The role of sodium depletion in hydrochlorothiazide-induced antidiuresis in Brattleboro rats with diabetes insipidus

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Summary
1. The mechanism of the antidiuretic effect of hydrochlorothiazide in diabetes insipidus was studied in anaesthetized Brattleboro rats with the hereditary hypothalamic form of the disease.
2. The antidiuresis caused by acute administration of hydrochlorothiazide followed an increase in sodium excretion and was associated with a significant fall in the plasma sodium concentration. There were concomitant falls in effective renal plasma flow and glomerular filtration rate.
3. When sodium depletion was prevented by adjusting the infusion of sodium chloride, the falls in plasma sodium concentration, effective renal plasma flow and glomerular filtration rate were abolished. Under these circumstances there was an increase in urine volume, which suggests that hydrochlorothiazide may inhibit fractional fluid reabsorption in the proximal convoluted tubule.
4. The results indicate that the antidiuresis caused by hydrochlorothiazide in diabetes insipidus results, at least in part, from falls in effective renal plasma flow and glomerular filtration rate. These in turn seem to be entirely secondary to the drug-induced sodium depletion.

Key words: antidiuresis, diabetes insipidus, hydrochlorothiazide, sodium depletion.

Abbreviations: GFR, glomerular filtration rate; ERPF, effective renal plasma flow.

Introduction
In 1959 Crawford & Kennedy showed that thiazide diuretics had a paradoxical antidiuretic effect in diabetes insipidus, and since then thiazides have been used successfully in the treatment of patients with either the pituitary or nephrogenic form of the disease. However, the mechanism by which thiazides exert their antidiuretic effect has never been fully elucidated. Although it is often stated that the antidiuresis is secondary to a drug-induced sodium depletion (Earley & Orloff, 1962; Skadhauge, 1966), experiments designed to test this, by combining thiazide treatment with the oral administration of sodium in an attempt to prevent the antidiuresis, have yielded conflicting results (Havard & Wood, 1961; Kennedy & Hill, 1963; Ramos, Rivera, Peña & Dies, 1967). It has been suggested, moreover, that thiazides may have a direct effect on the renal vasculature, causing a reduction in renal blood flow (Havard & Wood, 1961; Ludens & Williams, 1970) and in glomerular filtration rate (Krause, Dume, Koch & Ochwadt, 1967; Fernandez & Puschett, 1973), which may account for at least some of the antidiuretic effect of these drugs. A reduction in glomerular filtration rate after thiazide administration is not, however, universally accepted (Earley & Orloff, 1962; Gillenwater, 1965; Ramos et al., 1967).

Previous investigations into the mechanism of action of thiazides have involved the participation of patients with diabetes insipidus (Harvard & Wood, 1961; Earley & Orloff, 1962; Kennedy & Hill, 1963; Ramos et al., 1967) or have used...
animals in which the condition has been induced by electrolytic lesions of the hypothalamus (Gillenwater, 1965; Skadhauge, 1966). All these studies are open to criticism in that the replacement of sodium during thiazide treatment has always been attempted merely by increasing the dietary intake, a manoeuvre which is inevitably rather imprecise. In addition the possibility that the results of those studies in animals with surgically induced diabetes insipidus may have been adversely influenced by factors associated with the surgery, such as damage to surrounding hypothalamic tissue or a reduction in glomerular filtration rate (Gillenwater, 1965), cannot be excluded. This problem can readily be overcome by the use of Brattleboro rats, which have a hereditary hypothalamic defect involving the inability to synthesize vasopressin.

In the present investigation we have re-examined the role of sodium depletion in relation to the antidiuretic effect of hydrochlorothiazide using anaesthetized Brattleboro rats with diabetes insipidus, infused intravenously so that sodium balance could be accurately adjusted. Some of these results have appeared in a preliminary communication (Laycock, Shirley & Water, 1977).

Materials and methods

Animals

Male and female Brattleboro rats with hereditary hypothalamic diabetes insipidus, weighing 180–240 g, were used in all experiments. Animals had free access to water and to a diet (Dixon’s FFG-M diet) containing Na 200 mmol/kg and K 184 mmol/kg.

Conscious rat experiments. In preliminary experiments to determine a suitable dose of hydrochlorothiazide, animals were placed in individual metabolism cages, with free access to food and water. After a habituation period of 48 h, each animal received a single subcutaneous injection of the sodium salt of hydrochlorothiazide (Merck, Sharp and Dohme) in doses ranging from 0.25 to 5.0 mg/100 g body weight (0.8–1.6 mmol/100 g) delivered in 0.2 ml of isotonic sodium chloride solution (155 mmol/l; saline). Urine was then collected for 9 h and the volumes were compared with those of control animals given a single subcutaneous injection of 0.2 ml of saline.

Anaesthetized rat experiments. Experimental animals were divided into three groups of eight rats (four male, four female). In order to prevent the volume depletion which would otherwise result from urinary water losses during subsequent surgery, each animal received two water loads (each 4 ml/100 g body weight) by gavage, separated by a 30 min interval. Ten minutes after the second water load, rats were anaesthetized by intraperitoneal injection of Inactin (Promonta, Hamburg) at a dose of 100 mg/kg body weight (0.4 mmol/kg). Thereafter rectal temperature was maintained at 37°C by means of a thermostatically controlled heating table. A tracheostomy was performed and the bladder catheterized. The arterial blood pressure was monitored via a catheter placed in the femoral artery; this catheter was also used to obtain arterial blood samples. Two catheters were inserted into a jugular vein. Unless otherwise stated, saline was infused through one jugular catheter at 1 ml/h and glucose solution (100 mmol/l) (Schnermann, Valtin, Thurau, Nagel, Horster, Fischbach, Wahl & Liebau, 1969) through the other, the rate of glucose infusion being adjusted so that the total volume of infusate equalled that of the urine.

Priming doses of 10 μCi of [3H]methoxy inulin and 6 μCi of p-aminom-[14C]hippuric acid (New England Nuclear Corp.) were given intravenously, followed by a sustaining infusion (contained within the saline) of 8 μCi of [3H]methoxy inulin and 12 μCi of p-aminom-[14C]hippuric acid/h.

After equilibration for 1 h, urine was collected in pre-weighed pots for a further hour, during which inulin and p-aminohippurate clearances were determined over two periods of 10 min. Blood samples (~80 μl) were obtained from the femoral artery at the mid-point of each clearance period.

Group 1 (control) animals were then given a subcutaneous injection of 0.2 ml of saline, and their infusion was maintained as before; rats of group 2 and group 3 each received a single subcutaneous injection of hydrochlorothiazide at a dose of 2.5 mg/100 g body weight (8 mmol/100 g) given in 0.2 ml of saline. Rats of group 2 continued to receive saline at a rate of 1 ml/h throughout the following 3 h. However, the rate of infusion of saline into group 3 rats was continuously adjusted in an attempt to ensure that the amount of Na infused equalled that excreted. The volume of saline to be infused in the first 10 min after the drug was estimated from a pilot experiment. Thereafter the rate of saline infusion during each 10 min period was adjusted so that the Na input matched the urinary Na excretion of the previous period.

In addition to the saline infusion, the rats of group 2 and group 3 each received an infusion of glucose (300 mmol/l). The rate of glucose infusion was again adjusted so that the total volume of fluid
infused in any period was the same as the volume of urine excreted. No glucose was detected in any urine sample. Urine was collected over 10 min periods for 3 h after the injections, and inulin and p-aminohippurate clearances were determined at the mid-point of each of the 3 h periods. Any experiments in which the mean blood pressure fell below 100 mmHg at any time were discounted.

Analyses

The Na and K concentrations of the urine, and the Na concentration of plasma samples, were measured with a dual-channel integrating flame photometer (Evans Electroselenium Ltd). Urine osmolality was measured cryoscopically (Advanced Instruments). Urine and plasma [3H]inulin and p-amino[14C]hippurate concentrations were determined by adding portions to Ready-Solv scintillation cocktail (Beckman Instruments Inc.), and radioactivity counting in a Packard Tri-Carb liquid scintillation spectrometer; no quench correction was necessary. Appropriate corrections for spill-over from the 14C channel into the 3H channel were made.

Values in the periods before the drug was given were compared with those in periods after the drug in the same animal by paired t-tests, unless otherwise stated. Values are given as mean ± 1 SEM.

Results

Experiments on conscious rats

The dose–response curve shows single injections of hydrochlorothiazide to have a marked, dose-dependent antidiuretic action in Brattleboro diabetes insipidus rats (Fig. 1). A dose of 2.5 mg/100 g body weight (8 μmol/100 g) was chosen to be administered in all subsequent clearance studies on anaesthetized animals.

Clearance studies on anaesthetized rats

Urine volume and osmolality, and urinary Na and K excretion were determined during the two half-hour periods immediately preceding the injection of either saline or hydrochlorothiazide, and for the following six half-hour periods. In addition, plasma Na and the clearances of inulin and p-aminohippurate were determined during the control period (average of two values per rat), and midway through each of the 3 h after the injection. During the control (pre-injection) hour there was no significant difference between control (group 1) rats and the rats (group 2 and group 3) subsequently given subcutaneous injections of hydrochlorothiazide, for any of the variables measured.

Urine volume and osmolality (Fig. 2). In control (group 1) rats injected with 0.2 ml of saline, urine volume and osmolality remained steady until the third hour after the injection, when there was a slight fall in urine volume (P < 0.05) and a rise in urine osmolality (P < 0.05).

In group 2 rats injected with hydrochlorothiazide [2.5 mg/100 g body weight (8 μmol/100 g)], although urine volume did not change significantly in the first post-injection hour, it thereafter...
fell markedly \((P < 0.001\) for each period). The hydrochlorothiazide caused an immediate rise in urine osmolality, which reached a value of 298 ± 28 mosmol/kg in the final period under study.

Increases in urine osmolality in group 3 rats (injected with hydrochlorothiazide, but given Na replacement) were similar to those of group 2 animals. However, in group 3 animals there was an increase in urine volume after hydrochlorothiazide treatment, which was statistically significant during the first \((P < 0.001)\), second \((P < 0.001)\) and third \((P < 0.02)\) post-injection periods.

**Urinary Na and K excretion** (Fig. 3). The only change observed in control (group 1) rats was a slight increase \((P < 0.05)\) in Na excretion in the first hour. In group 2 animals, rapid but transient rises in urine Na and K excretion were observed in response to hydrochlorothiazide injection, with both Na and K excretion returning to values indistinguishable from pre-injection values within 2 h of the injection.

Rapid rises in urine Na and K excretion were also observed in group 3 animals. The increase in Na excretion was significantly greater than that measured in group 2 animals, even during the first post-injection period \((P < 0.05,\) unpaired \(t\)-test). Furthermore, although by the third hour K excretion was not significantly different from pre-injection values, Na excretion remained greatly elevated. Even in the final period under study, the amount of Na excreted was 4-6-fold the control value. It is thus clear that replacement of the sodium losses incurred as a result of hydrochlorothiazide treatment effectively prevented any fall in GFR and ERPF.

Plasma sodium concentrations (Table 1). The Na concentrations of plasma removed during the inulin and \(p\)-aminohippurate clearance periods were determined in six animals from each group. There were no significant changes throughout the period of study in group 1 or group 3 animals. In marked contrast, in group 2 rats the plasma Na

**Glomerular filtration rate and effective renal plasma flow** (Fig. 4). In control animals (group 1) there was a slow decline in both glomerular filtration rate (GFR; measured by inulin clearance) and effective renal plasma flow (ERPF; \(C_{\text{PAH}}\)) during the period of study, the decrease in GFR becoming significant during the third post-injection hour \((P < 0.002)\). By contrast, in group 2 animals there were marked falls in GFR and ERPF after the hydrochlorothiazide injection, these being significant even within the first hour \((P < 0.05, P < 0.02\) respectively).

Inulin and \(p\)-aminohippurate clearances in group 3 rats were remarkably similar to those in group 1 and there was no significant difference at any time between the two groups of animals. It is thus clear that replacement of the sodium losses incurred as a result of hydrochlorothiazide treatment effectively prevented any fall in GFR and ERPF.

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**Fig. 3.** (a) Sodium and (b) potassium excretion (mean values ± SEM) in Brattleboro diabetes insipidus rats after a single subcutaneous injection of saline (control animals), hydrochlorothiazide or hydrochlorothiazide with intravenous Na replacement. For further details see the legend to Fig. 2.

**Fig. 4.** (a) Glomerular filtration rate (GFR) and (b) \(p\)-aminohippurate clearance \((C_{\text{PAH}})\) in Brattleboro diabetes insipidus rats after a single subcutaneous injection of saline (control animals), hydrochlorothiazide \((\Delta)\) or hydrochlorothiazide with intravenous Na replacement \((\triangle)\). Values are given as mean ± SEM. Injections (0.2 ml in all cases) were given at arrow. The dose of hydrochlorothiazide was 2.5 mg/100 g body wt. \(C_{\text{PAH}}\) was measured in only five rats from each group.
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TABLE 1. Plasma sodium concentrations
Values are given as mean ± SEM. The number of rats in each group was six. For further details see the legend to Fig. 2. Significantly different from pre-injection hour (paired t-test): *P < 0.05; **P < 0.01.

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<th>Pre-injection hour</th>
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<td>Hour 1</td>
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<tr>
<td>Control</td>
<td>152 ± 4</td>
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<tr>
<td>Hydrochlorothiazide</td>
<td>148 ± 2</td>
<td>143 ± 4</td>
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<tr>
<td>Hydrochlorothiazide + Na replacement</td>
<td>153 ± 3</td>
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Concentration had fallen significantly in the second (P < 0.05) and third (P < 0.01) hours after hydrochlorothiazide injection.

Discussion

There is still a great deal of controversy regarding the precise site(s) of action of thiazide diuretics (Thurau, 1966; Clapp & Robinson, 1968; Kunau, Weller & Webb, 1975). However, because they are known to reduce free water clearance, it is thought that their principal effect is to inhibit salt reabsorption in some region of the so-called 'diluting segment' of the nephron. Such an effect, which could involve the ascending limb of the loop of Henle, the distal convoluted tubule or the collecting duct, would adequately explain the increase in urine osmolality after hydrochlorothiazide administration, but could not in itself account for the antidiuretic response after hydrochlorothiazide injection.

Several previous studies have investigated the mechanism of this antidiuretic effect of thiazides, but such an explanation was not clearly demonstrated. In view of the antidiuretic effect of chlorothiazide in vasopressin-resistant diabetes insipidus (Crawford & Kennedy, 1959) and of the report by Earley & Orloff (1962) that chlorothiazide does not inhibit osmotic water movement across a vasopressin-sensitive membrane, it seems unlikely that thiazides have an action like antidiuretic hormone on the distal nephron. Nor can their antidiuretic effect be explained by an increase in aldosterone secretion (Gillenwater, 1965; Skadhauge, 1966) or by an effect on the medullary osmotic gradient (Baer, Brooks, Noll & Beyer, 1962). On the basis of indirect evidence, it has been suggested by several investigators (Earley & Orloff, 1962; Gillenwater, 1965; Skadhauge, 1966) that the antidiuretic effect of thiazides is secondary to the natriuresis and consequent Na depletion induced by the drugs, though how such a Na depletion would produce an antidiuresis is not clear.

Our investigation differed from previous studies in that we gave hydrochlorothiazide as a single subcutaneous injection, rather than administering it chronically over several days. In preliminary experiments on conscious animals we found that the acute administration of hydrochlorothiazide (2.5 mg/100 g body weight) produced a marked antidiuresis in diabetes insipidus rats within a few hours. We were therefore able subsequently to assess directly the role of Na depletion in the antidiuretic response by using anesthetized diabetes insipidus rats which were either allowed to become Na depleted as a result of the natriuretic effect of the drug, or were kept in Na balance after hydrochlorothiazide injection by the addition of sodium chloride to the infusion.

In the anesthetized animals, a single injection of hydrochlorothiazide resulted in a considerable reduction in urine flow and an increase in osmolality (Fig. 2). When Na depletion was prevented, however, not only was the antidiuretic effect of the drug abolished, but there was an increase in urine volume for the first 90 min. This indicates, first, that the acute antidiuretic effect of hydrochlorothiazide in diabetes insipidus rats must be entirely dependent on Na depletion, and secondly, that the direct (natriuretic) effect of hydrochlorothiazide may not be limited to the diluting segment of the nephron. Because of the minimal volume of water reabsorbed from the distal nephron during maximal water diuresis, the urine flow rate in animals with diabetes insipidus is related to the volume of unreabsorbed fluid leaving the proximal tubule. Provided that GFR remains unchanged, therefore, a rise in urine volume should indicate reduced fluid reabsorption in the proximal tubule. Some studies have already suggested that certain thiazide diuretics may inhibit reabsorption
in the proximal tubule (Edwards, Baer, Sutton & Dirks, 1973; Fernandez & Puschett, 1973; Kunau et al., 1975), although others contradict this (Berliner, Dirks & Cirksena, 1966; Lant, Baba & Wilson, 1967). However, in the two last-named studies urinary Na losses were not replaced, so that a consequent extracellular fluid volume reduction, resulting in increased fractional fluid reabsorption (Weiner, Weinman, Kashgarian & Hayslett, 1971), could not be discounted. Our results with hydrochlorothiazide strongly suggest an inhibitory effect on the proximal tubule, though they cannot be considered conclusive, for the raised osmolality of fluid in the collecting duct after hydrochlorothiazide will reduce the osmotic gradient across the collecting duct wall, which could affect the small amount of water reabsorption known to occur here even in the absence of antidiuretic hormone (Jamison, Buerkert & Lacy, 1971).

As would be expected, the administration of hydrochlorothiazide to diabetes insipidus rats caused a marked increase in Na excretion. However, only if the urinary Na losses were replaced was this increased Na excretion maintained throughout the period of study: in group 2 animals, in which urinary Na losses were not replaced, the consequent Na depletion resulted in a return of Na excretion to control values within 2 h of the injection. The natriuresis after hydrochlorothiazide injection was associated with a small increase in K excretion in each group, which may indicate that the region of the nephron in which hydrochlorothiazide inhibits salt reabsorption is proximal to the site of K secretion. Since no attempt was made to balance this extra K loss, animals given hydrochlorothiazide underwent a slightly greater K depletion than did control animals, as K was absent from all infusion fluids. However, it is unlikely that this contributed to the antidiuresis of group 2 rats, since group 3 animals, which were more K depleted, had no antidiuresis.

It is clear that the Na losses in group 2 animals must have significantly reduced body Na. With established values used for extracellular Na in diabetes insipidus rats (Friedman & Friedman, 1965), and a normal relationship assumed between extracellular Na and total exchangeable Na, the total amount of exchangeable Na in group 2 rats was reduced by 5-5% 1 h after hydrochlorothiazide injection, and by 7-0% after 2 h, but there was then no further loss. Paralleving these acute changes in body Na, plasma Na concentration had fallen significantly in the second and third hours. Although total body water was kept constant, the fall in extracellular Na would presumably result in a redistribution of body water so that extracellular fluid volume would also be reduced. Thus although there can be little doubt that the antidiuresis was a consequence of Na depletion it is impossible to determine from these results whether the reduction in plasma Na concentration played a role per se, or whether the antidiuresis resulted solely from the shrinkage in extracellular fluid volume.

Since the distal nephron in diabetes insipidus rats has only a limited permeability to water, the antidiuresis observed after hydrochlorothiazide administration to group 2 animals must reflect a reduction in the delivery of filtrate from the proximal nephron. This could result from either a reduction in GFR or an increase in fractional reabsorption in the proximal tubule, or from a combination of both. We found that in group 2 rats there were significant reductions in ERPF and GFR only 30 min after the hydrochlorothiazide injection (Fig. 4), at which time total exchangeable Na had been reduced by 2-9%. On the other hand, when Na depletion was prevented, no changes in ERPF and GFR were observed. These findings make it unlikely that hydrochlorothiazide has a direct effect on renal plasma flow or GFR; the observed decreases in group 2 rats are more likely to result from the Na depletion induced by the drug.

There is little doubt that the observed falls in GFR could account for much of the antidiuresis. An effect on GFR, however, may not be the complete explanation. Micropuncture studies have shown that severe, chronic Na depletion can result in significant increases in proximal tubular reabsorption (Weiner et al., 1971; Stein, Osgood, Boonjarern, Cox & Ferris, 1974). Without micropuncture evidence, however, it is not possible to determine whether an increase in fluid reabsorption by the proximal tubule contributed to the antidiuresis observed in our studies.

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References


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