Endothelin B Receptor Antagonism in the Rat Renal Medulla Reduces Urine Flow Rate and Sodium Excretion

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It is well established that activation of endothelin B (ETB) receptor induces natriuresis and diuresis and thus reduces blood pressure. However, the site of action of ETB receptor is debatable. The present study was undertaken to address the role of renal medullary ETB receptor in renal excretory function. In volume-expanded Sprague-Dawley rats, infusion of the ETB antagonist A192621 at 0.5 mg/kg/hr to the renal medulla induced an immediate and significant reduction of urine flow rate that was 87.5% ± 7.1%, 68% ± 20%, and 58.3% ± 15.5% of the control value at 10, 30, and 60 mins, respectively ($n = 5$, $P < 0.05$ at each time point). Following intramedullary infusion of A192621, urinary sodium excretion remained unchanged during the first 20 mins but started to decline thereafter with a maximal effect at 60 mins. Changes in urinary excretion of potassium and chloride followed the same pattern of changes as for urinary sodium. In contrast, urinary osmolality gradually and significantly increased (control: 419 ± 66; A192621 at 60 mins: 637 ± 204 mOsm/kg H_2O, $P < 0.05$). Over a 60-min period of intramedullary infusion of A192621, none of the hemodynamic parameters examined, including mean arterial pressure, renal blood flow, or medullary blood flow, were affected. These data suggest that: (i) intramedullary blockade of ETB receptor produces antidiuresis and antinatriuresis independently of hemodynamic changes, and (ii) the immediate response to intramedullary blockade of ETB receptor is the reduction of water excretion followed by the reduction of sodium excretion.
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Key words: endothelin receptor; renal medulla; diuretic; natriuresis; renal hemodynamics

Introduction

Emerging evidence suggests that endothelin B (ETB) receptor plays an important role in the regulation of sodium balance and blood pressure. Much of the evidence comes from pharmacologic studies using ETB receptor antagonists. At the whole-animal level, ETB blockade produces hypertension that is exaggerated during high salt intake (1). Hoffman *et al.* (2) show that the diuretic and natriuretic responses to the endothelin-1 (ET-1) precursor big ET-1 can be inhibited by ETB blockade. Vassileva *et al.* (3) observe that ETB blockade attenuates the natriuretic responses induced by increases in renal perfusion pressure. Recent studies provide genetic evidence in support of the role of ETB receptor in regulation of blood pressure. In this regard, mice heterozygous for ETB receptor exhibit an increase in blood pressure as compared with wild-type controls (4). Further evidence is obtained from spotting lethal (sl) rats carrying naturally occurring deletion of the ETB receptor gene. In this study, introduction of transgene expression of ETB receptor in intestine using a dopamine-hydroxylase (DBH) rescues the lethal intestinal phenotype, distal intestinal aganglionosis. DBH-ETB;$ETB^{sl/sl}$ rats develop severe hypertension when fed a high-salt but not a low-salt diet (5). These findings strongly suggest that ETB receptor functions as a depressor/natriuretic factor involved in stabilization of blood pressure.

The mechanisms of ETB action appears to be complex. Activation of ETB receptor on vascular smooth muscle mediates ET-1–induced vasoconstriction, whereas activation of ETB receptor on vascular endothelium produces a depressor response via release of nitric oxide and/or prostaglandins (6). ETB receptor on vascular endothelium is also involved in clearing circulating ET-1, thereby reducing endothelin A receptor–mediated vasoconstriction (7). Renal medulla is another important site of expression and action of ETB receptor. The highest concentrations of immunoreactive ET-1 and ETB receptor (8, 9) are found in renal medulla. Activation of ET(B) receptor by ET-1 or ET-3 inhibits transport function in the collecting duct (8). In

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isolated perused cortical collecting duct, ET-1 inhibits salt and water transport (10). Although there is no direct evidence that ET-1 affects sodium transport in isolated perused inner medullary collecting duct (IMCD), it has been shown that in cultured IMCD cells, ET-1 inhibits Na^+/K^+ ATPase activity (11). Activation of ETB receptor stimulates the release of nitric oxide and prostaglandins, both of which are known to decrease tubular transport and increase medullary blood flow (7, 12, 13). Dissection of the complex actions of ETB receptor in different tissues requires site-specific approaches that allow manipulation of ETB activity in a tissue-specific manner.

The intramedullary interstitial infusion technique was developed a decade ago by Lu *et al*. (14). This technique provides a unique tool to obtain access to the renal medulla *in vivo*. Thus, the present study employed this technique to deliver the ETB antagonist A192621 to the renal medulla and study its impact on renal excretory function.

Material and Methods

Animals. Sprague-Dawley rats (body wt 250–300 g, male) were from Simonsen Laboratories (Gilroy, CA). Animals were maintained on normal sodium diet and water *ad libitum*. All animal procedures were approved by the University of Utah Institutional Animal Care and Use Committee.

Surgical Preparation. The intramedullary infusion technique was employed to deliver the ETB antagonist A192621 (Abbott Laboratories, Abbott Park, IL) to renal medulla. The surgical procedures are performed as previously described (15). Briefly, rats were anesthetized with isoflurane and placed on an automatic temperature regulated surgical table to maintain body temperature at 37°C. Polyethylene catheters were placed in the jugular vein for fluid infusion, in the carotid artery for direct blood pressure measurement, and in the ureter for urine collection. Blood pressure was recorded using a pressure transducer (Abbott Critical Care System, Salt Lake City, UT). A combination probe (Bioanalytical Systems, Inc., West Lafayette, IN) was inserted 4 mm into the kidney to be positioned at the renal medulla for intramedullary infusion. A fiber optic probe for laser-Doppler flowmetry (Perimed, Järfälla, Sweden) was inserted 4 mm underneath the kidney surface for measurement of medullary blood flow (MBF). A transonic flow probe (Transonic System Inc., Ithaca, NY) was paced around the left renal artery for measurement of total renal blood flow (RBF). A data acquisition system (DATAQ Instruments, Akron, OH) was used to collect and analyze data on blood pressure, RBF, and MBF.

Experimental Protocols. Experiments were performed in rats undergoing saline diuresis. Animals received initial intravenous infusion of 1% bovine serum albumin (BSA; Sigma, St. Louis, MO) in saline at 3 ml/100 g/hr during and shortly after the surgery. When urinary flow rate reached approximately 50 μl/min, the infusion rate was changed to 1 ml/100 g/hr for the rest of the experimental period. Intramedullary infusion of vehicle (2% Tween20 in saline) was given at 0.5 ml/hr. When urine flow rate became stable for 1 hr, the intramedullary infusion was switched to A192621 at 0.5 mg/kg/hr. Sampling of urine was performed at every 10-min interval. The following parameters were continuously monitored: urine flow rate, urinary sodium excretion, MBF, RBF, and blood pressure. The placement of the intramedullary catheter was confirmed at the end of each study by infusion of a blue dye (Evan blue) into the catheter and viewing of the tip after postmortern hemisection of the kidney. Animals with inappropriate placement of the catheter were taken off the study.

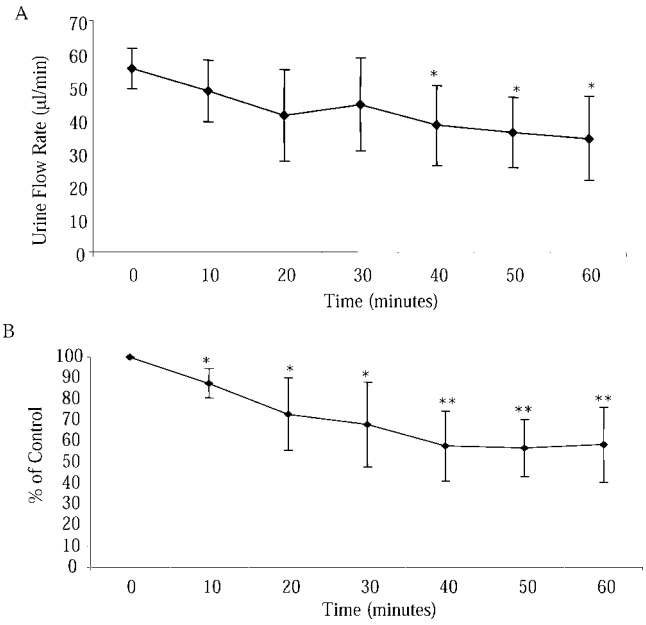

Figure 1. (A) Urine flow rate before and after intramedullary infusion of A192621. (B) Changes in urine flow rate relative to the control. When urine flow was stable for 1 hr (~50 μl/min), A192621 was infused at 0.5 mg/kg/hr through a catheter placed in the renal medulla of Sprague-Dawley rats. Urine was collected at an interval of every 10 mins. $^*P < 0.05$; $^{**}P < 0.01$ vs. control. $n = 5$ in each group.

Analytic Methods. Urinary sodium and potassium and chlorine concentrations were determined by ionselective electrodes. Urinary osmolality was measured by Osmette II (Precision Systems Inc., Natick, MA).

Statistical Analysis. Statistical analysis for blood pressure measurements was performed by ANOVA and Bonferroni tests. Values shown represent means ± SE. *P* values less than 0.05 are considered statistically significant.

Results

Effect of Intramedullary Infusion of the ETB Receptor Antagonist A192621 on Urine Flow. Experiments were performed in a volume-expanded state that was induced by intravenous infusion of 1% BSA in saline initially at 3 ml/100 g/hr during the surgery and then at 1 ml/100 g/hr during the experimental period. Over an 1-hr control period, urinary flow rate was maintained at 55.6 ±

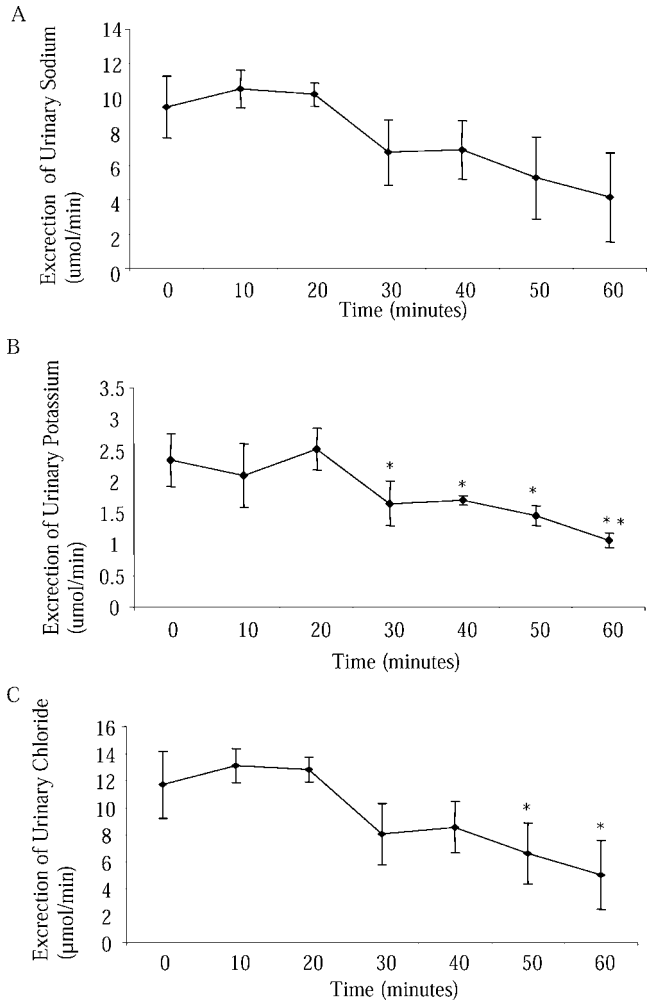

Figure 2. Changes in urinary excretion of sodium (A), potassium (B), and chloride (C) in response to intramedullary infusion of A192621. Experimental protocols were the same as described in Figure 1.

6.2 μl/min. Infusion of the ETB antagonist A192621 at 0.5 mg/kg/hr to the renal medulla induced an immediate and significant reduction of urine flow rate (Fig. 1). Intramedullary infusion of A192621 for 10 mins reduced urine flow rate to 48.8 ± 9.4 μl/min (87.5% ± 7.1% of control). Urinary flow rate dropped to 41.3 ± 13.9 μl/min (72.8% ± 17.4% of control) at 20 mins, 44.7 ± 14.1 μl/min (68% ± 20.1% of control) at 30 mins, 38.3 ± 12.1 μl/min (57.7% ± 14.6% of control) at 40 mins, 36 ± 10.6 μl/min (56.7% ± 11.9% of control) at 50 mins, and 34.3 ± 12.7 μl/min (58.3% ± 15.5% of control) at 60 mins. In addition to the absolute values of urine flow rate, values relative to the control group were also shown to eliminate the variations seen in individual animals.

Effect of Intramedullary Infusion of A192621 on Urinary Excretion of Electrolytes and Urinary Osmolality. Following intramedullary infusion of A192621, urinary sodium excretion remained unchanged during the first 20 mins but started to decline thereafter. A reduction of urinary sodium excretion was noticed at 30

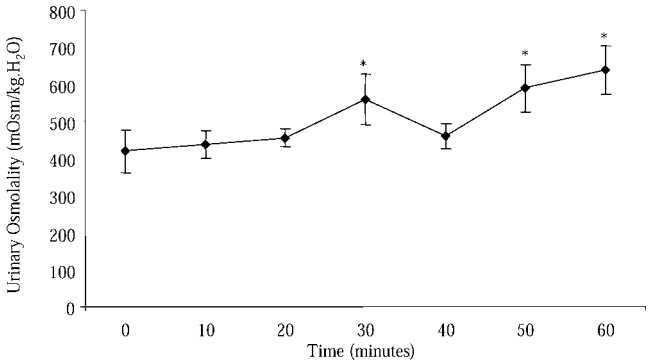

Figure 3. Changes in urinary excretion of sodium (A), potassium (B), and chloride (C) in response to intramedullary infusion of A192621. Experimental protocols were the same as described in Figure 1. $^*P < 0.05$; $^{**}P < 0.01$ vs. control. $n = 5$ in each group.

mins (71.9% of control) and was maximal at 60 mins. Similar patterns of changes were observed for urinary potassium and chloride. Urinary sodium, potassium, and chloride were 44%, 45.1%, and 42.8% of controls at 60 mins (Fig. 2).

Following intramedullary infusion of A192621, urinary osmolality gradually and significantly increased (control 419 ± 66, A192621 at 60 mins 637 ± 204 mOsm/kg H_2O, $P < 0.05$; Fig. 3).

Effects of Intramedullary Infusion of A192621 on Hemodynamic Parameters. We monitored hemodynamic parameters, including mean arterial pressure (MAP), RBF, and MBF, before and during intramedullary infusion of A192621. As shown in Fig. 4, none of these parameters were significantly affected by intramedullary infusion of A192621.

Discussion

ETB receptor exhibits natriuretic and diuretic properties, but the site of action of this receptor still remains to be determined. The present study employed the intramedullary infusion technique to evaluate the effects of site-specific inhibition of ETB receptor in the renal medulla on renal excretory function. In a saline diuresis state, intramedullary infusion of the selective ETB receptor antagonist A192621 led to an immediate and significant reduction of urine flow rate followed by a reduction in urinary salt excretion. In contrast, none of the hemodynamic parameters examined, including blood pressure, RBF, and MBF, were significantly affected by this maneuver. These observations demonstrate that selective blockade of ETB receptor in the renal medulla induces antidiuresis and antinatriuresis independent of an effect on renal hemodynamics.

The intramedullary interstitial infusion technique was developed a decade ago by Lu *et al.* (14). The technique was initially validated by examination of distribution of radioactivity infused radiolabeled (^{14}C) calcium antagonist clentazem. As a result, more than 90% of the total radioactivity was restricted to the medulla (outer zone plus

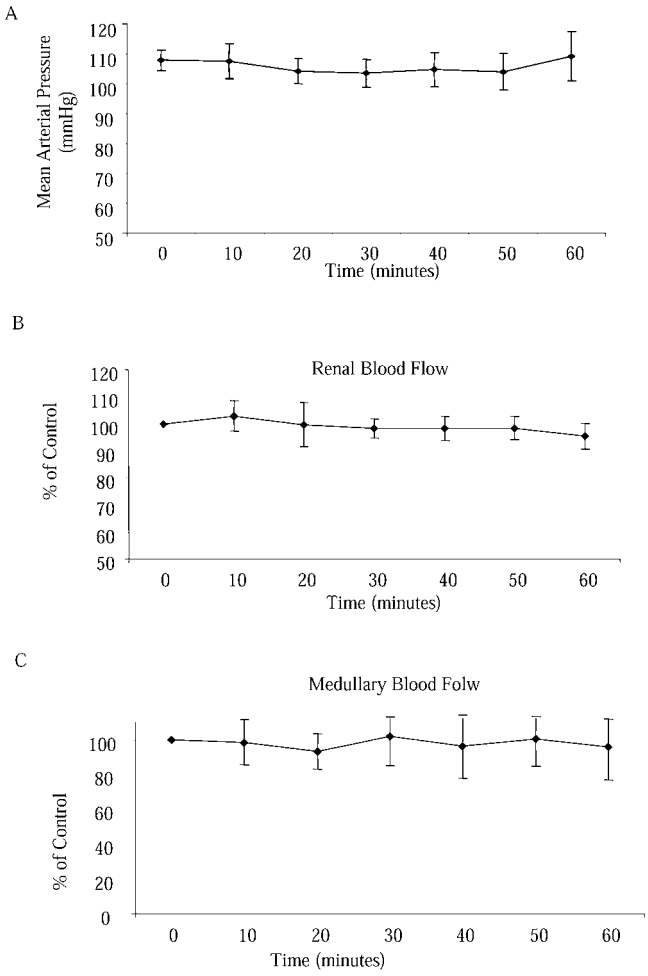

Figure 4. Renal blood flow (A) and medullary blood flow (B) and mean arterial pressure (C) did not significantly change after intramedullary infusion of A192621. Experimental protocols were the same as described in Figure 1. $n = 5$ in each group.

inner zone plus papilla) (16). This technique has been successfully employed to study influence of medullary blood flow on sodium balance and blood pressure regulation (17–19). It is considered that because of the efficient countercurrent exchanger in the vasa recta circulation, the substances infused to this region accumulated selectively in the medullary interstitial space. In the present study, intramedullary infusion of A192621 induced significant reductions of urine flow and salt excretion but did not significantly affect blood pressure or RBF, suggesting local action of the compound.

Our data reveal unparalleled changes in urine flow rate and urinary salt excretion, with the reduction of the former being more rapid than that of the latter after intramedullary infusion of A192621. This observation suggests that ETB receptor may play a primary role in regulation of water excretion rather than sodium excretion. This notion is further supported by the observation that intramedullary infusion of A192621 induced a significant increase in urinary osmolality. There is a large body of evidence supporting a role of renal medullary ETB receptor in regulation of water transport in the distal nephron. In this regard, ET-1 reduces vasopressin-stimulated osmotic water permeability in isolated perfused rat IMCD via ETB receptor (20, 21). In cultured IMCD cells, ETB receptor mediates the inhibitory effect of ET-1 and -3 on vasopressin-dependent accumulation of cAMP and release of PGE2 (22). It is unclear, however, whether ETs, via ETB receptor, regulate sodium transport in IMCD. It is reported that ET-1 inhibits Na^+/K^+ ATPase activity in cultured IMCD cells, but it is uncertain whether this phenomenon occurs *in vivo* (11).

In addition to the direct effects on tubular transport in the distal nephron, activation of ETB receptor in the renal medulla may indirectly affect tubular transport by an influence on renal medullary hemodynamics. There is evidence that ETB receptor is found in renal vascular endothelial cells (23) as well as in renal medullary interstitial cells (24) in addition to tubular cells (25, 26). Systemic blockade of ETB receptor reduces MBF associated with antidiuresis and antinatriuresis (3). Additionally, ETB receptor is coupled with nitric oxide and/or prostaglandins, both of which play an important role in regulation of microcirculation in the renal medulla (12, 15, 27–30). It is unclear why we were unable to detect changes in MBF after intramedullary infusion of A192621. Single-fiber laser-Doppler flowmetry is a nonquantitative and rather insensitive technique for blood flow measurement. It is possible that the modest changes in MBF after infusion of A192621 may be below the detection limit of the technique used. Another explanation is that the antidiuresis and antinatriuresis induced by A192621 might be the consequence of changes of tubular transport but not of local hemodynamics.

Is it possible that the antidiuresis and natriuresis induced by intramedullary infusion of A192621 are caused by increased circulating ET-1 levels? This possibility is very unlikely for the following reasons. Despite the increased circulating ET-1 levels after systemic blockade of ETB receptor, urinary ET-1 excretion, usually used as an index of intrarenal ET-1 production, is not affected (1). Further evidence suggests that kidney does not clear ET-1 from the circulation (6). In line with these observations, we found that intramedullary infusion of A192621 did not significantly affect blood pressure or RBF.

The present study employed the intramedullary infusion technique to manipulate ETB receptor activity selectively in the renal medulla without an obvious effect on systemic hemodynamics. Our results for the first time provide a direct evidence for a role of renal medullary ETB receptor in promoting water and salt excretion in a saline-induced volume expansion state. We further observe that ETB receptor blockade in the renal medulla induces an immediate reduction of urine flow that precedes the reduction of salt excretion, suggesting a dissociation of water and salt regulations by ETB receptor. The mechanism of action of ETB receptor in the renal medulla is not known, but our data

favor direct effects on tubular transport. It is important to note that our results support an important role of renal medullary ETB receptor in renal handing of salt and water but do not exclude the potential contribution of ETB receptor from systemic vascular endothelium.

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