Acute increases of renal medullary osmolality stimulate endothelin release from the kidney

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Boesen EI, Pollock DM. Acute increases of renal medullary osmolality stimulate endothelin release from the kidney. Am J Physiol Renal Physiol 292: F185–F191, 2007. First published August 15, 2006; doi:10.1152/ajprenal.00021.2006.—Experiments conducted in vitro suggest that high osmolality stimulates endothelin production and release by renal tubular epithelial cells. Whether hyperosmotic solutions exert similar effects in vivo is unknown. Therefore, we tested the hypothesis that increasing renal medullary osmolality enhances urinary excretion of endothelin in anesthetized rats. Isosmotic NaCl (284 mosmol/kgH2O) was infused either intravenously (1.5 ml/h) or into the renal medullary interstitium (0.5 ml/h) during a 1-h equilibration period and 30-min baseline urine collection period, followed by either isosmotic or hyperosmotic NaCl (921 or 1,664 mosmol/kgH2O iv; 1,714 mosmol/kgH2O into renal medulla) for two further 30-min periods. Compared with isosmotic NaCl, infusion of hyperosmotic NaCl into the renal medulla significantly increased the endothelin excretion rate (P < 0.05; from 0.30 ± 0.02 to 0.49 ± 0.03 fmol/min). Intravenous infusion of hyperosmotic NaCl also significantly increased endothelin excretion rate in a concentration-dependent manner (from 0.79 ± 0.07 to 1.77 ± 0.16 fmol/min and 0.59 ± 0.04 to 1.11 ± 0.08 fmol/min for 1,664 and 921 mosmol/kgH2O, respectively). To differentiate between effects of osmolality and NaCl, similar experiments were performed using mannitol solutions. Compared with isosmotic mannitol, medullary interstitial infusion of hyperosmotic mannitol (1,820 mosmol/kgH2O) significantly increased endothelin excretion rate (P < 0.05; from 0.54 ± 0.03 to 0.94 ± 0.12 fmol/min). Thus exposing the renal medulla to hyperosmotic concentrations of either NaCl or mannitol stimulates endothelin release in vivo, consistent with medullary osmolality being an important regulator of renal endothelin synthesis.

While such a dietary salt-mediated regulation of renal endothelin expression appears beneficial, the mechanism underlying this effect is unknown. One possibility is interstitial osmolality. High-NaCl intake has recently been reported to increase outer medullary osmolality in vivo (4). Studies conducted in vitro on freshly isolated or cultured renal tubular or collecting duct cells suggest that extracellular osmolality modulates endothelin production and release by these cells. For example, raising the osmolality of culture media with NaCl from isosmotic to 400–690 mosmol/kgH2O increased ET-1 release from rat medullary thick ascending limbs (mTAL) (4), and rat, rabbit, or porcine inner medullary collecting duct (IMCD) cells (10, 19). However, other studies have shown opposing results, with increased osmolality by NaCl inhibiting ET-1 release from rat IMCD (7) and Madin-Darby canine kidney cells (17). The reasons for these divergent results are not clear, and it is not known whether hyperosmotic NaCl solutions stimulate endothelin release in vivo. Therefore, we proposed acute intravenous and renal medullary interstitial infusions of hyperosmotic NaCl solutions enhance urinary excretion of endothelin in anesthetized rats. Furthermore, we investigated whether the stimulus to increase endothelin excretion was osmolality per se or NaCl specifically.

METHODS

Male Sprague-Dawley rats (Harlan Laboratories, Indianapolis, IN) were used in this study (n = 5–9 per group), which was approved in advance by the Medical College of Georgia Institutional Animal Care and Use Committee. In separate sets of experiments, rats received infusions either intravenously or directly into the renal medulla.

Surgical preparation: intravenous infusion. Once anesthetized (100 mg/kg ip thiothixate-barbital; Sigma, St. Louis, MO), rats were 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 by fluorescein isothiocyanate-inulin dissolved in phosphate-buffered NaCl (Sigma; 20 mg/ml at 0.6 ml/h). Mean arterial pressure was measured via a catheter inserted into a femoral artery, with data recorded using a Powerlab data-acquisition system. A catheter was inserted into a femoral vein for infusion of NaCl solutions of different osmolalities. Isosmotic (0.9%) NaCl was infused at 1.5 ml/h via the femoral vein once the catheter was in place. In a separate group of rats (for intravenous mannitol infusion), isosmotic NaCl was instead infused at 0.5 ml/h via the femoral vein and rats received isosmotic NaCl at 0.6 ml/h via the jugular vein rather than fluorescein isothiocyanate-inulin in phosphate-buffered NaCl. Urine was collected from the bladder, facilitated by a catheter inserted via a suprapubic incision.

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**RESULTS**

**Intravenous infusion of NaCl.** Urinary endothelin excretion rate was similar in the three groups during the baseline isotonic NaCl infusion period (Fig. 1A). Intravenous infusion of hyperosmotic NaCl solutions produced significant increases in the rate of endothelin excretion, an effect that appeared to be concentration dependent, with a higher endothelin excretion rate observed during infusion of NaCl at 1,664 compared with 921 mosmol/kgH₂O (Fig. 1A). In contrast, there was no significant change in endothelin excretion rate during continued infusion of isotonic NaCl.

There were no significant differences in baseline urine flow or Na⁺ excretion rate between the three groups (Fig. 1, B and C). As expected, urine flow and Na⁺ excretion significantly increased above baseline levels after commencing infusion of hyperosmotic NaCl, with this effect reaching statistical significance sooner and being of greater magnitude in rats receiving the solution with highest osmolality (1,664 mosmol/kgH₂O). Urine flow and Na⁺ excretion rate did not significantly change over time in rats receiving isotonic NaCl. There was no significant change in glomerular filtration rate over time in any group (data not shown).

Mean arterial pressure remained stable over the course of the experiment in rats receiving isotonic NaCl or NaCl at 921 mosmol/kgH₂O (Fig. 1D). Both groups of rats that went on to

**Fig. 1.** Effect of intravenous infusions of NaCl on systemic pressure and renal function of anesthetized rats. Data shown are endothelin excretion rate (UETV; A), urine flow (V; B), Na⁺ excretion rate (UNa⁺; V; C), and mean arterial pressure (MAP; D) measured during sequential 30-min periods in rats receiving intravenous infusions of isotonic (284 mosmol/kgH₂O) and hyperosmotic (921 and 1,664 mosmol/kgH₂O) NaCl at baseline followed by infusions of NaCl at 284, 921, or 1,664 mosmol/kgH₂O (n = 7, 9, and 9, respectively, except for Na⁺ excretion rate where n = 7, 6, and 6). 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*Ptime). NS, not statistically significant. Post hoc contrasts: *P < 0.05 vs. baseline for same group; †P < 0.05 vs. 284 mosmol/kgH₂O group at specified time point; ‡P < 0.05 vs. 0- to 30-min time point in same group; §P < 0.05 vs. 921 mosmol/kgH₂O group.
receive hyperosmotic NaCl had slightly lower mean arterial pressures during the baseline isosmotic NaCl infusion period compared with rats that continued to receive isosmotic NaCl throughout the experiment \((P < 0.05)\). Blood pressure rose during infusion of NaCl at 1,664 mosmol/kgH2O to reach the same level as in the isosmotic NaCl group during the final 30-min period.

**Renal medullary infusions.** During infusion of isosmotic or hyperosmotic NaCl into the renal medulla of the left kidney, mean arterial pressure was similar in both groups of rats and remained stable across the three collection periods (Fig. 2A). The rate of endothelin excretion from the left (infused) kidney was similar in both groups at baseline and remained stable in rats continuing to receive isosmotic NaCl throughout the experiment (Fig. 2B). Infusion of hyperosmotic NaCl (1,714 mosmol/kgH2O) into the renal medulla produced a significant increase in endothelin excretion rate within the first 30 min of infusion, which was maintained during the second 30 min of hyperosmotic NaCl infusion. Left kidney urine flow and Na+ excretion rate were also similar in both groups at baseline and remained stable in rats continuing to receive isosmotic NaCl (Fig. 2, C and D). Both urine flow and Na+ excretion increased markedly within the first 30 min of commencing infusion of hyperosmotic NaCl, with Na+ excretion rising further during the second 30-min period (Fig. 2D). In contrast, the rate of endothelin excretion, urine flow, and rate of Na+ excretion from the right (untouched) kidney remained constant throughout the experiment and were similar in both groups of rats (Fig. 2, E, F, and G). There was no significant difference between the two groups in endothelin concentration measured in plasma collected at the end of the experiment, although plasma Na+ concentration was slightly but significantly higher in rats receiving hyperosmotic NaCl (Table 1). Tissue endothelin concentrations, measured in the cortex, outer and inner medulla of the left kidney, were not significantly different between the two groups of rats (Table 1).

To determine whether osmolality or NaCl concentration was the stimulus for endothelin release, the experiment was repeated in separate groups of rats using mannitol as the osmo-
molyte rather than NaCl. Infusion of hyperosmotic mannitol (1,820 mosmol/kg H2O) into the renal medulla of the left kidney significantly increased endothelin excretion rate for both left and right kidneys during the final 30-min period (Fig. 3, B and E), both compared with the two previous periods in that group and compared with isosmotic mannitol infusion at the same time point. Left kidney urine flow and Na⁺ excretion rate and right kidney urine flow were also significantly increased during the final 30-min period by the hyperosmotic mannitol infusion (Fig. 3, C, D, and F). Right kidney Na⁺ excretion rate increased (P<0.05; Fig. 3G), and mean arterial pressure decreased (P<0.01; Fig. 3A) slightly but significantly over time in both groups of rats.

Effect of intravenous mannitol infusion on endothelin excretion. As a control experiment for possible spillover of mannitol into the systemic circulation from the medullary interstitial infusion, two further groups of rats were prepared as described for intravenous infusion of NaCl. Both groups of rats received infusions of isosmotic NaCl at 0.5 ml/h via the femoral vein during the 1-h equilibration and 30-min baseline urine collection periods. One group continued to receive isosmotic NaCl during the two further 30-min urine collection periods, whereas the other group received a solution of 0.9 M mannitol dissolved in isosmotic NaCl via the femoral vein at 0.5 ml/h. Intravenous infusion of 0.9 M mannitol produced a significant increase in urinary endothelin excretion rate during the final 30-min period, both compared with baseline in that group and compared with infusion of isosmotic NaCl alone at the same time point (P<0.05; Fig. 4A). Both groups underwent a small but significant increase in mean arterial pressure over time (P<0.001; Fig. 4D), but there were no significant differences between the two groups in mean arterial pressure, urine flow, or Na⁺ excretion, and no significant changes in urine flow or Na⁺ excretion were observed in either group (Fig. 4).

DISCUSSION

These experiments tested whether infusions of hyperosmotic NaCl solutions stimulated renal endothelin release in vivo. We found that infusion of hyperosmotic NaCl either directly into the renal medulla or intravenously enhanced urinary endothelin excretion in anesthetized rats. This confirms the earlier findings of most (e.g., Refs. 4, 10, 19) but not all (7) studies conducted with isolated tubular and collecting duct cells in vitro and is consistent with the effects of high dietary salt intake on urinary endothelin excretion observed in our laboratory (15, 16) and by others (3).

Importantly, we showed that the signal to enhance endothelin release in vivo appears to be high osmolality rather than high NaCl concentration specifically. In our study, infusion of either hyperosmotic NaCl or mannitol solutions directly into the renal medulla enhanced endothelin excretion. Previous studies conducted in vitro have yielded divergent findings on whether effects on endothelin release from tubular epithelial cells are solute specific, as well as whether increases in media osmolality stimulate or inhibit endothelin production. For example, one study conducted on IMCD cells reported increases in ET-1 release in response to hyperosmolality by NaCl, no effect of hyperosmolality by mannitol solutions, and inhibition of ET-1 production by hyperosmotic urea solutions (19). Another study reported that both urea and NaCl increase ET-1 production by the same cell type, although urea appeared to provide a less potent stimulus than NaCl (10), whereas yet another study reported that all three solutes decreased ET-1 production by IMCD cells (7). The reasons for these differing results are unclear but may relate to differences in the length of time the cells spent in vitro before commencing the experiment or differences in cell culture conditions.

As our results suggest that medullary osmolality may be a key factor in the control of renal endothelin production, it might be expected that urinary endothelin excretion would be increased under conditions in which medullary osmolality is increased. Recently, it was reported that rats maintained on a high-salt diet displayed an increase in outer medullary tissue osmolality (4). Although urinary endothelin excretion rate was not measured in that particular study, there is ample evidence in the literature to suggest that a high-salt diet increases endothelin excretion. Another condition known to increase medullary osmolality is dehydration. Little is currently known about the effect of dehydration on urinary endothelin excretion. One study (7) reported that the urinary ET-1 excretion rate of dehydrated rats was much lower than that of rats on a high-salt diet; however, a euvolemic group was not included in this study for comparison. In another study, urinary excretion of ET-1 by female rats deprived of water for 48 h was significantly reduced compared with that of euvolemic female rats (9). Thus, under circumstances such as dehydration, the apparently positive influence of medullary osmolality on endothelin release may perhaps be overridden by other factors. Medullary osmolality is, after all, unlikely to be the sole regulator of renal endothelin production.

The signaling mechanism by which high extracellular osmolality translates into an increase in endothelin production and/or release is currently unknown. Increases in extracellular osmolality act as a stimulus for the transcription of a variety of genes in cells of the renal medulla. For example, upon exposure of cells to hyperosmotic conditions, the transcription factor OREBP/TonEBP translocates to the nucleus where it binds to osmotic response elements on a number of osmoprotective genes (14). Induction of these genes enables cells to accumulate organic osmoles and increase production of HSP70, protecting the cells against osmotic stress (14). Whether similar signaling events are involved in the enhanced endothelin release from renal tubular epithelial cells observed over hours in response to increased ambient osmolality in vitro (4, 10, 19) or the increase in urinary endothelin excretion observed in response to high-salt intake in vivo (3, 15, 16) is currently unknown. The rapid onset of the increase in endothelin excretion observed in the present study may preclude involvement of a change in preproendothelin gene expression. Another possibility is that cell shrinkage upon exposure to hypertonic solutions, such as those used in this study, might somehow trigger release of endothelin from tubular and collecting duct cells. Whether such changes in cell volume trigger endothelin release is currently unknown. Another possible stimulus for endothelin release from tubules is an effect of fluid shear resulting from increased tubular fluid flow rate. Endothelin gene expression and peptide secretion from endothelial cells can be enhanced acutely by shear stress (12, 13) or strain (18). Whether endothelin release by tubular cells is similarly affected appears to be unknown. The mechanism(s) involved in
enhanced endothelin production and release in response to increased ambient osmolality or increased dietary NaCl intake awaits further investigation.

Arguing in favor of the medullary interstitial hypertonic NaCl infusion exerting a localized effect on endothelin secretion, the rate of endothelin excretion from the infused left kidney increased markedly, whereas there was no significant change in the rate of endothelin excretion from the untouched right kidney. In contrast, infusion of mannitol into the medullary interstitium of the left kidney resulted in enhanced endothelin excretion by both kidneys. A possible explanation for the effect on the right kidney is that some of the infused mannitol was recirculated to the right kidney, where it provided a stimulus for endothelin release. Previous studies using the technique of medullary interstitial infusion have reported that a portion of the substances infused does indeed recirculate to the contralateral kidney (8). We would...
expect that although some Na⁺ may also have been recirculated during medullary interstitial infusion of hyperosmotic NaCl, due to the differences in the way the body handles Na⁺ and mannitol, a much greater amount of mannitol may have distributed into the systemic circulation. As we are not equipped to measure plasma mannitol concentrations, we infused mannitol intravenously in separate groups of rats at a concentration half of that of what was infused into the renal medulla. Our finding, shown in Fig. 4A, that intravenous infusion of mannitol does enhance urinary endothelin excretion lends further support to the possibility that the increase in endothelin excretion observed in the contralateral kidney during medullary interstitial infusion of hyperosmotic mannitol was due to mannitol entering the systemic circulation.

Another possible explanation for the enhanced urinary endothelin excretion from the contralateral kidney is that perhaps infusion of hyperosmotic mannitol into the medullary interstitium triggered a renorenal reflex, stimulating diuresis in the contralateral kidney. The increased endothelin excretion might then have been due to an as yet undescribed neural effect or may have occurred secondary to the increase in urine flow. However, since little is currently known regarding whether increased urine flow per se acts as a stimulus for endothelin release, and we have no data to confirm or rule out the possible activation of renorenal reflexes under the experimental conditions in question, this explanation must be regarded as purely speculative.

One of the aims of this study was to determine whether osmolality or NaCl specifically affected endothelin excretion. Mannitol was chosen for use as a second osmolyte since it does not contain either Na⁺ or Cl⁻. One caveat and limitation regarding the use of mannitol is that the body handles mannitol and Na⁺ differently, with Na⁺ moving more freely across cell membranes than mannitol. When infused into the medullary interstitium, the mannitol may therefore have remained trapped or become concentrated to a greater degree than the infused NaCl, thus producing a greater increase in medullary osmolarity. Therefore, although we infused NaCl and mannitol solutions of similar osmolalities into the renal medulla, the osmol challenge presented by the two chemicals may be different.

Another finding of this study was that infusions of hyperosmotic NaCl and mannitol solutions into the renal medulla increased urine flow and Na⁺ excretion rate. The increase in Na⁺ excretion rate associated with infusion of hyperosmotic NaCl was much greater than that associated with infusion of hyperosmotic mannitol. This was expected considering that in one experiment an ~1.7 M NaCl solution was infused into the kidney, whereas in the other experiment only mannitol dissolved in distilled water was infused. In the case of intravenous infusion of NaCl at 1,664 mosmol/kgH₂O, the ~14-mmHg increment in blood pressure over the course of the experiment may also have contributed to the natriuretic and diuretic response. Although these and other factors may have contributed to the enhanced urine production and Na⁺ excretion observed during infusion of hyperosmotic solutions, these findings are nonetheless consistent with the released endothelin exerting autocrine/paracrine actions within the kidney to promote natriuresis and diuresis (6). Whether and to what extent the enhanced endothelin release observed in response to increases of medullary interstitial osmolality contributed to the enhanced Na⁺ and water excretion cannot be determined from the current results. Thus the functional consequences of the enhanced endothelin release observed in response to renal medullary interstitial infusion of hyperosmotic solutions remain to be determined. However, such a sequence of events would provide a pathway allowing the kidney to sense an increase in NaCl intake [increased renal medullary osmolality (4)], enhance the production of a natriuretic agent (endothelin), and rid the body of a NaCl load.

In summary, infusion of hyperosmotic NaCl solutions directly into the interstitial space of the renal medulla enhances urinary endothelin excretion. This effect appears to be mediated by the osmolality of the infused solution rather than NaCl concentration per se as infusion of a hyperosmolar mannitol solution also stimulated endothelin excretion. The current study thus provides some of the first in vivo evidence supporting a role for renal medullary osmolality in controlling renal endothelin synthesis and release.

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REFERENCES


