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The effect of hydrochlorothiazide on the composition of renal papillary interstitial fluid in the rat

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Abstract—The effect of chronic hydrochlorothiazide administration on the composition of renal papillary interstitial fluid was investigated in the rat. Hydrochlorothiazide alone had no effect on papillary composition. However, when the sodium depletion that usually accompanies thiazide treatment was minimized by allowing the rats access to hypertonic NaCl solution, hydrochlorothiazide administration resulted in significant reductions in papillary osmolality, and sodium and potassium concentrations. It is suggested that the effects of hydrochlorothiazide on the renal papilla might be mediated by thiazide-induced hypokalaemia, and that under normal circumstances these effects are masked by the concomitant sodium depletion.

It is generally accepted that thiazide diuretics do not influence the osmotic gradient of the renal medulla (Baer et al 1962); early attempts to locate the renal site of action of thiazides considered this to be evidence against an effect on salt transport in the medullary loop of Henle (Goldberg 1973). However, a problem encountered in most studies of diuretics is that the sodium and volume depletion associated with diuretic treatment might bring about changes which could obscure any direct effects of the drug itself.

In the present investigation we have re-assessed the influence of thiazides on the composition of the renal medulla by determining the osmolality and electrolyte concentrations of renal papillary interstitial fluid in the rat during chronic hydrochlorothiazide treatment, both in the presence and absence of sodium depletion. Prevention of sodium depletion was achieved by allowing the rats access to hypertonic NaCl solution (Shirley et al 1987).

Materials and methods

Experimental protocol. Male Long-Evans rats, 180–200 g at the start of the study, were kept in metabolic cages and maintained on a standard diet (Na and K contents 100 and 60 mmol kg^{-1} dry weight, respectively). Animals were divided into four groups: two control groups (8 per group), which remained on the same dietary regime throughout, and two thiazide-treated groups (9 per group), which, after habituation and control periods (4 and 3 days, respectively), received hydrochlorothiazide (Merck, Sharp & Dohme) in the food at a concentration of 35 mg kg^{-1} dry weight (providing a daily dose of approximately 2 mg kg^{-1} body weight). One control group and one thiazide-treated group had access to 0·46 M NaCl solution as well as drinking water. Control rats with access to saline drank only small quantities of it (< 1 mL day^{-1}), but the thiazide-treated group drank just sufficient to prevent any significant drug-induced sodium deficit (Olesen 1982; Shirley et al 1987). In contrast, the thiazide-treated group which did not have access to saline went into negative sodium balance on the first day of thiazide administration and remained sodium-depleted (approximately 1 mmol per animal, with respect to controls) throughout the treatment (Walter & Shirley 1986).

After the initial control period, rats were kept for 7–10 days on their respective regimes and were then anaesthetized with Inactin (Byk Gulden, Konstanz; 120 mg kg^{-1} b.w, i.p.) and prepared surgically for micropuncture experiments, the results of which have been reported separately (Walter & Shirley 1986; Shirley et al 1987).

To obtain a sample of renal papillary interstitial fluid, the left renal pedicle was clamped, the kidney excised and the papilla quickly removed. Papillary interstitial fluid was obtained using the method of sequential centrifugation (Shirley et al 1982). As soon as the renal pedicle had been clamped, a blood sample for analysis was taken from the femoral artery.

Analyses. The osmolality of papillary fluid was determined using a nanolitre osmometer (Clifton Technical Physics, Hartford, New York, USA). Papillary Na and K concentrations were measured by helium glow photometry (Aminco, Silver Spring, Maryland, USA). Packed cell volumes were determined in microhaematocrit tubes, plasma electrolyte concentrations were measured by flame photometry (IL, model 543), and plasma protein concentration by the method of Lowry et al (1951).

Results are presented as means ± s.e.m. Statistical comparisons were made using Student's unpaired *t*-test.

Results

Results obtained from the two control groups (with and without access to 0·46 M NaCl solution) were indistinguishable and have therefore been pooled and designated 'control'.

Results from the analysis of papillary interstitial fluid samples are shown in Fig. 1. It can be seen that in rats treated with hydrochlorothiazide and not allowed access to saline there were no statistically significant changes in papillary osmolality or in

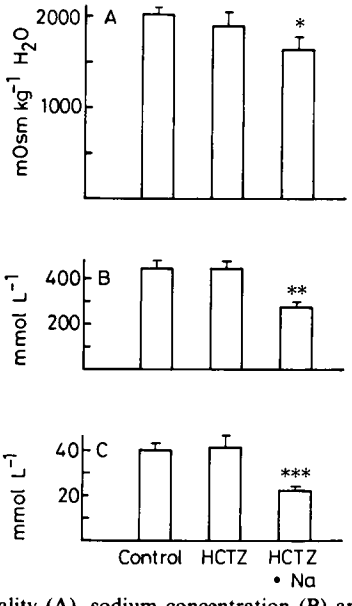

FIG. 1. Osmolality (A), sodium concentration (B) and potassium concentration (C) of renal papillary interstitial fluid in control rats (n = 16) and in rats treated with hydrochlorothiazide (HCTZ), with and without sodium replacement (n = 9 in each group). Values are means + s.e.m. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with the corresponding control value.

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Na or K concentrations, when compared with control animals. However, in rats treated with the same dose of hydrochlorothiazide but given access to saline there were significant reductions in papillary osmolality and in Na and K concentrations.

Table 1 shows that rats treated with hydrochlorothiazide and not allowed access to saline had a higher packed cell volume and a higher plasma protein concentration than control animals.

Table 1. The effect of hydrochlorothiazide (HCTZ) treatment on packed cell volume and plasma composition. Values are means ±s.e.m.

	Control (n = 16)	HCTZ (n = 9)	HCTZ + Na replacement (n = 9)
PCV (%)	48·2 ± 0·4	50·3 ± 0·6†	47·9 ± 0·5
Plasma protein ($g\ L^{-1}$)	52·7 ± 1·0	56·5 ± 1·4*	52·2 ± 2·0
Plasma Na ($mmol\ L^{-1}$)	144 ± 1	139 ± 1†	143 ± 1
Plasma K ($mmol\ L^{-1}$)	4·0 ± 0·1	3·4 ± 0·1‡	3·3 ± 0·1‡

*$P < 0·05$; †$P < 0·01$; ‡$P < 0·001$ compared with the corresponding control value.

These changes were accompanied by reductions in the plasma concentrations of sodium and potassium. In contrast, in thiazide-treated rats which had access to saline, packed cell volume, plasma protein concentration and plasma sodium were very similar to corresponding values in control rats; plasma potassium, however, was reduced.

Discussion

Since the investigation by Baer et al (1962), it has been accepted that thiazide diuretics have no effect on electrolyte concentrations in the renal medulla and papilla. In the present study we have re-investigated this question using the method of sequential centrifugation of the renal papilla to obtain samples of papillary interstitial fluid with minimal processing of the tissue (Shirley et al 1982). Control values for rat papillary interstitial electrolyte concentrations were found to be similar to those obtained by Beck et al (1984) using electron microprobe analysis. We were able to confirm that chronic hydrochlorothiazide treatment was without effect on the osmolality or sodium and potassium concentrations of the papillary interstitium, but only when no attempt was made to prevent the sodium depletion associated with thiazide treatment. When sodium depletion was prevented (or at least minimized) by allowing rats access to 0·46M NaCl solution, hydrochlorothiazide was found to cause significant reductions in papillary osmolality and in papillary sodium and potassium concentrations.

0·46M NaCl solution has been shown to be just strong enough to dissuade rats from drinking significant quantities under normal circumstances; however, if the rats are subjected to procedures which tend to cause sodium depletion, they drink just sufficient saline to prevent the depletion (Richter 1936; Olesen 1982). In the present study, control rats given access to saline drank less than 1 $mL\ day^{-1}$, whereas those given hydrochlorothiazide increased their saline intake to approximately 20 $mL\ day^{-1}$. The availability of 0·46 M NaCl solution thus allows investigation of the chronic effects of thiazide without the complicating factor of sodium depletion. Although infusions of saline following injections of diuretic might serve the same purpose, this would only allow the acute effects of the diuretic to be studied; moreover such a procedure is difficult to perform accurately and runs the risk of causing acute volume expansion. Evidence has been presented elsewhere that hydrochlorothiazide-treated rats allowed access to saline are *not* volume expanded (Shirley et al 1987). This is exemplified in the present study by the values for packed cell volume and plasma protein concentration which were similar to those of control animals. In contrast, in thiazide-treated rats without access to saline, increases in packed cell volume and plasma protein concentration, together with a fall in plasma sodium concentration, were observed, changes which are consistent with a sodium deficit and extracellular volume depletion.

The mechanism of the effect of hydrochlorothiazide on the renal papilla of sodium-replete rats is not clear. There is no evidence that this drug can directly influence transport in the ascending limb of Henle (Schlatter et al 1983); its principal site of action appears to be the early distal tubule (Costanzo 1985). Although there is some indication that hydrochlorothiazide can inhibit sodium reabsorption in the collecting duct (Wilson et al 1983), a more likely explanation for the reduced papillary solute concentrations might be an *indirect* inhibition of transport in the ascending limb of Henle, mediated by the thiazide-induced hypokalaemia. There is good evidence that hypokalaemia or the associated reduction in peritubular potassium concentration can affect salt transport in this region of the nephron. Gutsche et al (1984), using a stop-flow technique applied to single nephrons, have demonstrated impaired NaCl transport from the thick ascending limb of Henle of potassium-depleted rats, an effect which was reversed by the restoration of plasma potassium to control levels.

If the hypokalaemia *were* responsible for the reduced papillary osmolality observed in sodium-replete rats during hydrochlorothiazide administration, then why did hydrochlorothiazide have no such effect on papillary composition in animals *not* allowed access to saline, despite a similar degree of hypokalaemia? The most likely answer is that factors associated with the thiazide-induced sodium deficit in the latter group might have masked any effect of hypokalaemia itself. This could explain why alterations in papillary composition are not usually observed during thiazide treatment.

In conclusion, the present investigation has demonstrated reductions in papillary osmolality and electrolyte concentrations in rats treated chronically with hydrochlorothiazide, but only when animals were kept in sodium balance. It is suggested that these changes might be mediated by thiazide-induced hypokalaemia, though possible contributions from other factors, such as alterations in medullary blood flow or in prostaglandin synthesis, cannot be discounted.

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