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THE DIPSOGENIC ACTIVITY OF PROLACTIN IN MALE AND FEMALE RATS

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SUMMARY

1. Ovine prolactin injected intravenously in doses of 10·0, 5·0, 1·0, 0·1 and 0 mg/kg body wt. caused neither drinking nor a change in urine output in normal water replete male and female rats.

2. The water intake of male rats subjected to 48 hr water deprivation was substantially increased after injection of prolactin.

3. The water intake of male and female rats injected I.P. with a hyperoncotic solution of polyethylene glycol (20 M) was significantly increased after injection of prolactin.

4. Prolactin was found to act synergistically with a subthreshold dose of angiotensin II amide to cause significant drinking and fluid retention.

5. The drinking responses of male and female rats injected I.V. with hypertonic saline were not modified by prolactin.

6. It is concluded that prolactin may act to increase the net water gain of animals suffering a deficit of the extracellular fluid space but is without effect on stimuli arising from deficits in the intracellular fluid space.

INTRODUCTION

For a long time the role of prolactin in mammalian physiology was believed to be limited to lactation but it is now realised that prolactin is involved in a great variety of mechanisms, often acting permissively to modify the effects of other hormones. Although it has been implicated in ion and water metabolism in lower vertebrates (Bern, 1975) recent studies on its role in fluid and electrolyte balance in mammals have led to controversial findings. Renal effects of prolactin have been reported in the rat (Lucci, Bengele & Solomon, 1975; Richardson, 1973), sheep (Burstyn, Horrobin & Marku, 1972) and rabbit (Lloyd, 1973). On the other hand no effect on renal clearance of water and electrolytes could be found after increasing prolactin levels by giving synthetic thyrotropin releasing hormone to human subjects (Berl, Brautbar, Ben-David, Czaczke & Kleeman, 1976; Baumann & Loriaux, 1976). Buckmann, Peake & Robertson (1976) reported that hyperprolactinaemic patients with small pituitary tumours showed a decrease in osmolar clearance following water loading but Baumann, Marynick, Winters & Loriaux (1977) found no such effect.

Prolactin has been shown to stimulate salt appetite (Burstyn, 1978; Burstyn *et al.*

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1972) but a primary effect on food intake has not been demonstrated (Fleming, 1977, 1976; Shari (Mishkinsky), Goldhaber & Sulman, 1975). Literature on the dipsogenic activity of prolactin is very sparce. Horrobin, Lloyd, Lipton, Burstyn, Durkin & Miururi (1971) reported that ovine prolactin, injected into humans, aroused thirst and salt appetite and Ensor, Edmondson & Phillips (1972) showed that ovine prolactin, injected into intact female rats was dipsogenic and antidiuretic. In this paper are presented the results of a study on the dipsogenic activity of ovine prolactin in water replete rats, in rats suffering deficits in their intra- and, or, extracellular fluid compartments, and in rats injected with angiotensin II.

METHODS

Animals. Wistar rats weighing between 200 and 224 g were obtained from a commercial breeder. One week or more after delivery the rats (forty-seven females and thirty-two males) were prepared with chronic I.V. cannulae and housed individually with free access to food (Purina rat chow) and water. Lighting was controlled to 12 hr light:12 hr dark and experiments were always started between 9.00 a.m. and 10.00 a.m. Oestrous cycling in the females was confirmed by daily vaginal smears made before the commencement of this study.

Technique for implantation of chronic I.V. *cannula.* The animals were anaesthetized with Na pentabarbitone (50 mg/kg body wt.). A silastic cannula (i.d. 0·51 mm, o.d. 0·94 mm) was implanted into the inferior vena cava so as not to obstruct blood flow. It was exteriorized at the nape of the neck, filled with heparinized saline and plugged with a short metal obturator. The details of this technique have already been described (Kaufman, 1980). The animals were allowed at least one week to recover from the surgery before being used for experiments.

Technique for I.V. *injection.* The rat was placed in a plastic restraining cage and the cannula was flushed through with heparinized saline (0·2 ml.). The test substance was then slowly injected (over a period of 1 min) and flushed through with heparinized saline (0·3 ml.). Control animals were treated in exactly the same manner except that no drug was injected. The animals rapidly became used to this procedure and would remain so calm that it was seldom necessary to close the back of the cage.

Chemicals and drugs. Polyethylene glycol (mol.wt. 20,000), obtained from Sigma Chemical Company, was dissolved in 0·9% saline (50% w/w). Synthetic valine^5-angiotensin II-amide (Hypertensin CIBA) was dissolved in sterile 0·9% saline to a concentration of 0·5 mg/ml. and stored at 4°C. Ovine prolactin, obtained from Sigma Chemical Company, was dissolved in $NaHCO_3$ buffer (0·01 M, pH 7·6) at a concentration of 10 mg/ml. It was then either stored frozen for later use at this concentration or further diluted and stored frozen in 1 ml. aliquots at a concentration of 1·0 mg/ml.

Measurement of water intake and urine output. The day before an experiment the animals were placed in metabolic cages with food and water. The water was contained in 50 ml. burettes fitted with standard rat drinking tubes. The next morning clean urine collection funnels and bottles were put in the cages, the animals were weighed and, after injection or other appropriate treatment, they were replaced in the cages with fresh drinking water but no food. Water intake and urine volume were measured as indicated in the experiment. Animals subjected to 48 hr water deprivation were treated in exactly the same manner except that there was food but no water available in the cages during the period of deprivation.

Experimental protocol. The data, both control and experimental, for any given experimental condition were drawn from rats tested throughout the period necessary to complete the series of experiments reported in this article. That is to say, the protocol was *not* designed so as to complete the first experiment before passing to the second, etc. This is very important since, in chronic animal experiments the responses may possibly change somewhat as the animal ages and increases in weight. Although the same rats were thus tested repeatedly, any given animal was never used more often than once a week.

Statistics. Results are expressed as mean $\pm$S.E. of mean. In graphs the S.E. of mean is delimited by the vertical bars. The significance of the difference between means was determined by Student's *t*-test or by the Duncan Multiple Range test. Levels of significance are indicated by asterisks: $*P < 0{\cdot}05$; $**P < 0{\cdot}01$; $***P < 0{\cdot}005$.

RESULTS

Prolactin in water replete rats

Normal satiated male and female rats were injected I.V. with prolactin in doses of 10, 5·0, 1·0, 0·1 and 0 mg/kg body wt. Water intake, urine output and weight change during the ensuing 6 hr were measured. There was no indication of either a dipsogenic or a renal effect in either male or female rats, at any of the doses of prolactin tested.

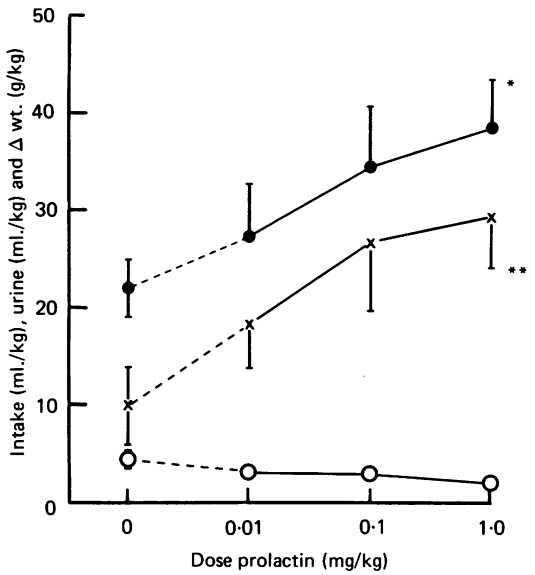

Fig. 1. Water intake (●), urine output (○) and change in body wt. (×) of male rats during the 3 hr following 48 hr water deprivation plus I.V. injection of prolactin, plotted as a function of the dose of prolactin. The figures shown above each point indicate the number of animals used to compute the mean ±S.E. of mean.

Prolactin in water deprived rats

Male and female rats were deprived of water, but not food, during 48 hr and their loss in body weight was recorded. They were then injected I.V. with prolactin in doses of 1·0, 0·1, 0·01 and 0 mg/kg body wt. Water intake, urine output and weight change during the ensuing 3 hr were measured. In dehydrated male rats there was a direct relationship between the amount of water drunk and the dose of prolactin injected (Fig. 1). Intake was nearly doubled from 22·0 to 38·5 ml./kg body wt. at the highest dose studied. At 1 hr the injected rats had already drunk significantly more than the controls but the time course of drinking thereafter was not modified by prolactin. Since, in the prolactin treated rats, the increased water intake was not accompanied by an increased urine output, body weight increased in parallel with water intake. Prolactin did not modify the drinking response of water deprived female rats.

Prolactin plus polyethylene glycol

Rats were injected I.P. (5 ml./kg body wt.) with a solution (50 % w/w) of polyethylene glycol (mol. wt. 20,000). Four hours later, when an ascites had formed of isotonic fluid drawn from the extracellular fluid space, prolactin was injected in doses of 1·0, 0·1 and 0 mg/kg body wt. Water was then offered and the intake and urine output during the next 2 hr was recorded. Total body weight was recorded just before injection and again 6 hr later at the end of the experiments. The peritoneal cavity was drained after the experiment and only those animals from which ascitic fluid could be recovered were included.

Total water intake over the 2 hr was significantly greater in the prolactin treated male rats than the controls. After just 30 min of drinking the animals injected with 1·0 mg prolactin/kg body wt. had already drunk three times as much as the controls but the ensuing rate of drinking was about the same in all three goups and had still not appreciably declined at the end of the experiments (Fig. 2). Urine output was negligible in all groups.

The results in the female rats were slightly different. In the first place non-prolactin treated females drank considerably more than similarly treated male rats. Secondly, although those animals injected with 0·1 mg prolactin/kg body wt. initially drank more than the controls, the increase was onlysignificant at 30 min and 1 hr after injection (Fig. 3). However, since all groups were virtually anuric, the net gain in body weight at the end of the experiment was significantly greater in the prolactin treated female rats ($+5 \pm 4$ g/kg body wt. ($n = 11$) compared with $-10 \pm$ g/kg body wt. ($n = 11$) for controls; $P < 0{\cdot}01$). The higher dose of prolactin did not significantly change the fluid intake, urine output or weight compared with the controls.

Prolactin plus angiotensin II

Rats were injected I.P. with angiotensin II-amide at doses $2{\cdot}4 \times 10^{-7}$ mole/kg body wt. and $9{\cdot}7 \times 10^{-7}$ mole/kg body wt. followed by I.V. injection of prolactin at 1·0, 0·1, 0·01 and 0 mg/kg body wt. Water intake, urine output and change in body weight were measured during the next 3 hr.

Male rats did not drink to the lower dose of angiotensin given alone or in combination with prolactin. Nor did prolactin modify their urine output or change in body weight. At the higher dose of angiotensin, prolactin possibly acted to increase water intake but the differences, in these experiments, were not significant. However, the significantly lower urine output coupled with a possibly increased water intake did result in a much reduced weight loss in those animals receiving 1·0 mg prolactin/kg body wt. (Table 1).

In female rats, drinking elicited by the lower doses of angiotensin was increased by prolactin. This action of prolactin was dose related with a highly significant maximal increase at 0·01 mg/kg body wt. (Fig. 4). Since urine output was independent of the dose of prolactin, the increased fluid intake was accompanied by a net gain in weight relative to the controls ($P < 0{\cdot}01$ for doses of 0·01 and 0·1 mg prolactin/kg body wt.).

At the higher dose of angiotensin, prolactin did not interact to produce a demonstrable dose related increase in drinking. Nor was there any significant effect on urine output or weight change.

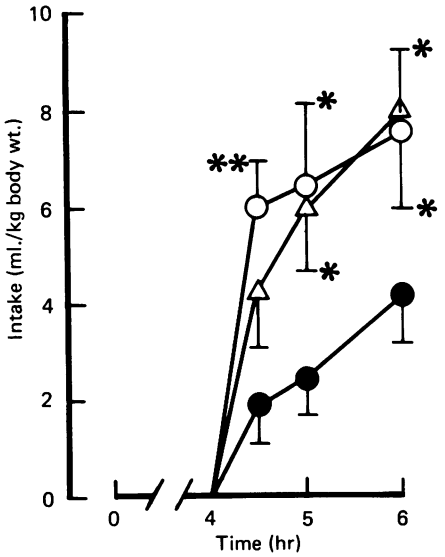

Fig. 2. Cumulative water intakes (mean ±S.E. of mean) of male rats following I.P. injection of a 50% solution of polyethylene glycol (mol.wt. 20,000, 5 ml./kg body wt.) plus I.V. prolactin in doses of 1·0 (○, $n = 12$), 0·1 (△, $n = 13$) and 0 (●, $n = 14$) mg/kg body wt. Access to water was denied during the first 4 hr following injection of the polyethylene glycol. The rats were then (at 4 hr) injected with prolactin, given access to water and the subsequent intake was measured during the next 2 hr.

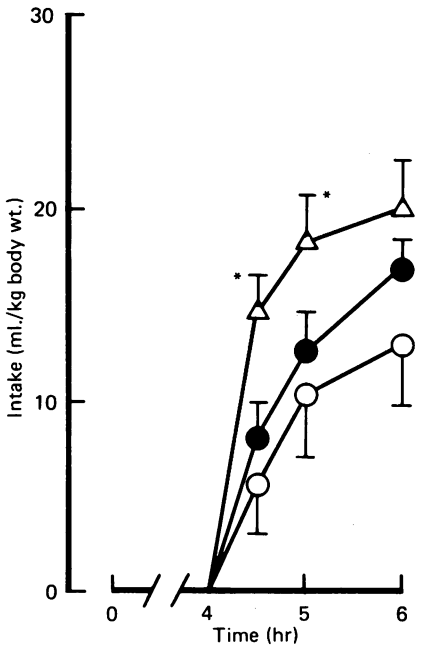

Fig. 3. Cumulative water intakes (mean±S.E. of mean) of female rats following I.P. injection of a 50% w/w solution of polyethylene glycol (mol.wt. 20,000, 5 ml./kg body wt.) plus I.V. prolactin in doses of 1·0 (○, $n = 10$), 0·1 (△, $n = 11$) and 0 (●, $n = 11$) mg/kg body wt. Access to water was denied during the first 4 hr following injection of the polyethylene glycol. The rats were then (at 4 hr) injected with prolactin, given access to water and the subsequent water intake was measured during the next 2 hr.

Prolactin plus hypertonic NaCl

Normal satiated male and female rats were injected I.V. with 2 M-NaCl (5 ml./kg body wt.) followed by prolactin in doses of 1·0, 0·1 and 0 mg/kg body wt. Prolactin did not significantly modify the water intake, urine output, weight change or time course of drinking.

TABLE 1. Water intake, urine output and weight change of male rats during the 3 hr following injection of angiotensin II plus prolactin

Dose AII (mole/kg body wt.)	Dose prolactin (mg/kg body wt.)	Water intake (ml./kg body wt.)	Urine output (ml./kg body wt.)	Wt. change (g/kg body wt.)	n
0	0	0·3±0·2	—	—	8
$9·7 \times 10^{-7}$	0·01	1·6±0·8	5·3±0·9	−27±7	8
$9·7 \times 10^{-7}$	0·1	2·5±1·0	6·4±1·9	−15±4	7
$9·7 \times 10^{-7}$	1·0	2·9±1·2	0·9±0·5*	−6±2**	8

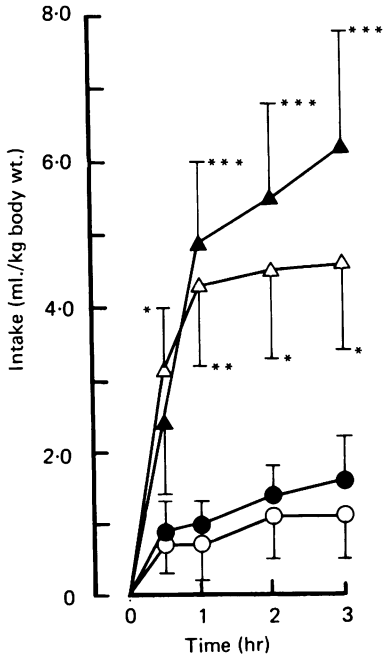

Fig. 4. Water intake (mean ±S.E. of mean) of female rats during the 3 hr following I.P. injection of angiotensin II amide $2·4 \times 10^{-7}$ mole/kg body wt.) plus I.V. prolactin in doses of 1·0 (○, $n = 8$), 0·1 (△, $n = 14$), 0·01 (▲, $n = 10$) and 0 (●, $n = 14$) mg/kg body wt.

DISCUSSION

Many of the confusing results concerning the renal actions of prolactin may have been due to vasopressin present as contaminant in the NIH preparation of ovine prolactin (Vorherr, Vorherr & Solomon, 1978; Cary, Johanson & Seif, 1977). It was for this reason that the Sigma preparation was chosen since, according to Keeler &

Wilson (1976), it contains only 2·5 ng vasopressin/mg prolactin compared with 20 ng/mg prolactin in the NIH preparation. Vasopressin, even if it is present as contaminant, should prove to be less of a problem in studies on drinking than in renal physiology since its dipsogenic activity in the rat, if any, seems to be slightly depressive (Fitzsimons, 1979). Moreover, the doses of vasopressin reported to elicit these effects are several order of magnitude greater than the largest possible dose given as a contaminant in this prolactin preparation.

Chen, Amenomori, Lu, Voogt & Meites (1970) report that the serum prolactin levels in lactating rats approach 300 ng/ml. Chen & Meites (1970) report circulating plasma levels of nearly 400 ng/ml. after injection of oestradiol benzoate into mature ovariectomized rats. We do not, however, know the physiological concentration of plasma prolactin at its target organ; if prolactin influences fluid balance at the level of the hypothalamus, the concentration in blood flowing from the pituitary to the effector cells in the hypothalamus is probably considerably higher than that in the general circulation. Assuming a plasma volume of 5 % body weight, it was calculated that the peak systemic plasma prolactin levels achieved in these experiments ranged from 200 ng/ml. to 200 μg/ml. The normal physiological concentration of the hormone thus falls within the range of doses which were tested.

In the present study it was established that, over a wide range of doses of prolactin given to non-stressed, water replete animals, there was no evidence in either male or female rats of a dipsogenic or diuretic response. This is contrary to the results of Ensor *et al.* (1972) who found injected prolactin to be dipsogenic and antidiuretic in intact female rats. However, although it was implied in the above mentioned paper that a range of doses was studied, the actual doses and the amounts drunk are not reported thus rendering it impossible to make valid comparison.

This is not to say that prolactin plays no role in fluid balance. It could act to sensitize the animal to a pre-existing body fluid deficit i.e. to reduce the threshold to drinking, and/or it could increase the quantity of water drunk. Thus prolactin (1·0 mg/kg body wt.) was found to increase the water intake of water deprived male rats from 22 to 38·5 ml./kg body wt., the effect being dose dependent (Fig. 1). The increased drinking was not however accompanied by an increase in urine output. This is suggestive of a possible renal effect of prolactin acting to retain water in previously dehydrated animals. In females, the water intake, which was already much higher than that of the males (S. Kaufman, unpublished), was not further augmented by prolactin. It is possible that, over the long period of water deprivation, secretion of the female rats' own prolactin was so stimulated that the addition of exogenous prolactin was not able to augment the water intake further. This hypothesis is supported by the findings of Ensor *et al.* (1972) that water deprivation in female rats is accompanied by a substantial drop in pituitary prolactin levels.

Water deprivation results in dehydration of both the intra- and extracellular fluid compartments of the body. These two components, which act via independent mechanisms to stimulate thirst (Fitzsimons, 1972) were studied separately to determine which was responsible for the increased drinking observed in water deprived males. Dehydration of the intracellular compartment was achieved by injecting the animals with hypertonic NaCl. Prolactin had no effect, in either males or females, on the drinking response to this stimulus. This suggests that in the water deprived animals, prolactin might have been acting on the pathways of extracellularly

induced thirst, but not on those of the intracellular component, to increase drinking. Indeed, prolactin did significantly increase the drinking of both male and female rats suffering an extracellular fluid deficit created by peritoneal dialysis with a hyperoncotic solution of polyethylene glycol (Figs. 2, 3). In considering the magnitude of these reported increases in fluid intake it is important to realize that the prolactin was given as one single bolus injection and that the plasma half-life is of the order of 5 min (Meites & Clemens, 1972). Under normal physiological conditions the prolactin levels would be expected to remain elevated for an extended period due to continued secretion by the pituitary. This could explain why, in the dehydrated and polyethylene glycol treated animals, an increased intake was observed only during the initial 30 min after injection of prolactin, the temporal pattern of intake being similar to that of the controls for the ensuing period of the experiment.

Angiotensin II, in addition to its hypertensive action, is one of the most potent dipsogens known and is believed to act through the pathways involved in extracellularly induced thirst (Fitzsimons, 1972). Extremely small doses (5 pmole) of angiotensin II provoke water intake when injected intracranially (Epstein, Fitzsimons & Rolls, 1970). However, very high doses are required when it is injected I.P. since, in the peripheral circulation, it is rapidly deactivated by angiotensinases before it can reach the proposed target receptors in the brain. The doses used and the intakes observed in the present study were virtually the same as those reported by Evered & Fitzsimons (1976). Prolactin elicited a significant water intake in female rats which had received a dose of angiotensin II that, alone, was not dipsogenic (Fig. 4). This phenomenon was dose dependent showing a maximal effect at 0·01 mg prolactin/kg body wt., the lowest dose tested. Urine output was independent of the dose of prolactin so that weight change parallelled water intake. This may be interpreted as antidiuretic activity since prolactin seemed to cause retention of the extra fluid that had been drunk thus keeping the animal in positive water balance. Prolactin did not significantly increase the drinking response of female rats injected with the higher dose of angiotensin. However, these rats were already drinking as much as the male rats receiving the low dose of angiotensin plus prolactin and it is possible that the particular receptors involved in angiotensin-induced drinking were saturated and the response was already maximal.

From this study one may conclude that prolactin, acting alone in a water replete animal, is neither dipsogenic nor antidiuretic. However, when there is a fluid deficit, prolactin may act either directly or through the action of other hormones to increase the amount of water drunk and, in some cases to reduce the urinary water loss. More specifically, only the drinking responses to stimuli arising from a deficit in the extracellular fluid space are modified; those induced by intracellular dehydration are unaffected by prolactin. Thus the conclusion that 'prolactin in the adult rat is not important in water retention' (Mattheij, 1977) is perhaps not justified since that author demonstrated only that changes in plasma osmolality did not result in measurable changes in plasma prolactin levels. This lack of correlation between intracellular dehydration and endogenous prolactin levels has also been reported by Baumann *et al.* (1977), Berl *et al.* (1976) and Adler, Gordon, Wartofsky & Franz (1975). On the other hand Relkin (1974) and Buckman & Peake (1973) have observed increases in plasma prolactin levels after injecting I.V. hypertonic NaCl and,

conversely, decreases after gastric or I.V. water loading. In considering these results it must be realized that there are inherent problems associated with sampling blood from unstressed animals, since stress of itself increases prolactin levels (Krulich, Hefio, Illner & Reid, 1974). Secondly, according to these present studies with exogenous prolactin, one probably would not expect changes in prolactin levels to be associated with intracellular dehydration. Thirdly, as mentioned before, changes in the plasma prolactin levels reaching the target cells in the brain may well not be reflected by changes in the levels in the systemic circulation. It was on this basis that the 'short loop' theory of autoregulation of prolactin secretion was formulated (Meites & Clemens, 1972). Thus the presence or absence of changes in systemic plasma prolactin levels is probably of less significance in the demonstration of a role for prolactin in fluid and electrolyte balance than might initially be assumed.

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