Cooperative role of ET$_\text{A}$ and ET$_\text{B}$ receptors in mediating the diuretic response to intramedullary hyperosmotic NaCl infusion

Erika I. Boesen and David M. Pollock
Vascular Biology Center, Medical College of Georgia, Augusta, Georgia

Submitted 11 January 2010; accepted in final form 10 September 2010

Boesen EI, Pollock DM. Cooperative role of ET$_\text{A}$ and ET$_\text{B}$ receptors in mediating the diuretic response to intramedullary hyperosmotic NaCl infusion. Am J Physiol Renal Physiol 299: F1424–F1432, 2010. First published September 15, 2010; doi:10.1152/ajprenal.00015.2010.—Acute intramedullary infusion of hyperosmotic NaCl, used to simulate a high-salt diet-induced increase of medullary osmolality, increases urine production and endothelin release from the kidney. To determine whether endothelin mediates this diuretic and natriuretic response, urine flow and Na$^+$ excretion rate were measured during acute intramedullary infusion of hyperosmotic NaCl in anesthetized rats, with or without endothelin receptor antagonism. Isomotic NaCl was infused into the left renal medulla during an equilibration period and 30-min baseline period, followed by hyperosmotic NaCl for two additional 30-min periods. Hyperosmotic NaCl infusion significantly increased urine flow of vehicle-treated rats (from 5.9 ± 0.9 to 11.1 ± 1.8 µl/min). Systemic ETB receptor blockade enhanced this effect (A-192621; from 7.7 ± 1.1 to 18.7 ± 2.9 µl/min; $P < 0.05$), ETA receptor blockade (ABT-627) had no significant effect alone, but the diuresis was markedly attenuated by combined ABT-627 and A-192621 administration (from 4.4 ± 0.7 to 5.4 ± 0.9 µl/min). Mean arterial pressures overall were not significantly different between groups. Surprisingly, the natriuretic response to hyperosmotic NaCl infusion was not significantly altered by systemic endothelin receptor blockade, and furthermore, intramedullary ETB receptor blockade enhanced the diuretic and natriuretic response to hyperosmotic NaCl infusion. ETA receptor blockade significantly attenuated both the diuretic and natriuretic responses to hyperosmotic NaCl infusion in ETB receptor-deficient sl/sl rats. These results demonstrate an important role of endothelin in mediating diuretic responses to intramedullary infusion of hyperosmotic NaCl. Moreover, these data suggest ET$_\text{A}$ and ET$_\text{B}$ receptors are both required for the full diuretic and natriuretic actions of endothelin.

THE RENAL ENDOTHELIN SYSTEM is important for control of salt and water excretion, and in particular for maintenance of normal blood pressure during high-salt intake. Urinary endothelin concentration, which reflects renal endothelin production (2), is increased by high-salt intake in rodents (3, 26) and correlates with sodium excretion in humans (17). In the healthy kidney, endothelin is thought to act as a diuretic and natriuretic agent, via inhibition of tubular Na$^+$ and Cl$^-$ transport (6, 8, 11, 25, 37), inhibition of vasopressin-induced water reabsorption by the collecting duct (21, 24, 30, 31), and possibly via ETB receptor-mediated increases in medullary blood flow (15, 32), which is proposed to have a pronatriuretic effect (7). The majority of the diuretic and natriuretic effects of endothelin appear to be mediated by the ETB receptor, and impairment of ETB receptor function at the whole animal level (10, 26) or specifically in the collecting duct (12) causes salt-sensitive hypertension. Together, these data suggest that high-salt intake triggers an increase in renal endothelin production, which in turn acts on ETB receptors to produce a natriuretic and diuretic response, thereby facilitating excretion of excess sodium. However, work from Kohan’s laboratory (13) suggests that both ET$_\text{A}$ and ET$_\text{B}$ receptors within the collecting duct operate in a possible synergistic manner to facilitate excretion of salt and water. These findings have been supported by more recent results from our own laboratory demonstrating that both ET$_\text{A}$ and ET$_\text{B}$ receptors contribute to the natriuretic response to intramedullary infusion of ET-1 in female rats (23).

The mechanism by which high-salt intake increases renal ET-1 production is still under investigation, but one possible signal is a change in medullary osmolality and/or associated increases in tubular flow rate. Outer medullary osmolality and tubular flow rate are increased by high-salt intake (16), and increases of culture media osmolality have been shown to stimulate endothelin release from medullary thick limb and collecting duct cells in some studies (16, 36) but not others (20). We previously demonstrated that exposing the renal medulla of rats to a hyperosmotic NaCl solution enhanced endothelin release in vivo (increased urinary endothelin excretion) (5). This enhanced endothelin release was accompanied by natriuresis and diuresis (5), consistent with the hypothesis that high-salt intake increases medullary osmolality, stimulating an increase in endothelin production and release, which facilitates excretion of the salt load. However, it has been suggested that increased luminal flow might act as a stimulus for endothelin release from collecting duct cells via a primary cilium Ca$^{2+}$-dependent mechanism (27). It therefore remains to be determined whether the endothelin released in response to intramedullary hyperosmotic NaCl infusion plays an active role in mediating the diuretic and natriuretic response or merely occurs secondary to increased luminal flow generated by some other mechanism.

The current study therefore sought to determine whether endothelin actively contributes to the diuretic and natriuretic response to intramedullary hyperosmotic NaCl infusion, and to ascertain which endothelin receptor subtype is involved in mediating the response.

METHODS

General procedures. Male Sprague-Dawley rats (n = 6–11 per group; Harlan Laboratories, Indianapolis, IN) and wild-type (WT) transgenic and ETB receptor-deficient (sl/sl) rats (n = 7–10, bred in-house) were used in these experiments (10). All protocols were in accordance with the Guide for the Care and Use of Laboratory Animals and were approved in advance by the Medical College of Georgia Institutional Animal Care and Use Committee. The surgical procedures were similar to our previous study (5). Rats were fasted overnight and then anesthetized (Sigma, St. Louis, MO; 100 mg/kg ip

Address for reprint requests and other correspondence: E. I. Boesen, Vascular Biology Center, Medical College of Georgia, 1459 Laney Walker Blvd., Augusta, GA 30912 (e-mail: eboesen@mcg.edu).
thiobutabarbitalone), placed on a heating table to maintain body temperature at 37°C throughout the experiment, and a tracheotomy was performed to facilitate breathing. A catheter was inserted into the jugular vein and 6.2% bovine serum albumin in phosphate-buffered NaCl was infused at 6 ml/h to 1.25% of the rat's body weight, followed thereafter by 0.9% NaCl at 1.5 ml/h to replace fluids lost and maintain euoeuolemia. Mean arterial pressure was measured via a catheter inserted into a femoral artery, with data recorded using a PowerLab data acquisition system. A midline incision was made and a stretched PE10 catheter was inserted 5 mm into the left kidney. Positioning of the catheter tip at the outer-inner medullary junction was confirmed at the end of each experiment by dissection. Once the catheter was in place, 0.9% (isosmotic) NaCl was infused directly into the renal medulla (0.5 ml/h). Urine was collected separately from the left (infused) and right (untouched) kidneys via catheters placed in each ureter. We found previously (5) that the effects of renal medullary interstitial infusion of hypertonic substances are largely confined to the kidney receiving the infusion, with very little to no effect on urine flow or sodium excretion observed in the contralateral kidney. Isosmotic (154 mM, or 284 mosmol/kgH2O) NaCl was infused into the renal medullary interstitium during the 1-h postsurgery stabilization period and 30-min baseline urine collection period. This was followed by two further 30-min urine collection periods during which rats either continued to receive isosmotic NaCl or the infusion was switched to hyperosmotic NaCl (0.9 M, or 1,669 mosmol/kgH2O) measured by freezing point depression). Urine samples were snap-frozen in liquid nitrogen and stored at −80°C until analysis.

Role of endothelin in response to hyperosmotic NaCl. Male Sprague-Dawley rats were randomized to one of four systemic treatment groups, with drugs being administered as an intravenous bolus (0.5 ml/kg via jugular vein catheter) 30 min before the end of the 1-h postsurgery stabilization period: 1) vehicle, 2) the selective ET\textsubscript{B} receptor antagonist A-192621 (10 mg/kg), 3) the selective ET\textsubscript{A} antagonist ABT-627 (5 mg/kg), or 4) A-192621 plus ABT-627 (both kindly provided by Abbott Laboratories). These doses are as high or higher than those administered previously in our laboratory (32) and by others (4). Urine was collected for 30-min periods at baseline (intramedullary infusion of isosmotic NaCl in all rats) and for two subsequent 30-min periods (isosmotic or hyperosmotic NaCl). At the completion of the experiment, a 0.3-mmol/kg bolus of ET-1 (American Peptide, Sunnyvale, CA) was given intravenously and the maximum decrease and increase in blood pressure were measured to verify appropriate receptor blockade. A-192621 alone or in combination with ABT-627 completely abolished the transient depressor response to ET-1 at 0.3 nmol/kg, which was on average −42 ± 2 mmHg in vehicle-treated rats. ABT-627 alone had no significant effect on the transient depressor response (−37 ± 2 mmHg), but when given alone or in combination with A-192621 significantly (P < 0.05) reduced the pressor response (to +11 ± 1 and +6 ± 1 mmHg, respectively) compared with the response in vehicle-treated rats (+18 ± 1 mmHg). A-192621 significantly enhanced the pressor response to ET-1 (to +44 ± 3 mmHg, P < 0.05). These findings are consistent with the antagonist treatments providing significant blockade of the intended endothelin receptors, for which the antagonists each show >1,000-fold selectivity for their targeted endothelin receptor subtype over the other subtype (33).

To further test the role of the ET\textsubscript{B} receptor in mediating the response to intramedullary hyperosmotic NaCl infusion, additional groups of rats underwent intramedullary NaCl solution infusions as described above, with or without the selective ET\textsubscript{B} receptor antagonist BQ-788 (EMD Biosciences, La Jolla, CA) included in the intramedullary infusion at 7 nmol/min. This dose of BQ-788 was identical to that administered via the renal artery previously by Just and colleagues (18) who observed complete blockade of ET\textsubscript{B}-dependent effects.

In separate experiments, male ET\textsubscript{B} receptor-deficient sl/sl rats and WT littermates underwent identical procedures to those described above but were either treated with vehicle or ABT-627 30 min before the end of the 1-h equilibration period and received intramedullary infusion of hyperosmotic NaCl for the final two 30-min urine collection periods. In addition, an adjustable ligature was placed around the aorta proximal to the renal arteries, enabling renal perfusion pressure (estimated as femoral arterial pressure) to be held constant at normotensive levels in all four groups. This measure was taken to preclude differences in arterial pressure confounding interpretation of results in ET\textsubscript{B}-deficient sl/sl rats, which display substantial reductions in blood pressure following ET\textsubscript{A} receptor blockade.

Role of endothelin in response to hyperosmotic mannitol. To further investigate whether endothelin plays a role in mediating the diuretic response to increased medullary osmolality, separate groups of male Sprague-Dawley rats received intramedullary infusions of isosmotic (0.3 M, or 297 mosmol/kgH2O) and hyperosmotic (1.6 M, or 1,496 mosmol/kgH2O) mannitol solutions with and without pretreatment with ABT-627 plus A-192621 for the same time periods as described above.

Assays. Osmolality of infused solutions was determined by freezing point depression (μOsmette model 5004 Automatic Osmometer, Precision Systems, Natick, MA). Urine electrolyte concentrations were determined by ion-sensitive electrodes (Synchron EL-ISE, Beckman Instruments, Brea, CA). Urine flow was determined gravimetrically.

Statistical analysis. Data from the three 30-min collection periods were analyzed by repeated-measures ANOVA with (Bonferroni) post hoc contrasts used when P\textsubscript{group*time} < 0.05. All other data were analyzed by one-factor ANOVA. A two-tailed Dunnett post hoc test was used to compare maximal pressor responses to ET-1 between vehicle and endothelin receptor antagonist-treated groups. Values are presented as means ± SE, with P < 0.05 considered statistically significant.

RESULTS

Excretory response to hyperosmotic NaCl during endothelin receptor blockade. Intramedullary hyperosmotic NaCl infusion produced a significant increase in urine flow from the left (infused) kidney of Sprague-Dawley rats (P\textsubscript{time} < 0.001; Fig. 1A). Unexpectedly, pretreatment with the selective ET\textsubscript{B} receptor antagonist A-192621 significantly enhanced the diuretic response, whereas selective ET\textsubscript{A} receptor blockade with ABT-627 did not significantly alter the response compared with vehicle pretreatment. Pretreatment with both A-192621 and ABT-627 significantly attenuated the diuretic response to hyperosmotic NaCl infusion. There was no effect of hyperosmotic NaCl infusion on urine flow of the right (untouched) kidney (Fig. 1B).

The increase in Na\textsuperscript{+} excretion from the left kidney in response to hyperosmotic NaCl infusion followed a similar pattern as urine flow, i.e., increased in response to infusion, although there were no statistically significant differences between antagonist-treated groups and control (Fig. 1C). Left kidney urine osmolality was measured where sample volume allowed, yielding n = 4–7 per time point in each group. There was no significant change in urine osmolality over time (P\textsubscript{time} = 0.4), although osmolality was significantly lower in A-192621-treated rats throughout the experiment compared with the other three groups (P < 0.05; data not shown). The right, noninfused kidney displayed significant increases in Na\textsuperscript{+} excretion only in the vehicle- and A-192621-treated rats (P < 0.05; Fig. 1D), but these changes were very small compared with the effects observed on the left (infused) kidney (Fig. 1C). Overall, mean arterial pressures were not significantly different between the four groups (P\textsubscript{group} = 0.9), ranging between 96 ± 5 and 103 ±
5 mmHg (Fig. 1E). The only minor but statistically significant differences in mean arterial pressures were between A-192621-treated and the two other endothelin receptor antagonist-treated groups at certain time points (P < 0.05 by post hoc contrast; Fig. 1E); however, there was no difference in mean arterial pressure between these and the vehicle group at any time.

In rats receiving infusion of isosmotic NaCl into the medullary interstitium of the left kidney, urine flow from the left kidney did not change over time but was at slightly but significantly different levels between groups (Fig. 2A). Overall, there was a significant decrease in urine flow from the right kidney over time (P<0.05; Fig. 2B), but this effect was not significantly different between the four groups (Pgroup*time > 0.05). Although there was no overall difference in Na+ excretion by the left or right kidneys between the four groups (Pgroup > 0.2), there were some small but statistically significant differences between particular groups at specific time points as indicated in Fig. 2, C and D. However, it should be noted that Na+ excretion did not change significantly over time either overall (Ptime > 0.2), nor within any of the four groups by post hoc contrast. Mean arterial pressure decreased slightly but significantly over time to a similar degree in all four groups (Ptime < 0.001; Fig. 2E).

As an additional approach to determining whether ETB receptor blockade enhanced the diuretic and natriuretic response to hyperosmotic NaCl infusion, separate groups of rats received intramedullary infusions of NaCl with or without the selective ETB receptor antagonist BQ-788 being included in the infused solution. BQ-788 was included in the infusate commencing 30 min before the beginning of the baseline collection period. Similar to rats treated intravenously with the ETB receptor antagonist A-192621, intramedullary ETB receptor blockade with BQ-788 increased urine flow and also enhanced the natriuretic response to hyperosmotic NaCl infusion in the left kidney (Fig. 3, A and C). There was no difference in urine flow or Na+ excretion from the right kidney, or in mean arterial pressure between the two groups, although right kidney Na+ excretion and mean arterial pressure increased slightly but significantly over time in both groups (Fig. 3).

**Intramedullary NaCl in ETB receptor-deficient rats.** The results described above revealed a surprising effect of ETB receptor blockade to enhance the diuretic response to hyperosmotic NaCl infusion in ETB receptor-deficient rats. Further studies are needed to determine the underlying mechanisms of this effect.

---

Fig. 1. Effect of endothelin receptor blockade on response to intramedullary infusion of hyperosmotic NaCl into the left kidney (LK) of anesthetized rats. Data shown are urine flow (V; A and B) and Na+ excretion rate (UNa+/V; C and D) for the LK (A and C) and right kidney (RK; B and D) and mean arterial pressure (MAP; E) measured during sequential 30-min periods in rats receiving intramedullary infusions of isosmotic (284 mosmol/kgH2O) NaCl at baseline followed by infusion of NaCl at 1,669 mosmol/kgH2O (n = 8, 7, 7, and 6 for vehicle, A-192621, ABT-627, and A-192621 + ABT-627 pretreatment groups, respectively). Repeated-measures ANOVA was used to test whether responses were affected by group (Pgroup), or time (Ptime), and whether responses differed between groups in a time-dependent manner (Pgroup*time). NS, not statistically significant. Post hoc contrasts: *P < 0.05 vs. vehicle for same time point; †P < 0.05 vs. A-192621 group at specified time point; ‡P < 0.05 vs. baseline in same group; §P < 0.05 vs. previous time point in same group.
osmotic NaCl infusion. In addition, these results suggest that rather than ET\textsubscript{B} receptors alone mediating the diuretic and natriuretic actions of endothelin in male rats, ET\textsubscript{A} and ET\textsubscript{B} receptors cooperate to facilitate salt and water excretion. To further examine the role of the ET\textsubscript{B} receptor and to test whether ET\textsubscript{A} receptors can indeed mediate diuretic and natriuretic responses, the effect of ETA receptor blockade on the response to intramedullary infusion of hyperosmotic NaCl was examined in WT and ET\textsubscript{B} receptor-deficient sl/sl rats. The ET\textsubscript{B} receptor-deficient sl/sl rat lacks functional ET\textsubscript{B} receptor expression other than that provided by a transgene containing a functional ET\textsubscript{B} receptor expressed under the control of the dopamine \(\beta\)-hydroxylase promoter and does not express detectable levels of ET\textsubscript{B} receptor mRNA in renal tubules or vasculature (10). Although the diuretic response to hyperosmotic NaCl infusion into the left kidney was not significantly affected by ABT-627 (\(P_{\text{group}} < 0.05\); Fig. 4A), urine flow from the right kidney was significantly lower in ABT-627-treated WT rats compared with vehicle-treated WT rats but urine flow did not change over time in either group (Fig. 4B). The right, noninfused kidney displayed significant increases in Na\textsuperscript{+} excretion only in the vehicle-treated rats, and only during the final 30-min period (Fig. 4D), but this was again a much smaller effect than that observed on the left kidney (Fig. 4C).

In contrast to pharmacological studies, ET\textsubscript{B} receptor-deficient rats did not display an exaggerated response to intramedullary infusion of hyperosmotic NaCl (Fig. 5, A and C). ETA receptor blockade in ET\textsubscript{B} receptor-deficient rats produced a reduction in urine flow from the left kidney across the three urine collection periods (\(P_{\text{group}} < 0.01\); Fig. 5A) and significantly attenuated the natriuretic response to hyperosmotic NaCl infusion (Fig. 5C). The effects of ABT-627 on the diuretic and natriuretic responses to intramedullary hyperosmotic NaCl infusion occurred independent of any change in renal perfusion pressure, which was maintained constant and equal between the two groups by means of the adjustable ligature placed around the aorta (Fig. 5E).
the experiment, both groups displayed small but statistically significant increases in urine flow but not Na\textsuperscript{+}/H\textsubscript{2}O\textsuperscript{+} excretion from the right kidney (Fig. 5, B and D).

Intramedullary hyperosmotic mannitol infusion. To further investigate the role of endothelin in mediating diuresis, urine flow and Na\textsuperscript{+} excretion were measured in Sprague-Dawley rats receiving intramedullary infusion of hyperosmotic mannitol with and without pretreatment with ABT-627 plus A-192621. When given alone, hyperosmotic mannitol produced a significant diuresis ($P_{\text{time}} < 0.001$; Fig. 6A), but not natriuresis (Fig. 6C). Both urine flow and Na\textsuperscript{+} excretion rate were significantly reduced by combined ET\textsubscript{A} and ET\textsubscript{B} receptor blockade ($P_{\text{group}} < 0.05$; Fig. 6) without inducing a significant difference in mean arterial pressure between groups ($P_{\text{group}} > 0.05$; Fig. 6E), indicating an important role for endothelin in mediating Na\textsuperscript{+} and water excretion.

DISCUSSION

The hypothesis that changes in medullary osmolality may act as a control mechanism for renal endothelin production has been studied both in vitro by others (16, 20, 36), and more recently, in vivo by our group (5). We previously demonstrated that intramedullary infusion of hyperosmotic NaCl increases...
urinary excretion of endothelin (5), suggesting that an increase in medullary osmolality, which has been reported to occur during high-salt intake (16), acts as a stimulus for endothelin release. The current study extends this finding, demonstrating that endothelin plays a critical role in the diuretic response induced by intramedullary hyperosmotic NaCl infusion.

The finding that endothelin participates in the diuretic response to hyperosmotic NaCl infusion, rather than urinary endothelin excretion merely increasing secondarily to increased urine flow, is not surprising given the reported pronatriuretic and -diuretic actions of this peptide. What was surprising is that ETB receptor blockade either systemically with A-192621 or locally with BQ-788 enhanced rather than attenuated the response to intramedullary hyperosmotic NaCl infusion. This finding is in contrast to the well-established role for ETB receptors functioning in a diuretic and natriuretic manner and so the mechanism for this effect is not evident. An alteration of reno-renal reflex sensitivity by ETB receptor blockade would not provide an explanation for this finding, since blocking ETB receptors in our study would be predicted to impair renal afferent nerve-induced inhibition of sympathetic outflow to the kidney (22). Moreover, urine flow from the right kidney did not change over the course of the exper-

Fig. 5. Effect of ETA receptor blockade on the response to intramedullary infusion of hyperosmotic NaCl in ETB receptor-deficient (sl/sl) rats. Data shown are V (A and B) and UNa/V (C and D) for the LK (A and C) and RK (B and D) and MAP (E) measured during sequential 30-min periods in rats receiving intramedullary infusions of isosmotic NaCl at baseline followed by infusion of hyperosmotic NaCl for 2 further 30-min periods; n = 7 per group. Statistical analyses are as per Fig. 1. Post hoc contrasts: *P < 0.05 vs. vehicle for same time point; †P < 0.05 vs. baseline in same group; ‡P < 0.05 vs. previous time point in same group.

Fig. 6. Effect of combined ETA and ETB receptor blockade on the response to intramedullary infusion of hyperosmotic mannitol into the LK of anesthetized rats. Data shown are V (A and B) and UNa/V (C and D) for the LK (A and C) and RK (B and D) and MAP (E) measured during sequential 30-min periods in rats receiving intramedullary infusions of isosmotic mannitol (297 mosmol/kgH2O) at baseline followed by infusion of hyperosmotic mannitol (1,496 mosmol/kgH2O) for 2 further 30-min periods; n = 10–11 per group. Statistical analyses are as per Fig. 1.
imment in either A-192621- or BQ-788-treated rats (Figs. 1B and 3B). Curiously, chronic lack of functional ETB receptors in sl/sl rats did not enhance the diuretic response to intramedullary hyperosmotic NaCl infusion. While on the one hand, this, too, is somewhat puzzling, the difference between pharmacological and genetic ablation of ETB receptors may be consistent with sl/sl rats having adapted to high levels of circulating ET-1 (10), whereas the acute blockade of ETB receptors in normal rats may produce an abrupt increase in ET-1 availability due to reduced binding to the ETB receptor (9), leading to enhanced activation of what appears to be prodiuretic ETA receptors. It is also possible that the smaller diuretic response to hyperosmotic NaCl infusion in the ETB-deficient rats compared with A-192621-treated Sprague-Dawley rats may be a consequence of the use of an adjustable clamp to equalize renal perfusion pressure between WT and ETB-deficient rats, thus shifting the ETB receptor-deficient rats down their pressure-natriuresis/diuresis curves.

Until recently (23), most data pointed toward anti-natriuretic and anti-diuretic actions of ETA receptors in the kidney. Activation of ETA receptors promotes renal vasoconstriction (18), and in the renal pelvis, ETA receptors suppress renal afferent sensory nerve activity, consequently promoting anti-natriuretic and anti-diuretic efferent sympathetic neural influences on the kidney (22). In addition, conditional knockout technology revealed that collecting duct ETA receptors enhance the anti-diuretic effect of vasopressin (14). In the current study, however, blockade of both ETA and ETB receptors clearly attenuated the diuretic response to hyperosmotic NaCl infusion in male Sprague-Dawley rats, and blockade of the ETA receptor in WT and ETB receptor-deficient rats also reduced urine flow independently of any effect on blood pressure. This effect of ABT-627 on urine flow did not reach statistical significance in Sprague-Dawley rats, possibly due to the analysis being underpowered to detect this effect. Together, these data indicate a role for the ETA receptor in promoting water excretion, a property that may become more obvious under conditions of abrogation of ETB receptor function, either by pharmacological or genetic means. Moreover, the diuretic properties of the ETA receptor run counter to the prevailing dogma that renal medullary ETB receptors are primarily responsible for the diuretic and natriuretic actions of endothelins while renal medullary ETA receptors do not promote Na\(^+\) and water excretion (1, 14, 19). Nonetheless, our findings across the different experimental groups suggest a cooperative role for the two endothelin receptors in control of urine flow, and perhaps of sodium excretion as well.

Recently, an ETA receptor nitric oxide synthase type 1 (NOS1)-dependent diuretic and natriuretic response to intramedullary ET-1 infusion was uncovered by our group in female but not male rats, with this response being particularly pronounced in female ETB receptor-deficient sl/sl rats (23). Our current data are consistent with ETA receptors also being capable of mediating the diuretic and natriuretic actions of endothelin in male rats, with ETB receptor blockade paradoxically enhancing diuresis and natriuresis during hyperosmotic NaCl infusion, and ETA receptor antagonist treatment producing marked effects on urine flow and Na\(^+\) excretion in ETB receptor-deficient rats. The apparent discrepancy in findings in male rats between the current study with intramedullary hyperosmotic NaCl infusion and the previous study with intramedullary infusion of ET-1 (23) may lie with the source of endothelin, i.e., endogenous vs. exogenous. It is likely that slightly different populations of endothelin receptors are being activated by the different experimental maneuvers used, uncovering a potential for ETB receptors in mediating natriuresis and diuresis in the current study, whereas this effect appeared to be counteracted by a reduction of renal medullary blood flow in the previous study with exogenous ET-1 infusion (23). Further experiments would be needed to discern whether changes in intrarenal hemodynamics may have contributed to the effects observed in our study.

Notably, ETA receptor antagonist treatment attenuated the natriuretic response to hyperosmotic NaCl infusion in ETB-deficient rats, whereas Na\(^+\) excretion was essentially unaffected by systemic ETA receptor or ETB receptor blockade in WT littermates and Sprague-Dawley rats. These observations suggest that factors other than endothelin are responsible for the bulk of the increase in Na\(^+\) excretion observed during hyperosmotic NaCl infusion. Indeed, if a large amount of paracellular Na\(^+\) flux occurred, this might obscure more modest effects of endothelin on Na\(^+\) excretion. We therefore examined whether hyperosmotic mannitol infusion elicited a natriuretic response and tested whether ETB and ETB receptor antagonist antagonism might affect this. We did not, however, observe natriuresis in response to hyperosmotic mannitol infusion. These results suggest that intramedullary infusion of hyperosmotic solutions stimulates diuresis more effectively than natriuresis. Furthermore, these data suggest that the increase in Na\(^+\) excretion observed during intramedullary infusion of hyperosmotic NaCl may arise via a process such as paracellular backleak of Na\(^+\) from the interstitium, rather than hyperosmolarity-induced stimulation of natriuresis per se. Together with the lack of significant effect of endothelin receptor blockade on the increased Na\(^+\) excretion associated with hyperosmotic NaCl infusion, these data would suggest that the natriuresis observed in response to hyperosmotic NaCl infusion is mediated by something other than pronatriuretic actions of hyperosmolarity-induced ET-1 release.

Curiously, the natriuretic response to hyperosmotic NaCl infusion was significantly blunted by ETA receptor blockade only in ETB receptor-deficient sl/sl rats, with urinary Na\(^+\) excretion rate of ABT-627-treated rats being less than half that of vehicle-treated rats (Fig. 6C). It is not clear whether the seemingly unique role for the ETA receptor in mediating the natriuretic response to hyperosmotic NaCl infusion in sl/sl rats represents ETA receptor-mediated compensation for the chronic loss of functional ETB receptors in sl/sl rats, or might be related to the previously identified existence of an ET-3 binding site unique to the inner medulla of sl/sl rats (29). This atypical ET-3 binding site was shown in competition binding assays to bind the ETA receptor antagonist A-127722, a racemic mixture of which ABT-627 is the (+)-enantiomer, with high affinity (29). However, this binding site does not appear to be present in either Sprague-Dawley or heterozygous sl/+ rats (29). Nothing is currently known regarding the identity or function of this novel ET-3 binding site. The mechanism by which ETA receptors promote diuresis and natriuresis and the cell types involved is not clear. It has been shown that ETA receptors stimulate NOS1 protein expression in inner medullary collecting duct (IMCD)-3 cells (28), and the ETA receptor-dependent diuretic and natriuretic
response to intramedullary ET-1 infusion in female rats can be blocked with a NOS1 inhibitor (23). In addition to what is thought to be a small population of ETA receptors on IMCD cells (34), ETA receptors are also present on renal medullary interstitial cells, which release prostaglandin E2 in response to ET-1 (35), potentially enhancing Na⁺ and water excretion. The relative roles of NO, prostaglandins, or other mediators and the specific cell types involved in the ETA receptor-mediated diuretic response await future investigation.

Our findings that blockade or absence of both ETA and ETB receptor subtypes was necessary to markedly attenuate the diuretic response to hyperosmotic NaCl infusion suggest that one endothelin receptor subtype is able to compensate for impairment of the other under acute conditions. In a way, these data echo the phenotypes of collecting duct-specific endothelin receptor knockout mice. Selective deletion of ETA receptors from the collecting duct has little effect on blood pressure or Na⁺ homeostasis.

interstitial cells, which release prostaglandin E2 in response to specific cell types involved in the ETA receptor-mediated response. These findings, together with recent findings of ETA receptors. These findings, together with recent findings of ETA and ETB receptors, however, provides a hypertensive phenotype similar to collecting duct-specific knockout of ET-1 itself (3, 13). Together, these studies support a cooperative role for ETA and ETB receptors in controlling blood pressure and salt and water homeostasis.

It was surprising that ETA and ETB receptor blockade did not significantly attenuate the natriuretic response to intramedullary infusion of hyperosmotic NaCl in Sprague-Dawley and WT rats given the large body of evidence implicating endothelin in the control of Na⁺ excretion. This suggests that the majority of the natriuretic response to intramedullary hyperosmotic NaCl infusion is due to factors other than the endothelin system, such as paracellular backleak. Indeed, factors other than the endothelin system may be called into play to help excrete the abrupt increase in intrarenal Na⁺ provided by intramedullary infusion of hyperosmotic NaCl, although at this point we have not yet investigated other mechanisms. Finally, it should be borne in mind that acute long-term exposure to a high-salt intake. Accordingly, the results of the current study do not negate previous studies by our group showing that the pronatriuretic and diuretic actions of endothelin are mediated by more than just the ETB receptor.

ACKNOWLEDGMENTS

The authors thank Dr. D. Kohan for invaluable scientific advice on this work.

GRANTS

This work was supported by a Post-Doctoral Fellowship and Beginning Grant-In-Aid from the American Heart Association (E. L. Boesen) and National Institutes of Health Grants HL-64776 and HL-74167 (D. M. Pollock).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


