The antidiuretic effect of chronic hydrochlorothiazide treatment in rats with diabetes insipidus: renal mechanisms

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Summary

1. Brattleboro rats with hereditary diabetes insipidus were maintained in metabolism cages for 12–14 days. During the final 5–7 days hydrochlorothiazide was added to the food of half the animals, resulting in a sustained antidiuresis. At the end of this time all rats were anaesthetized and their renal function was investigated.

2. Water, sodium and potassium excretion rates during anaesthesia were similar to their respective values during the final period in metabolism cages.

3. Total glomerular filtration rate and superficial nephron filtration rate were similar in untreated and thiazide-treated animals. Fractional fluid reabsorption in proximal convoluted tubules, as measured by tubular fluid/plasma inulin concentration ratios in late surface convolutions, was moderately increased in the thiazide-treated rats, and was associated with a small reduction in the volume of fluid delivered to more distal nephron segments.

4. The osmolality of renal papillary interstitial fluid from thiazide-treated rats was considerably greater than that from untreated animals. There was also a small increase in papillary fluid sodium concentration.

5. It is concluded that the mechanism of the sustained antidiuresis during chronic hydrochlorothiazide administration in diabetes insipidus differs from that of the acute response. The changes in proximal tubular function during chronic thiazide treatment only partially account for the reduction in urine volume; it seems probable that the raised papillary osmolality, by enhancing water reabsorption at sites beyond the proximal tubule, makes a greater contribution to the antidiuresis.

Key words: antidiuresis, diabetes insipidus, hydrochlorothiazide, kidney papilla, kidney proximal tubule.

Abbreviation: DI, diabetes insipidus.

Introduction

The antidiuresis which results from the administration of thiazide diuretics to patients or animals with diabetes insipidus is poorly understood. Not only has there been uncertainty regarding the role of sodium depletion in mediating the response (examined in the preceding paper [1]), but the renal mechanisms themselves have been, until recently, largely a matter for speculation [2]. Using micropuncture techniques in rats with diabetes insipidus (DI), we have demonstrated previously that the acute antidiuretic response to hydrochlorothiazide results from a fall in glomerular filtration rate (GFR) accompanied by a rise in fractional reabsorption by proximal tubules. These events lead to a marked reduction in the delivery of fluid to more distal nephron segments [3].

The present experiments were designed to investigate the renal mechanisms operating during longer-term treatment with hydrochlorothiazide in Brattleboro rats with hereditary DI. Although it is often assumed that any reduction in urine flow in DI results solely from a reduced delivery of fluid to the distal nephron [2], there is little doubt that some water reabsorption occurs in the collecting duct even in the absence of
antidiuretic hormone [4], and the extent of this reabsorption will be dependent on the osmolality of the medullary and papillary interstitium. In the present study, therefore, in addition to measuring proximal tubular function, we examined papillary interstitial fluid solute concentrations. Some of these results have appeared in a preliminary communication [5].

Materials and methods

Male Brattleboro rats with hereditary hypothalamic diabetes insipidus, weighing 220–270 g, were used. Until the day of the experiment, all animals were housed in metabolism cages (Metabowl; Jencons Ltd, Leighton Buzzard, U.K.) for a period of 12–14 days, with free access to distilled water and food (CRM diet; Labsure Foods, Poole, U.K.). For the final 5–7 days, hydrochlorothiazide (Merck, Sharp and Dohme) was added to the food of half the rats (thiazide-treated rats) at a dose of 350 mg/kg dry weight (1.17 mmol/kg), as previously described [11], which resulted in a sustained antidiuresis. The other animals remained on the control diet (control rats).

At 08.00–08.30 hours on the day of the experiment, each rat was given a water load by stomach tube, the volume administered being equal to the hourly water intake of the animal during the previous 24 h. Fifteen minutes later, animals were anaesthetized with Inactin (Promonta, Hamburg), delivered intraperitoneally at a dose of 100–120 mg/kg body weight (0.4–0.5 mmol/kg), and were prepared for micropuncture essentially as described previously [31]. In all animals isotonic sodium chloride solution (154 mmol/l; saline) was infused throughout at a rate of 1.3 ml/h, whilst glucose solution (150 mmol/l: control rats; 300 mmol/l: thiazide-treated rats) was infused through a second jugular catheter. The glucose infusion, which was begun as soon as it was estimated that the water load had been excreted, was adjusted so that the total infusion rate matched the urine flow rate.

After completion of operative procedures, all animals received intravenously 1 ml of saline, for replacement of surgical losses, followed by $^{3}H$inulin (The Radiochemical Centre, Amersham, U.K.) as previously described [3]. The saline infusion of five of the nine thiazide-treated rats contained hydrochlorothiazide at a concentration (0.6 mmol/l) which provided an equivalent dose to that received in the food before anaesthesia. The results from these animals were indistinguishable from those of the four thiazide-treated rats which did not receive a hydrochlorothiazide infusion.

Halfway through a 1 h equilibration period, lissamine green (50 µl of a 5% solution) was injected intravenously, for determination of proximal tubule transit time [6] and identification of distal tubule segments.

After the equilibration period, urine was collected for 3 h, during which time inulin clearance was determined three times and collections were made from late surface loops of proximal tubules [3]. In most rats, a timed collection from a distal tubule was also made. In all cases the site of collection was confirmed by injection of Microfil (Canton Biomedical Products, Boulder, CO, U.S.A.) and subsequent dissection [3].

Small femoral arterial blood samples (~40 µl), for measurement of plasma inulin concentration, were taken at the midpoint of each clearance determination and at the start and end of the micropuncture period.

Immediately all micropuncture had been completed, a second proximal tubular transit time was measured, and 15 min later the hilum of the left kidney was clamped, and the kidney quickly removed. The papilla was exposed by an eccentric longitudinal section, excised, and a sample of papillary interstitial fluid obtained by a modification of the method of sequential centrifugation [7]. The papilla was centrifuged, under cooled paraffin oil, at 200 g for 10 min, then at 2000 g for a further 10 min. Centrifugation began within 40 s of clamping the renal hilum.

Immediately the hilum had been clamped, a large arterial blood sample (~1 ml) was taken for measurement of packed cell volume and analysis of plasma.

Papillary fluid samples were also obtained from Brattleboro rats not used for micropuncture studies. These rats were not anaesthetized, but were killed by stunning and exsanguination, the left kidney being removed immediately after stunning.

Analyses and calculations

The osmolality and Na and K concentrations of urine and plasma samples, the packed cell volumes of blood samples and plasma protein concentrations were measured as described previously [1]. The Na and K concentrations of tubular and papillary fluid samples were determined with a helium glow photometer (Aminco, Silver Spring, MD, U.S.A.), and papillary fluid
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Measurements and calculations for the determination of total and nephron filtration rates were performed as previously described [3]. Fluid delivery from the proximal convoluted tubule to more distal nephron segments (‘distal delivery’) was measured with calibrated constriction pipettes. Absolute proximal tubular fluid reabsorption was taken to be the difference between nephron filtration rate and ‘distal delivery’.

It was assumed that in addition to Na⁺ and K⁺ and their associated anions, the only solute present in significant quantities in papillary interstitial fluid was urea, and ‘urea concentration’ was therefore calculated according to the equation:

\[
\text{urea concentration (mmol/l)} = \frac{\text{osmolality (mosmol/kg of water)}}{2(\text{[Na}^+ \text{]} + \text{[K}^+ \text{]})} \text{ (mmol/l)}
\]

Statistical significance was determined by Student’s unpaired t-test. All values are given as means ± SEM.

Results

Overall renal function and blood pressure

Throughout the period of micropuncture, urine flow rate in hydrochlorothiazide-treated rats was considerably lower than in untreated (control) animals, and urine osmolality was correspondingly higher (Fig. 1). Sodium excretion from the left kidney was 40 ± 13 and 42 ± 9 μmol/h in control and thiazide-treated rats respectively; potassium excretion was 58 ± 4 and 52 ± 2 μmol/h. Na and K excretion rates were not significantly different between the two groups.

Although these figures apply to the micro-punctured (left) kidney, values for urine volume and solute excretion from the non-punctured right kidney were similar to those from the left. Overall excretion rates were similar to values obtained when the animals were housed in metabolism cages before anaesthetization [1].

Mean arterial blood pressure was the same in the two groups of animals, being 114 ± 2 and 114 ± 4 mmHg in control and thiazide-treated rats respectively.

Proximal tubular function

Values for total kidney GFR, superficial nephron filtration rate and proximal tubular reabsorption are shown in Table 1. It can be seen that in contrast to the marked fall in GFR which occurs during the period immediately after thiazide administration [3], neither total GFR nor single nephron GFR was reduced in animals given hydrochlorothiazide chronically. Nor was there a significant difference between the two groups when nephron filtration rate was determined from distal tubular collections (control rats: 32.2 ± 1.8 nl/min, n = 8; thiazide-treated rats: 31.4 ± 1.9 nl/min, n = 8). Fractional fluid reabsorption in the proximal convoluted tubule, measured by tubular fluid/plasma inulin concentration ratios in late surface convolutions, was significantly increased in thiazide-treated rats, and there was a small but significant reduction in the volume of fluid delivered to nephron segments beyond the proximal convoluted tubule (‘distal delivery’, Table 1). Mean proximal tubular transit time in thiazide-treated rats (11.2 ± 0.4 s) was significantly greater than in control animals (9.9 ± 0.3 s, P < 0.02).
Table 2. Renal papillary fluids from untreated and hydrochlorothiazide-treated DI rats

Each group contained nine animals. Values (mean ± SEM) are those obtained from the left (micropunctured) kidney. Statistical significance of results (comparison with control values): * P < 0.001; ** P < 0.002; *** P < 0.01.

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<tr>
<th></th>
<th>Control</th>
<th>Hydrochlorothiazide</th>
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<tr>
<td>Papillary osmolality (mosmol/kg)</td>
<td>451 ± 25</td>
<td>692 ± 37*</td>
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<tr>
<td>Papillary Na (mmol/l)</td>
<td>165 ± 8</td>
<td>212 ± 14***</td>
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<tr>
<td>Papillary K (mmol/l)</td>
<td>22.0 ± 2.0</td>
<td>22.0 ± 2.2</td>
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<tr>
<td>Calculated papillary urea (mmol/l)</td>
<td>79 ± 23</td>
<td>224 ± 31**</td>
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Blood and plasma analyses (Table 3)

It can be seen that in accordance with observations in the preceding paper [1], there was some evidence of haemoconcentration in the thiazide-treated group in that packed cell volume was significantly raised. The apparent rise in plasma protein concentration, however, was not statistically significant.

Plasma Na concentration and osmolality were reduced in thiazide-treated rats, but the mean value for plasma K concentration did not differ significantly from that of control animals.

Discussion

The present investigation was undertaken to assess, by using micropuncture, the renal mechanisms responsible for the antidiuresis caused by chronic thiazide administration in DI. It should be emphasized that during micropuncture the urinary excretion rates of water, sodium, potassium and total solute, in both control and thiazide-treated animals, were similar to their respective values before anaesthesia [1].

In previous experiments we have investigated the mechanism of the antidiuresis after acute hydrochlorothiazide administration [3]. The present findings, when hydrochlorothiazide was administered chronically, indicate that the long-term mechanisms differ in certain respects. In contrast to the marked fall in GFR which occurs during the first few hours after thiazide administration [3], chronic hydrochlorothiazide treatment was not associated with a reduction in overall GFR. Nor was the single nephron filtration rate of superficial tubules reduced significantly. Taking the two investigations together, therefore, it would appear that hydrochlorothiazide administration has a biphasic effect on GFR, causing an initial reduction which is followed by a return to normal values. This finding may go some way towards explaining previous disagreements over GFR changes during thiazide treatment [2, 8, 9].

In view of the reduced extracellular volume (ECFV) of thiazide-treated DI rats [1], and of the suggestion that the proposed tubulo-glomerular feedback mechanism might have an increased sensitivity in sodium-conserving conditions [10], some measurements of nephron filtration rate were made with collections from distal tubules. These also failed to show a difference between the two groups of rats.

Although there was no statistically significant change in absolute proximal tubular reabsorption during thiazide treatment in the present experi-
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ments, fractional proximal reabsorption showed a small but significant increase, resulting in a modest reduction in the volume of fluid escaping proximal reabsorption and being delivered to the pars recta. These findings are consistent with the small increase in proximal tubular transit time seen in thiazide-treated rats. The increase in fractional fluid reabsorption by the proximal convoluted tubule could have resulted from a number of factors associated with chronic thiazide treatment which have been documented in either the present or the preceding paper [11]. These include increases in packed cell volume [11] and plasma protein concentration [12] and reductions in plasma Na [13] and K [14] concentrations. In addition, it might be expected that the thiazide-induced reduction in extracellular fluid volume [1] would result in raised angiotensin levels, which could also increase proximal tubular reabsorption [15].

The reduction in distal delivery found during chronic hydrochlorothiazide treatment may well have contributed to the observed antidiuresis. However, in contrast to the marked reduction in distal delivery seen in the acute antidiuretic response to thiazide, when the volume of fluid delivered to the pars recta in superficial nephrons was halved [3], the reduction during chronic hydrochlorothiazide administration was relatively minor. Since the degree of antidiuresis was nevertheless similar in the two situations, it seems reasonable to suppose that the reduction in distal delivery was not the only mechanism operating chronically. A possible explanation is found in the results of the papillary interstitial fluid analyses. In control DI rats, papillary osmolality was very low, in keeping with previous findings in these animals [16], and the calculated urea concentration (~70 mmol/l) was similar to directly determined values [17]. Chronic thiazide treatment resulted in a marked elevation of osmolality, accompanied by a significant increase in Na concentration. The most striking increase, however, was in the calculated urea concentration. This latter finding is in accord with that seen in other situations in which papillary osmolality is raised in DI rats (e.g. during dehydration or vasopressin treatment) [17, 18].

These data for papillary interstitial fluid solute concentrations, obtained in both anaesthetized and unanaesthetized animals, may appear to contradict those of Baer et al. [19], who found no effect of thiazides on the medullary sodium gradient of rats in which DI had been induced surgically. However, these workers did not measure osmolality, which increases to a much greater extent than does Na concentration. Furthermore, Baer et al. found the Na concentration of the renal papilla of DI rats before thiazide treatment to be indistinguishable from that in rats without DI, so their results clearly apply to a different state from that usually present in DI.

The thiazide-induced increase in medullary osmolality seen in the present investigation might enhance water reabsorption from the descending limb of Henle, which, together with the small reduction in delivery from the proximal tubules, could lead to a reduction in the volume of fluid reaching the distal tubule. Nevertheless, this does not necessarily imply a reduced rate of fluid entry into the collecting ducts, since the primary effect of thiazides is to inhibit reabsorption from the distal tubule [20]. It seems clear, however, that the raised osmotic concentration of the medullary interstitium could make a significant contribution to the antidiuresis by increasing the vasopressin-independent reabsorption of water from the collecting ducts [4]. It should be noted that the osmotic gradient between the fluid in the papillary interstitium and that within the papillary collecting ducts (the osmolality of the latter being measured as urine osmolality during the final hour of micropuncture) was similar in hydrochlorothiazide-treated and untreated DI rats (692 vs 351 mosmol/kg of water and 451 vs 126 mosmol/kg of water respectively). It therefore seems unlikely that hydrochlorothiazide increases the water permeability of the collecting ducts.

In summary, we suggest that the antidiuretic response to chronic hydrochlorothiazide administration in DI rats is mediated by a moderate reduction in the delivery of fluid from the proximal convoluted tubule and, probably more importantly, by a rise in inner medullary osmolality, favouring increased water reabsorption from the collecting duct.

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References


