Influence of Renal Nerves and Sodium Balance on the Acute Antidiuretic Effect of Bendroflumethiazide in Rats with Diabetes Insipidus

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Accepted for publication May 23, 1997

ABSTRACT

To examine the role of the renal nerves and sodium depletion for the acute antidiuretic response to bendroflumethiazide (BFTZ; 25 µg/hr) in rats with diabetes insipidus (DI), renal clearance experiments were performed in the following groups of conscious, chronically instrumented male Brattleboro rats with vasopressin-deficient DI: Control (n = 7), BFTZ (n = 9), BFTZ + sodium replacement (n = 7) and BFTZ + chronic bilateral renal denervation (n = 6). Urine flow rate and urinary sodium concentration were measured drop-by-drop with a sodium-sensitive electrode and by collection of urine in vials placed on an electronic balance. This allowed computer driven, servo-controlled, independent i.v. replacement of sodium and fluid losses, respectively. Mean arterial pressure, glomerular filtration rate (GFR) and proximal tubular water and sodium handling, assessed by lithium clearance (C_Li), were stable in the control group. BFTZ produced a marked antidiuretic response (ΔV = -79%; ΔUrine osmolality = +218%) associated with decreases in GFR (-28%), C_Li, (-62%), free water clearance (-100%) and plasma Na (-5 mM). Fractional water reabsorption was increased by 19% in the proximal tubules and by 7% in segments beyond. Sodium replacement did not modify the fall in GFR or the antidiuresis, but partly prevented the increase in fractional proximal water reabsorption. Bilateral renal denervation did not affect the response to BFTZ. We conclude that the acute antidiuretic effect of BFTZ is independent of sodium balance and renal nerve activity and is elicited by a reduction in GFR accompanied by an increase in fractional water reabsorption in the proximal tubules and in the distal nephron.

In 1959, Crawford and Kennedy reported that treatment with chlorothiazide could lower the urine flow in patients and rats with DI. Since then, several experimental studies have addressed the question of the mechanism of this “paradoxical” antidiuretic effect of the thiazide diuretics. The predominant hypothesis, as stated in authoritative pharmacology textbooks, is that the sodium depletion associated with diuretic treatment causes a reduction in the flow rate of tubular fluid leaving the proximal tubules (Hays, 1992; Ives and Warnock, 1995). This hypothesis is supported by studies in rats with DI, demonstrating a decrease in proximal tubular fluid output, measured by micropuncture or C_Li, during HCTZ-induced antidiuresis (Walter et al., 1979; Shirley et al., 1982; Thomsen and Schou, 1973) and the observation that replacement of urinary sodium losses could prevent the acute antidiuresis induced by HCTZ (Shirley et al., 1978; Walter et al., 1979).

However, other studies are not readily compatible with the above mentioned hypothesis. Walter and Shirley (1983) reported that although HCTZ produced a negative sodium balance along with a decreased urine flow rate in DI rats, dietary sodium restriction caused even more pronounced sodium losses without significant changes in urine flow rate. This suggest that sodium depletion is not essential for the antidiuresis induced by HCTZ. Furthermore, in a recent study we observed that the antidiuresis associated with chronic BFTZ administration showed no significant correlation with the changes in distal delivery, as measured by C_Li (Gronbeck et al., submitted for publication).

The conflicting data on the role of sodium balance for the antidiuretic effect of thiazides in rats with DI could be related to the opposite actions of these diuretics on sodium and water excretion in DI. Thus, when urinary volume losses are replaced by conventional technique, the delay in down-regulation of volume infusion, to match the decrease in urine flow, might induce volume expansion that in turn could abolish the antidiuresis. Therefore, the aim of this study was to examine

ABBREVIATIONS: DI, diabetes insipidus; BFTZ, bendroflumethiazide; HCTZ, hydrochlorothiazide; GFR, glomerular filtration rate; MAP, mean arterial blood pressure; C, renal clearance; V, urine flow; P, plasma concentration; U, urine concentration; DD, distal delivery; FE, fractional excretion; FR, fractional reabsorption; [Na⁺], sodium concentration; HCT, hematocrit; C_Li, lithium clearance.
the role of sodium depletion for the acute antidiuretic response to BFTZ in conscious Brattleboro DI rats maintained under conditions with constant total body water. To make this possible, we developed a computer-driven system that allowed immediate and independent servo control of sodium and water balances. BFTZ was chosen because this drug, in contrast to HCTZ, has no effect on the renal carbonic anhydrase (Beyer and Baer, 1961; Boer et al., 1989) and therefore would not be expected to inhibit fluid reabsorption in the proximal tubules.

It has been suggested that the increase of fractional tubular water reabsorption after acute administration of HCTZ in DI rats could be mediated by the renal nerves (Walter et al., 1979). To test this hypothesis, the antidiuretic response to BFTZ was examined in an additional group of animals with chronic bilateral renal denervation.

Materials and Methods

Animals and physical environment. Adult male Brattleboro rats with hereditary hypothalamic diabetes insipidus were purchased from Harlan Sprague-Dawley Inc., Indianapolis, IN. Average weight on the day of the experiment was 292 ± 4 g. Rats were kept individually in a temperature (22-24°C) and moisture (60%) controlled room with a 12-hr light-dark cycle (lights on from 7.00 a.m. to 7.00 p.m.). The rats were fed a standard diet (Altromin no. 1314, Altromin International, Lage, Germany) containing 29% protein, 150 mmol/kg of Na and 290 mmol/kg of K. Three days before the experiment, the diet was changed to a diet containing 12 mmol/kg of Li as lithium citrate. Tap water was available ad libitum.

Surgical preparation. One week before the experiment, the animals were anesthetized with halothane/N₂O. Using aseptic surgical techniques, sterile medical grade Tygon catheters were advanced into the abdominal aorta and the inferior caval vein via the femoral vessels. A sterile chronic suprapubic bladder catheter was implanted into the bladder. All catheters were produced and fixed as described by Petersen et al. (1991). After instrumentation, the rats were housed individually and given free access to 1.5% NaCl in addition to tap water for 3 days. After a recovery period of 5 to 6 days, the rats were acclimatized to restriction by daily training sessions in the restraining cages. The duration of each daily session was gradually increased from 2 to 4 hr/day. During training sessions, the rats were given 150 mM glucose (6 ml/hr) i.v. to prevent dehydration.

In one group, bilateral renal denervation was performed through flank incisions. The adventitia of the renal vein and artery were carefully stripped off under a dissection microscope using 25 magnification. All visible nerves were cut and the vessels were clamped with 10% phenol in 95% ethanol. With this procedure, we have previously found renal norepinephrine content to be reduced to less than 5% of control levels (Petersen and DiBona, 1992).

Experimental design. Four groups of rats were studied: group 1. Control (n = 7); group 2, BFTZ (n = 9); group 3. BFTZ + Na⁺ replacement (n = 7); group 4. BFTZ + renal denervation (n = 6).

In all groups, water balance was maintained during administration of vehicle or BFTZ. Group 1 was given vehicle only and sodium balance was maintained. Group 2 was given i.v. infusion of BFTZ (25 µg/hr) and allowed to loose sodium. Group 3 was given BFTZ with sodium replacement. Group 4 was given BFTZ without sodium replacement 1 wk after bilateral renal denervation.

Clearance protocol. At 9 a.m. the rat was placed in a restraining cage and the permanent catheters were unplugged and connected to extension lines. Through the Baxter Uniflow pressure transducer (Bentley Laboratories, Uden, Holland), a continuous intraarterial infusion of 150 mM glucose containing 20 units/ml of heparin (LEO Pharmaceuticals, Ballerup, Denmark) at a rate of 0.25 ml/hr was given to keep the arterial catheter open. Intravenous infusion of 150 mM glucose added [3H]-inulin (3.14 µCi/ml; Amersham, Buckinghamshire, UK; batch 137 or 139; specific activities 1.13 or 1.80 Ci/mmol) and LiCl (6 µmol/ml) were administered at 4 ml/hr for 15 min, followed by 1 ml/hr throughout the experiment. To prevent dehydration, the computer-driven pump was adjusted to deliver 6.75 ml/hr of 150 mM glucose before BFTZ administration. Thus, during equilibration and control periods, the total infusion rate was 8 ml/hr. After a 1-hr equilibration period and two control periods of 30 min each, i.v. infusion of BFTZ (25 µg/hr) or vehicle (0.5 µg/hr) was started. Urine was collected in 30-min periods throughout. Arterial blood samples of 200 µl were drawn 15, 105, 195 and 285 min after start of the first control period. Blood pressure and heart rate were measured continuously using Hugo Sachs (Hugo Sachs GmbH, Hugstetten, Germany) pressure and heart rate couplers. Signals were displayed on a Watanabe Instruments WR3101 Linearcorder Mark VII (Watanabe Instruments Corp., Tokyo, Japan).

The servo-control system. The system for simultaneous servo-control of water and sodium balance is a further development of the computer-driven system for servo-controlled fluid replacement that we have described and used previously (Burgess et al., 1993; Bak et al., 1993; Hasbak et al., 1994; Jonassen et al., 1995). From the bladder catheter, urine passed a Na⁺-sensitive electrode that performed one measurement of urinary [Na⁺] per second (NOVO-biocatalytic, Waltham, MA). Urine was collected in vials arranged on an autosampler placed on an electronic balance (Sartorious model LC 3200 D, Göttingen, Germany). The autosampler was operated by a photocell that allowed change of the vial without touching the balance. Data on urine production (weight on the scale) and urinary [Na⁺] were sampled continuously on an IBM compatible computer, which in turn controlled the infusion rates of two independent infusion pumps (Harvard model 22, Scandidact, Kvistgaard, Denmark) which delivered 150 mM glucose and 150 mM NaCl, respectively. Urinary output of sodium and fluid were integrated over 5 min, thus allowing a 5-min delay in changes of sodium and glucose infusion rates. The computer program was written in LabView (National Instruments, Hørsholm, Denmark) and developed in collaboration with Bio Data (Copenhagen, Denmark).

Before each experiment, the sodium electrode was carefully calibrated with standard solutions containing 2, 10, 50 and 100 mM NaCl in 5 mM KCl. When not in use, the electrode was perfused with 2 mM NaCl in 5 mM KCl. After the experiment, the computer calculated sodium excretion was compared with the sodium excretion based on measurements of urinary [Na⁺] by atomic absorption spectrophotometry.

Analytical procedures. Urine volumes were determined gravimetrically. Concentrations of Na⁺ and Li⁺ in plasma and urine were determined by atomic absorption spectrophotometry, using a Perkin-Elmer model 2380 atomic absorption spectrophotometer (Perkin-Elmer, Almed, Denmark). 3H-[H]-inulin concentrations in urine and plasma were determined by liquid scintillation counting, using a Packard Tri-Carb liquid scintillation analyzer, model 2250CA (Packard Instruments, Gieve, Denmark). Fifteen µl of the sample and 285 µl of water were mixed with 2.5 ml of Ultima Gold (Packard Instruments, Gieve, Denmark) before counting. Urine and plasma osmolalities were determined on a vapor pressure osmometer (model 5100C, Wescor Inc., Logan, UT).

Determination of whole kidney norepinephrine content. Rats were anesthetized with halothane/N₂O and both kidneys were extirpated and quickly frozen in isopentane on dry ice and stored at -80°C until analysis. Kidneys were thawed at 0°C in ice-cold 0.2 M perchloric acid (both kidneys/15 ml) and homogenized by an Ultra-Turrax T-25 homogenizer at maximum speed for 1 min. During homogenization, the temperature was kept at 0 to 4°C by an ice-cooling jacket. The homogenates were centrifuged at 10,000 × g for 10 min at 4°C, and the supernatant was stored at -20°C until analysis. Five-ml aliquots of supernatant were added 150 µl of 5 µM 3,4-dihydroxy-phenylalanine (Sigma, D 7012; Sigma Chemical Co., St. Louis, MO) as internal standard and catecholamines were extracted.
by conventional aluminum oxide extraction. Norepinephrine and 3,4-dihydroxy-benzylamine were separated and quantitated by high-performance liquid chromatography using electrochemical detection. The instrumentation consisted of a Hewlett-Packard TI-Series 1050 liquid chromatograph equipped with a 250 × 4 mm I.D. RP-18 Higbar, Supersphere LiChroCART (5 μm) RP-18 analytical column protected by a 20 × 4 mm I.D. guard column (both from E. Merck, GmbH, Germany). A 100-μl sample was injected and separation was performed at ambient temperature with the buffer described by Petersen and DiBona (1992), using 6.94 methanol:0.1 M KH2PO4, 0.1 mM EDTA and 4 mM heptane sulfonic acid as the ion pairing agent. Flow of mobile phase was 1.7 ml/min. Catecholamines were detected by a Hewlett-Packard programmable electrochemical detector (HP 1049A) by applying an oxidation potential of +750 mV. The analytical sensitivity for norepinephrine and DHBA was less than 0.5 pmol. The analytical coefficient of variation for norepinephrine was 1.6% within assay and 4.7% between assays.

**Calculations and statistics.** Renal clearances (C), fractional excretions (FE) and fractional reabsorptions (FR) were calculated by the standard formula:

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C = U \cdot V / P; \quad FE = C / GFR; \quad FR = 1 - C / GFR
\]

where V is the urine flow rate, U is the urine concentration, and P is the plasma concentration. Free water clearance was calculated as: \( V - C_{\text{uw}} \). \( C_{\text{uw}} \) was used as a marker for GFR and \( C_{Li} \) as marker for the delivery of tubular fluid from the pars recta of the proximal tubules (Thomsen 1990; Boer et al. 1995). Based on the assumption that in the present experimental setting, \( C_{Li} \) was a valid estimate of distal delivery (DD), the following expressions for the segmental water handling were calculated:

- Fractional proximal water excretion (FEprox): \( C_{Li} / C_{In} \)
- Fractional proximal water reabsorption (FRprox): \( 1 - C_{Li} / C_{In} \)
- Absolute proximal water reabsorption (APR): \( C_{In} - C_{Li} \)
- Fractional distal water excretion (FEdist): \( V / C_{Li} \)
- Fractional distal water reabsorption (FRdist): \( 1 - V / C_{Li} \)
- Absolute distal water reabsorption (ADR): \( C_{Li} - V \)

Overall statistical comparisons were performed by one-way analysis of variance (between groups), one-way analysis of variance for repeated measures (within group), or two-way analysis of variance for repeated measures for two-way classified data (group and time). Individual comparisons within or between groups were performed by subsequent use of Scheffe’s test for simultaneous multiple comparisons. Statistical analyses were performed using the program Statistica version 5.1 (Statsoft Inc., Tulsa, OK). Data are expressed as mean ± S.E.M. Differences were considered significant at \( P < .05 \).

**Results**

Figure 1 shows the time-course of changes in GFR, urine flow, urine osmolality, and free water clearance in the four groups, and figure 2 shows the simultaneous changes in lithium clearance and its derived variables. Before interventions, all renal variables were similar in the four groups, and all rats showed marked polyuria (V = 50-60 μl/min/100g body weight ~72-86% body weight/24 hr).

**Time control experiment (group 1).** In the vehicle-treated control group, where total body water and total body sodium were kept constant with the servo-control system, no significant changes were observed in GFR, urine flow rate, urine osmolality, free water clearance, \( C_{Li} \) or derived variables.
Antidiuretic response to BFTZ (group 2). DI-rats treated with BFTZ with servo-control of total water balance, but without replacement of sodium, showed a rapid and distinct antidiuretic response, which reached a steady state after 3 to 4 hr of BFTZ infusion. The urine flow rate was reduced to the level observed in normal rats and the urine became hypertonic. Free water clearance became slightly negative. This antidiuretic response to BFTZ was associated with marked decreases of GFR and CLi. There was a statistically significant decrease in fractional water excretion from the proximal tubules (CLi/CIn) as well as from the distal nephron (V/CLi).

Table 1 summarizes data on segmental tubular water handling at the time of maximal BFTZ-induced antidiuresis, i.e., during the final two clearance periods, in comparison with the time control group. The relative decrease in urine flow (-79%) exceeded the relative decrease in distal delivery (-62%) which in turn exceeded the relative decrease in GFR (-28%). Fractional water reabsorption, i.e., reabsorption as a percentage of the delivery, was increased both in the proximal tubules (from 72.5 ± 3.0% to 86.1 ± 2.5%; P < .01) and in the distal nephron (from 85.1 ± 1.0% to 90.7 ± 2.2%; P = .059) during maximal antidiuresis.

Effect of sodium replacement on the antidiuretic response to BFTZ (group 3). Replacement of BFTZ-induced sodium losses did not modify the BFTZ-induced changes in GFR, urine flow, urine osmolality or free water clearance (fig. 1). However, sodium replacement partly prevented the BFTZ-induced decrease in CLi and FELi (P , .001 vs. group 2; fig. 2). Thus, during maximal antidiuresis the increase in fractional proximal water reabsorption was decreased by sodium replacement [maximal FRprox: group 1: 72.5 ± 3.0%; group 2: 86.1 ± 2.5%; group 3: 78.5 ± 1.9%; (P < .001 vs. group 2)]. In contrast, sodium replacement had no effect on BFTZ-induced stimulation of fractional distal water reabsorption (1-V/CLi).

**TABLE 1**

| Segmental tubular water handling in control rats and in rats during BFTZ-induced antidiuresis; figures indicate mean values ± S.E.M. for the last two clearance periods; flow rates and reabsorption rates are expressed as μl/min/100 g body weight; fractional reabsorptions are expressed as % |
|---------------------------------------------------|-------------------|-----------------|-----------------|-------------------|
|                      | Group 1 Control | Group 2 BFTZ | Absolute Change | Relative Change |
|                      | (n = 7)          | (n = 9)        | Induced by BFTZ | Induced by BFTZ | P               |
| Filtered water (GFR) | 967 ± 34         | 701 ± 53       | -266            | -28%             | <.001           |
| Absolute proximal water reabsorption (GFR-Ci) | 700 ± 35         | 599 ± 42       | -101            | -14%             | .095            |
| Absolute distal water reabsorption (Cil-V)      | 227 ± 26         | 94 ± 22        | -133            | -59%             | <.001           |
| Fractional proximal water reabsorption (1-Cil/GFR)| 72.5 ± 3.0       | 86.1 ± 2.5     | +13.6%          | +19%             | .003            |
| Fractional distal water reabsorption (1-V/Cil)  | 85.1 ± 1.0       | 90.7 ± 2.2     | +5.6%           | +7%              | .059            |
| Urine flow (V)   | 39 ± 4           | 8 ± 1          | -31             | -79%             | <.001           |

Mean values ± S.E.M. are indicated. P refers to comparisons by unpaired t test.
Effect of renal denervation on the antidiuretic response to BFTZ (group 4). Chronic bilateral renal denervation did not affect BFTZ-induced changes in GFR, or renal tubular handling of sodium, lithium or water (figs. 1-3). The efficiency of renal denervation was examined by determination of norepinephrine in both kidneys after experiments. The norepinephrine content was 1020 ± 181 pmol/g kidney (n = 5) in control kidneys and not detectable (<2 pmol/g kidney weight; n = 5) in denervated kidneys.

Natriuretic response to BFTZ. Figure 3 shows the natriuretic response to BFTZ in the four groups. When sodium losses were not replaced (groups 2 and 4), low dose BFTZ infusion produced a weak and transient natriuretic response. FE\textsubscript{Na} increased from a baseline level of 0.5% to a peak value of 1.2% within 30 to 60 min and returned to control levels within 3 hours. When urinary sodium losses were replaced (group 3) the natriuretic response was augmented and preserved until the end of the 4-hr infusion of BFTZ, and the cumulated natriuresis was doubled. Table 2 indicates the changes in cumulated sodium balance and P\textsubscript{Na} in the four groups of rats after 4 hr infusion of BFTZ. In the time control group, cumulated sodium balance was not different from zero. Relative to cumulated sodium excretion, the degree of sodium replacement was 102 ± 8% in this group. In both groups where BFTZ were administered without sodium replacement, BFTZ induced significant cumulative sodium losses of about 0.5 mmol. In group 3 that received BFTZ with sodium replacement, the cumulated sodium balance was not different from zero, but significantly different from the negative sodium balance in group 2 (P < .05). The efficiency of the sodium servo-control system to maintain sodium homeostasis was also reflected in the plasma sodium concentration. Na replacement thus prevented the 4 to 5 mM decrease in P\textsubscript{Na} observed in groups 2 and 4 without sodium replacement.

Overall correlations. Figure 4 indicates correlations between individual changes in urine flow rate vs. changes in GFR (fig. 4A) and C\textsubscript{Li} (fig. 4B) in all four groups. The changes were calculated as the mean of the two last clearance periods minus the mean of the two control periods. Irrespective of treatment, changes in urine flow rate correlated significantly with changes in GFR (r = 0.75; P < .001) as well as with changes in C\textsubscript{Li} (r = .84; P < .001). Figure 5A shows that changes in FR\textsubscript{prox} (1-C\textsubscript{Li}/C\textsubscript{In}) correlated significantly with changes in GFR (r = 0.82; P < .001) whereas there was no significant correlation between changes in FR\textsubscript{dist} (1-V/C\textsubscript{Li}) and C\textsubscript{Li} in the four groups (fig. 5B).

Mean arterial blood pressure and hematocrit. During baseline conditions, MAP were similar in groups 1 to 3 (110 ± 1 mm Hg) but lower in group 4 (98 ± 3 mmHg; P < .05 vs. all other groups). Baseline HCT values were similar in the four groups. Administration of vehicle or BFTZ did not affect MAP or HCT in any group.

Discussion

The main purpose of this study was to elucidate the influence of sodium balance for the acute antidiuretic response to thiazide diuretics in rats with DI. The results indicate that a low dose of BFTZ, which only produced a weak natriuretic response, reduced free water clearance to zero and normalized urine flow rate within a few hours. By using a computer-driven, servo-control system that allowed independent control of total body sodium and water, the infusion rate of fluid could be reduced along with BFTZ-induced antidiuresis, and simultaneously, BFTZ-induced sodium losses could be accurately replaced by increasing the infusion rate of saline. These experiments clearly demonstrated that the antidiuretic response to BFTZ was preserved after replacement of BFTZ-induced sodium losses.

In two early studies, Shirley and coworkers (Shirley et al., 1978; Walter et al., 1979) examined the role of sodium balance by giving a maximal antidiuretic dose of HCTZ (25 mg/kg) s.c. to anesthetized Brattleboro rats kept in water balance by preoperative oral water loads, and after induction of anesthesia, by replacement of urinary water losses with 300 mM glucose. Sodium replacement was given as a fixed amount of isotonic saline during the first 10-min period and thereafter by adjusting the saline infusion rate to match urinary sodium losses as measured concurrently by flame photometry. In this experimental setting, sodium replacement completely prevented the HCTZ-induced decreases in GFR, distal delivery and urine flow rate, and in fact converted the antidiuretic response to an increase in urine flow. The reason for the discrepancy between our results and the early studies by Shirley and coworkers is difficult to establish with certainty, but major differences in experimental set-up could be of importance. Most importantly, the sodium and volume replacement technique used in the previous studies was not as accurate as the technique used in our study. The manual replacement technique used by Shirley et al. (1978)
involves a risk of extracellular fluid volume expansion, because of the delay in down-regulation of volume infusion along with the decrease in urine flow. Moreover, HCTZ, due to its action on the carbonic anhydrase enzyme, may exert an acute inhibitory effect on proximal tubular sodium reabsorption which has not been observed with BFTZ (Boer et al., 1989; Beyer and Baer, 1961). Finally, in contrast to the present experiments which were performed in chronically instrumented, conscious rats, the studies by Shirley and coworkers were performed during anesthesia and involved major acute surgical procedures which are known to affect renal function and the responses to diuretics (Petersen et al., 1991; Petersen et al., 1996). In our study, the accuracy of the computer-driven servo-control of total body sodium and water balances was confirmed by comparing the computer readings with the actual urinary output. In addition, the efficacy of the sodium servo to maintain sodium balance was indicated by the observation that the decrease in PNa in response to BFTZ administration was prevented when the servo-control loop was activated.

The second hypothesis for the antidiuretic action of thiazides in DI that was examined in this study was related to the possibility that sodium depletion associated with diuretic administration could elicit an increase in renal sympathetic nerve activity, similar to what has been observed after furosemide administration, which in turn could elicit the decrease in GFR and stimulate proximal tubular fluid reabsorption (Walter et al., 1979; Petersen et al., 1991; Petersen et al., 1996).
and DiBona, 1994). The present results showed that bilateral renal denervation did not modify the effects of BFTZ on GFR or tubular sodium and water handling in DI rats. The total degree of renal denervation was confirmed by the absence of norepinephrine in denervated kidneys. These data suggest that the renal nerves are not essential for the antidiuretic action of BFTZ in rats with DI. Considering that sodium depletion would be the stimulus for increased renal sympathetic nerve activity, the fact that neither sodium replacement nor renal denervation affected the antidiuretic response to BFTZ, strongly supports the notion that a volume homeostatic neurogenic reflex mechanism is not involved in the antidiuretic action of BFTZ in rats with DI.

Although our study did not provide any detailed information about the mechanisms responsible for the antidiuretic response to BFTZ in rats with DI, the data allowed us to dissociate the components of the antidiuresis, in terms of changes in filtered and segmentally reabsorbed water. During maximal antidiuresis, urine flow rate was reduced by 79%, associated with a 28% decrease in GFR and a 62% decrease in distal delivery (C_{\text{Li}}). Fractional water reabsorption was increased from 73 to 86% in the proximal tubules and from 85 to 91% in the distal nephron (table 1). In comparison, Shirley and coworkers (Shirley et al., 1978; Walter et al., 1979) reported that acute HCTZ administration to anesthetized rats with DI caused a 40% reduction in effective renal blood flow, a 36% reduction in GFR and a 50% reduction in distal delivery, as measured by micropuncture. Thus, the available data indicate that the acute antidiuretic response to thiazide diuretics in DI is elicited primarily by a reduction in GFR and hence in the delivery of tubular fluid to the renal tubules, with only a moderate increase in fractional water reabsorption. This is consistent with the findings of previous studies in normal rats (Leyssac et al., 1994) and DI rats (Grønbeck et al., 1997), which showed that acute thiazide administration to DI rats causes a 40% reduction in effective renal blood flow, a 36% reduction in GFR and a 50% reduction in distal delivery, as measured by micropuncture.

The mechanism by which low-dose BFTZ-infusion in our study decreased the GFR in vasopressin-deficient rats is not known. Because BFTZ has no direct effect on fluid reabsorption in the proximal tubules, it would not be expected to activate the tubulo-glomerular feedback mechanism, as has been demonstrated for acetazolamide (Leyssac et al., 1994). It is noteworthy, however, that the fall in GFR occurred without any changes in MAP and that it was not prevented by sodium replacement or renal denervation. Interestingly, acute administration of a 10-fold higher dose of BFTZ (250 \mu g/hr) had no discernible effect on the GFR in normotensive rats (Jonassen et al., 1995). Whatever the mechanism, these data suggest a specific role for vasopressin in maintaining the GFR during acute administration of BFTZ. In contrast, chronic administration of BFTZ (2 mg daily) or HCTZ (7 mg daily) did not influence the GFR in rats with vasopressin-deficient DI (Grønbeck et al., submitted; Shirley et al., 1983). It therefore seems that the action of thiazide diuretics on the GFR in rats with DI is biphasic: An initial decrease in GFR, followed by return to normal during prolonged therapy.

Another component of the antidiuretic response to BFTZ-infusion was the significant increase in FR_{\text{prox}} that correlated with the reduction in GFR and was partly prevented by sodium replacement. Similar opposite changes in GFR and FR_{\text{prox}} have been observed in normal rats in response to partial constriction of the aorta above the renal arteries (Thomsen et al., 1981) or administration of cyclosporin A (Dieperink et al., 1986). Thus, the increase in FR_{\text{prox}} observed in this study may be considered as a normal intrarenal adaptation to the fall in GFR. However, it should be mentioned that even in absence of changes in GFR, chronic thiazide treatment stimulates fluid reabsorption in the proximal tubules (Thomsen and Schou, 1973; Walter and Shirley, 1986; Lunau et al., 1994), and the resultant decrease of C_{\text{Li}} is the mechanism behind the clinically important drug interaction between thiazide diuretics and lithium (Petersen et al., 1974). Therefore, the well-documented fall in distal delivery (and C_{\text{Li}}) observed during chronic thiazide administration seems to be elicited by an increase in absolute proximal water reabsorption, rather than by a fall in GFR.

The third component of the antidiuretic response to BFTZ was an increase in fractional distal water reabsorption, which was independent of changes in distal delivery of tubular fluid, sodium balance and renal nerve activity. In relative terms, FR_{\text{dist}} increased only from 85 to 91% of distal delivery. However, even small changes in fractional distal water reabsorption will affect the urine flow rate considerably and the generation of hypertonic urine is due to relatively small amounts of water being reabsorbed in the medullary collecting ducts. The mechanism underlying this flow-independent increase of distal water reabsorption during thiazide treatment is not known. In a recent study we found that chronic BFTZ administration does not produce any changes in total expression or intracellular distribution of the vasopressin-stimulated water channels (AQP2) in the collecting ducts of rats with vasopressin-deficient DI (Grønbeck et al., submitted). In absence of vasopressin, an increase in fractional distal water reabsorption could be due to enhancement of the renal corticomedullary osmotic gradient. This explanation was proposed by Shirley et al. (1982) who reported that the interstitial osmolality in the papilla rose from 451 to 692 mosmol/kg during long-term administration of HCTZ. However, the mechanism by which thiazide treatment per se might increase the corticomedullary osmotic gradient in DI rats is unclear, and the above mentioned study did not provide conclusive evidence as to whether the increased papillary interstitial osmolality in rats with DI was a cause or a consequence of the antidiuresis. In normal rats, thiazide treatment has no discernible effect on medullary interstitial osmolality (Baer et al., 1962; Walter and Shirley, 1986). In fact, HCTZ was shown to impair maximal urinary concentrating ability in Wistar rats in response to water deprivation (Steven and Skadhauge, 1969). Thus, although thiazide diuretics have been used for the treatment of DI for more than thirty years, the mechanism by which these drugs enhance tubular water reabsorption is still elusive.

In conclusion, the antidiuretic response to acute low-dose administration of BFTZ in rats with vasopressin-deficient DI is preserved in rats with chronic bilateral renal denervation and during i.v. replacement of BFTZ-induced sodium losses. These results suggest that the antidiuretic action of BFTZ in rats with DI is not mediated by a volume homeostatic neurogenic reflex mechanism. Acute low-dose administration of BFTZ produced a decrease in GFR associated with increased fractional water reabsorption both in the proximal tubules and in the distal nephron segment. Although in the proximal
tubules, the increase in fractional water reabsorption was related to the reduced delivery of glomerular filtrate, the increased fractional distal water reabsorption was independent of the changes in distal delivery. We suggest that BFTZ exerts a stimulatory effect on distal water reabsorption which is independent of changes in sodium balance, tubular flow rate, renal nerve activity and vasopressin.

References


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