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THE RENAL ACTIONS OF OXYTOCIN IN THE CONSCIOUS RAT

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The renal actions of oxytocin were studied in the conscious unrestrained rat infused with 0.077 M saline at a rate of 150 μl/min. During the control period volume and sodium excretion reached stable equilibria, the rates being equal to those infused. Administration of oxytocin at 200 pmol/min produced plasma oxytocin levels of 26.0±2.1 pmol/l and caused a significant diuresis and natriuresis. Renal responses could also be seen with a lower dose of 30 pmol/min which produced plasma levels of 5.1±0.5 pmol/l while a dose of 15 pmol/min which produced no significant increase in the plasma oxytocin had no renal effect. It appears that oxytocin has a natriuretic action in concentrations within the physiological range.

Key words: *oxytocia, natriuresis, diuresis,*

INTRODUCTION

While the role of vasopressin in fluid balance is clear, that of the other neurohypophyseal hormone, oxytocin is not. Oxytocin is known to be released in the rat by an increase in plasma osmolality (1) and by a reduction in blood volume (2). Dehydration is also known to cause oxytocin release (3) and it has been suggested that this contributes to the natriuresis associated with dehydration, as this phenomena is seen to occur not only in the normal rat, but also in the Brattleboro rat (4). These observations have given new impetus to the idea that oxytocin may play a part in the mechanisms of sodium regulation.

The renal actions of oxytocin are, however, far from clear. Oxytocin has been reported to have a diuretic action in some studies (5) and an antidiuretic action in others (6). This may in part be explained by the presence or absence

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of vasopressin in the experimental preparations used, as oxytocin is seen to be antidiuretic in the water loaded ethanol anaesthetised rat (6) and in the Brattleboro rat (7); additionally oxytocin has been shown to act as a partial agonist of AVP (8). Oxytocin has also been shown to be natriuretic (9, 10), the increase in sodium excretion occurring both in the presence and absence of vasopressin and independently of the rate of urine flow (1, 8, 11).

The doses required to produced natriuresis in many studies were very high, which would indicate that this natriuresis is not of physiological importance However, when oxytocin and vasopressin were administered together to the neurohypophysectomised rat a natriuresis was produced with very low doses of the hormone (12). Since these studies, like most others, were performed in anaesthetised animals in which both hormone release and renal function would be affected (13, 14), these observations are now being extended to the conscious rat. The initial studies on the effects of vasopressin in the conscious rat have been reported (15), and here we described our observations on the renal actions of oxytocin. Initial reports of this work have been presented (16).

METHODS

Animals. All experiments were carried out on male Sprague Dawley rats (250—300 g) allowed food (R & M Maintenance diet No 1, Special Diet Services Ltd. Witham, Essex, U. K.) and water ad libitum and housed under conditions of constant temperature and humidity. They were maintained under conditions of 12h light/12h dark with the lights on at 06.00h

Renal studies. Each rat was anaesthetised with sodium methahexitone (Brietal, Eli. Lily, Indianapolis, U.S.A.) in a dose of 45 mg/kg body weight. The right jugular vein was cannulated with a saline filled polythene tube (O. D. 0.96 mm: I. D. 0.58 mm; PP5, Portex Ltd, Hythe, Kent, U. K.) which was subsequently guided through the subcutaneous tissue and exposed at the nape of the neck. The end of the cannula was sealed with a small pin and the cannula coiled into the small pocked of a fabric jacket around the forelimbs (17). The rats were left to recover overnight in individual housing cages.

The following morning (0.8.30—10.00) the rats were placed in individual metabolism cages (N. K. P., Dartford, Kent, U. K.) An infusion punmp (Watson Marlow, model 502) was set to deliver 150 μl/min of warmed, sterile 0.077 M saline via an extension line, which was fed through the top of the cage and weighted to allow the rats free movement without access to the cannula.

The rats were infused for an equilibration period ot at least 3.5 h ending in a spontaneous urination. During this phase the urine voided was collected as a single sample and subsequently each spontaneous urination was timed and collected into a preweighed plastic tube. This infusion continued for at least 45 min, again ending at a spontaneous urination. Following this control period the infusate was switched to one of identical ionic composition, but containing synthetic oxytocin (Syntocinon, Sandoz Pharmaceuticals, Middlesex U. K.) delivered to the rat at a rate of 0, 15 30 or 200 pmol/min. After 60 min of hormone administration, the animals were returned to hormone free infusate for a further 1.5 h, ending after a spontaneous urination.

Determination of plasma hormone levels. Parallel groups of rats were infused as described above. At the end of the 60 min of hormone infusion or comparable time in the controls, the infusion line was disconnected and the rats were decapitated. Trunk blood was collected into chilled heparinised tubes and a sample was also collected into a microhaematocrit tube for the determination of packed cell volume (PCV, Hawksley & Sons Ltd, Lancing, Sussex, U. K.). The plasma was separated and aliquots taken for electrolyte and hormone determinations. Blood samples were also collected from rats which been infused for only 3.5 h and from non infused groups.

Urine and plasma analyses. Urine volumes were determined gravimetrically. Urinary and plasma sodium and potassium concentrations were deteremined by flame photometry (Corning model 410 C, Corning, Halsted. Essex U. K.). Plasma osmolality was determined by depression of the freezing point (Advanced Digimatic osmometer model 3D Needham Heights M. A. U.S.A.) and chloride using a chloride meter (Corning model 625).

Each infusion period was divided into 15 min intervals. The mean urine flow and electrolyte excretion for each interval was calculated from the relative contribution of each each urine sample (or part of a sample which was produced during 2 intervals) to that interval.

Hormone assay. Plasma samples were extracted using silica columns (Sep-pak C50 Millipore M. A. U.S.A.) as previously described (18). Samples were then assayed for vasopressin against the First International Standard for vasopressin (77—501) and for oxytocin against the Fourth International Standard for oxytocin (76—575). All samples were processed in the same assay to avoid inter-assay variation, the intra-assay variations being 7.7 and 4.1% respectively. The limit of detection of the vasopressin, assay was 0.05 pmol/l and for the oxytocin assay 0.8 pmol/l.

Data presentation. Values are presented as the means and standard error of the mean. As previously described for the renal studies the final 30 min of the control period was used to determine control excretion rates for each rat and was compared by paired Student's t-tests to the final 30 min of the hormone period (15). All other analysis was carried out by unpaired Student's t-tests or Spearman's rank correlation test. Significance was recorded at the 5% level.

RESULTS

After surgery and recovery, the rats had lost only $0.06 \pm 0.27\%$ of their original body weight. Non-infused rats which had been implanted with cannulae had plasma vasopressin and oxytocin concentrations of 0.6 ± 0.1 and 3.9 ± 1.0 pmol/l ($n = 8$) respectively.

Behaviour during infusion.

During the infusion the rats spent most of the time at rest, apart from brief activity for urination which occurred at 5—10 min intervals. Normally this was associated with grooming. The rats largely ignored the cannulae which did not impede movement or normal behaviour.

Fluid balance during saline infusion.

During the equilibration period the rats retained 4.7±2.1% of the infused volume and 6.2±9.0% of the infused sodium (n = 38). Rats decapitated after the 3.5 h of equilibration had plasma vasopressin and oxytocin concentrations of 2.0±0.4 and 2.8±1.0 pmol/l (n = 6) respectively which were not

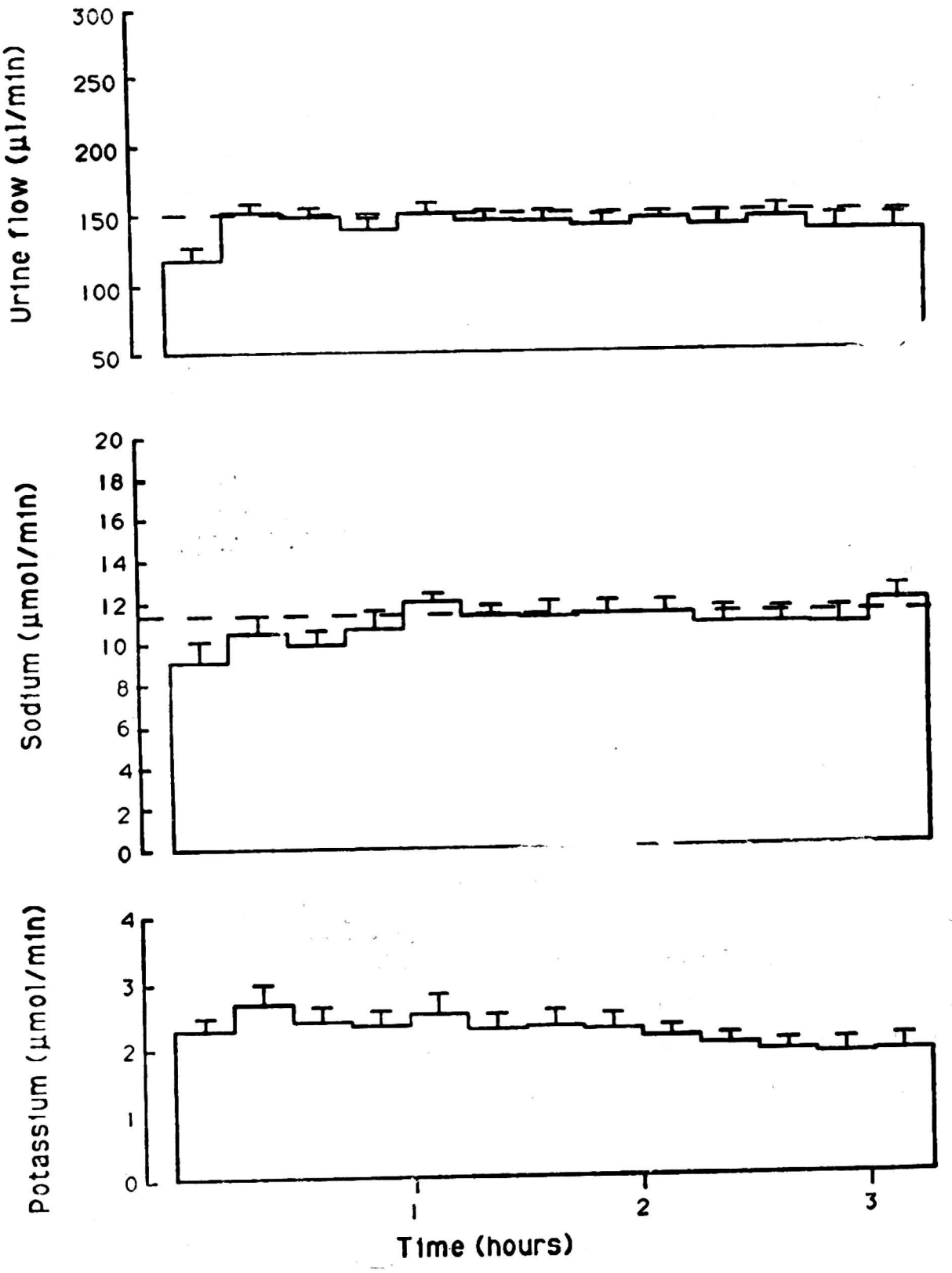

Fig. 1. Urine volume and sodium and potassium excretion during infusion of 0.077 M sodium chloride at a rate of 150 µl·min (n = 11). Values presented are the mean ±S.E. for 15 min collection periods. Rates of infusion are shown by dashed lines.

significantly different from those measured in the non-infused controls. Similarly PCV, plasma sodium, chloride and osmolality were not significantly altered by 3.5 h of infusion.

During the experimental period rats infused with saline retained a further 5.2±2.0% of the fluid and 10.6±2.5% (n = 7) of the sodium infused. The packed cell volume was significantly lower at 36.3±0.8% representing a 7.4% increase in blood volume. The fluid retained over this period was approximately isotonic, and thus there was no significant change in plasma sodium or osmolality. A significant decrease in plasma vasopressin was seen in the controls, the levels becoming undetectable, but the fall in oxytocin to 2.8± 0.4 pmol/l (n = 7) was not significant.

During control periods the rate of excretion approximated the rate of infusion allowing for the small amount of fluid and sodium retained as hown in *Fig. 1*. The rate of volume and sodium excretion remained stable over the course of the infusion period whilst that for pottassium tended to decrease.

The effects of oxytocin infusion on urine volume and composition.

Infusion of 7.5 pmol/min of oxytocin produced a small, but not statistically significant increase in plasma oxytocin concentration as compared to the controls and, as with all the doses of oxytocin used, plasma vasopressin concentrations were undetectable. This lowest infucion rate produced no significant change in the rate of urine flow or sodium excretion, and hence no significant alteration in the amount of fluid and sodium retained, the packed cell volume and plasma electrolyte concentrations remaining the same as those in the control.

Infusion of 30 pmol/min of oxytocin produced plasma oxytocin concentrations of 5.06±0.5 pmol/l (n = 6). This led to a significant diuresis with a mean flow of 170.1±4.49 µl/min as compared to the control of 139.1± 7.4 µl/min (n = 9) and also to a significant increase in sodium excretion of 11.6±0.7 µmol/min as compared to the control rate of 8.7±0.7 µmol/min. The higher rate of fluid excretion continued after the cessation of hormone infusion and the rats showed a net loss of fluid over the course of the infusion of 5.3±3.2%. Sodium excretion returned to normal at the end of hormone infusion.

The highest rate of oxytocin infusion of 200 pmol/min produced plasma oxytocin concentrations of 26.0±2.1 pmol/l (n = 6) and a significant increase in urine flow *Fig. 2* from 139.6±6.1 µl/min to 186.0±9.07 µl/min. The flow rate remained significantly elevated after the cessation of hormone infusion so that the overall volume retained was again significantly lower than in the controls. There was also a significant increase in the rate of sodium excretion from 9.7±0.6 to 14.8±0.6 µmol/min which also continued into the recovery

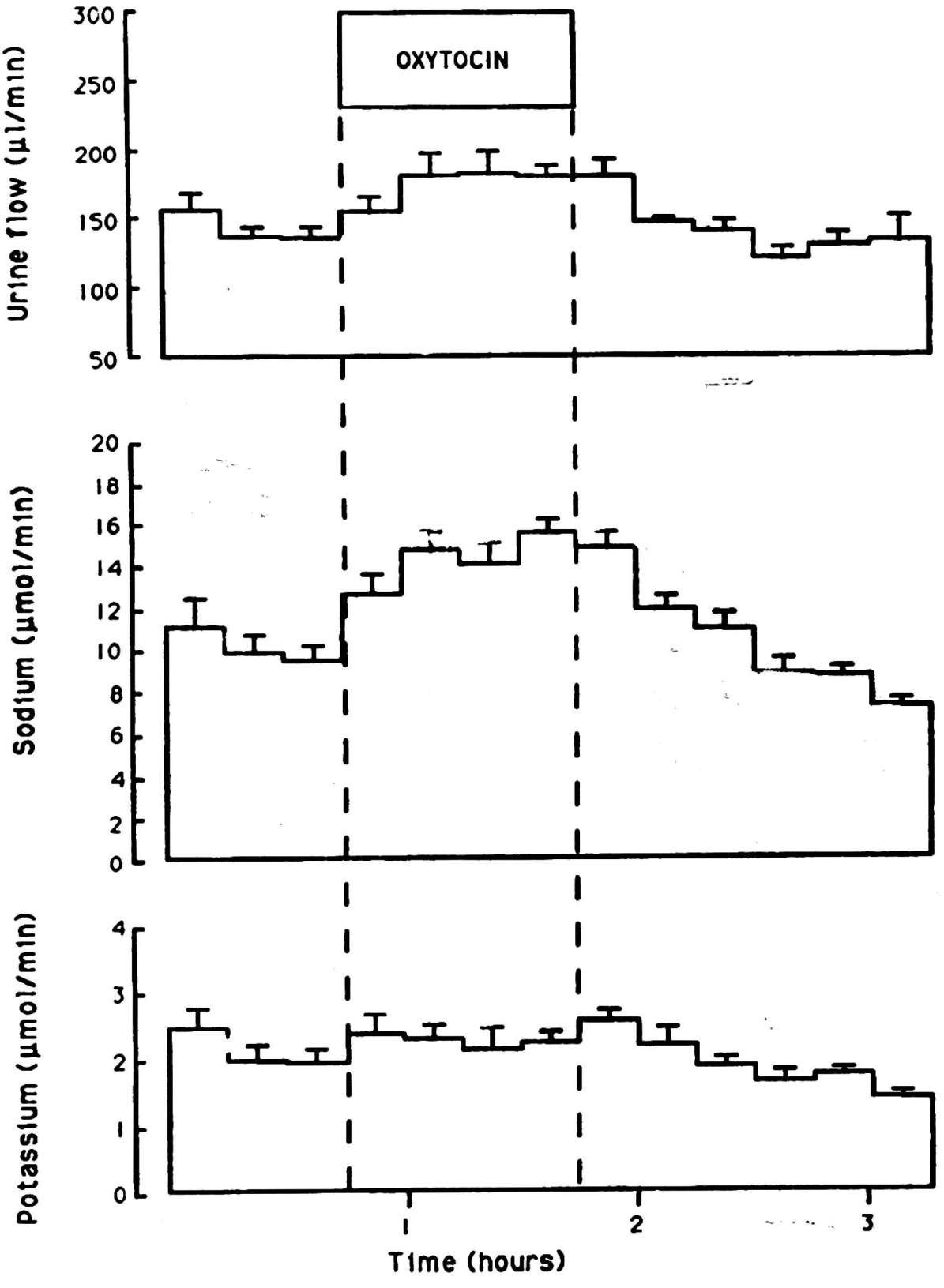

Fig. 2. The effect of oxytocin administration at 200 pmol/min on urine flow and sodium and potassium excretion (n = 9). Values presented are the mean ±S. E. for 15 min collection periods.

period. This was followed by a period of "rebound" when the rate of excretion fell below that of the control. Overall the amount of sodium retained was significantly less than in the control infusion being 2.7±2.2% of that infused. The rate of potassium excretion remained constant throughout infusion of this dose of hormone, not declining as during control infusions. There was a significant correlation between the rate of urine flow and the rate of sodium excretion with all the doses of oxytocin employed ($P < 0.001$).

DISCUSSION

The possible role of oxytocin in the male has been a matter of speculation for a number of years. Apart from its possible role in sexual behaviour and sperm transport (19), no physiological role has been found to account for the consistent presence of the hormone in plasma. There is increasing evidence to suggest that in some species it may contribute to salt and water balance. Secretion of oxytocin in the rat is affected by the hydrational state of the animal. Increasing the extracellular sodium concentration results in increased firing rate of putative oxytocinergic neurones and increased hormone secretion (20). A linear relationship has been found between the release of oxytocin and the plasma osmolality (1) similar to that for vasopressin. Oxytocin is also released by increased cerebrospinal fluid sodium concentrations (21). Hypovolaemia has been found to stimulate oxytocin release (2) and as would be expected from these observations dehydration, too, results in increased circulating concentrations of oxytocin in the rat, although not in species such as the goat (22). Interestingly dehydration reverses the normal daily variations in oxytocin secretion seen in the euhydrated rat. Plasma oxytocin concentrations normally increase over the light hours of the 24 h cycle whereas during dehydration they fall over this phase despite a general upward trend (23). The relative elevation of oxytocin during the dark phase of the 24 hour cycle would appear to be associated with the relatively high intake of sodium in the absence of water. Consistent with this suggestion is the finding that oxytocin secretion is increased following ingestion of food (24).

The levels of oxytocin achieved on dehydration (23) were found in this study to be effective in promoting a natriuresis. The idea that oxytocin may promote sodium excretion is not new. Sawyer in 1952 working on rats (25) and Abrahams and Pickford in 1954 working on dogs (26) reported that oxytocin administration resulted in a natriuresis. These observations were confirmed by Balment et al (1), who subsequently demonstrated that in the acutely neurohypophysectomised rat oxytocin acts synergistically with vasopressin to promote salt excretion (12). The natriuretic response is also seen in Brattleboro rats (27) which is consistent with the suggestion that increased plasma oxytocin may be responsible for the increased sodium excretion seen during dehydration (4). While these earlier studies were performed on anaesthetised animals, the present investigation confirms that oxytocin is also natriuretic in the conscious animal. The increase in sodium excretion with even the highest dose was relatively modest. However, natriuretic responses to vasopressin that we have observed in animals from the Sprague Dawley colony eployed were smaller that that previously reported (15), suggesting a slight difference in technique, or variability in colony may affect fluid balance as has been previously reported (28).

The mechanisms underlying the oxytocin induced natriuresis have yet to be identified. Increased salt loss following infusion of the other neurohypophyseal hormone, vasopressin, has been attributed to altered tubular handling of sodium (29) and changes in filtered load (30). Lote, Thewles, and Wood (31) were able to exclude atrial natriuretic factor and prostaglandin E_2 as essential mediators of the response. Similar mechanisms would be expected for oxytocin. Receptors for oxytocin have been described in the glomerulus (32) and an oxytocin induced increase in the glomerular filtration rate (GFR) has been described in rats with and without hereditary diabetes insipidus (33, 34). The increase in GFR does not rule out the possibility of a further tubular action of oxytocin although neither the location nor the type of receptor involved is known. The natriuretic response of vasopressin may occur via a V subtype receptor (35) and whether there is partial agonist action of the oxytocin at this receptor is unknown. Chan (36) reported that the natriuretic response to oxytocin is blocked by an antagonist of the hormone, [Pen-Phe(Me)^{2}Thr^{4}Ora] oxytocin Steir, Manning and Sawyer (37) found that the natriuretic potency of a small series of oxytocin analogues did not parallel their oxytocic, pressor or antidiuretic activities in rats. The blood volume of the rats was slightly expanded in the present studies and this could cause a natriuresis (38) but, the greatest natriuresis occurred with the smallest retention of volume. We have shown that the rate of flow was correlated to the rate of natriuresis in these studies. The diuresis brought about by oxytocin probably occurred as a result of the partial agonist effect at the V_2 receptor, its concentration being sufficient to displace the residual vasopressin present.

In summary oxytocin, in concentrations seen in altered hydrational states, produces a natriuresis and a diuresis in the conscious rat indicating that the hormone may have a role in salt and water balance. The mechanisms underlying the response remain to be established.

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