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THE ANTIDIURETIC ACTION OF HYDROCHLOROTHIAZIDE IN THE HYDRATED RAT

BY S. E. DICKER AND M. GRACE EGGLETON

From the Departments of Pharmacology and Physiology, University College London

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In patients suffering from nephrogenic diabetes insipidus, who are resistant to the action of vasopressin, it is now well established that the normally diuretic compounds derived from thiazide act as antidiuretic agents. It is also agreed that this action is dependent on the maintenance of a negative sodium balance, but there is no generally accepted explanation of the mechanism responsible for these effects. Cutler, Kleeman, Dowling & Maxwell (1960), finding a 30% reduction in filtration rate following chlorothiazide administration in four such patients, concluded that its action was 'probably due to altered body sodium and renal haemodynamics'; but Earley & Orloff (1962), who measured inulin clearance in three patients, found a fall (13–15%) in only two out of six estimations when hydrochlorothiazide was given.

Since the rat has been shown to react like man, in the sense that the diuretic action of chlorothiazide is transformed into an antidiuretic action when diabetes insipidus is induced by bilateral hypothalamic electrolytic lesions (Kennedy & Crawford, 1961; Crawford, Frost, Welsh & Terry, 1962), this species has been used for further investigation of the problem.

METHODS

Unanaesthetized rats were used. All experiments were performed in the morning. Since the laboratory temperature was variable, comparison between animals receiving hydrochlorothiazide and controls not receiving the drug were usually made at the same time. The dose of hydrochlorothiazide was 0·1 mg/100 g body weight given by gavage in 1 ml. water/100 g body weight; control animals were given 1 ml. water/100 g. Urine samples were collected individually during $\frac{1}{2}$ or 1 hr periods over $2\frac{1}{2}$ or 3 hr and pooled from groups of three animals for analysis.

Unless otherwise stated the animals were deprived of food over-night but allowed free access to water until the onset of the experiment; these are referred to in the text as '*non-hydrated*'. In experiments on *dehydrated* animals, they were deprived of both food and water for about 18 hr before the experiment. In experiments on *hydrated* animals, 4 ml. water/100g body weight was given by stomach tube immediately after the dose of drug or control solution, at the beginning of the period of urine collection.

To produce sodium depletion the animals were fed on a diet containing 20–30 m-equiv

Na/kg in contrast with 225 m-equiv Na/kg in the normal diet. Sodium depletion of the animals was also attained by the use of another thiazide derivative, Enduron (Methyclothiazide, Abbott), which the makers claim to have 50 times the potency of hydrochlorothiazide. Enduron was more convenient for our purpose than hydrochlorothiazide, since the latter forms a suspension in water which settles fairly rapidly whereas the former is soluble. 100 μg/l. of this drug was added to the animals' drinking water for a week before an experiment.

Endogenous creatinine excretion was accepted as a relative measure of filtration rate in the 2½ or 3 hr period. It was measured by the Jaffe reaction. Total urine and plasma osmolarity was measured with the Advance osmometer ('Advanced Instruments, Inc.' U.S.A.), and sodium and potassium by flame photometer.

In a few experiments acidic mucopolysaccharides were determined by the turbidity of chondroitin sulphate (beef trachea) on reaction with cetyltrimethylammonium bromide (CTAB) (di Ferrante, 1956) and hyaluronidase by reduction of this turbidity. A linear relation between optical density and concentration of chondroitin sulphate was obtained over the range 0–150 μg, and urine acidic mucopolysaccharides were expressed as μg chondroitin sulphate. 0·5 ml. urine + 0·5 ml. citric acid-phosphate buffer (pH 4·0) containing 0·15 M-NaCl were incubated at 37° C for 10 min, cooled, and 2 ml. CTAB (2·5 % wt./vol. in 2 % NaOH containing 0·15 M-NaCl) added. The resulting turbidity was measured at 400 mμ. For measurement of hyaluronidase (H-ase), the 0·5 ml. buffer contained 125 μg chondroitin sulphate, the general procedure being the same as that for measurement of acidic mucopolysaccharides. A correction was made for the latter present in the urine samples, and H-ase expressed as percentage reduction in turbidity. In neither estimation did incubation for 30 min give significantly different values from those obtained at 10 min.

The pooled results of each experiment are expressed in terms of excretion/100 g rat/hr. The example in Table 1 of the results of an individual experiment on three rats will show most readily how this was calculated. Water clearance was calculated as urine flow—osmolar U/P × V. From plasma obtained by decapitation, values of 287 m-osmole/l. at 1 hr, 295 at 1½ hr, 305 at 2 hr and 303 m-osmole/l. at 2½ hr were obtained after 1 ml. (0·1 mg) hydrochlorothiazide/100 g and 4 ml. water/100 g, and a value of 305m-osmole/l. from a control animal. For calculation of water clearance, a value of 300 m-osmole/l. plasma was used throughout.

TABLE 1. An individual experiment made on a group of three rats weighing 769 g. 5 ml. water with 0·1 mg hydrochlorothiazide/100 g rat was given at time 0

Time (hr)	Urine volume		Urine osmolarity		Water clearance
	Total ml.	ml./100 g	m-osmole/l.	μ-osmole/100 g	ml./100 g
0 –1	6·9	0·9	338	304	−0·12
1–1½	12·0	1·56	175	272	+0·65
1½–2	10·5	1·37	151	206	+0·68
2–2½	9·6	1·25	147	184	+0·64
Total over 2½ hr		5·08		966	+1·84
Average/100 g/hr		2·03		386	+0·74

RESULTS

In both dehydrated and non-hydrated rats, hydrochlorothiazide produces its typical diuretic effect accompanied by a pronounced increase of sodium excretion, as may be seen from the results given in Table 2. This increase in urine flow occurs in spite of an increase in free water reabsorption.

In animals hydrated with 5 ml. water/100 g, on the other hand, although sodium excretion is still markedly increased by hydrochlorothiazide, the accompanying decrease in free water clearance is sufficient to produce a reduction in urine flow (Table 3). It was thought that the antidiuretic

TABLE 2. The effect of hydrochlorothiazide (HYD) on non-hydrated rats and on rats deprived of water for 18 hr. In this and succeeding tables the numbers in brackets indicate the number of experiments performed in each category; the standard error of the mean is indicated.

	Dehydration		Non-hydration	
	1 ml. water/100 g (4)	1 ml. water + 0·1 mg HYD/100 g (7)	1 ml. water/100 g (4)	1 ml. water + 0·1 mg HYD/100 g (6)
Urine flow (ml./100 g/hr)	0·391 ± 0·086	0·485 ± 0·078	0·780 ± 0·100	1·025 ± 0·030
Osmolarity (μ-osmole/100 g/hr)	154 ± 12	247 ± 23	217 ± 18	313 ± 20
Sodium (μ-equiv/100 g/hr)	8·4 ± 4·1	49·7 ± 11·5	18·4 ± 3·1	64 ± 3·6
Potassium (μ-equiv/100 g/hr)	8·8 ± 3·5	18·9 ± 4·0	16·0 ± 5·3	18·3 ± 3·7
Creatinine (mg/100 g/hr)	0·166 ± 0·017	0·163 ± 0·011	0·163 ± 0·008	0·171 ± 0·009
Water clearance (ml./100 g/hr)	−0·121 ± 0·050	−0·339 ± 0·070	+0·015 ± 0·130	− 0·21 ± 0·06

effect might be enhanced if a negative sodium balance was first achieved, but this proved not to be the case. When the sodium depletion had been attained by giving Enduron in the drinking water for a week, and the animals then hydrated, free water clearance was decreased from 1·3 ml./100 g/hr in controls (Table 3) to 1·18 ml./100 g/hr (Table 4). In-

TABLE 3. The effect of hydrochlorothiazide (HYD) on rats hydrated with 5 ml. water/100 g

	5 ml. water/100 g (6)	5 ml. water + 0·1 mg HYD/100 g (6)
Urine flow (ml./100 g/hr)	2·10 ± 0·103	1·88 ± 0·071
Osmolarity (μ-osmole/100 g/hr)	208 ± 17	325 ± 36
Sodium (μ-equiv/100 g/hr)	12·8 ± 2·7	57·7 ± 7·4
Potassium (μ-equiv/100 g/hr)	6·8 ± 0·8	18·2 ± 2·1
Creatinine (mg/100 g/hr)	0·182 ± 0·003	0·185 ± 0·0045
Water clearance (ml./100 g/hr)	1·365 ± 0·076	0·797 ± 0·077

cidentally, the effects of Enduron persisted for at least a week after the drug had been stopped, for two of the five experiments were done at this time, with entirely consistent results. When hydrochlorothiazide with 5 ml. water/100 g was given to these animals, free water clearance was still further diminished to 0·77 ml./100 g/hr, a value indistinguishable from that attained by hydrochlorothiazide alone in normal animals (Table 3).

These changes can be appreciated more readily when presented in graphic form and this has been done in Fig. 1. The changes in osmolar and sodium excretion parallel each other, the drain of body sodium by daily dosage with Enduron leading to diminished excretion when the rats were water-loaded or given hydrochlorothiazide. Potassium excretion on the other hand is increased both by Enduron and hydrochlorothiazide individually and, when given together, their effect is additive. The change in water clearance cannot be attributed to change in creatinine output.

The possibility that the decrease in free water clearance induced by hydrochlorothiazide might be mediated in the same way as that induced by ADH was considered, but measurement of H-ase by reduction of viscosity of hyaluronic acid seemed of dubious value, since rats' urine contains protein and comparison is made between boiled and unboiled samples. In the two experiments in which such measurements were made,

TABLE 4. The effect of sodium depletion and hydrochlorothiazide (HYD) in hydrated rats

	Enduron (1 week)		Na-low diet (2 weeks)
	5 ml. water/100 g (5)	5 ml. water + 0·1 mg HYD/100 g (5)	5 ml. water + 0·1 mg HYD/100 g (2)
Urine flow (ml./100 g/hr)	1·85 ± 0·118	1·8 ± 0·103	1·94 ± 0·08
Osmolarity (μ-osmole/100 g/hr)	192 ± 8·6	323 ± 12·3	320 ± 10
Sodium (μ-equiv/100 g/hr)	10·3 ± 1·8	51·1 ± 8·0	35·7 ± 2·7
Potassium (μ-equiv/100 g/hr)	13·2 ± 2·5	37·9 ± 9·2	23·3 ± 0·9
Creatinine (mg/100 g/hr)	0·195 ± 0·008	0·21 ± 0·006	0·186 ± 0·014
Water clearance (ml./100 g/hr)	+ 1·186 ± 0·09	+ 0·769 ± 0·078	+ 0·815 ± 0·005

H-ase appeared to be present in the urine of hydrated rats and was only slightly and not significantly greater in that from rats receiving hydrochlorothiazide.

Another method of estimation, by reduction of the turbidity of chondroitin sulphate and CTAB, was unaffected by the presence of protein and yielded the results shown in Fig. 2. As blank values (boiled urine) were not obtained for all samples, the amounts of H-ase do not represent absolute values. It will be seen from Fig. 2, however, that although small amounts were sometimes present in the urine of animals receiving water alone at the onset of diuresis, for similar rates of urine flow appreciably more was present in those receiving hydrochlorothiazide. This excretion of H-ase was accompanied by excretion of small amounts of acidic mucopolysaccharides, averaging < 5 μg/ml. in those receiving water alone and .8 μg/ml. in those receiving hydrochlorothiazide. In four groups of dehydrated animals receiving 1 ml. water/100 g, with or without hydrochlorothiazide, acidic mucopolysaccharides averaged 80 μg/ml. (60–95 μg/ml.) and reduction in turbidity 49 % (46–58 %).

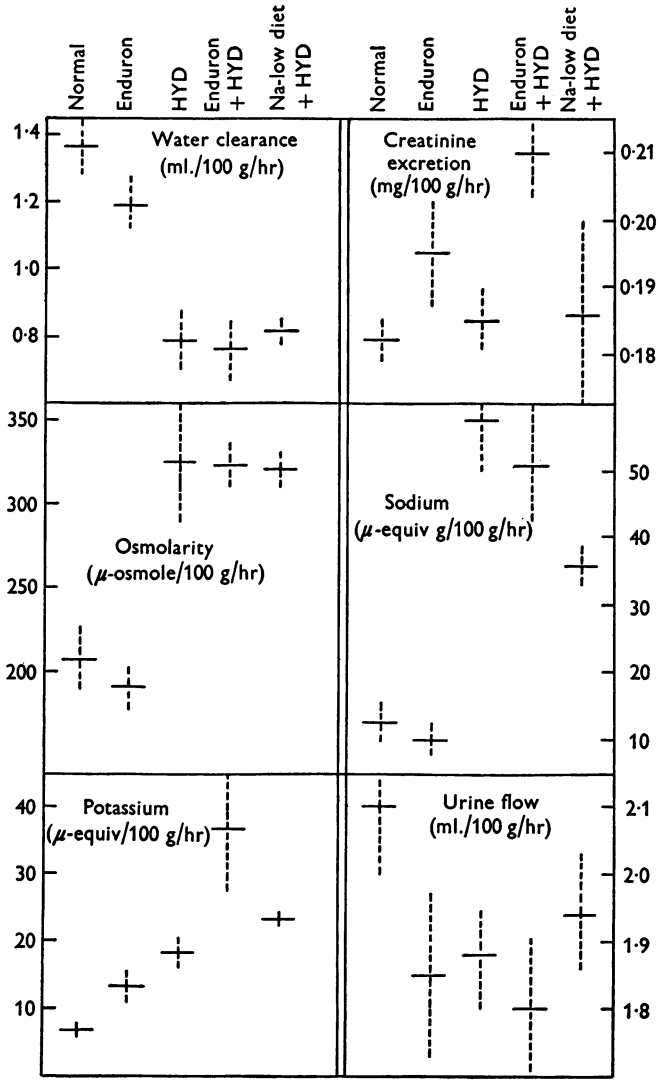

Fig. 1. The effect of various agents on renal function in rats hydrated with 5 ml. H_2O/100 g. The broken vertical lines indicate the standard error of the means. (Hydrochlorothiazide is indicated by HYD.)

DISCUSSION

While this manuscript was in preparation, our attention was drawn to a paper by Dies & Rivers (1962) who have shown conclusively that the antidiuretic action of benzothidiazine, another derivative of the thiazide group of drugs, in water diuresis in man, is due to a decrease of free water clearance. This present work confirms and extends their conclusions to

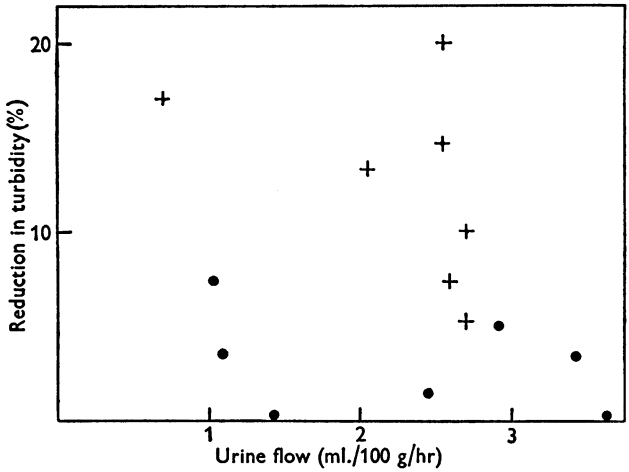

Fig. 2. The effect of hydrochlorothiazide on the urinary excretion of hyaluronidase. ● = 1 ml. H_2O/100 g rat; + = 0·1 mg hydrochlorothiazide in 1 ml. H_2O/100 g rat.

rats, in whom free water clearance was reduced by hydrochlorothiazide whether the animals were hydrated, non-hydrated or dehydrated.

There is some evidence that the diuretic action of chlorothiazide and its derivatives is due to its blocking the action of aldosterone (Kennedy & Crawford, 1961) and the balance of evidence favours its action as being on the distal tubule. This would result in an osmotic diuresis and is observed in the non-hydrated and dehydrated rats. In the hydrated animals, however, the decrease in free water clearance is sufficient to mask this effect and a decrease in rate of urine flow occurs.

In the light of modern views on the mechanisms of concentration and dilution of urine, a decrease of free water clearance would occur when the collecting tubules, normally impermeable in the hydrated animal, become permeable to water. ADH is thought to act in this way, by causing liberation of H-ase which depolymerizes the cement substance between the cells of the collecting tubules. Excretion of small amounts of the enzyme under the action of hydrochlorothiazide, together with the appearance of acidic mucopolysaccharides, suggests that reduction of free water clearance by the drug may be of the same nature as that effected by ADH. There is no evidence at present to indicate whether this might be a direct action on the renal tubule walls or whether indirect, via hypothalamic centres or post-pituitary, by liberation of ADH.

SUMMARY

1. Hydrochlorothiazide decreases free water clearance and increases sodium excretion in hydrated, non-hydrated and dehydrated rats.

2. In the two latter groups of animals, the second effect is dominant and an osmotic diuresis occurs.

3. In the hydrated animals, the decrease in free water clearance overrides the osmotic effect and a decrease of urine flow results.

4. This is accompanied by an increased excretion of H-ase and acidic mucopolysaccharides. Both are excreted in much greater amount by dehydrated rats.

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