24 μEq/min during ADM infusion. Urine flow tended to increase (P = 0.08) from 7.0 ± 0.5 to 8.1 ± 0.6 μl/min during ADM infusion and continued to increase to 9.7 ± 1.5 μl/min after the infusion was stopped. Renal plasma flow increased from 3.22 ± 0.22 to 3.82 ± 0.20 ml/min/g kidney wt during ADM infusion and continued to increase to 4.14 ± 0.22 ml/min/g kidney wt after the ADM infusion was stopped. Glomerular filtration rate averaged 1.11 ± 0.07 ml/min/g kidney wt during baseline and did not significantly change during or after ADM infusion. These results indicate that a constant infusion of adrenomedullin, at a dose that results in a minimal hypotensive effect increases renal plasma flow and urinary sodium excretion in the rat.**
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Adrenomedullin (ADM) is a newly discovered peptide that possesses hypotensive and vasodilatory properties (1–2). The ADM molecule has 52 amino acids in humans, a C-terminal tyrosine amide structure, and one disulfide bond, and shows slight sequence homology with calcitonin gene–related peptide (CGRP) and amylin (2). Rat ADM consists of 50 amino acids and is similar but not identical to the human peptide (3).

The hypotensive effect of ADM is pronounced and dose dependent (1, 4–7). Intravenous bolus administration of ADM (1.0 nmol/kg) decreases mean arterial pressure approximately 40 mm Hg for 10 min in the anesthetized rat (1). ADM has minimal effects on cardiac performance in the isolated rat heart preparation (8) and appears to lower arterial pressure by inducing vasodilation in several major vascular beds including lung, heart, kidney, mesenteric, and hindquarters (4, 5, 9). ADM-induced vasodilation has been shown to be nonadrenergic and noncholinergic (10). There is some evidence to suggest that ADM activates CGRP receptors in view of the observations that CGRP receptor blockade inhibits ADM-induced vasodilation in some isolated vascular beds (10), hamster cheek pouch microvasculature, and rat skin (11). However, specific high-affinity (K_d:1.3 × 10^{-8} M) binding sites for ADM, distinct from CGRP receptors, have been identified in cultured vascular smooth muscle cells (VSMC) from the rat (12, 13). Recently, CGRP receptor blockade inhibited the regional hemodynamic effects induced by exogenous CGRP administration in the conscious rat,

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however had no effect on the vascular effects of exogenously administered ADM (5). CGRP blockade also had no effect on the hypotensive response to ADM in the anesthetized rat (6).

The role of ADM in the regulation of renal function is currently under investigation. ADM has been immunohistochemically localized to glomeruli, cortical, and distal tubules in the dog (14). Renal vasodilation, diuresis, and natriuresis has been reported during intrarenal infusions of ADM in the dog (14–16). The diuresis and natriuresis have been proposed to be due to increases in glomerular filtration rate (14) and reductions in tubular sodium reabsorption (14, 15). ADM caused a dose-dependent vasodilation in the isolated, perfused rat kidney, a response that appeared to be dependent upon activation of CGRP receptors (6). In the conscious rat, however, the dose-dependent renal vasodilation induced by ADM was not dependent upon activation of CGRP receptors (5). Recently, bolus injections of ADM lowered blood pressure, increased renal blood flow and glomerular filtration rate, and produced natriuresis in a dose-dependent fashion in the anesthetized rat (7). The renal vasodilation was shown to be partially dependent upon nitric oxide synthesis and mediated by decreases in both afferent and efferent arteriolar resistances (7). In consideration of the marked hypotensive effects of systemic bolus injections of ADM (4–7), the objective of the current study was to investigate the effects of a continuous infusion of ADM that minimally lowers arterial pressure on renal hemodynamics and excretory function in the rat.

Materials and Methods

All of the experiments described in this study conform to the guide for the care and use of laboratory animals published by the U.S. National Institutes of Health (NIH publications No. 85-23, revised 1985) and were approved by the University of North Dakota Animal Care Committee.

Male Sprague-Dawley rats were obtained from the in-house colony located in the Center for Biomedical Research at the University of North Dakota. The rats were maintained on a 12:12-hr light:dark cycle, standard laboratory rodent chow (PMI Feeds, Inc., St. Louis, MO), and tap water *ad libitum*. The rats were divided into two groups of eight and surgically prepared for the measurement of renal hemodynamics and urinary electrolyte excretions. The rats were anesthetized with Inactin (100 mg/kg ip; Research Biochemicals, International, Natick, MA). A catheter (PE-50) was implanted into a jugular vein for a bolus injection of 10% inulin containing 800 μl of 20% p-aminohippurate (PAH) followed by a continuous infusion of 5% inulin containing PAH at a rate of 20 μl/min. Another jugular catheter was inserted for subsequent infusion of ADM or 0.9% saline as vehicle. A carotid artery was catheterized for the continuous measurement of mean arterial pressure (MAP) and heart rate (HR) and for the collection of arterial blood samples. The bladder was catheterized (PE-90) via a small midline incision for the collection of free-flow urine samples. A tracheostomy was performed to facilitate respiration. Rectal temperature was monitored and maintained at 37° ± 1°C.

Following at least a 30-min surgical equilibration period and coincident with at least 60-min of inulin/PAH infusion, two consecutive 20-min renal clearance periods were obtained to establish baseline conditions for renal hemodynamics, and fluid and electrolyte excretions (C_1 and C_2). An arterial blood sample (~120 μl) was taken at the beginning and end of each urine collection and the volume replaced with an equal volume of 0.9% saline. At the conclusion of C_2 an intravenous infusion was begun at a rate of 10 μl/min. This continuous infusion was for 40 min and urine was collected for the entire duration of this clearance period (INF). In preliminary experiments, we determined that this infusion would compensate for the 100 μl of dead space in the venous line and deliver ADM at 10 μl/min for the last 30 min of the period. The ADM-infused rats therefore received a continuous infusion of 0.9% saline (400 μl) over 40 min, the last 30 min delivering 5 μg (167 ng/min) of ADM (ADM_{11-50}, Peninsula Laboratories). We chose the 5-μg dose for these infusion experiments in order to minimize the effects of rapid and/or marked reductions in blood pressure produced by bolus injections of this amount of the peptide in the rat (4–7). Also, in additional preliminary experiments, 1 μg of ADM infused according to this protocol had no effect on blood pressure or urinary sodium excretion and 10 μg of ADM lowered MAP to levels well below 100 mm Hg. The vehicle animals received the same total volume (400 μl) of 0.9% saline at the same rate. At the end of the infusion period (INF) the ADM or vehicle infusion was terminated and two consecutive 20-min clearance periods (R_1 and R_2) were obtained. At the end of R_2 a terminal arterial blood sample was taken (2 ml) for the measurement of plasma sodium and potassium concentrations. The animals were killed with an overdose of anesthetic plus a saturated KCl solution. The kidneys were removed, decapsulated, blotted dry, and weighed.

Plasma electrolytes were measured by flame photometry. Inulin and PAH concentrations in urine and plasma were measured by the anthrone (17) and Waugh and Beal (18) methods, respectively. PAH clearances were not corrected for the renal extraction of PAH. Plasma protein concentration was measured by refractometry. Statistical analysis was performed with a two-way analysis of variance (ANOVA) with repeated measures on one-factor followed by a least significant difference (LSD) post-hoc test whenever appropriate. Student's *t* test for unpaired data was used to compare group data that did not involve repeated measurements. Data are presented as mean ± SEM. Significance was set at the $P < 0.05$ level.

Results

Body weights, left and right kidney weights, plasma sodium and potassium concentrations, baseline Hct, and plasma protein concentrations were not different ($P > 0.05$) between the rats infused with ADM or saline (Table I). Hct and plasma protein concentration decreased to the same degree in the ADM and vehicle infused rats probably due to the infusion of clearance markers and replacement of arterial blood samples (v/v) with 0.9% saline.

The effects of ADM or saline infusion on MAP are presented in Figure 1. Baseline MAP averaged 116 ± 3 and 112 ± 4 mm Hg in the vehicle- and ADM-infused rats, respectively, during C_1. Infusion of ADM resulted in a small but significant decrease in MAP of approximately 10 mm Hg that returned toward baseline levels after the ADM infusion was stopped. MAP also decreased in the vehicle-infused rats by about 8 mm Hg. However, MAP was significantly lower in the ADM-infused rats throughout the infusion protocol. The decrease in MAP was accompanied by a significant increase in heart rate from 366 ± 10 during C_1 to 384 ± 9 bpm during ADM infusion, which remained elevated at 390 ± 10 and 385 ± 11 bpm during R_1 and R_2, respectively. Heart rate in the vehicle group averaged 352 ± 14, 354 ± 10, 361 ± 9, and 364 ± 12 bpm during these periods.

The effects of ADM or vehicle infusion on renal fluid and electrolyte excretions are presented in Figure 2 (Panel A–C). Despite the hypotensive effect of ADM infusion, urinary sodium excretion ($U_{Na^+}V$) shown in Panel A, increased greater than 3-fold during the infusion and remained elevated during both periods after the infusion was stopped (R_1 and R_2). $U_{Na^+}V$ averaged 0.35 ± 0.06 and 0.28 ± 0.06 μEq/min in the two groups of rats during C_1 and these values were not different ($P > 0.05$). ADM infusion increased $U_{Na^+}V$ to 0.81 ± 0.17 μEq/min during INF. $U_{Na^+}V$ remained elevated after the ADM was stopped and averaged 1.14 ± 0.35 and 1.04 ± 0.23 μEq/min in R_1 and R_2, respectively. $U_{Na^+}V$ actually peaked in R_1 after the termination of ADM infusion. There tended to be a slight increase in $U_{Na^+}V$ in the vehicle group following the saline infusion reaching a maximum of 0.61 ± 0.16 μEq/min in R_2, but this increase was not significant ($P > 0.05$). Urinary potassium excretion ($U_{K^+}V$) averaged 1.76 ± 0.17 and 1.94 ± 0.22 μEq/min in the vehicle and ADM infused groups, respectively during C_1 (Fig. 2B). $U_{K^+}V$ significantly increased during ADM infusion to 2.75 ± 0.24 μEq/min and remained elevated at 2.65 ± 0.23 and 2.44 ± 0.14 μEq/min during R_1 and R_2, respectively. $U_{K^+}V$ increased above baseline following vehicle infusion in R_1 and R_2. Unlike the sustained natriuretic response, $U_{K^+}V$ was significantly elevated in the ADM group above the vehicle group only during the ADM infusion. Urine flow (V) averaged 7.0 ± 0.5 μl/min in the ADM group during baseline (Panel C) and tended to increase to 8.1 ± 0.6 μl/min during ADM infusion and remained elevated at 9.7 ± 1.5 and 8.8 ± 0.9 μl/min during R_1 and R_2. These changes in V did not quite reach statistical significance ($P = 0.08$). In the vehicle-infused rats, V averaged 6.5 ± 0.5 μl/min and did not change throughout the protocol.

Table I. Systemic Variables

	ADM infused ($n = 8$)	Saline infused ($n = 8$)
BW (g)	306 ± 14	337 ± 13
LKW (g)	1.31 ± 0.08	1.37 ± 0.04
RKW (g)	1.32 ± 0.07	1.38 ± 0.05
P_{Na^+} (mEq/l)	146 ± 0.7	146 ± 0.5
P_{K^+} (mEq/l)	3.7 ± 0.1	3.7 ± 0.1
Hct (%)		
C_1	49 ± 0.6	49 ± 0.8
R_2	45 ± 0.6[a]	47 ± 1.5[a]
P_{Pr} (g/dl)		
C_1	5.2 ± 0.1	5.2 ± 0.1
R_2	4.1 ± 0.1[a]	4.2 ± 0.1[a]

Note. BW, body weight; LKW and RKW, left and right kidney weights, respectively; P_{Na^+} and P_{K^+}, plasma electrolytes; Hct, hematocrit; P_{Pr}, plasma protein concentration; C_1 and R_2, control and recovery periods, respectively.

[a] $P < 0.05$ R_2 vs C_1.

The effects of ADM on renal hemodynamics are shown in Figure 3. Renal plasma flow, as measured by the renal clearance of PAH (C_{PAH}), averaged 3.26 ± 0.18 and 3.01 ± 0.18 ml/min/g kidney wt in the ADM and vehicle infused rats during C_1 (Panel A). C_{PAH} significantly increased during ADM infusion to 3.82 ± 0.20 ml/min/gkw and remained elevated during R_1 at 4.14 ± 0.22 ml/min/g. C_{PAH} did not change ($P > 0.05$) in the rats that were infused with saline. GFR averaged 1.12 ± 0.09 and 1.01 ± 0.04 ml/min/g kidney wt in the ADM-infused and vehicle rats during C_1 (Panel B). GFR in both groups remained stable throughout the protocol. The increase in RPF in the ADM infused rats with no change in GFR resulted in a decrease in filtration fraction from 0.34 ± 0.01 in C_1 to 0.31 ± 0.02 during ADM infusion and reached statistical significance at 0.28 ± 0.02 during R_1. There was no effect of saline infusion on filtration fraction which averaged 0.34 ± 0.02 during C_1.

Discussion

The results of the current study demonstrate that a constant infusion of ADM that produces a modest systemic hypotensive effect can increase renal plasma

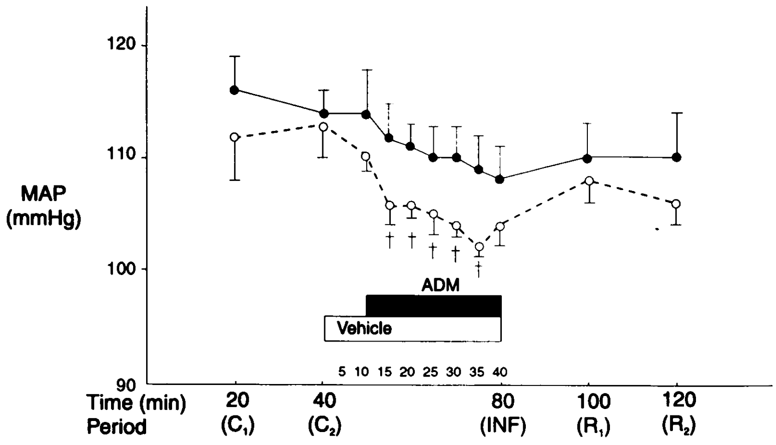

Figure 1. The effects of ADM or vehicle infusion on mean arterial blood pressure (MAP) in the rat. C_1 and C_2, baseline values prior to infusion; INF, infusion period (see text for details); R_1 and R_2, postinfusion periods; closed circles are saline vehicle–infused rats ($n = 8$); open circles are ADM-infused rats ($n = 8$); data are mean ± SEM; †significant difference between the two groups at the $P < 0.05$ level.

flow and urinary sodium excretion in the rat. These results confirm previous observations of a natriuretic effect of ADM during intrarenal infusions in the dog (14, 15) and following bolus injections in the rat (7), and extend these observations to include an experimental setting in which ADM administration produced only a minimal reduction in blood pressure. In the current study, a continuous infusion of ADM into anesthetized rats was utilized in an attempt to determine the renal effects of ADM without the effects of a rapid and marked decrease in mean arterial pressure produced by bolus administration of the peptide (1, 4–7). ADM, infused at a dose of 167 ng/min, lowered mean arterial pressure by approximately 10 mm Hg during the infusion to a nadir of 102 ± 1 mm Hg at 25 min into the infusion. Thus, the antinatriuretic effects of a reduction in systemic blood pressure which would tend to offset a natriuretic effect of ADM were minimized in the current study.

The natriuretic effect of ADM was pronounced and long lasting. Urinary sodium excretion increased greater than 3-fold during the infusion period and remained elevated for 40 min after the infusion was stopped (Fig. 2A). These results are in agreement with

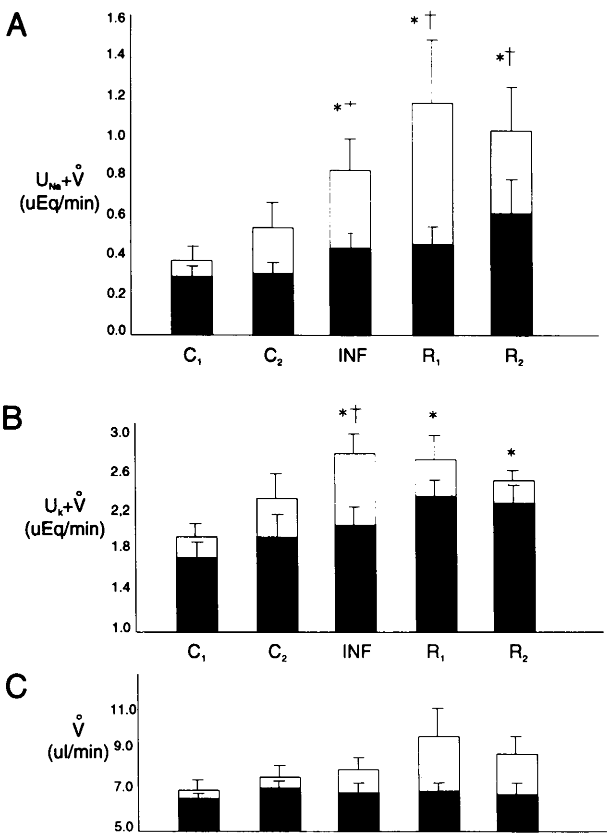

Figure 2. The effects of ADM or vehicle infusion on renal fluid and electrolyte excretion in the rat. (A) U_{Na}+$\mathring{V}$, urinary sodium excretion. (B) U_K+$\mathring{V}$, urinary potassium excretion. (C) $\mathring{V}$, urine flow. Shaded bars, vehicle-infused rats ($n = 8$); open bars, ADM-infused rats ($n = 8$). Time points and periods are identical to those in Figure 1. Data are mean ± SEM. *$P < 0.05$ vs C_1; †$P < 0.05$ ADM vs vehicle groups.

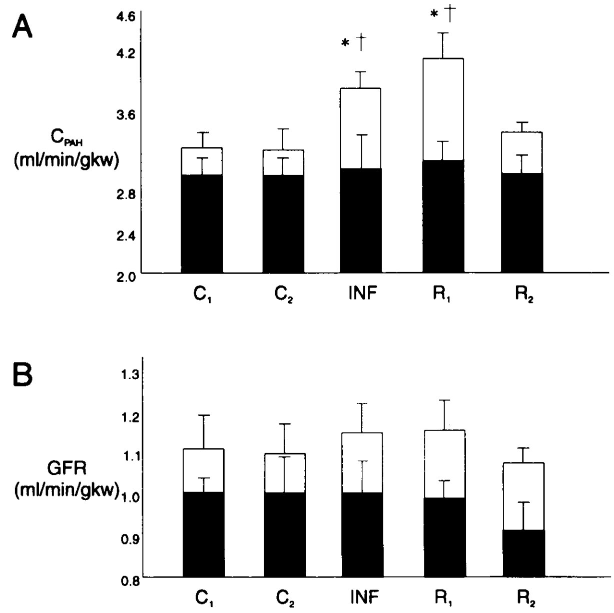

Figure 3. The effects of ADM or vehicle infusion on renal hemodynamics in the rat. (A) C_{PAH}, PAH clearance as an index of renal plasma flow; GFR, glomerular filtration rate; kw, kidney weight. Symbols and animal numbers are as described in Figure 2.

recent observations of natriuresis during intrarenal infusion of ADM in the dog (14–16), and following intravenous bolus injections that produced significant hypotensive effects in the anesthetized rat (7). The present results clearly demonstrate that ADM possesses natriuretic properties when circulating levels are elevated with a constant infusion protocol. The prolonged effect of the ADM infusion on sodium excretion (R_1 and R_2) could be a result of several factors. First, this response could have resulted from previously reported long-lasting effects of the peptide on sodium excretion. In that study, intrarenal infusion of 20 ng/kg/min of ADM in the dog resulted in an increase in renal blood flow and sodium excretion that lasted for 60 min after the infusion was terminated (15). A similar response is reported here in the anesthetized rat. Since plasma levels of ADM achieved in this study were not measured and the degradation rate half-life and clearance characteristics of ADM have not been determined, it is difficult to predict the plasma level profile changes resulting from this infusion protocol. Therefore, it is possible that the infusion of ADM, designed for maximum natriuresis with minimal hypotension, may have produced maximum steady-state ADM plasma levels during the R_1 and R_2 periods with resulting peak natriuretic effects in these periods.

The mechanism of ADM-induced natriuresis in the rat is not readily apparent, but the hemodynamic results of the current study suggest a possible tubular site of ADM action within the kidney. Renal plasma flow, as measured by PAH clearance, increased approximately 17% during ADM infusion and remained approximately 27% elevated during the period immediately after the infusion was terminated (Fig. 3A). These results confirm vasodilatory effects of ADM in the isolated rat kidney (6), *in vivo* in the rat following bolus injections (5–7), and during intrarenal infusion of ADM in the dog (14–16). The mechanism of renal vasodilation appears to be related, at least in part, to activation of the nitric oxide pathway (7, 16), although blockade of nitric oxide can sometimes only marginally affect the vasodilatory actions of ADM (5). The role of nitric oxide in the renal vascular and possibly tubular responses to ADM remains to be elucidated. Some investigators have observed marked increases in GFR associated with ADM-induced natriuresis (7, 14). However, as in the current experiments, other studies have shown natriuresis without increases in GFR during ADM administration (15). Decreases in fractional distal sodium reabsorption, estimated by the lithium clearance technique, have been reported in the dog (14). Also, in addition to glomeruli, ADM has been localized in the cortical distal nephron and medullary collecting duct of the canine kidney (14) and the inner and outer medullary collecting ducts in the rat (19). These results, taken together suggest that the natriuresis may be related to a presently undefined, direct or indirect, renal tubular action of this newly discovered peptide. The reason for divergent effects of ADM on GFR is presently unknown but may involve methodological differences between these studies involving dose and route of ADM administration. The mechanisms, either direct or indirect and glomerular or tubular, by which ADM increases urinary sodium excretion remain to be defined. The results of this study provide further evidence that ADM is a potent renal vasodilator with significant natriuretic properties.

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