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THE EFFECT OF CHLORPROPAMIDE ON WATER BALANCE IN PITRESSIN-TREATED BRATTLEBORO RATS

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1 The daily administration of a 5% glucose solution to the heterozygous Brattleboro rat produced an experimental model in a comparable state of polydipsia and polyuria to the homozygous rat with diabetes insipidus (DI).

2 The effect of chlorpropamide on water metabolism was then examined in both the homozygous DI rat treated with submaximal doses of pitressin tannate in oil (Pitressin), and in the glucose-hydrated heterozygous rat with and without simultaneous pitressin therapy.

3 A dose-response curve for chlorpropamide (5, 10, and 20 mg/24 h) in DI rats treated with Pitressin (25 mu/24 h) indicated that the drug decreased fluid intake further, but only by a maximum of 13.8% (at the 20 mg/24 h dose of chlorpropamide), differing markedly from results obtained in patients with diabetes insipidus. A second experiment in which chlorpropamide (5 mg/24 h) was administered to DI rats treated with Pitressin (either 25 or 50 mu/24 h) confirmed the lack of any significant drug-effect on water metabolism in these animals.

4 Chlorpropamide (20 mg/24 h), when administered alone or simultaneously with a submaximal dose of Pitressin (25 mu/24 h), had no obvious effect on the fluid intake of glucose-hydrated heterozygous rats. The absence of any action by chlorpropamide on water metabolism was confirmed in these experimental animals using 5 mg/24 h of the drug together with Pitressin (either 25 or 50 mu/24 hours).

5 Indirect evidence for the slower growth-rate in the DI rat being due to an insufficient daily calorific intake was obtained from the study on glucose-hydrated heterozygous rats.

Introduction

Since the fortuitous discovery that chlorpropamide, a hypoglycaemic drug, could act as an antidiuretic (Arduino, Ferraz & Rodriguez, 1966), the drug has been used with apparent success in the treatment of many patients with vasopressin-sensitive diabetes insipidus (e.g. Webster & Bain, 1970).

Certain possible mechanisms of action for the drug have been listed (Wales & Fraser, 1971), and include a primary antidipsic effect acting centrally in the hypothalamic region (Bergmann, Zerachia & Gutman, 1968), a central effect resulting in an increased secretion of vasopressin (Miller & Moses, 1970a), and a potentiating effect of the antidiuresis induced by small, submaximal amounts of circulating vasopressin (Berndt, Miller, Kettyle & Valtin, 1970; Miller & Moses, 1970b; Zweig, Ettinger & Earley, 1971). A direct action of chlorpropamide on the renal tubules in a manner analogous to that of vasopressin would appear to be unlikely from *in vitro* studies on toad bladder preparations (Inglefinger & Hays, 1969: Mendoza, 1969) and from *in vivo* studies on homozygous Brattleboro rats with hereditary hypothalamic diabetes insipidus (DI) (Berndt *et al.*, 1970; Miller & Moses, 1970b; also Jones & Lee, personal communication).

In 1970, Ettinger & Forsham pointed out that there appeared to be a large difference between the antidiuresis observed in chlorpropamide-treated patients with DI (up to ten-fold decreases in urine volume) and the relatively minute antidiuresis induced by the drug even in the presence of submaximal amounts of vasopressin, in studies on toad bladder preparations (e.g. Mendoza, 1969) and in DI rats (e.g. Berndt *et al.*, 1970).

The present study was therefore undertaken to verify whether chlorpropamide had a significant effect on water metabolism in Pitressin-treated homozygous DI rats, comparable to the effect observed in the vasopressin-sensitive patient with DI. As a control the glucose-hydrated heterozygous rat was used, since it was considered to be a better model for comparison with the homozygous

rat with DI. In addition, polydipsic heterozygous rats might provide evidence for any antidipsic action of the drug chlorpropamide.

Methods

Brattleboro rats were classified as homozygous or as heterozygous by the criteria of Lee & Williams (1972), which were urine volume/24 h and urine osmolality. Rats were placed either individually in metabolism cages (E.K. Bowman, London NW5), or in groups of four in standard plastic cages (see individual experiments). All animals ate Dixon's FFG-M diet (18.6% protein, 58.4% carbohydrate, 1.96% fat, 0.5% Na^+, 0.73%K^+), either as powder or as pellets. Glucose solutions (5%) for the heterozygous rats were prepared fresh each day and replaced daily. All fluid volumes were measured to the nearest ml and urine osmolalities were estimated with an Advanced Digimatic Osmometer (Model 3D). Injections were all given subcutaneously into the dorsal surface of the experimental animals, the injection-area being rotated frequently. Vasopressin was administered as Pitressin tannate in oil (Pitressin, containing a mixture of porcine and bovine vasopressins in unspecified proportion). Two of the doses administered (25 and 50 mu Pitressin/24 h) were considered as submaximal from the dose-response curve obtained by Laycock & Williams, (1973). Chlorpropamide, from 10 ml vials containing 50 mg/ml, was also injected daily into the experimental animals, the injection volume being proportional to the dose.

Glucose experiments in the heterozygous rats

Experiment 1a Eight female adult heterozygous rats weighing between 234 and 281 g at the beginning of the experiment, were placed in individual metabolism cages. After a 6 day habituation period, measurements of water intake, urine volume and urine osmolality were made daily for the subsequent 4 day control period, at the end of which the drinking water was replaced by a 5% glucose solution. Measurements of the three variables were made daily for the 14 days of this treatment period.

Experiment 1b Eight female adult heterozygous rats weighing between 202 and 275 g were placed in standard plastic cages, four rats to each cage. One group (A) of rats were given tap-water and food *ad libitum* daily for 17 days, while the second group (B) was given 5% glucose solution and food *ad libitum* over the same period. At the end of this 17 day period, group B received tap-water while group A received the 5% glucose solution daily, for the next 17 day period. Two further 17 day treatment periods then ensued, during which the two treatments were again exchanged, so that at the end of the experiment each group of rats had received tap-water or glucose solution for two 17 day periods each. Food and fluid intakes were estimated daily throughout the experiment. The calorific value of the diet (FFG-M) was calculated to be roughly 3 Kcal/g, while that of glucose was assumed to be 4 Kcal/g (1 Kcal $\approx$ 4.2 KJ).

Experiment 1c Sixteen adult female heterozygous rats weighing between 240 and 306 g, were placed in standard plastic cages, four rats to each cage. Throughout the experiment each group of rats (A, B, C and D) were offered 1000 ml 5% glucose solution daily. After a 7 day control period, each of the sixteen rats received Pitressin (50 mu/24 h) for 4 successive days. After a 3 day recovery period, the sixteen rats received Pitressin (100 mu/24 h) for 4 days. Treatment with Pitressin (200 and 400 mu/24 h) for 4 day periods also followed intervening 3 day recovery periods.

A period of 8 days elapsed during which the rats received fresh tap-water daily. After this period, the rats were given 5% glucose solution for 5 days during which fluid intakes rose to values measured in the initial glucose-control period. For the following 4 days, Pitressin (800 mu/24 h) was administered to each of the sixteen rats.

Chlorpropamide experiments in DI rats

Experiment 2a Sixteen adult DI rats, eight male and eight female, were placed in standard plastic cages, four rats of the same sex to each cage. The male rats weighed between 212 and 316 g, and the female rats weighed between 192 and 252 g. After a 6 day control period, all sixteen rats received Pitressin (25 mu) daily for 4 days. After a recovery period of 3 days, the DI rats received Pitressin (25 mu) and chlorpropamide (5 mg) daily for the subsequent 4 days. After a further 3 day recovery phase the sixteen rats were injected with Pitressin (25 mu) and chlorpropamide (10 mg) daily for 4 days followed by another 3 day recovery phase. Finally, each rat was treated with Pitressin (25 mu) and chlorpropamide (20 mg) daily for 4 days. Water intakes were measured daily for each group of rats.

Experiment 2b Eight female adult DI rats weighing between 224 and 236 g were placed in individual metabolism cages. The experiment lasted for 4 weeks, treatment being applied for 4 days each week with an interval of 3 days between

successive treatments. Animal and time effects were balanced by a Latin Square treatment schedule. The two Standard treatments (S1 and S2) consisted of Pitressin (25 and 50 mu/24 h) respectively. The two Test treatments (T1 and T2) consisted of the same two doses of Pitressin respectively, combined with chlorpropamide (5 mg/24 hours).

	Rat 1	Rat 2	Rat 3	Rat 4
Period 1	S1	S2	T1	T2
Period 2	T2	T1	S2	S1
Period 3	S2	S1	T2	T1
Period 4	T1	T2	S1	S2

Fluid intakes were estimated daily, together with urine volume and urine osmolality.

Chlorpropamide experiments in glucose-hydrated heterozygous rats

Experiment 3a Eight female adult heterozygous rats weighing between 270 and 360 g were placed in two standard plastic cages, four rats to each cage. The rats were given 5% glucose solution and food *ad libitum* daily. Each glucose-hydrated heterozygous rat received chlorpropamide (20 mg) daily for the subsequent four days. After a 3 day recovery phase, during which the eight rats were maintained on 5% glucose solution, a second 4 day treatment period was started. Each rat this time received chlorpropamide (20 mg) and Pitressin (25 mu) daily. Fluid intakes were estimated daily throughout the experiment.

Experiment 3b Sixteen female adult heterozygous rats weighing between 241 and 302 g were placed in standard plastic cages, four rats to each cage. The rats were maintained on 5% glucose solution and food *ad libitum* throughout the experiment which lasted for 4 weeks. The experiment was again based on a Latin Square treatment schedule (see Experiment 2b). The doses of Pitressin and chlorpropamide given in the four treatments (S1, S2, T1 and T2) were identical to those described in experiment 2b. Fluid intakes were estimated daily throughout the four treatment periods.

Statistics

Significant differences were calculated by Student's *t* test. Probabilities referred to as 'significant' are $P<0.05$; 'not significant' differences are $P>0.05$. Analyses of variance were performed on measurements made in the Latin Square experiment with DI rats, according to Bailey (1969).

Results

Glucose experiments in the heterozygous rats

Experiment 1a The effect of replacing the drinking water of heterozygous rats with 5% glucose solution on the fluid volumes, urine osmolality and solute output is shown in Figure 1. Fluid intakes increased from approximately 30 ml/24 h in these rats to values over 140 ml/24 hour. Urine volumes increased correspondingly from some 15 ml/24 h to values over 100 ml/24 hour. The difference between daily fluid intake and urine output increased from approximately 15 ml to over 30 ml when the rats started drinking the glucose solution. This input-output increase could be accounted for partly by increased experimental inaccuracy due to increased fluid loss from the drinking bottles when the rats eagerly lick the nozzles.

There was a decrease in urine osmolality from over 1400 mosmol/kg during the period of treatment with the 5% glucose solution. Although fluid intake, urine volume and urine osmolality measurements approached values for DI rats within 2 days, there was a gradual tendency for the three parameters to drift even closer to the DI values throughout the 14 days of glucose treatment. On the 14th day (day 18, Fig. 1), the mean fluid intake was nearly 160 ml/24 h, the mean urine volume had risen to about 110 ml/24 h and the mean urine osmolality had decreased to 200 mosmol/kg H_2O.

The mean daily solute output of the heterozygous rats rose from about 18 mosmol/24 h in the control period (days 1 to 4, Fig. 1) to about 24 mosmol/24 h for the next 6 days while on 5% glucose. On the 7th day of glucose-treatment, an unaccountable decrease in the solute output to 13.4 mosmol/24 h occurred. This decrease was followed by a gradual return to 24 mosmol/24 h over the following 5 days.

Experiment 1b The calorific intakes of female heterozygous rats drinking tap-water (controls) or 5% glucose solution were calculated from the measurements of food and fluid intake, measured daily. The control values for these measurements and the mean calorific intakes are given in Table 1a. The results of the effects of 5% glucose solution on fluid, food and calorific intake of the same heterozygous rats are summarized in Table 1b. Periods 1 and 2 are the two 17 day periods each group was on that particular treatment.

The means with s.e. mean for the control water intakes of both groups (A and B) during periods 1 and 2 (see Table 1b) were not significantly different from each other. The mean volume of

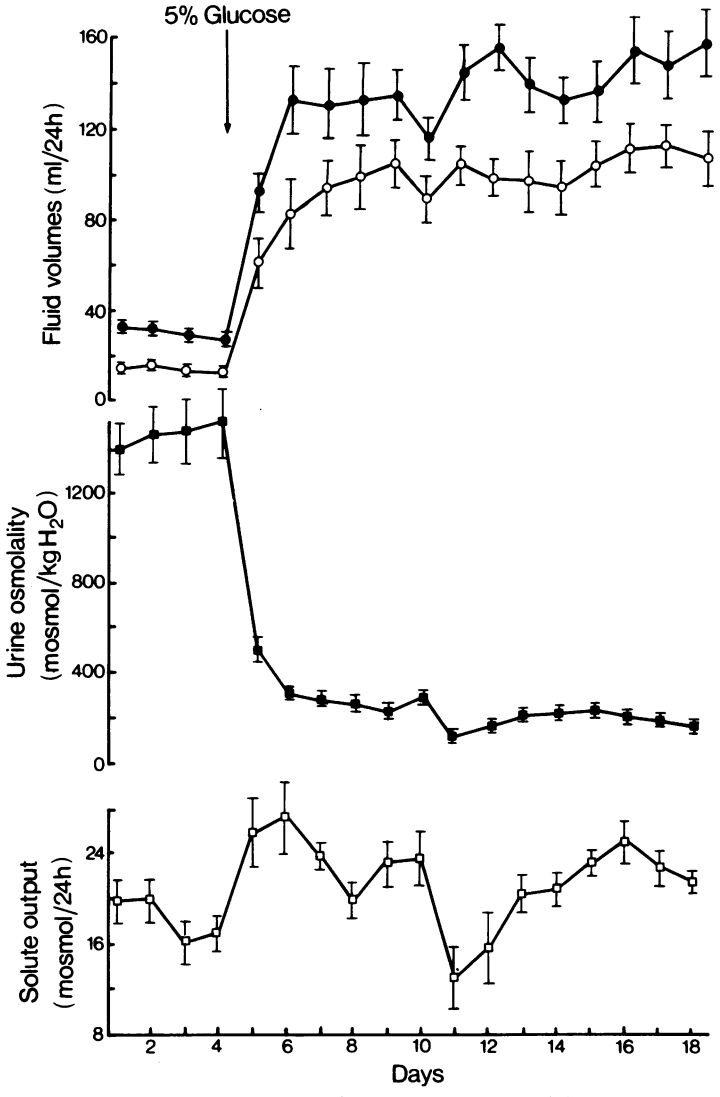

Fig 1 The effect of 5% glucose on the fluid intake (•) and urine volume (○), urine osmolality (■), and solute output (□) of eight heterozygous Brattleboro rats. The standard errors are represented by vertical bars through each of the mean values.

water drunk by each heterozygous rat ranged from 33.15 ± 2.72 to 37.70 ± 2.16 ml/24 hours. The means with s.e. mean for the control food intakes of both groups during the same periods 1 and 2 were also not significantly different from each other, ranging from 17.35 ± 0.83 to 19.72 ± 0.94 g/24 hours. From the mean values obtained for food intake, the mean daily calorific intake of heterozygous rats was calculated. The mean daily calorific intakes for the two groups of rats did not differ significantly from each other, ranging from 52.05 ± 2.49 to 59.16 ± 2.82 Kcal/24 h (see Table 1a).

In Table 1b the means with s.e. mean for the fluid, food and calorific intakes of the two groups of heterozygous rats while on 5% glucose solution and food *ad libitum*, can be compared. The mean fluid intakes during periods 1 and 2 within each group did not differ significantly whereas the two means for group A were both significantly higher than those for group B (compare 190.93 ± 17.87 and 214.91 ± 10.97 with 138.63 ± 8.04 and 141.66 ± 6.62 Kcal/24 hours). It is interesting to note that a similar relationship between mean fluid intakes of the two groups was also present during the control periods on tap-water, although the

differences then were not significant; (i.e. replacing the tap-water with the 5% glucose increased the difference in fluid intakes between the two groups sufficiently to make it significant). The mean food intakes with s.e. mean for groups A and B while on 5% glucose solution are also shown in Table 1b; no significant difference existed between them. The calculated calorific intakes were not significantly different from each other, although the means for group B were both slightly lower than those for group A (69.58 ± 4.46 and 67.96 ± 6.72 Kcal/24 h compared with 78.68 ± 8.46 and 76.73 ± 5.82 Kcal/24 hours). The mean calorific intake was significantly greater when the heterozygous rats were drinking 5% glucose solution than when they were drinking tap-water (compare mean values, Tables 1a and b). Thus, even though food intake decreased when on 5% glucose solution, the total daily calorific intake increased significantly.

Experiment 1c The effect of various doses of Pitressin on the fluid intake of heterozygous rats drinking 5% glucose solution is shown in Figure 2. The mean fluid intake decreased from 195.1 ± 37.6 ml/24 h to 138.6 ± 8 ml/24 h with Pitressin (50 mu) daily for 4 days, corresponding to a 29% decrease. The doses of Pitressin (100, 200, 400 and 800 mu/24 h) for 4-day treatment periods reduced the mean fluid intake by 42.7, 59.9, 70.4 and 82.6%, respectively. The large standard error of the mean for the Pitressin (200 mu/24 h) dose was mainly due to the exceptionally high fluid intake measurement made on the first day of the 4 day treatment period. This was due to a delayed response in 4 of the 16 experimental animals. Extrapolation of the curve to the dose of Pitressin (1000 mu/24 h) would give fluid intakes of approximately 30 ml/24 h which can be considered as normal water-intake values for untreated heterozygous rats.

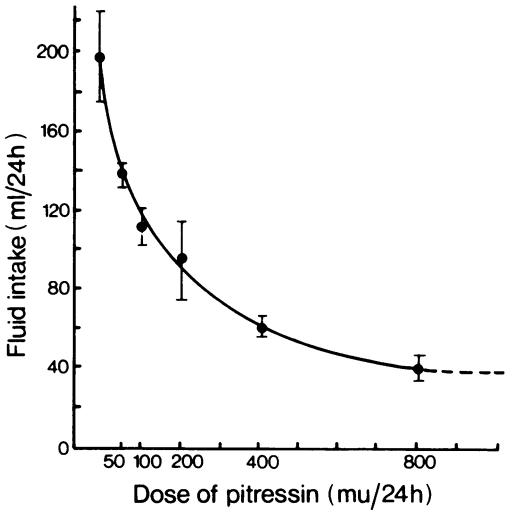

Fig. 2 The effect of graded doses of Pitressin tannate in oil (Pitressin) on the fluid intake of glucose-hydrated heterozygous Brattleboro rats. Each point (•) represents the mean fluid intake of 16 animals over a 4 day treatment period. The standard errors are represented by vertical bars through each of the mean values.

Table 1 Mean fluid intake (ml/24 h), food intake (g/24 h) and calculated calorific intake (Kcal/24 h) for the two groups (A and B) of heterozygous Brattleboro rats drinking (a) tap water and (b) 5% glucose solution, given together with s.e. mean.

Group	*Period*	*Fluid intake (ml/24 h)*	*Food intake (g/24 h)*	*Calculated calorific intake (Kcal/24 h)*
(a) Tap water				
A	1	37.70 ± 2.16	18.38 ± 1.17	55.14 ± 3.51
	2	36.69 ± 2.79	17.82 ± 0.67	53.46 ± 2.01
B	1	33.15 ± 2.72	19.72 ± 0.94	59.16 ± 2.82
	2	34.88 ± 0.91	17.35 ± 0.83	52.05 ± 2.49
Mean values		35.57 ± 2.27	18.30 ± 0.92	54.88 ± 2.76
(b) Glucose solution (5%)				
A	1	190.93 ± 17.87	13.50 ± 1.63	78.68 ± 8.46
	2	214.91 ± 10.97	11.25 ± 1.21	76.73 ± 5.82
B	1	138.63 ± 8.04	13.95 ± 0.95	69.58 ± 4.46
	2	141.66 ± 6.62	13.21 ± 1.80	67.96 ± 6.72
Mean values		172.02 ± 11.71	12.77 ± 1.44	72.72 ± 6.53

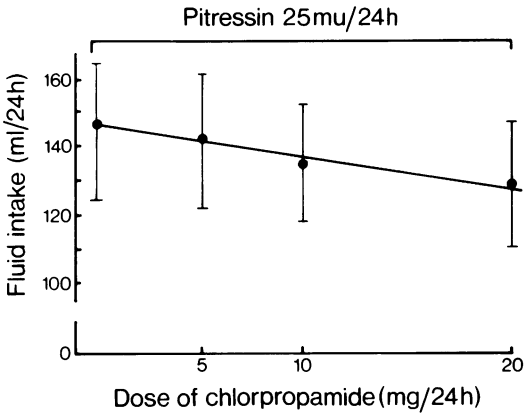

Fig. 3 The effect of various doses of chlorpropamide on the fluid intakes of DI rats treated simultaneously with Pitressin tannate in oil (Pitressin 25 mu/24 hours). Each point (•) represents the mean fluid intake of 16 animals over a 4 day treatment period. The standard errors are represented by vertical bars through each of the mean values.

Chlorpropamide experiments in DI rats

Experiment 2a After the initial control period of 6 days, the 16 DI rats were treated with Pitressin (25 mu/24 h) for 4 days during which the mean water intake decreased from the control mean value of 224 ml/24 h to 146 ml/24 h, corresponding to a 34.7% reduction. The effect of graded doses of chlorpropamide up to 20 mg/24 h on DI rats treated with a small submaximal amount of Pitressin, is shown in Figure 3.

After the 3 day recovery phase, treatment with Pitressin (25 mu/24 h) was resumed for the subsequent 4 days, together with chlorpropamide (5 mg/24 hours). The mean water intake decreased to 142 ± 19 ml/24 h corresponding to a further reduction of 2.7%. After the following 3 day recovery phase, chlorpropamide (10 mg/24 h) were administered to each DI rat, together with Pitressin (25 mu/24 h) for the next 4 days. The mean water intake decreased to 135 ± 18 ml/24 h corresponding to a reduction of 7.5%. The last dose of chlorpropamide (20 mg/24 h) when administered daily for 4 days together with Pitressin (25 mu/24 h) reduced the water intake to a mean value of 126 ± 18 ml/24 h, corresponding to a total decrease of only 13.8%.

Experiment 2b The results for fluid intake, urine volume, urine osmolality and solute output measurements obtained from the experiment to determine the effect of chlorpropamide on Pitressin-treated DI rats are given in Figure 4. An analysis of variance indicated that significant differences were present for the first three variables listed (Fig. 4a, b and c), but not for solute output (Figure 4d). S1 and S2 refer to Pitressin (25 and 50 mu/24 h), respectively, while T1 and T2 refer to the same two doses of Pitressin, respectively, combined with chlorpropamide (5 mg/24 hours). It can be seen from Fig 4a that the mean fluid intakes for days 2, 3 and 4 for treatments S2, T1 and T2 were always significantly lower than their respective day-1 values, whereas for S1 only the day 4 mean was significantly reduced. No significant differences existed between the overall means for treatments S1 and T1, nor between those for treatments S2 and T2 (141.3 ± 7.5 with 129.0 ± 7.5 ml/24 h, and 105.8 ± 7.5 with 100.5 ± 7.5 ml/24 h, respectively). The urine volumes showed exactly the same daily trends as the fluid intakes for treatments S2, T1 and T2 (Figure 4b). In this case, urine volumes with S1 decreased significantly on days 2 and 4, with reference to day 1. There was no significant effect of chlorpropamide on the response to Pitressin at either of the two doses of hormone used (107.8 ± 7.0 with 104.0 ± 7.0 ml/24 h for S1 and T1, respectively, and 80.0 ± 7.0 with 74.5 ± 7.0 ml/24 h for S2 and T2, respectively).

With regard to urine osmolality there was a significant increase by day 2 for treatments S2, T1 and T2, and this was maintained. The response to treatment S1 showed no significant variation over the 4 day treatment period (see Figure 4c). It can be seen that there is no overall significant difference between the Pitressin alone, and Pitressin combined with chlorpropamide (5 mg/24 h), at the two doses of hormone administered (309.5 ± 22.3 with 304.2 ± 22.3 mosmol/kg H_2O for treatments S1 and T1, respectively, and 396.0 ± 22.3 with 423.4 ± 22.3 mosmol/kg H_2O for treatments S2 and T2, respectively). The general trends for solute output were similar for all four treatments (Fig. 4d), significant changes occurring on days 2 and 4 for treatment T2 only. The means with s.e. means for S1, S2, T1 and T2 over the 4 day experimental period were 31.52 ± 1.94, 28.16 ± 1.94, 28.31 ± 1.94 and 26.45 ± 1.94 mosmol/24 h, respectively. No significant drug-effect was observed at either of the two doses of Pitressin used (25 and 50 mu/24 h).

From Fig. 4 it can be seen that, while there are general trends induced by chlorpropamide, the action of the drug on water metabolism remains dubious in the Pitressin-treated DI rat.

Chlorpropamide experiments in glucose-hydrated heterozygous rats

Experiment 3a In Fig. 5, the mean (± range) of the fluid intakes of glucose-hydrated heterozygous

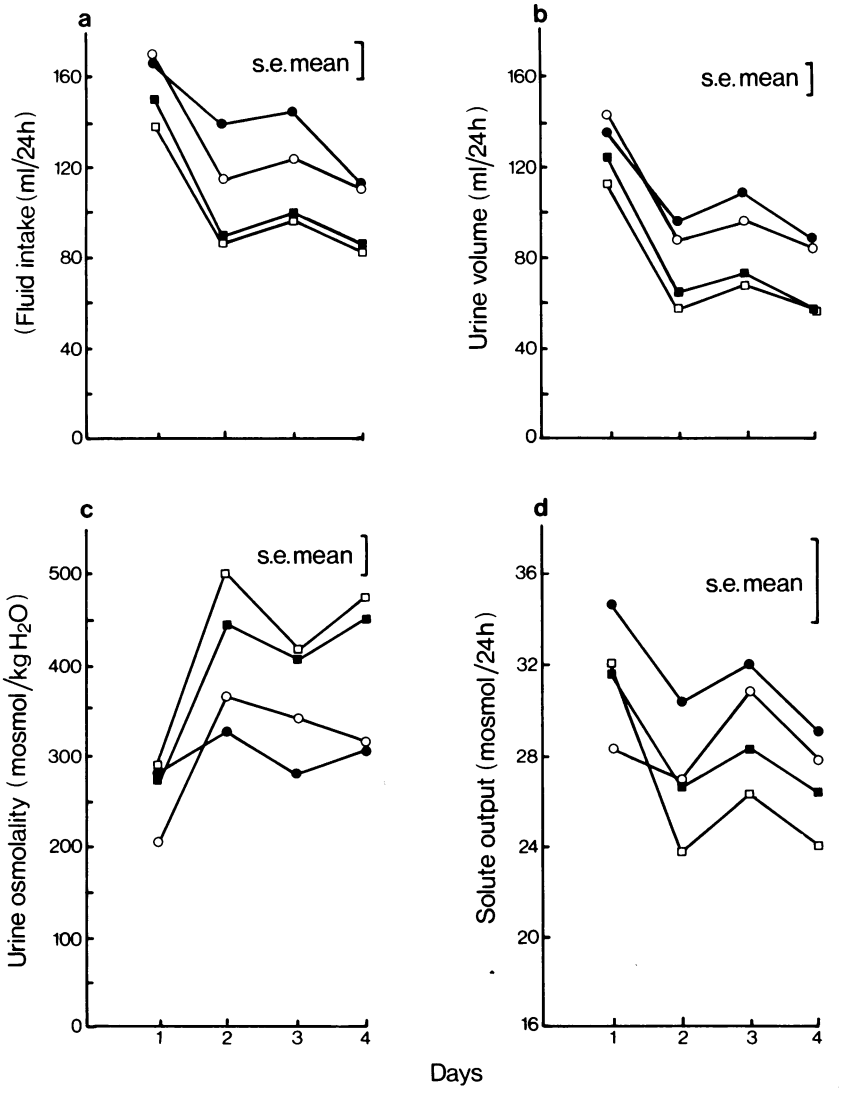

Fig 4 The effect of treatments S1, S2, T1 and T2 (see text) on (a) the fluid intake (b) urine volume (c) urine osmolality and (d) solute output of 8 DI rats. The four treatments are represented by the following symbols: (●) for S1, (■) for S2, (○) for T1 and (□) for T2. Each point represents a daily mean value. Standard errors (s.e. mean) are represented on each of the figures.

rats during 7 control days is given, such that the line indicates the lower limit of the control fluid intakes.

The daily treatment of each of the experimental animals with chlorpropamide (20 mg/24 h) for four days failed to reduce fluid intake (days 1 to 4, Figure 5). When treatment with the same daily dose of chlorpropamide was combined with the simultaneous administration of Pitressin (25 mu/24 h), a decreased fluid intake was observed (days 1^1 to 4^1, Figure 5). Comparison of the means for the control (113.7 ml/24 h) and this treatment (84.0 ml/24 h) periods showed a decrease of only 26.1%.

Experiment 3b The results of the Latin Square experiment in which glucose-hydrated heterozygous rats were used to determine the effect of chlorpropamide (5 mg/24 h) on two submaximal doses of Pitressin are given in Table 2.

No significant differences were found between the treatment means, probably due to the relative-

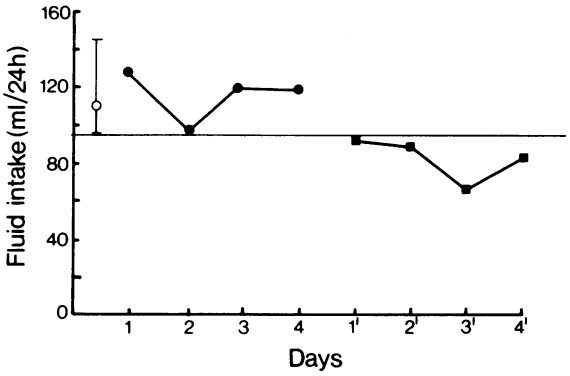

Fig. 5 The effect of chlorpropamide 20 mg/24 h (●), and the same dose of chlorpropamide combined with Pitressin Tannate in Oil 25 mu/24 h (■) on the daily mean fluid intakes of 8 glucose-hydrated heterozygous Brattleboro rats. The median control value is given (○), together with its range, to indicate the degree of variation of control values. The horizontal line represents the lowest control value obtained.

ly large standard errors. An insignificant increase in fluid intake was measured at the lower dose of Pitressin used (compare S1 and T1 treatment means ± s.e. mean) while the drug at the higher dose of hormone induced an insignificant decrease in fluid intake in these glucose hydrated heterozygous rats (compare treatment means ± s.e. mean for S2 and T2). From these results, it did not appear that chlorpropamide decreased fluid intakes significantly in the presence of Pitressin (either at 25 or 50 mu/24 hours).

Discussion

It is now generally accepted that chlorpropamide is of therapeutic value in the control of diabetes insipidus (DI) in man, although the mechanism of action of this drug is still in dispute. However, in the DI rat, which in the past has been used as its own control, the drug is unable to control water excretion, even when small, submaximal doses of vasopressin are administered simultaneously. The present experiments have attempted to determine what action (if any) the drug has on the water balance of these animals. In this investigation the heterozygous Brattleboro rat made polydipsic by the administration of glucose was used as a control as it more closely simulated the state of the DI rat, in that (1) both the DI rat and the control polydipsic heterozygous rat have similar fluid intake and excretory rates; (2) both rats should have similar metabolic rates; (3) an antidipsic

Table 2 The mean fluid intake for each group (A, B, C and D) of glucose-hydrated heterozygous rats with treatments S1, S2, T1 and T2 (see text).

Treatments	*Days*	*Daily Means (ml) Group A*	*Daily Means (ml) Group B*	*Daily Means (ml) Group C*	*Daily Means (ml) Group D*	*Daily Means (ml/24 h)*	*Treatment Means with s.e. mean (ml/24 h)*
S1	1	64.25	125.00	112.50	139.00	110.2	106.1 ± 25.9
	2	61.25	111.50	114.75	145.00	108.1	
	3	82.50	88.25	99.25	125.25	98.8	
	4	78.25	96.00	120.75	135.25	107.6	
S2	1	70.00	98.75	122.50	132.00	105.8	98.6 ± 18.9
	2	82.50	81.25	117.50	119.00	100.1	
	3	80.00	97.50	87.00	124.50	97.3	
	4	92.25	91.75	81.00	100.00	91.3	
T1	1	103.75	118.50	151.25	143.75	129.3	131.5 ± 31.9
	2	146.25	119.50	119.50	139.50	131.2	
	3	174.00	104.00	120.00	103.50	125.4	
	4	221.50	97.50	109.00	131.75	139.9	
T2	1	88.75	101.50	86.50	113.50	97.6	92.5 ± 8.5
	2	92.00	98.75	86.25	99.00	94.0	
	3	87.50	91.25	80.25	83.00	85.5	
	4	84.50	90.75	99.00	97.50	92.9	

The total daily means and the overall treatment means (with s.e. mean) are shown. S1 and S2 represent the two standard treatments of Pitressin (25 and 50 mu/24 h) respectively; T1 and T2 represent the same two doses of Pitressin/24 h respectively, combined with chlorpropamide (5 mg/24 hours).

effect of chlorpropamide might be more readily apparent in the glucose-hydrated heterozygous rat; and (4) similar conditions of papillary osmolality apply.

It is important to note that the daily administration of Pitressin (1000 mu/24 h) to the DI rat failed to increase its urine concentration above that of the untreated heterozygous rat (about 1400 mosmol/kg), and even the additional stimulus of dehydration for 72 h only increased urine osmolality insignificantly to a median value of 1500 mosmol/kg (Lee & Williams, 1972). While the normal urine concentration of the heterozygous rat is generally lower than that of the Long Evans or Wistar strains of rats (usually over 2000 mosmol/kg H_2O) the exogenous administration of Pitressin (1000 mu/24 h) failed to increase further the urine osmolality (Laycock & Williams, 1973). However, Lee & Williams, (1972), did observe that the stimulus of 72 h dehydration to the heterozygous rat was sufficient to raise drastically the urine osmolality to a maximum value of 4560 with a mean of 3300 mosmol/kg. Thus, while urine concentrating ability is present in these animals with a stimulus such as dehydration, the renal response to vasopressin is less impressive.

Although the glucose-induced polydipsic heterozygous rat drinks comparably large volumes of fluid for a different reason to the DI rat, nevertheless it provides a useful model for comparison. It is interesting to note that the administration of various doses of Pitressin to the polydipsic heterozygous rat induced decreases in fluid intake (see Fig. 2) comparable to the decreases in urine volume observed in DI rats (Laycock & Williams, 1973), but at the lower doses (e.g. 50 mu/24 h) the resulting decrease was greater in the DI rat (56%) than the glucose-hydrated heterozygous rat (30%). Unfortunately, the mode of action of the hormone in the polydipsic animal is uncertain in that it may induce reduced fluid intake simply by a renal mechanism (i.e. retention of water, resulting in an expansion of the ECV) or by an additional hypothalamic action. If only the renal action operated, water retention would occur first and a gain in body weight would be observed; but no significant weight gain was reported by Laycock (1973) who pointed out that such measurements may involve large errors.

It was noted in the present investigation that the polydipsic rat was relatively insensitive to Pitressin, as such large doses (1000 mu/24 h) were required to restore urine osmolality to that of the untreated heterozygous animal. This resistance to the action of the hormone is well documented in the overhydrated subject (de Wardener & Herxheimer, 1957), and in patients with DI (Alexander, Filbrin & Fruchtman, 1959). The impaired response is unlikely to be due to an osmotic diuresis since no glucose (less than 0.1 g%) could be detected in the urine (Laycock, unpublished observation). A favoured suggestion to account for the lack of effect of vasopressin is that the tubular responsiveness to the antidiuretic hormone (ADH) has changed through variable hydration of the tissues, including the nephron (Epstein, Kleeman & Hendrikx, 1957). Atherton, Evans, Green & Thomas, (1971) studied the extent to which the influence of hydration and solute excretion on the urinary responses to ADH could be accounted for by differences in renal medullary composition. They concluded that differences in the papillary osmolality under the prevailing conditions could account for the differences in the maximal capacity to elaborate concentrated urine. It is appreciated that as the hormone was given in an oily solution it is possible that the percentage reabsorbed may be less than that assumed; i.e. the excess may not be real. However, indirect evidence which suggests that the hormone was being absorbed in sufficient quantities was provided by Laycock & Williams, (1973), who estimated the urinary excretions of 'vasopressin' in DI rats treated with various doses of Pitressin. From this good, indirect evidence, it would therefore appear that even when the hormone is administered in this manner, the polydipsic rat is relatively insensitive.

The measurement of changes in water intake and/or in water excretion cannot by themselves indicate the primary action of any substance. In the case of antidiuretic hormone (ADH) either parameter can be used to determine its antidiuretic potency since it is accepted that the hormone acts by reducing water excretion, and as water intake is significantly correlated to water excretion (Laycock & Lewis, unpublished observation). Chlorpropamide by itself does not alter fluid exchange in the DI rat, but in combination with Pitressin it induces an additional fall in water intake and/or urine flow, as measured in the present experiments. From these observations it seems likely that an action of the drug is to enhance the antidiuretic action of Pitressin. From the results presented in this paper, this potentiating action did not appear to be of any significance in the control of water balance in the DI rat. For instance, chlorpropamide (20 mg/24 h) to each DI rat enhanced the effect induced by Pitressin (25 mu/24 h) by only 13.8%. The dose of 20 mg/24 h of the drug to a rat weighing on an average approximately 250 g corresponds to some 1000 mg of the drug administered to a patient with DI (i.e. twice the maximum therapeutic dose; see Webster & Bain, 1970), and yet the response in the DI rat (or the polydipsic heterozygous rat) is

minute compared to that observed in the human patient. While from this investigation a potentiating action by the drug is one possible mechanism (although small) in agreement with other workers using DI rats (Berndt *et al.* 1970; Miller & Moses, 1970b), *in vitro* toad bladder preparations (Inglefinger & Hays, 1969; Mendoza, 1969), mammalian kidney preparations (Zweig, *et al.*, Ettinger & Earley, 1971) and in patients with DI (Miller & Moses, 1969), from the available evidence this mechanism is unlikely to be of primary importance.

An alternative mechanism of action for chlorpropamide is that it could act on the hypothalamus, either by stimulating the synthesis and/or release of vasopressin, or by inhibiting thirst. As chlorpropamide had no effect on the fluid intake of polydipsic heterozygous rats it would seem that a thirst-acting mechanism is excluded. However, it is appreciated that a direct comparison between this experimental animal and polydipsic man may not be valid. The drug also had no effect on the DI rat, but the assumption that the only defect in these animals is a failure to synthesize vasopressin may not be correct. Once again, one cannot exclude an antidipsic action of the drug in man.

The present investigation with polydipsic rats would also indicate that chlorpropamide does not stimulate the synthesis and/or the release of vasopressin, contrary to the suggestion of Ettinger & Forsham, (1970) and of Moses, Numann, Friedman & Miller (1971). However, a possible explanation could be that the doses of chlorpropamide administered were not large enough to stimulate the neurohypophysis to release sufficient quantities of vasopressin. Morton (1973) showed by radioimmunoassay, that the level of the hormone in venous plasma of 17 normal subjects ranged from 4-8 pg/ml while in patients with DI it ranged from 1.4-5.5 pg/ml. This would suggest that the level of circulating vasopressin is critical for differentiation between the normal and the DI state. It is therefore possible that in the polydipsic rat, doses of chlorpropamide greater than 20 mg/24 h might have resulted in the release of sufficient hormone to inhibit the diuresis. However at these higher doses, the problem of inducing hypoglycaemia then becomes difficult to overcome. For this reason, chlorpropamide (20 mg/24 h) was the maximum dose administered. It is unlikely that any significant fall in blood glucose would have been induced at this dose, since Miller & Moses (1970b) showed that of the doses used, in a dose-response curve, the 20 mg/100 g body weight dose decreased the blood glucose levels from 115 to 75 mg/100 ml in DI rats; this dose of chlorpropamide is between two to three times higher than the maximum dose used in the present experiments.

Another aspect of interest is the difference between the calorific intakes of homozygous DI rats, heterozygous rats and the glucose-hydrated heterozygous rats. The food intake of the DI rat is 3 to 4 g/24 h greater than that of a heterozygous rat of similar body weight, corresponding to an increased calorific intake of 9 to 12 Kcal/24 h (Laycock, 1973). Despite the increased daily calorific intake, the DI rat has a slower growth rate and fails to deposit as much fat at maturity as its heterozygous counterpart (Valtin, Sawyer & Sokol, 1965). From the present study the mean calorific intake of the heterozygous rat increases significantly from 54.88 ± 2.76 to 72.72 ± 6.53 Kcal/24 h (an increase of some 18 Kcal/24 h) when the fluid intake induced by glucose reaches a volume similar to that of the DI rat on tap water. The lower calorific intake of the DI rats, compared with that of the glucose-hydrated heterozygous rat, may account for their slower weight gain.

Thus this investigation carefully assesses whether chlorpropamide potentiates the antidiuretic action of vasopressin. While there is a potentiating effect, as shown by other workers, this action is small, unlike the conclusion of the previous reports. In addition, these results showed that chlorpropamide had no antidipsic effect in the polydipsic heterozygous rat, but unfortunately this action cannot be excluded as a possible mechanism in man. Likewise, stimulation by the drug of vasopressin release from the neurohypophysis cannot be excluded in man, although the present evidence indicates that this mechanism is unlikely in the polydipsic animal. As yet, the reason for the discrepancy which exists between the efficiency of the drug in patients with DI, and the virtual lack of effect in the DI rat, remains uncertain.

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