Br. J. Pharmac. (1977), **59**, 11–16

POTENTIATION OF THE RESPONSE TO VASOPRESSIN (PITRESSIN) BY TREATMENT WITH A COMBINATION OF CHLORPROPAMIDE AND CHLOROTHIAZIDE IN BRATTLEBORO RATS WITH HEREDITARY HYPOTHALAMIC DIABETES INSIPIDUS

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1 The effect of a combination of chlorpropamide and chlorothiazide in Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI) treated with low doses of vasopressin (Pitressin tannate in oil) was investigated with particular reference to the time course of response from the initiation of treatment.

2 Analysis of the relationship between water intake and body weight indicated no real correlation and body weight accounted for only 4.4% of the variation in water intake. It was therefore decided to use whole body responses as the index in preference to the response per unit body weight.

3 The daily administration of 5 mg chlorpropamide combined with chlorothiazide in the drinking water (4 mg/l) to Pitressin-treated DI rats potentiated the response to small doses of vasopressin (25 and 50 mu Pitressin/24 hours). Water intake was reduced by the drug combination by an average of 12.35 ml/24 h, but only on the second day of treatment was the decrease of any real magnitude (30 ml/24 h but otherwise 9 ml/24 h or less). Analysis of urine volume measurements gave similar results to those obtained for water intake and the potency ratio measured in terms of free water clearance was 1.26 (agreeing closely with the ratio for water intake which was 1.24).

4 A reduction in the solute excretion was observed only in those DI rats treated with the higher dose of Pitressin (50 mu/24 h) combined with the two drugs.

5 Possible reasons for the discrepancy between the effect of the combination of chlorpropamide and chlorathiazide on water metabolism in the DI rat and the DI patient are discussed.

Introduction

In studies on the effect of chlorpropamide in Brattleboro rats with hereditary diabetes insipidus (DI) it has been shown that the drug appears to potentiate the antidiuresis induced by small amounts of exogenous vasopressin (Berndt, Miller, Kettyle & Valtin, 1970; Miller & Moses, 1970). Laycock, Lee & Lewis (1974) recently showed that while the potentiating effect of chlorpropamide did exist in these experimental animals treated with a small amount of vasopressin (25 mu Pitressin/24 h) this effect was minimal and could not be compared with the sometimes dramatic reductions in water excretion observed in many patients with diabetes insipidus (e.g. Arduino, Ferraz & Rodriguez, 1966; Wales & Fraser, 1971).

Chlorothiazide, on the other hand, has been shown to have an antidiuretic effect independent of vasopressin in an uncontrolled trial on patients with DI (Crawford, Kennedy & Hill, 1960), rats with experimental DI (Skadhauge, 1966) and in Brattleboro DI rats (Laycock, 1973). In the present study, the effect of a combination of the two drugs, chlorpropamide and chlorothiazide, has been measured in the Brattleboro DI rat treated with low doses of Pitressin to examine whether the antidiuresis is significantly improved by such a therapy. In addition, the time course of the changes in water intake, urine volume and solute output were studied, in order to determine the possible mechanism (or mechanisms) involved in potentiating the actions of the antidiuretic hormone, vasopressin.

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Methods

The general methodology used for this experiment has been described by Laycock *et al.* (1974). Sixteen

female adult Brattleboro DI rats weighing between 175 and 240 g were divided into four equal groups (A, B, C and D). Rats in groups A and B were placed in individual netabolism cages, while those in groups C and D were placed in individual normal plastic cages allowing water intake measurements only.

A 2 × 2 bioassay was performed on each group of rats simultaneously using a Latin square treatment schedule within each group to balance animal and time effects. Each one of four treatments was given for four days, during which water and/or urine measurements were made daily, and this was followed by an interval of three days before the consecutive treatment of the schedule. (A pilot study had previously shown that this interval was adequate to eliminate residual effects from the various treatments.) The standard drug vasopressin was used in the form of Pitressin tannate in oil; this was injected subcutaneously into the dorsal surface of each rat in doses of 25 and 50 mu/24 h (Treatments S1 and S2). The responses to these doses were known to be submaximal (Laycock & Williams, 1973). Treatments T1 and T2 consisted of the same two doses of Pitressin respectively, combined with the two drugs under investigation. These were chlorpropamide, also injected subcutaneously at a dose of 5 mg/24 h, and chlorothiazide which was added to the drinking water at a concentration of 4 mg/l so that the daily dose to each rat was around 0.5 mg.

This experimental design was therefore completed in four weeks and provided data for a 4 × 4 Latin square which for water intake was replicated four times so that there was information about the effect of housing the animals in metabolism cages. For each animal/treatment combination there were four sequential daily observations and these provided information about any trend during treatment.

Results

Choosing an index of response

While it is common practice to express water intake or urine output per 100 g body weight, no evidence of a correlation between body weight and these two volumes has been presented in the literature. When no correlation exists, an index of response where water intake or urine output is expressed per 100 g body weight becomes inappropriate for such a derived index must inevitably have a higher error variance (Lewis, 1974). It is therefore necessary to determine a suitable response index for this particular experiment.

The initial and final body weights of the 16 animals used in the experiment together with the total water intake for the 16 days of observation are given in Table 1. Every animal received each one of the four treatments during this period and the relationship between these totals should be little affected by the treatments. The range of the water intake values during the experiment was highest on Treatment S1 at 157.5 ml/24 h and lowest on Treatment T2 at 105.9 ml/24 hours. There was almost zero correlation ($r = 0.007$) between the water intakes and the average of the initial and final body weights for each animal. This point was explored in more detail by using analysis of covariance to determine the correlation

Table 1 The initial and final body weights of the sixteen DI rats used in the experiment together with the total water intake for each rat over the 16 days of treatment (see text)

		Initial weight	*Final weight*	*Total water intake (16 days)*
Group A	1	225	215	2199
	2	220	217	1831
	3	240	245	2123
	4	240	250	2343
Group B	1	205	220	2387
	2	190	200	1606
	3	175	200	2124
	4	200	210	2184
Group C	1	210	220	2078
	2	235	232	2118
	3	235	245	1887
	4	215	225	2846
Group D	1	225	220	2055
	2	215	210	2954
	3	195	202	889
	4	220	242	1227

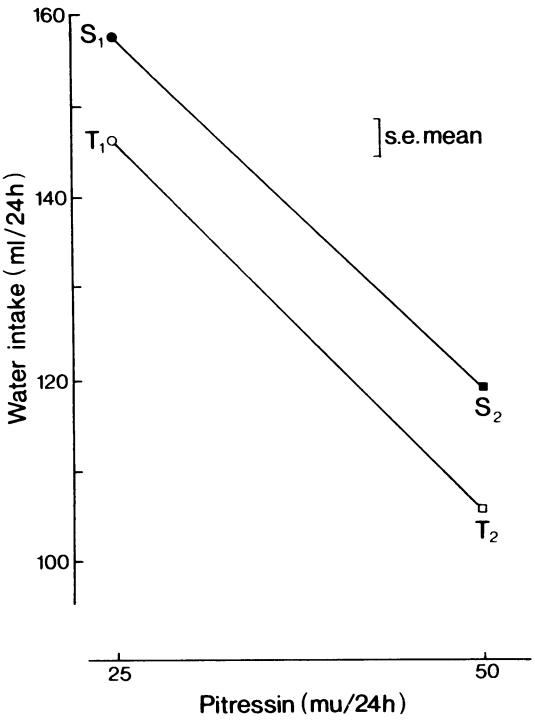

Figure 1 The 2 x 2 assay for the mean water intake of 16 Brattleboro DI rats treated with 25 and 50 mu Pitressin/24 h (treatments S1 and S2) and with the same two doses of Pitressin/24 h combined with the two drugs chlorpropamide and chlorothiazide (treatments T1 and T2).

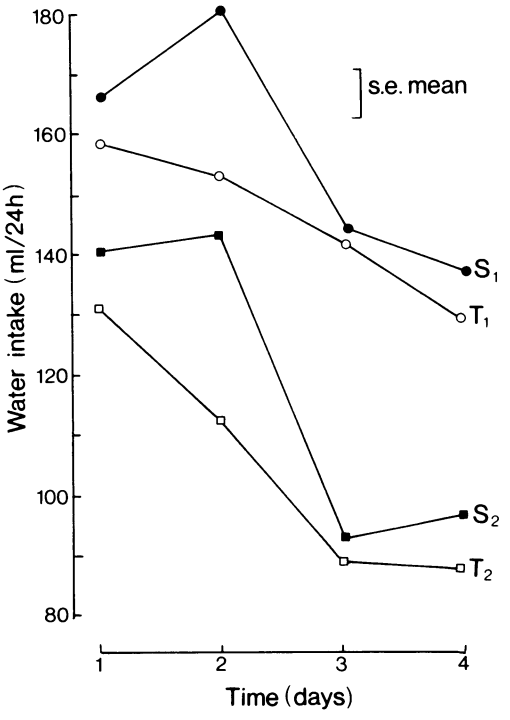

Figure 2 The mean daily water intake of the 16 Brattleboro DI rats on each of the four days of treatment with 25 and 50 mu Pitressin/24 h (treatments S1 and S2) and the same two doses of Pitressin/24 h combined with the two drugs chlorpropamide and chlorothiazide (treatments T1 and T2).

within groups after adjusting for any differences in treatment response. It was shown that the degree of correlation was still not significant and body weight accounted for only 4.4% of the variation in water intake. It was therefore decided to use whole body responses as the index in preference to the response per unit body weight.

The effect of the drug combination on water intake

Water intake measurements were available for all four groups of animals, and the overall response is shown in Figure 1. This assay satisfied the requirement of similarity (parallelism of the log-dose response lines, $P=0.80$), and the effect of doubling the dose of vasopressin was to decrease water intake by 39.3 ml/24 h ($P<0.001$). The effect of the combination of the two drugs chlorpropamide and chlorothiazide decreased water intake by 12.35 ml/24 h ($P<0.01$), giving a potency ratio of 1.24 (95% confidence limits: 1.06, 1.50). On each day of treatment the drug effect reduced water intake (see Figure 2) but only on the second day was it of any real magnitude (30 ml/24 h but otherwise 9 ml/24 h or less).

The effect of the drug combination on urine and solute outputs

Values for the urine and solute output were available for the eight animals in groups A and B and analysis of the urine output gave similar results to those obtained from water intake measurements (Table 2), the daily urine volume being on average 22.7 ml less than the daily water intake.

The daily solute output was calculated from the known urine volumes and urine osmolalities. The effect of the four treatments (S1, S2, T1 and T2) upon the solute excretion of DI rats is shown in Figure 3. A considerable degree of interaction exists and there is therefore no evidence for similarity between the treatment responses. On the basis of the results, only treatment T2 had any material effect on the solute output, eliminating the increase on day 2 (Figure 3b)

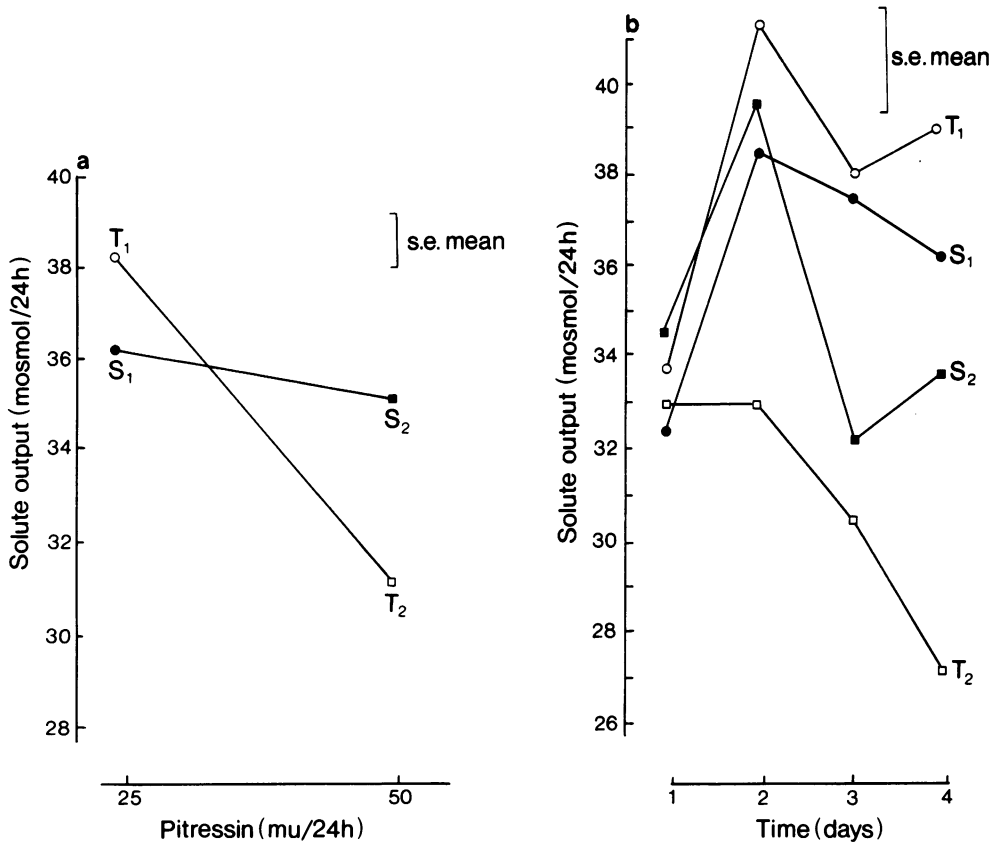

Figure 3 (a) The 2 x 2 assay for the mean total solute output of eight Brattleboro DI rats treated with the four treatments S1, S2, T1 and T2 (see legend for Figure 1). (b) The mean solute excretion of the same DI rats on each of the four days of treatment with S1, S2, T1 and T2.

and yielding a more progressive reduction in solute excretion over the 4 days (Figure 3a) than the other three treatments.

The relationship between urine volume and solute excretion is shown in Figure 4a. The insertion of an iso-osmotic line with a slope equal to the reciprocal of 0.3 mosmol/kg (assuming a rat plasma osmolality of 300 mosmol/kg) permitted rapid graphical determination of the response in terms of free-water clearance, thus avoiding an otherwise tedious calculation. The vertical distance from the zero free-water clearance line in Figure 4b for the four treatments is equal to the vertical distance from the iso-osmotic line in Figure 4a. The potency ratio for this assay is 1.26.

Table 2 The mean differences between water intake and urine output values for the eight rats in groups A and B (see text) for the four treatments used in the experiment

	Treatment				
Days	*S1*	*S2*	*T1*	*T2*	*Mean*
1	20.88	17.25	31.38	14.75	21.09
2	22.63	20.63	24.13	21.88	22.31
3	25.25	18.25	27.88	20.13	22.88
4	26.62	21.63	28.50	21.75	24.63
Mean	23.84	19.44	27.97	19.66	22.73

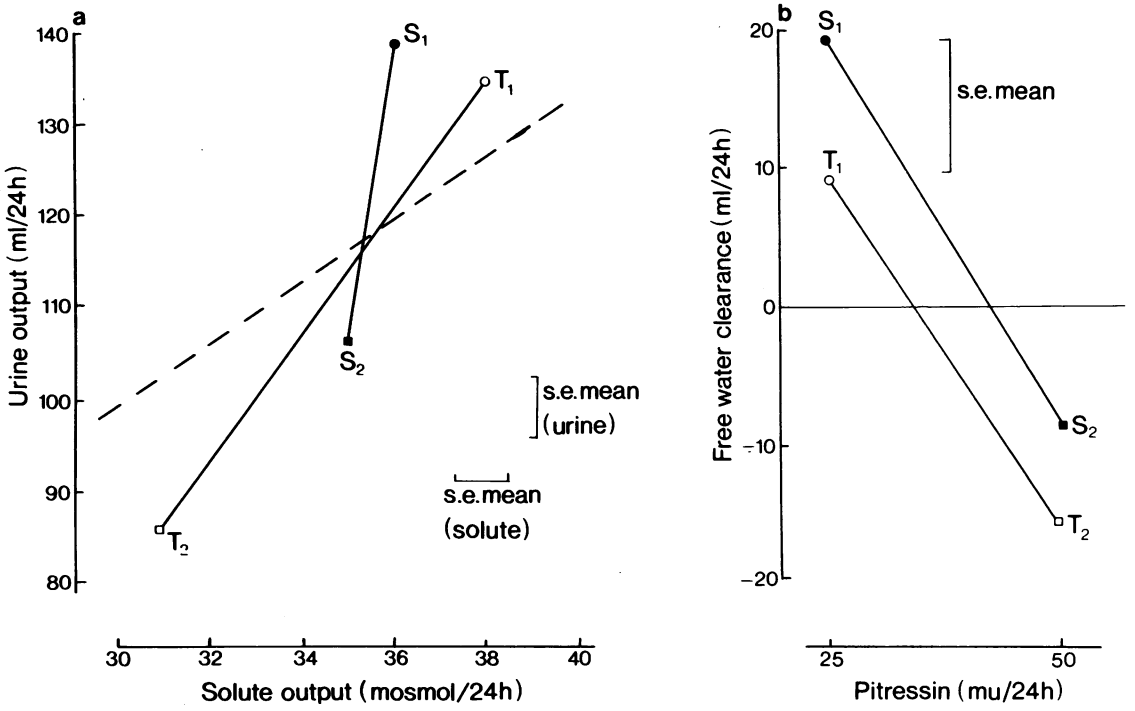

Figure 4 (a) The relationship between the mean overall urine and solute excretion of eight Brattleboro DI rats with the four treatments S1, S2, T1 and T2 (see legend to Figure 1). The dashed line represents the estimated iso-osmotic line (see text). (b) The overall effect of the four treatments (S1, S2, T1 and T2) on the free-water clearance in the eight DI rats.

Discussion

In studies upon patients suffering from diabetes insipidus, both chlorpropamide and various thiazides have been shown to exert an antidiuretic effect. Webster & Bain (1970) reported complete control with chlorpropamide alone in six out of eleven patients suffering from either idiopathic diabetes insipidus or a secondary form stemming from a known hypothalamic or pituitary lesion. The remaining five patients were controlled by a combination of chlorpropamide and a thiazide. These authors concluded that chlorpropamide alone excited a powerful effect in controlling the diuresis in vasopressin-deficient patients. Their results also showed an enhancement of the effect of chlorpropamide by the addition of the thiazide (hydrochlorothiazide) although fully controlled studies were not made.

In the present experiments, the effect of a combination of chlorpropamide and chlorothiazide on the water balance of Brattleboro rats with hereditary hypothalamic DI treated with small doses of Pitressin was examined. The 5 mg/24 h dose of chlorpropamide injected into each DI rat in this experiment compared with doses generally administered to DI patients (250–500 mg/24 h) when calculated on a surface-area ratio basis. The quantity of chlorothiazide ingested by each rat (average weight 220 g) daily was of the order of 0.5 mg/24 h, which dose using the same surface-area ratio would correspond to some 20 mg/24 h to an average DI patient weighing some 70 kg. Such a dose of chlorothiazide would be unlikely to have any direct antidiuretic effect in treating a patient with DI, on its own, so that it was hoped that if it also had an antidiuretic effect via the chlorpropamide-Pitressin system, such an effect might be detectable in the present experiment. However, the potentiation of the vasopressin-induced antidiuresis by the drug combination was shown to be significant, but surprisingly small when compared to the clinically relevant effect observed in many DI patients. The administration of the two drugs chlorpropamide and chlorothiazide with 50 mu Pitressin/24 h (treatment T2) no longer produced an increased solute output on any of the four days of treatment but gave a progressive reduction in output over the four days.

A response expressed in terms of free water clearance gives a very similar overall potency ratio (1.26 against 1.24 for water intake) but with slightly

more evidence of interaction between the two treatments (shown by the slight convergence of the response lines). On this basis, water intake is the preferred response variable for bioassay.

Another feature of interest concerned the daily trend of the drug-effect, most clearly shown for the water intake measurements (Figure 2). The drug effect was present on all four days of treatment, but only on the second day of treatment was it of real importance. Since the effect due to the vasopressin alone increased to reach a maximum on the third or fourth day of treatment, these time trends could indicate that the combination of drugs might increase the velocity with which the concentrating ability of the kidney is stimulated by the action of vasopressin. One possible mechanism could be to increase the medullary interstitial fluid concentration which is a prerequisite for the maintenance of the countercurrent multiplier loop.

To conclude, the administration of a combination of chlorpropamide and chlorothiazide in the present doses to Brattleboro DI rats treated with small doses of Pitressin is insufficient to restore their water balance to levels which are comparable to those observed in normal rats. This observation differs from the reported extremely successful effect of comparable doses of these drugs in DI patients (e.g. Webster & Bain, 1970). However, there is evidence of a dose-dependent action of vasopressin on solute excretion in these experimental animals which is modified by combination with the other drugs. Since the possibility of a renal defect in the Brattleboro DI rat cannot be discounted, further studies using this experimental model are necessary.

J.F.L. is grateful for the support of the MRC during the completion of this work. The Pitressin tannate in oil was kindly donated by Parke, Davis and Company, and the chlorpropamide by Pfizer Limited.

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(Received December 1, 1975. Revised August 12, 1976.)