A micropuncture study of proximal tubular function after acute hydrochlorothiazide administration to Brattleboro rats with diabetes insipidus

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Summary

1. Renal function in anaesthetized Brattleboro rats with hereditary hypothalamic diabetes insipidus was studied with micropuncture techniques before, and 1–3 h after, a single injection of hydrochlorothiazide.

2. In rats given hydrochlorothiazide and kept in sodium and water balance, total glomerular filtration rate and superficial nephron filtration rate were similar to values in control animals, whereas fractional fluid reabsorption in the proximal tubule (as evidenced by tubular fluid/plasma inulin concentration ratios) was slightly, but significantly, reduced. This suggests that hydrochlorothiazide may have a small direct inhibitory effect on proximal tubular reabsorption.

3. When rats were given hydrochlorothiazide and the resultant extra urinary sodium losses were not replaced, there was a marked antidiuresis. In these animals total glomerular filtration rate was reduced by 23% and superficial nephron filtration rate by 27% when compared with values in control rats. Fractional proximal tubular fluid reabsorption increased significantly whereas absolute proximal fluid reabsorption was unaffected.

4. It is concluded that the reduction in body sodium which follows acute hydrochlorothiazide administration over-rides any inhibitory effect of the drug on proximal tubular reabsorption, and leads instead to an increase in fractional fluid reabsorption at this site. This effect, combined with the fall in glomerular filtration rate, results in a greatly reduced delivery of fluid to the more distal nephron segments, and is probably largely responsible for the observed antidiuresis.

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

Abbreviation: GFR, glomerular filtration rate.

Introduction

The administration of thiazide diuretics to patients with diabetes insipidus results in a paradoxical antidiuresis (Earley & Orloff, 1962; Skadhauge, 1973). We have previously shown, in Brattleboro rats with hereditary hypothalamic diabetes insipidus, that the antidiuresis which follows the acute administration of hydrochlorothiazide is entirely secondary to the natriuresis and consequent sodium depletion induced by the drug (Shirley, Walter & Laycock, 1978). Moreover, significant falls in renal plasma flow and glomerular filtration rate (GFR) were found to accompany the sodium depletion. It seems probable that these effects might account for at least part of the antidiuresis, since a fall in GFR, if it were to reduce the rate of fluid entry into the distal segments of the nephron, would not only reduce urine volume directly, but could also, by virtue of the reduced tubular flow rate, have the additional effect of increasing the proportion of water reabsorbed from the fluid entering the collecting duct (del Greco & de Wardener, 1956; Berliner & Davidson, 1957).
A reduced rate of glomerular filtration may not be the only factor involved in the antidiuretic response: studies of severe, chronic sodium depletion imply that the proximal tubule might also play a role (Stein, Osgood, Boonjarern, Cox & Ferris, 1974). As yet, however, no information is available concerning proximal tubular function during thiazide-induced diuresis. The purpose of the present investigation, therefore, was to assess, with micropuncture techniques, the role of the proximal convoluted tubule in the antidiuretic response to hydrochlorothiazide in rats with diabetes insipidus. Some of the results have appeared in a preliminary communication (Laycock, Shirley & Walter, 1978).

Materials and methods

All experiments were performed on male Brattleboro rats with hereditary hypothalamic diabetes insipidus, weighing 200–250 g. Until the start of each experiment, animals had free access to food and water.

In view of reports that rats with diabetes insipidus are deficient in adrenal glucocorticoids (Mohring, Mohring, Dauda & Haack, 1974; Vinson, Goddard & Whitehouse, 1977), each rat was given an intramuscular injection of 5 mg of hydrocortisone (Glaxo Ltd, Greenford, Middlesex, U.K.) before the experiment (Schnermann, Valtin, Thurau, Nagel, Horster, Fischbach, Wahl & Liebau, 1969). Thirty minutes later, rats were given by stomach tube the first of two water loads (each 3 ml/100 g body weight), which were separated by a 30 min interval. Fifteen minutes after the second water load, rats were anaesthetized with Inactin (Promonta, Hamburg) delivered intraperitoneally at a dose of 100 mg/kg body weight (0.4 mmol/kg). Rectal temperature was then maintained at 37°C. A tracheostomy was performed and appropriately sized catheters were placed in the bladder, a femoral artery and a jugular vein. Two jugular catheters were used, one for infusion of isotonic sodium chloride solution (154 mmol/l: saline), and the other for infusion of glucose solution (100 mmol/l).

The left kidney was exposed by a subcostal incision, freed from the adrenal gland and perirenal fat, and immobilized in a Perspex cup rigidly attached to the operating table. The kidney surface was bathed with paraffin oil heated to 37°C. The left ureter was cannulated fairly close to the pelvis. To minimize resistance to urine flow, which might otherwise have affected renal function (Cortell, Davidman, Gennari & Schwartz, 1972; Vande- walle & Bonvalet, 1976), a wide-bore catheter (PP 240; Portex Plastics, Hythe, Kent, U.K.), with its tip pulled out just sufficiently to fit into the ureter, was used for this purpose.

Infusions were begun as soon as the jugular vein had been cannulated (approximately 25 min after anaesthetic administration). Unless otherwise stated, saline was infused at a rate of 1 ml/h, and the rate of infusion of the glucose solution was adjusted so that the total volume of infusate equalled that of the urine. Urine flow rate was measured every 10–15 min. No urine sample was found to contain glucose.

A priming dose of 80 μCi of [3H]inulin (The Radiochemical Centre, Amersham, Bucks, U.K.) was given intravenously, followed by a sustaining infusion (contained within the saline) of 80 μCi of [3H]inulin/h. During a 1 h equilibration period, proximal tubular transit time of lissamine green was determined (Steinhäuser, 1963). During a subsequent control hour, inulin clearance was measured and timed collections were made from late surface loops of proximal convoluted tubules with sharpened micro-pipettes (external tip diameter 8–10 μm) filled with water-equilibrated mineral oil. For each collection an oil block 4–5 tubule diameters in length was maintained in a constant position just distal to the site of puncture. After initial gentle suction it was found that in the majority of cases fluid flowed into the pipette spontaneously without need for further aspiration, although in a few cases some slight additional intermittent suction was required. Collection times were 3–8 min. Before collection, puncture sites were identified by injection of a very small oil droplet, which was allowed to flow down the tubule: if the pipette was in a final surface loop or if there was only one additional loop, then the puncture site was taken to be 'late'. Subsequent confirmation of the collection site was made after injection of Microfil (Canton Biomedical Products, Boulder, Colorado, U.S.A.). The kidney was stored overnight in distilled water at 4°C, then partially digested in NaOH (5 mol/l) for 15–30 min, and the silicone rubber-filled tubules were dissected out.

At the end of the control hour, animals were divided into three groups. Group 1 (control) animals received a subcutaneous injection of 0.2 ml of saline and were maintained on the same infusions as before. Group 2 rats were given a single subcutaneous injection (in 0.2 ml of saline) of hydrochlorothiazide (Merck, Sharp and Dohme) at a dose of 25 mg/kg body weight (80 μmol/kg), a...
dose previously shown to have a maximal anti-
diuretic effect (Shirley et al., 1978). These animals
were also maintained on the same infusions as
before, except that, in an attempt to ensure that all
groups received similar amounts of glucose, the
concentration of the glucose solution was raised to
300 mmol/l 2 h after the hydrochlorothiazide
injection. Group 3 animals were given the same
dose of hydrochlorothiazide, but the saline propor-
tion of their infusions was thereafter adjusted
so that the amount of sodium infused equaled the
urinary sodium excretion.

After the saline or hydrochlorothiazide injection,
urine was collected for a further 3 h. During the
final 2 h (1–3 h after the injection) at least two
more inulin clearances were performed and timed
micropuncture collections were again made from
late surface convolutions of proximal tubules (not
the same tubules as those punctured during the
control period). Small femoral arterial blood
samples (~80 µl) for determination of plasma
osmolality and inulin, Na+ and K+ concentrations
were taken regularly throughout each experiment,
and always at the mid-point of each clearance
period.

At the end of the 3 h post-injection period, a
second proximal tubular transit time was measured.

Analyses

The Na+ and K+ concentrations of urine and
plasma samples were measured with a dual-channel
integrating flame photometer (Evans Electo-
selenium Ltd), and the Na+ and K+ concentrations
of tubular fluid samples with a helium glow photo-
meter (Aminco, Silver Spring, Maryland, U.S.A.).
Urinary osmolality was measured with an Advanced
osmometer (model 3D) and plasma and tubular
fluid osmolality with a nanolitre osmometer
(Clif-
Tubular fluid volumes were measured in previously
calibrated pipettes. The [3H]inulin radioactivity of
plasma, urine and tubular fluid samples was
determined in Aquasol 2 scintillation cocktail (New
England Nuclear, Boston, Mass., U.S.A.) in a
Packard Tri-carb liquid scintillation spectrometer.
No quench correction was necessary. The packed
cell volumes of all blood samples taken were
measured in microhaematocrit tubes.

Calculations

Glomerular filtration rate was taken to be the
clearance of [3H]inulin. Single-nephron glomerular
filtration rate was calculated from the tubular fluid
inulin/plasma inulin concentration ratio (TF/P
inulin) and the volume of tubular fluid collected per
min (V TF), according to the expression:

\[ \text{nephron filtration rate} = \frac{\text{TF}}{\text{P inulin}} \times V_{TF}. \]

Absolute proximal tubular fluid reabsorption was
calculated from the following expression:

\[ \text{nephron filtration rate} \times (1 - \frac{P}{TF \text{ inulin}}). \]

Results

It has been shown previously that the antidiuretic
effect of a single injection of hydrochlorothiazide
into rats with diabetes insipidus begins after
approximately 1 h (Shirley et al., 1978). This was
also observed in the present study. Presentation of
all data has therefore been restricted to a single
value for each group during the control hour and a
single value for each group during the period 1–3 h
after the injection of saline or hydrochlorothiazide
(experimental period). Values given apply only to
the kidney that was exposed for micropuncture.
However, urine was also collected from the non-
exposed (right) kidney throughout, and urine
volumes and Na+ and K+ excretion rates of the two
kidneys were found not to differ significantly.

Fig. 1(a) shows the volumes of urine collected
from the left kidney in the three groups of rats both
before and 1–3 h after injection of saline or hydro-
chlorothiazide. Values during the control period
were similar to those found in our previous investi-
gation with these animals. During the experimental
period (1–3 h after the injection) there was a
marked antidiuresis in group 2 animals (given
hydrochlorothiazide and allowed to become sodium-depleted), whereas in the other two groups
urine volume remained high, confirming that the
antidiuresis was dependent on depletion of body
sodium. Urine osmolality rose in those animals
given hydrochlorothiazide (Fig. 1b). Increases in
osmotic excretion were due entirely to increased
urinary Na+ and (to a much smaller extent) K+
excretion after administration of the drug.

Total kidney GFR is shown in Fig. 1(c).
FIG. 1. (a) Urine volume, (b) urine osmolality and (c) GFR in anaesthetized Brattleboro rats with diabetes insipidus before (control period, C) and 1–3 h after (experimental period, E) a single subcutaneous injection of saline (group 1, ●), hydrochlorothiazide (group 2, △) or hydrochlorothiazide with intravenous sodium replacement (group 3, ▲). Injection volumes were 0.2 ml; the dose of hydrochlorothiazide was 25 mg/kg body weight. Values (mean ± SEM) apply to the left kidney only. Each group contained eight to ten animals.

Although GFR fell in all three groups during the experimental period, the decreases measured in group 1 (control) and group 3 (hydrochlorothiazide + sodium replacement) animals were relatively small; the GFR of group 2 animals during this period was significantly less than in the other two groups (P < 0.01, P < 0.002 respectively).

Micropuncture data are shown in Fig. 2. It can be seen that the pattern of change in superficial nephron filtration rate in the three groups (Fig. 2a)
was similar to that of total GFR, the nephron filtration rate of group 2 rats during the experimental period being significantly less than that of group 1 ($P < 0.02$) or group 3 ($P < 0.002$) animals.

Fractional fluid reabsorption in the proximal tubule, as measured by TF/P inulin concentration ratios in late proximal surface convolutions, was similar in the three groups during the control period (Fig. 2b). During the experiment the TF/P inulin concentration ratio in group 1 animals did not change significantly. In group 2 animals, however, there was a rise in the TF/P inulin ratio to a value significantly higher than in the other two groups ($P < 0.001$ in each case), indicating that fractional proximal tubular reabsorption had increased. During the experimental period the TF/P inulin ratio in group 3 animals (given hydrochlorothiazide but kept in sodium balance) was slightly less than that in control (group 1) animals, the difference just achieving a level of statistical significance ($P < 0.05$).

The combination of a fall in nephron filtration rate and a rise in fractional fluid reabsorption after hydrochlorothiazide administration and subsequent sodium depletion meant that absolute proximal reabsorption was unchanged in group 2 animals when compared with that in control rats, whereas the volume of fluid delivered to nephron segments distal to the proximal convolutions was greatly reduced (Fig. 2c, d).

Proximal tubular transit times were determined 30 min before the control period and at the end of the experimental period. The initial transit times in groups 1, 2 and 3 respectively were (means ± SEM) 11.1 ± 0.3 s ($n = 9$), 10.7 ± 0.4 s ($n = 10$) and 11.0 ± 0.4 s ($n = 8$), values not significantly different from one another. Transit times changed little in groups 1 and 3, being 12.7 ± 0.7 s and 11.8 ± 0.3 s respectively at the end of the experimental period, but in those rats allowed to become sodium depleted after the drug (group 2), proximal tubular transit time increased to 16.2 ± 0.5 s, a value significantly higher than in the other two groups ($P < 0.002, P < 0.001$ respectively).

Tubular fluid/plasma osmolality ratios and Na$^+$ and K$^+$ concentration ratios in the three groups of rats were determined during both the control and experimental periods. There were no significant differences between the groups with regard to any of the three parameters during either period, and all values were close to unity. Thus proximal tubular fractional reabsorptions of solute and water were at all times equivalent.

Changes in packed cell volume and in plasma Na$^+$ concentration and osmolality are shown in Table 1. Packed cell volume in group 1 animals when compared with that in control rats, whereas the volume of fluid delivered to nephron segments distal to the proximal convolutions was greatly reduced (Fig. 2c, d).

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Changes in packed cell volume and in plasma Na$^+$ concentration and osmolality are shown in Table 1. Packed cell volume in group 2 animals during the experimental period was significantly greater than in the other groups. Although plasma Na$^+$ concentration during the experimental period was lower in group 2 rats than in the other groups, the differences between groups were not statistically significant. However, the reduced plasma osmolality in group 2 rats did just reach statistical significance when compared with the other groups.

Table 1 also shows that plasma K$^+$ concentration was unaffected by any of the procedures. The low plasma K$^+$ values obtained accord with the belief that rats with diabetes insipidus are hypokalaemic (Möhring et al., 1974).

### Table 1. Packed cell volume and plasma Na$^+$, K$^+$ and osmolality values

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Hydrochlorothiazide (n = 10)</th>
<th>Hydrochlorothiazide + sodium replacement (n = 8)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>(Group 1)</td>
<td>(Group 2)</td>
<td>(Group 3)</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>C 45.8 ± 2.0</td>
<td>N.S. 45.5 ± 1.6</td>
<td>N.S. 44.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>E 44.8 ± 2.0</td>
<td>&lt;0.05 49.3 ± 0.6</td>
<td>&lt;0.001 43.3 ± 0.9</td>
</tr>
<tr>
<td>Plasma Na$^+$ (mmol/l)</td>
<td>C 148 ± 3</td>
<td>N.S. 147 ± 3</td>
<td>N.S. 146 ± 5</td>
</tr>
<tr>
<td></td>
<td>E 146 ± 3</td>
<td>N.S. 141 ± 3</td>
<td>N.S. 150 ± 5</td>
</tr>
<tr>
<td>Plasma K$^+$ (mmol/l)</td>
<td>C 3.1 ± 0.1</td>
<td>N.S. 2.9 ± 0.1</td>
<td>N.S. 3.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>E 3.2 ± 0.2</td>
<td>N.S. 3.0 ± 0.2</td>
<td>N.S. 3.0 ± 0.3</td>
</tr>
<tr>
<td>Plasma osmolality (mosmol/kg)</td>
<td>C 299 ± 9</td>
<td>N.S. 287 ± 8</td>
<td>N.S. 294 ± 4</td>
</tr>
<tr>
<td></td>
<td>E 294 ± 6</td>
<td>0.05 277 ± 4</td>
<td>&lt;0.05 306 ± 10</td>
</tr>
</tbody>
</table>

* $P$ values refer to comparisons between group 1 and group 2 rats.
† $P$ values refer to comparisons between group 2 and group 3 rats.
Discussion

In a previous investigation it was shown that the antidiuresis which occurs within 2 h of administration of hydrochlorothiazide to rats with diabetes insipidus could be prevented if the animals were kept in sodium balance (Shirley et al., 1978). The present experiments, using a similar procedure, fully confirmed our previous findings: the antidiuresis was dependent on some degree of sodium depletion. The actual amount of sodium lost in group 2 animals, when compared with control rats, averaged 185 μmol/100 g body weight 1 h after hydrochlorothiazide administration, a similar value to that found previously. There was little further loss after the first hour, since only if urinary sodium losses were replaced (as in group 3 rats) did the drug-induced increase in sodium excretion persist. Both plasma Na⁺ concentration and plasma osmolality appeared to fall in group 2 rats after the drug (Table 1), although when compared with values in the other two groups only the decrease in osmolality was statistically significant.

As discussed previously (Shirley et al., 1978), the fall in body sodium should not simply result in decreased plasma Na⁺ concentration and osmolality, but also in a consequent reduction in extracellular fluid volume. In fact evidence for a shrinkage of plasma volume, probably indicative of a general reduction in extracellular fluid volume, was obtained in the present study from the increase in packed cell volume in group 2 rats. It therefore seems probable that the antidiuresis observed in this group resulted in some way from a reduction in extracellular fluid volume, although a separate effect of the fall in plasma osmolality itself cannot be ruled out.

During the experimental period, whole-kidney GFR and superficial nephron filtration rate in rats given hydrochlorothiazide but kept in sodium balance (group 3 animals) were similar to values in control (group 1) rats. Thus the drug itself did not appear to affect GFR directly. However, in sodium-depleted (group 2) animals total kidney GFR was approximately 23% less than in the other two groups, and superficial nephron filtration rate was approximately 27% less. The fall in total GFR will almost certainly have contributed to the antidiuresis of group 2 rats. Since the fall in superficial nephron filtration rate was slightly greater than that in total GFR, and since most of the nephrons are of the superficial type, it seems possible that the reduction in total GFR could largely be accounted for by that found in the superficial nephrons alone. In other words, the sodium depletion may have resulted in a redistribution of the glomerular filtrate away from the superficial nephrons. However, although the present results provide some hint of this possibility, they are certainly not conclusive.

The increase in proximal tubular transit time in sodium-depleted (group 2) animals, together with the increased TF/P inulin ratio in late proximal convolutions, provides strong evidence that fractional fluid reabsorption in the proximal tubules of group 2 animals was increased after the drug. In view of the fall in GFR, this result might have been expected, since several studies in sodium-replete animals have shown that by artificially reducing GFR (by constriction of the aorta or renal artery) an increase in fractional proximal tubular reabsorption can be elicited (Brenner, Bennett & Berliner, 1968; Landwehr, Schnermann, Klose & Giebisch, 1968). The present results in sodium-depleted rats differ from those of the above-mentioned studies, however, in that in our experiments the increase in fractional reabsorption relative to the GFR reduction was such that absolute proximal fluid reabsorption remained unchanged (compared with control animals). Thus there was a complete absence of glomerulotubular balance, whereas in the studies of Brenner et al. (1968) and Landwehr et al. (1968) some degree of glomerulotubular balance remained after the reduction in GFR. Therefore in the present investigation the sodium depletion itself must have enhanced proximal tubular reabsorption. It is conceivable that the rise in arterial packed cell volume may have been a contributory factor, since haemocencentration has been shown to increase tubular reabsorption (Nashat, Scholfield, Tappin & Wilcox, 1969; Schrier & Earley, 1970). It is possible, however, that the increase in packed cell volume may not itself have played a role, but may simply have been indicative of a rise in the plasma concentration of other agents responsible for the increased reabsorption. An obvious possibility is that the concentration of plasma proteins may have increased. There is considerable evidence that a raised concentration of plasma proteins in the peritubular capillaries can increase fractional fluid reabsorption in the proximal tubule (Spitzer & Windhager, 1970; Brenner & Troy, 1971), although it should be borne in mind that more recently the importance of peritubular plasma protein concentration in the control of proximal tubular function has been called into question (Conger, Bartoli & Earley, 1976; de Wardener, 1978).
At least two further factors may have been involved in the enhanced fractional proximal fluid reabsorption observed in the sodium-depleted rats. Some investigators believe that proximal tubular reabsorption is partly controlled by the renal sympathetic nerves (Bello-Reuss, Trevino & Gottschalk, 1976; Di Bona, 1977) and evidence has also been obtained for a role of the renin–angiotensin system at this site (Harris & Young, 1977). It is possible that the decreased extracellular fluid volume in group 2 rats could have increased renal sympathetic nervous activity and/or the plasma concentration of angiotensin II, either of which may have contributed to the increased fractional reabsorption in the proximal tubule. It is conceivable also that these two systems may have been involved in the reduction in GFR.

It is important to emphasize that the present results were obtained from acute studies only. Whether the same degree of sodium depletion induced over a longer period of time by the chronic administration of hydrochlorothiazide, as in the clinical situation, would have the same effect on either GFR or proximal tubular reabsorption in rats with diabetes insipidus remains to be seen. An increase in proximal fractional fluid reabsorption after a similar degree of sodium depletion produced by chronically feeding a low-sodium diet to non-diabetic Sprague–Dawley rats has been observed by Weinman & Eknoyan (1975), although other studies of chronic sodium depletion in non-diabetic animals have required a much greater degree of depletion to produce a discernable effect (Stein et al., 1974).

In the present investigation, as a result of the reduction in nephron filtration rate and the rise in proximal fractional fluid reabsorption in sodium-depleted (group 2) animals, the rate of fluid delivery from the final surface convolutions of the proximal tubule to more distal segments was approximately 8 ml/min, compared with approximately 15 ml/min in control animals. If the changes in the surface tubules were reflected throughout the nephron population as a whole, the absolute volume required to be reabsorbed in the distal segments of the nephron (in order to account for the observed urine flow rates) would actually be less in the sodium-depleted rats than in control animals. In fact, of course, it cannot be assumed that the deeper nephrons behave similarly to the superficial ones, so it may be that at least some enhancement of fluid reabsorption in the distal nephron is necessary. In this respect it seems likely that the reduced tubular flow rate in the distal nephron will favour increased water backflux in the collecting duct, which occurs even in the absence of anti-diuretic hormone (Berliner & Davidson, 1957; Jamison, Buerkert & Lacy, 1971).

When animals were given hydrochlorothiazide but kept in sodium balance (group 3 rats), there was no increase in fractional fluid reabsorption in the proximal tubule. Instead, late proximal TF/P inulin ratios actually fell slightly (but significantly) when compared with values in control rats. This finding, together with our previous demonstration of an increased urine flow rate in diabetes insipidus rats during the period immediately after the drug’s administration (Shirley et al., 1978), provides further evidence that, in addition to its likely distal effects (Kunau, Weller & Webb, 1975), hydrochlorothiazide may have a direct inhibitory effect on proximal tubular reabsorption. Only when animals are prevented from becoming sodium-depleted is this inhibitory effect revealed.

In conclusion, the acute anti-diuretic response to hydrochlorothiazide administration in anaesthetized rats with diabetes insipidus is a consequence of the resulting sodium depletion: by producing a fall in total and superficial nephron GFR, combined with an increase in fractional fluid reabsorption in the proximal tubule, the sodium depletion results in a greatly reduced delivery of fluid to the loop of Henle and distal nephron. This reduced distal delivery appears to be largely responsible for the observed antidiuresis.

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