Functional Role of ET$_B$ Receptors in the Renal Medulla

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Abstract—Experiments were conducted to determine the influence of ET$_B$ receptors in the control of renal medullary function. The acute relation between renal perfusion pressure (RPP) and natriuresis was examined in anesthetized rats treated with the ET$_B$ antagonist A-192621 (10 mg/kg IV). In A-192621–treated rats, sodium excretion (UNaV) was 0.4±0.1, 0.6±0.3, and 2.7±0.5 μmol/min at RPP of 80±1, 107±1, and 144±5 mm Hg, respectively. In control rats, UNaV averaged 0.8±0.4, 3.4±1.2, and 8.1±1.7 μmol/min at RPP of 77±2, 115±5, and 137±3 mm Hg, respectively. For normal and high RPP, UNaV was significantly lower in A-192621–treated rats compared with control rats. Additional experiments determined the effects of Big ET-1 (10 pmol/kg per minute) on intrarenal blood flow. Medullary blood flow (MBF) and cortical blood flow were measured in anesthetized rats by single-fiber, laser Doppler flowmetry. Cortical blood flow significantly decreased in response to Big ET-1 in rats on a normal or high salt diet. Big ET-1 significantly increased MBF in rats on a high salt diet, whereas there was no change in MBF in rats on a normal salt diet. These results demonstrate that medullary vasodilation produced by Big ET-1 is more prominent in rats on a high salt diet and are consistent with a contribution of ET$_B$-mediated events in the natriuretic response to high salt intake. Taken together, these findings support the hypothesis that endothelin plays an important role in regulating sodium excretion through activation of ET$_B$ receptors. (Hypertension. 2003;41:1359-1363.)

Key Words: endothelin • receptors, endothelin • natriuresis • blood flow

Cowley, Roman, and colleagues have provided much of the evidence demonstrating that changes in blood flow and renal tubular reabsorption within the renal medulla contribute significantly to the relation between blood pressure and changes in sodium and water excretion. The renal medulla is a major site of endothelin production and is the location with the highest concentration of endothelin B (ET$_B$) receptors. The ET$_B$ receptor has several distinct functions on both intrarenal hemodynamics and tubular reabsorption within the renal medulla that may play a role in arterial pressure regulation. Hoffman and colleagues recently observed that the diuretic and natriuretic responses to the ET-1 precursor Big ET-1 can be inhibited by an ET$_B$ receptor antagonist. These investigators have also reported that Big ET-1 vasodilates the renal medullary circulation despite cortical and systemic vasoconstriction. In addition, several studies have provided evidence that renal tubular ET$_B$ receptors serve to inhibit reabsorption of sodium in the thick ascending limb and collecting duct.

Our laboratory recently completed a study examining the effects of salt intake on the response to chronic ET$_B$ receptor blockade. The chronic hypertension produced by ET$_B$ receptor blockade was exaggerated in animals on a high salt diet. The high concentration of ET$_B$ receptors in the medulla and their described effects on the medullary circulation and on sodium and water reabsorption led us to hypothesize that the ET$_B$ receptors may play a role in the pressure natriuresis and diuresis phenomenon. Studies from our laboratory have also shown that ET$_B$ receptors are upregulated in the kidneys of DOCA-salt hypertensive rats and that urinary ET-1 excretion is significantly increased in rats on a high salt diet. Taken together, these observations have led us to propose that ET$_B$-mediated vasodilation may be an important component of the functional response to increased dietary salt intake.

Experiments in the present study were conducted to test the hypothesis that ET$_B$ receptor function is necessary to maintain the normal pressure-natriuresis relation. Acute pressure natriuresis curves were generated before and after receptor ET$_B$ blockade in anesthetized rats. Experiments were also conducted to determine the ability of Big ET-1 to produce medullary vasodilation during conditions of high salt intake.

Methods

Pressure-Natriuresis Studies

The effect of ET$_B$ receptor blockade on the relation between acute changes in renal perfusion pressure and urinary sodium and water excretion was determined in male Sprague-Dawley rats (220 to 250 g, Harlan Laboratories). Rats were anesthetized with Inactin (50 mg/kg IP) and surgically prepared for renal clearance measurements as previously described. Ligatures were placed around the upper mesentery and the celiac arteries and the aorta just proximal and distal to the renal arteries to control renal perfusion pressure. Glomerular filtration rate (GFR) was measured by intravenous infusion of saline containing [1H]inulin (4 μCi/h per 100 g; American Pharmacia Biotech).

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After surgical preparation, rats were given an intravenous bolus injection of either vehicle (saline) or the ET₄ antagonist A-192621 (10 mg/kg). A-192621 is selective for the ET₄ receptor (IC₅₀=8.2 µmol/L for ET₄, and 6.4 µmol/L for ET₃), with a half-life of >6 hours.¹⁰ Fifteen minutes later, renal perfusion pressure was set sequentially at low (~80 mm Hg), normal (~110 mm Hg), and high (>130 mm Hg) levels and was maintained at each level for 30 minutes. Urine was collected during the final 20 minutes of each period.

Renal Blood Flow Autoregulation Studies

Animals were prepared as described above except that renal blood flow (RBF) was measured with an ultrasonic probe placed on the renal artery. Renal perfusion pressure was controlled by an adjustable constricting ligature placed on the abdominal aorta. Renal perfusion pressure was reduced in increments of 10 mm Hg for 1 to 2 minutes starting at spontaneous arterial pressure down to 70 mm Hg. After generating 2 to 3 control autoregulatory curves, rats were given A-192621 and 15 minutes later, a series of 2 to 3 autoregulatory curves were again generated.

Intrarenal Blood Flow Studies

A final series of experiments were conducted to determine whether a high salt diet would influence the renal hemodynamic response to Big ET-1 infusion. Sprague-Dawley rats were maintained on either a normal sodium (0.8% NaCl) or high sodium (10% NaCl) diet for 1 week. Rats were then anesthetized and surgically prepared as described above. Medullary blood flow and cortical blood flow (MBF and CBF, respectively) were measured by single-fiber, laser Doppler flowmetry, based on the technique of Mattson et al.¹¹ After a 30-minute period of recovery from surgery, a 30-minute control period was obtained. Rats were then given an intravenous bolus of either saline or A-192621. Fifteen minutes after the antagonist was given, Big ET-1 was infused for 1 hour at a dose 10 pmol/kg per minute (20 µL/min). Control groups received vehicle (0.9% NaCl). The function of the laser Doppler fibers was verified at the beginning of the experiment by recording the changes of the regional blood flow in response to compression of the aorta above the renal arteries. Positioning of the fibers was verified at the end of the experiments by dissection.

Analytical and Statistical Analysis

Urine sodium concentrations were measured with the use of ion-selective electrodes (EL-ISE; Beckman Instruments). ANOVA or ANOVA for repeated measures was used along with means comparison contrasts to determine differences between individual means among groups (SuperANOVA, Abacus Concepts).

Results

Pressure-Natriuresis Studies

Adjusting renal perfusion pressure to 77±2, 115±5, and 137±3 mm Hg in untreated, control rats increased urine flow from 11.3±2.8 to 23.2±5.2 to 52.8±8.7 µL/min, respectively (Figure 1). In rats treated with the ET₄ antagonist, A-192621, the pressure-diuresis relation was blunted, with renal perfusion pressures of 80±1, 107±1, and 144±5 mm Hg associated with urine flow rates of 9.4±1.3, 12.7±2.2, and 28.7±4.6 µL/min, respectively. For a given target range of arterial pressure, urine flow rate after antagonist treatment was significantly lower only at the highest renal perfusion pressure.

Sodium excretion in control rats averaged 0.8±0.4, 3.4±1.2, and 8.1±1.7 µmol/min at the low, normal, and high renal perfusion pressures, respectively (Figure 1). In A-192621-treated rats, the pressure-natriuresis curve was shifted to the right and the slope of the curve was decreased when compared with the curve for the vehicle-treated rats. Sodium excretion was 0.4±0.1, 0.6±0.3, and 2.7±0.5 µmol/min at the low, normal, and high renal perfusion pressures, respectively, in A-192621–treated rats. For the 2 higher levels of renal perfusion pressure, sodium excretion was significantly lower in rats treated with A-192621 compared with control rats.

For the high renal perfusion pressure, GFR was not significantly different between control and A-192621–treated rats (Figure 1). However, at the low and normal target range of pressures, GFR was significantly lower in A-192621–treated rats compared with control rats: 1.0±0.23 versus 2.0±0.13 mL/min at the low pressure and 1.6±0.26 versus 2.7±0.42 mL/min at the normal pressure range.

RBF Autoregulation

Whole-kidney RBF autoregulatory responses were determined before and after treatment with the ET₄ antagonist A-192621 (Figure 2). Spontaneous arterial pressure averaged 106.8±3 mm Hg during the baseline period and was significantly increased after administration of A-192621 (120.6±4 mm Hg, P<0.05). As renal perfusion pressure was decreased sequentially from the spontaneous pressure to 70 mm Hg in 10 mm Hg decrements, RBF was maintained fairly steady during the control period. After treatment with the ET₄ antagonist, baseline RBF was significantly reduced; however, RBF autoregulatory capability appeared to be maintained efficiently.

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Figure 1. Urine flow rate, sodium excretion, and GFR at low, normal, and high renal perfusion pressures in rats pretreated with ET₄ antagonist A-192621 (10 mg/kg IV) or vehicle (1 mL/kg). *P<0.05 vs vehicle-treated rats. Vehicle-treated group, n=9; group pretreated with ET₄ antagonist A-192621, n=11. Number of the rats in which GFR data were obtained was 5 and 6 for vehicle and A-192621 groups, respectively.
Intrarenal Blood Flow Studies

Intravenous infusion of Big ET-1 significantly increased mean arterial pressure to a similar extent in rats on a high salt diet compared with those on a normal salt diet (Figure 3A). Administration of the ET$_B$ receptor antagonist A-192621 produced a significant increase in the magnitude of the response to Big ET-1. This increase was not influenced by increased salt intake (Figure 3B). Within the kidney, Big ET-1 decreased CBF to a similar extent in rats on normal and high salt diets (Figure 4A). Prior administration of A-192621 exaggerated the effects of Big ET-1 on CBF. These changes again were not influenced by dietary salt. In contrast, changes in MBF were highly dependent on dietary salt levels (Figure 4B). Big ET-1 had no effect on MBF in rats on a normal salt diet with or without ET$_B$ receptor blockade. However, in rats on a high salt diet, Big ET-1 produced a significant increase in MBF that was completely blocked by ET$_B$ receptor blockade. In fact, MBF significantly decreased after Big ET-1 infusion in rats on a high salt diet given the ET$_B$ receptor antagonist. In separate groups of time control experiments, an identical protocol was used but without Big ET-1 infusion (Figure 5). A-192621 produced a significant decrease in both CBF and MBF in rats on a normal salt diet. These results are consistent with changes in total RBF, as observed in autoregulation experiments. No changes in either CBF or MBF were observed in rats given only saline over the time course of these experiments.

Discussion

Results from the current study provide several lines of evidence to support the hypothesis that ET$_B$ receptors play an important role in the control of renal medullary function. We observed that the acute loss of ET$_B$ receptor function severely blunts the normal relation between renal perfusion pressure and sodium and water excretion. In addition, we observed that Big ET-1 at 10 pmol/kg per minute significantly increased MBF in rats on a high salt diet but not in rats on a normal salt diet. This finding is consistent with the idea that the functional response to a high salt diet includes increased capacity for ET$_B$-induced medullary vasodilation. The current study extends the previous work from our laboratory demonstrating that long-term ET$_B$ receptor blockade results in hypertension that is exacerbated by high salt intake. Furthermore, we have observed that ET$_B$ receptors within the kidney are upregulated in a model of high salt hypertension, the DOCA-salt–treated rat. This model is characterized by elevated endothelin synthesis and ET$_A$-dependent hypertension. Together, these studies provide evidence that endothelin may participate in the long-term adaptation of the pressure-natriuresis relation and suggest that endothelin plays a role in regulating arterial pressure during conditions of high salt intake. Further support for this hypothesis is demonstrated by the observation that renal ET-1 production, as assessed by urinary ET-1 excretion, was increased during high salt intake. The current study extends these previous studies to suggest the mechanism for endothelin control of pressure natriuresis is through ET$_A$-dependent vasodilation.

The ET$_B$ receptor is known to function in a variety of ways. Many of the effects of ET$_B$ receptor blockade can be explained by increased ET-1 survival and subsequent ET$_A$ receptor activation because of the reported clearance function of the ET$_B$ receptor. However, there is considerable evidence that ET$_B$ receptors located on renal tubular epithelium inhibit sodium and water reabsorption, whereas those located on vascular endothelium mediate vasodilation. Both of these mechanisms appear to account for the natriuretic and diuretic actions attributed to ET-1 and the ET$_B$ receptor. Although we cannot exclude the possibility that the effects of ET$_B$ blockade on pressure natriuresis can be explained by increased ET$_A$ receptor function, the change in Big ET-1–mediated changes in MBF are consistent with a role for ET$_A$-mediated vasodilation in the response to changes in renal sodium excretion. It is also important to note that whereas ET$_B$ receptor blockade increases circulating ET-1 levels, urinary ET-1 excretion, often used as an index of intrarenal ET-1 production, increases with high salt intake but does not change during ET$_B$ receptor blockade. Furthermore, the kidney does not clear ET-1 from the circulation. These findings again argue that the vasodilatory and tubular actions of ET$_B$ receptors are responsible for the changes in the renal pressure-natriuresis relation during ET$_B$ receptor blockade.
It is important to note that all of the effects of Big ET-1 require conversion to ET-1 because the renal vasoconstrictor, diuretic, and natriuretic effects of Big ET-1 are blocked by the endothelin-converting enzyme phosphoramidon. In the present study, we used Big ET-1 because we have previously observed that Big ET-1 produces a larger natriuretic and diuretic effect compared with ET-1 at doses that produce similar increases in arterial pressure. This is probably related to limited conversion of Big ET-1 to ET-1 in the cortical circulation and the larger decreases in GFR produced by ET-1. We have also previously observed that ETA receptor blockade will completely block the pressor response to Big ET-1, yet the diuretic actions are unaffected. These observations are consistent with the ETb receptor mediating the diuretic actions of ET-1.

One of the main findings of our study is that ETb receptor blockade shifts the pressure diuresis and natriuresis curves to the right, so that higher renal perfusion pressures are needed for the same amount of sodium to be excreted. To assess whether changes in GFR could account for the change in the pressure-natriuresis relation, we determined the effect of ETB blockade on GFR when renal perfusion pressure was maintained at 3 different levels. For the low and normal renal perfusion pressures, we found that ETb blockade significantly decreased GFR but only at the 2 lower pressures. This result may be due to vasoconstriction associated with ETb blockade. That is, reduced ETb-dependent vasodilation and reduced clearance of ET-1, resulting in increased ETA-dependent vasoconstriction. Therefore, reduced GFR could account for some but not all of the effect of ETb blockade on the acute pressure-natriuresis relation. Despite reducing baseline renal blood flow, the results also indicated that ETA blockade did not impede the ability of the kidney to autoregulate RBF. These hemodynamic results coupled with reduced pressure natriuresis during ETb receptor blockade are consistent with a role for ETb receptors in modulating renal medullary function. As discussed, our results suggest that changes in the pressure-natriuresis and diuresis relation could be attributed to vasoconstrictory and/or tubular effects of ETb receptors. Nonetheless, these results alone are not the definitive link between ETb receptors and control of medullary function, since they could be explained simply on the basis of increased ETA receptor activation through reduced ET-1 clearance.

Using a moderately low dose of Big ET-1, we observed significant decreases in renal CBF with little change in MBF.

Figure 4. Changes in CBF (A) and MBF (B) in response to A-192621 (10 mg/kg IV) and Big ET-1 (10 pmol/kg per minute IV), n=5 to 8 in each group. *P<0.05 vs baseline before Big ET-1; †P<0.05 vs rats treated with A-192621 on similar salt diet.

Figure 5. Changes in CBF (A) and MBF (B) in response to A-192621 (10 mg/kg IV) or saline (0.9% NaCl). n=5 in each group. *P<0.05 vs initial flow; †P<0.05 vs rats treated with A-192621 on similar salt diet.
discern a vasodilatory effect that was blocked by the ETB antagonist. These results indicate that the vasodilatory capability of Big ET-1 is enhanced by a high salt diet and provide more compelling evidence that ETB function is related to salt intake. It has been previously established that Big ET-1 can produce ETB-dependent medullary vasodilation and that the diuretic and natriuretic effects of Big ET-1 are also ETB-dependent.3,4 We can therefore hypothesize that medullary ETB receptors function to enhance the excretion of salt and water under conditions of high salt intake through vasodilation within the renal medulla.

**Perspectives**

Our results support an important role for endothelin and the ETB receptor in the regulation of arterial pressure through the participation in pressure natriuresis and diuresis events. We further conclude that control of MBF is an important mechanism for this regulatory activity. Specifically, we propose that ETB receptors facilitate pressure natriuresis through increases in MBF. These actions work in concert with direct effects of ETB receptor activation on tubular reabsorption. Nonetheless, the complex nature of ETB receptor-mediated actions on both tubular and vascular sites will require further investigation into the mechanism of how ETB receptor activation controls salt handling within the kidney.

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