Role of Sodium Depletion in Acute Antidiuretic Effect of Bendroflumethiazide in Rats with Nephrogenic Diabetes Insipidus

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ABSTRACT
The mechanisms underlying the acute antidiuretic response to bendroflumethiazide (BFTZ; 0.25 mg/h for 3 h) in rats with nephrogenic diabetes insipidus (NDI) was investigated. NDI was induced in conscious chronically instrumented female Wistar rats either by chronic lithium administration (40–60 mmol Li/kg of diet for 4 weeks) or by acute infusion of V2 antagonist OPC-31260 (0.2 mg/h). Renal clearance experiments were performed in conscious rats instrumented with permanent catheters. During experiments total body water content was held constant by i.v. replacement of urine production (V) with 150 mM glucose. One group in addition received i.v. replacement of urinary sodium losses. In both models of NDI, BFTZ-induced antidiuresis was associated with a decrease in the delivery of tubular fluid to the distal nephron, as measured by lithium clearance (CLi). Both the antidiuresis and the decrease in CLi could be prevented by sodium replacement. BFTZ did not affect distal water handling as measured by V/CLi. BFTZ did not induce antidiuresis in normal rats with water diuresis. It is concluded that in rats with NDI, thiazide-induced antidiuresis can be entirely explained by a fall in distal delivery of tubular fluid related to sodium depletion. This contrasts the response in rats with central diabetes insipidus, where thiazides in addition increase distal water reabsorption.

Thiazide (TZ) diuretics exert an antidiuretic action in central diabetes insipidus (CDI) (Crawford and Kennedy, 1959) as well as in nephrogenic diabetes insipidus (NDI) (Forrest et al., 1974). The exact mechanism behind this “paradoxic” antidiuretic response remains elusive. The major hypothesis proposed by several investigators and cited in authoritative textbooks (Ives, 2001) suggests that TZ-induced contraction of the extracellular fluid volume leads to a baroreceptor-mediated reduction in the flow of tubular fluid output delivered to the diluting segment (distal delivery) and hence production of reduced amounts of a more concentrated urine. This is supported by studies showing that TZ treatment reduces distal delivery in normal and CDI rats as measured by micropuncture (Weinman and Eknoyan, 1975; Shirley et al., 1982; Walter and Shirley, 1986) or indirectly by lithium clearance (Thomsen and Schou, 1973; Petersen et al., 1974; Lunau et al., 1994). Furthermore, in anesthetized CDI rats sodium replacement prevented the antidiuresis as well as the reduction in distal delivery induced by hydrochlorothiazide (HCTZ) (Shirley et al., 1978; Walter et al., 1979).

However, a number of observations in Brattleboro rats with CDI are not entirely consistent with the above-mentioned hypothesis: 1) sodium depletion induced by sodium restriction alone is not accompanied by antidiuresis, and the antidiuresis induced by HCTZ administration ceases when the drug is withdrawn despite sustained sodium depletion (Walter and Shirley, 1983); 2) the antidiuresis induced by chronic bendroflumethiazide (BFTZ) administration is not related to changes in lithium clearance (Grønbeck et al., 1998); and 3) in our laboratory BFTZ-induced antidiuresis in conscious diabetes insipidus rats could not be prevented when sodium losses were replaced by use of a computerized servo-control system (Spannow et al., 1997). For these reasons we concluded that at least in rats with CDI, sodium depletion cannot be the sole mechanism eliciting TZ-induced antidiuresis, i.e., that thiazides must exert some additional effects on distal nephron water reabsorption. This is consistent with the findings of Cesar and Magaldi (1999) that HCTZ added to the perfusion fluid in vitro stimulated water permeability in inner medullary collecting ducts isolated from Brattleboro rats.

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ABBREVIATIONS: ADH, antidiuretic hormone; BFTZ, bendroflumethiazide; DI, diabetes insipidus; C, renal clearance; CDI, central diabetes insipidus; ERPF, effective renal plasma flow; GFR, glomerular filtration rate; HCTZ, hydrochlorothiazide; NDI, nephrogenic diabetes insipidus; V, urine flow rate; TZ, thiazide; AQP2, aquaporin-2.
duct intracellular distribution of AQP2 water channels, it seems unlikely that thiazides should exert an antidiuretic hormone-like action on the collecting duct cells (Grenbeck et al., 1998).

Whereas most studies on the mechanism of TZ-induced antidiuresis have been carried out in Brattleboro rats with CDI, the major clinical application of thiazides as antidiuretics is in patients with NDI, which can either be congenital (Bichet et al., 1997) or induced by drugs such as lithium (Jackson and Dousa, 1990). In rats with lithium-induced NDI, BFTZ treatment (10 mg/kg/day) caused a two-thirds reduction in urine flow associated with generation of slightly hypertonic urine (Christensen, 1976). A similar antidiuretic response was observed when chlorothiazide was administered to patients with lithium-induced polyuria (Forrest et al., 1974). Nevertheless, so far no study has addressed the mechanism of the antidiuretic response to thiazides in NDI.

The objective of the present study was to investigate the role of sodium depletion for TZ-induced antidiuresis in NDI. We used two different animal models: rats with NDI induced by chronic lithium administration, and rats with NDI induced by acute i.v. infusion of the vasopressin V2 receptor antagonist OPC-31260. For comparison, we also examined the response of TZ in rats undergoing water diuresis. Clearance experiments were conducted in conscious rats instrumented with permanent catheters, and the renal hemodynamic and excretory responses to i.v. infusion of BFTZ (0.25 mg/h) were assessed with and without replacement of urinary sodium losses.

Experimental Procedures

Animals and Physical Environment

Female Wistar rats [Crl:(W)BR; 210–230 g] were purchased from Charles River, Sulzfeld, Germany. The animals were housed in a temperature- (22–24°C) and moisture (40–70%)-controlled room with a 12-h light/dark cycle (light on from 6:00 AM to 6:00 PM). All animals were given free access to tap water and a pelleted rat diet containing approximately 140 mmol/kg of sodium, 275 mmol/kg of potassium, and 23% protein (catalog no. 1310; Altromin International, Lage, Germany).

Animal Preparation

One week before the renal clearance experiments all rats were anesthetized with 1% halothane in a gas mixture of N2O/O2 (2/1) and chronic medical grade Tygon catheters were implanted into the abdominal aorta and into the inferior caval vein via a femoral artery and vein. A silicone-coated stainless steel suprapubic urethral catheter was implanted into the urinary bladder and sealed with a screw after flushing the bladder with 0.6 mg/ml ampicillin (Anhype; Nycomed Pharma, Oslo, Norway). Catheters were produced, fixed, and sealed as previously described (Petersen et al., 1991). All surgical procedures were performed during aseptic conditions. To relieve postoperative pain, rats were treated with 0.2 mg/kg of b.wt. i.p. buprenorphin, twice daily for 2 days (Anorfin; GEA A/S, Copenhagen, Denmark). After instrumentation, the animals were housed individually.

Rats with NDI Induced by Lithium Treatment

The rats were fed lithium-containing rat pellets containing 40 mmol/kg of lithium for the first 2 weeks, thereafter 60 mmol/kg. Lithium was added as lithium citrate. The rats were given tap water ad libitum and free access to solid sodium chloride to prevent lithium toxicity (Thomsen et al., 1974). The treatment period was 4 to 5 weeks to induce maximal polyuria and impairment of renal concentrating ability (Christensen et al., 1982). Daily water intake was measured as an index of polyuria.

Rats with NDI Induced by Acute V2 Receptor Blockade

In this model, polyuria was induced by i.v. infusion of the V2 receptor antagonist OPC-31260 at a rate of 0.2 mg/h from the beginning of the clearance experiment (Jonassen et al., 1998).

Rats with Water Diuresis

The rats were infused with 150 mM glucose at a rate of 8 ml/h from the beginning of the clearance experiment.

Renal Clearance Technique

The renal hemodynamic and tubular response to BFTZ was examined by clearance techniques in conscious rats. Prior to the clearance experiments all rats were adapted to the restraining cage used for these experiments by training them for two periods of 2 h each on separate days. Clearance of [14C]tetraethylammonium bromide was used as a marker for the effective renal plasma flow (Rasmussen et al., 1990), clearance of [3H]inulin as a marker for glomerular filtration rate (Shalmi et al., 1991) and clearance of lithium as a marker for distal delivery (Thomsen and Shirley, 1997). Whereas the former substances were administered in tracer amounts by constant i.v. infusion, lithium was added in small amounts (12 mmol/kg) to the diet of control rats for 2 to 3 days prior to experiments to avoid acute effects on renal function (Shalmi and Thomsen, 1989).

Clearance experiments were started at 8:00 AM and clearance markers were infused at a constant rate (1.5 ml/h) throughout experiments. A 90-min equilibration period was followed by 2 × 30-min control periods and 6 × 30-min experimental periods. BFTZ was infused i.v. with a constant rate of 0.25 mg/h (0.5 ml/h). All infusions were made through the venous catheter. The arterial catheter was used for blood samples (300 μl each being immediately replaced with heparinized donor blood) and for monitoring of mean arterial blood pressure during experiments.

Study Design and Clearance Protocols

For both models of NDI, three separate subgroups were investigated.

Time-Control Subgroup. Lithium-pretreated rats were given constant infusion of 150 mM glucose (8 ml/h) during equilibration and control periods and switched to servo-controlled replacement of urinary volume losses during the rest of the experiment. In the group receiving acute administration of V2 antagonist, servo-controlled volume replacement with 150 mM glucose was on from the beginning of the experiment and maintained throughout. The water-loaded group received constant infusion of 150 mM glucose (8 ml/h) during equilibration and control periods and were switched servo-controlled replacement of urinary volume losses during the rest of the experiment.

BFTZ-Treated Subgroup. These rats were treated like the time-control rats but at the end of the second control period, BFTZ infusion was started.

BFTZ-Treated Subgroup with Sodium Replacement. This subgroup was treated like the BFTZ rats, but in addition urinary sodium losses were replaced (Spannow et al., 1997) by a servo system during BFTZ infusion.

Analytical Methods

Electrolytes in samples of plasma and urine were determined by atomic absorption spectrometry, using a PerkinElmer series 2380 or a PerkinElmer Analyst 300 atomic absorption spectrometer. [3H]Inulin and [14C]tetraethylammonium were determined by double label scintillation counting in a Packard 2250 CA liquid scintillation counter. Urinary osmolality was determined by freezing point depression, using an Advanced model 3CII freezing depression osmometer.
Drugs and Chemicals

Bendroflumethiazide was a gift from Leo Pharmaceuticals, Copenhagen, Denmark. It was dissolved in 150 mM glucose (0.5 mg/ml) by addition of 0.1% ethanolamine. OPC-31260 was a gift from Otsuka Pharmaceutical Co., Tokushima, Japan. It was dissolved in 150 mM glucose (0.2 mg/ml). [3H]Inulin was purchased from Amersham (Hillerod, Denmark) (catalog no. TRA324). [14C]Tetraethylammonium bromide was purchased from Dupharma A/S (Kastrup, Denmark) (catalog no. NEC298). Other chemicals were of analytical grade from standard suppliers.

Calculations

Renal clearances (C) and fractional excretions (FE) of the clearance markers were calculated by the standard formula C = U · V/P; FE = C/GFR, where V is urine flow rate, U is concentration in urine, and P is plasma concentration. Lithium clearance was used as an index of distal delivery (Thomsen and Shirley, 1997). Thus, V/C_Li signifies the fraction of distal delivery being excreted as urine (fractional distal water excretion).

Results

In the sodium-replaced groups the cumulated sodium balances during the 3 h with BFTZ infusion were $-23 \pm 14$ μmol in the lithium rats and $+5 \pm 26$ μmol in the rats with V2 receptor blockade, indicating that sodium losses were completely compensated for by the servo-control system.

Rats with NDI Induced by Chronic Lithium Administration (Fig. 1). During the days before the clearance

Fig. 1. Studies in rats with NDI induced by chronic lithium administration. Urinary sodium excretion (U_{NaV}), V, urine osmolality (U_{osm}), C_Li, urine flow as percentage of distal delivery (V/C_Li), ERPF, and GFR during two control periods (0–60 min) and during infusion of vehicle or BFTZ with or without servo control of the sodium balance (60–240 min). Control period 2 versus two last experimental periods (paired Student’s t test): *p < 0.05, **p < 0.01, ***p < 0.001.
experiment, rats treated with lithium for 4 to 5 weeks showed polyuria corresponding to a daily water consumption of 70 to 100% of their body weight. The plasma lithium concentration was 0.54 ± 0.06 mmol/l. During experiments, the urine flow rate was maintained by infusion of 150 mM glucose solution (8 ml/h, corresponding to 85% of the body weight per 24 h) in equilibration and control periods, and thereafter at a rate controlled by the volume servo. As seen from Fig. 1, the urine flow rate remained stable throughout in the time control group when the volume servo was activated.

Administration of BFTZ without sodium replacement induced a transient natriuresis followed by a decline in urine flow rate. BFTZ-induced antidiuresis was associated with significant decrease in distal delivery as measured by $C_{\text{Li}}$. Distal water handling, as measured by $V/C_{\text{Li}}$, did not change significantly. Although BFTZ tended to lower effective renal plasma flow (ERPF) and GFR, neither of these changes reached statistical significance.

When urinary sodium losses were replaced (group BFTZ + Na), the antidiuresis as well as the associated decrease of $C_{\text{Li}}$ were prevented.

Rats with NDI Induced by Acute $V_2$ Receptor Blockade (Fig. 2). In this model, polyuria was induced by i.v. infusion of the $V_2$ receptor antagonist OPC-31260 along with servo-controlled replacement of urinary volume losses with 150 mM glucose solution. As reported previously (Jonassen et al., 1998), this procedure induced a urine flow rate of about 100 μl/min/100 g of b.wt., which in the time control group remained stable throughout.

In this model, too, BFTZ without sodium replacement induced transient natriuresis followed by a fall in urine flow rate and an associated rise in urine osmolality. This was associated with a significant fall in $C_{\text{Li}}$, whereas $V/C_{\text{Li}}$, ERPF, and GFR remained unaltered.

Sodium replacement (group BFTZ + Na) enhanced BFTZ-induced natriuresis and prevented the fall in urine flow rate and $C_{\text{Li}}$.

Rats with Water Diuresis (Fig. 3). Water diuresis was induced by infusion of 150 mM glucose solution (8 ml/h for

Fig. 2. Studies in rats with NDI induced by acute $V_2$ receptor blockade. The renal variables and treatments are the same as described in legend to Fig. 1.
150 min) and maintained by the volume servo. In the time control group, the urine flow rate was stabilized at about 80 μl/min/100 g of b.wt. with a urine osmolality of about 100 mosmol/kg. BFTZ induced natriuresis without significant changes in urine flow rate, leading to an increase in urine osmolality. EPRF, GFR, and \( C_{\text{fci}} \) remained unaltered whereas \( V/C_{\text{fci}} \) decreased. Because BFTZ did not cause antidiuresis in water diuretic rats, the group with sodium replacement was omitted.

Discussion

In this article we investigated the role of sodium depletion for the acute antidiuresis induced by BFTZ in two different rat models of NDI, using computer-controlled independent replacements of urinary losses of sodium and water. The major new observation is that in NDI, whether induced by lithium or acute \( V_2 \) receptor blockade, BFTZ-induced antidiuresis can be completely prevented by sodium replacement. This contrasts our recent findings in rats with CDI (Spannow et al., 1997) using an identical protocol.

Mechanism of Antidiuresis Induced by BFTZ in Rats with NDI. In this study we chose two different models of drug-induced NDI: long-term lithium administration known to inhibit vasopressin \( V_2 \) receptor-stimulated cyclic AMP formation in the collecting duct principal cells (Christensen et al., 1985), and acute blockade of \( V_2 \) receptors with the selective vasopressin antagonist OPC-31260. In both models, polyuria is due to inability of vasopressin to stimulate insertion of AQP2 water channels in the luminal collecting duct membrane (Marples et al., 1995; Christensen et al., 1998).

A consistent finding in both models of NDI was that BFTZ induced antidiuresis was paralleled with a fall in distal delivery as measured by lithium clearance. Since the total body volume was held constant by the volume servo, sodium depletion must have caused a redistribution of body fluid from the intravascular compartment to extravascular, probably intracellular compartments. It is well known that neurohu-

![Fig. 3. Studies in rats with diabetes insipidus due to infusion of fluid (water diuresis). The renal variables and treatments are the same as described in legend to Fig. 1 with the only exception that a BFTZ + Na group was omitted because BFTZ did not cause antidiuresis.](image-url)
moral mechanisms are stimulated during contraction of the intravascular volume. Low-pressure baroreceptors in the atria and high-pressure baroreceptors in the aorta and sines react to sensed underfilling of the circulation, causing a reflex increase in renal sympathetic nerve activity. The latter reduces distal delivery by contraction of the afferent arteriole, leading to a reduction in GFR, and by direct stimulation of the proximal tubular sodium reabsorption. Since in addition sodium replacement completely prevented both the fall in distal delivery and the antidiuresis, our results provide strong evidence for the hypothesis that in NDI, thiazide antidiuresis is caused exclusively by a reduction in distal delivery related to thiazide-induced sodium depletion.

Although currently the clinical use of thiazides as antidiuretics is mainly or exclusively in patients with NDI, this is the first study elucidating the mechanism of the antidiuresis in this condition. This may be due to the fact that animal models of NDI have not been available, whereas the Brattleboro rat has been available for many years as the standard model of CDI (Valtin, 1967).

Interestingly, it now appears that the mechanism whereby thiazides provoke antidiuresis is not identical in CDI and NDI. In our previous study in Brattleboro rats with CDI (Spannow et al., 1997), BFTZ, in addition to lowering distal delivery, induced a significant decrease of V/Ci, signifying stimulation of distal water reabsorption. Furthermore, in this model sodium replacement was unable to prevent the antidiuresis, as was bilateral renal denervation. These results suggested that in CDI, thiazides stimulate distal water reabsorption by a mechanism not involving volume depletion, renal nerves, or AQP2 water channels (Grenbeck et al., 1998). Such an effect could be mediated via enhancement of the cortico-medullary osmotic gradient as proposed previously (Shirley et al., 1982). However, this mechanism could not explain the recent observation that HCTZ increased water permeability in isolated perfused collecting tubules (Cesar and Magaldi, 1999).

Response to BFTZ in Rats with Water Diuresis. Rats with water diuresis induced by sustained water loading develop polyuria due to suppression of AVP release. It is well known that water diuretic individuals will not respond to thiazide administration with antidiuresis, and this was confirmed in the present study. BFTZ induced natriuresis and negative sodium balance both in water diuretic rats and in NDI rats, but distal delivery was only decreased in the latter. One major difference between diabetes insipidus and water diuresis is the state of body hydration: whereas diabetes insipidus is usually associated with negative water balance, as signaled by plasma hyperosmolality (Valtin, 1967), individuals with water diuresis are per definition overhydrated. Thus, the explanation for the absence of an antidiuretic response to thiazides in water diuretic rats could simply be that the overhydration prevents TZ-induced contraction of the intravascular volume, and thereby the baroreceptor-mediated reduction in distal delivery. It seems physiologically reasonable that the compensatory fall in distal delivery in response to thiazide-induced sodium depletion is more readily activated in states with volume deficiency than in conditions with volume excess.

An interesting observation in the water diuretic rats was that the in this model, too, sodium losses appeared to be transient in spite of a constant delivery of tubular fluid from the proximal tubule. This indicates that in this specific model, compensatory sodium reabsorption in response to thiazides must occur in the distal part of the nephron, i.e., in the loop of Henle or the collecting ducts.

In conclusion, the present study indicates that in rats with nephrogenic diabetes insipidus the acute antidiuretic response to BFTZ administration can be fully explained by a reduction in distal delivery of tubular fluid related to urinary sodium depletion, supposed to decrease the intravascular fluid volume in the face of a constant total body volume. In contrast with the observation in rats with central diabetes insipidus, BFTZ does not affect distal water handling in rats with nephrogenic diabetes insipidus.

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References


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