Contribution of endogenous oxytocin to sodium excretion in anaesthetized, surgically operated rats

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Abstract

In order to determine the possible role of endogenous oxytocin in controlling electrolyte and water excretion in animals whose renal function is being assessed by invasive techniques, rats were anaesthetized and subjected to micropuncture surgery. Clearance measurements were made in the presence and absence of the potent oxytocin receptor antagonist d(CH_2)$_5$[Tyr(Me)^2, Thr^4, Orn^8, Tyr(NH_2)^9]-vasotocin. In rats infused with vehicle alone, glomerular filtration rate (GFR), sodium excretion and urine flow rate remained stable. In contrast, in antagonist-treated rats GFR was modestly reduced ($P<0{\cdot}05$), and there were large falls in both absolute and fractional sodium excretion ($P<0{\cdot}01$ in each case) and absolute and fractional water excretion ($P<0{\cdot}05$ in each case), indicating effects on both filtered load and fractional tubular reabsorption.

The antinatriuresis was not accompanied by a change in the fractional excretion of lithium, suggesting that proximal tubular function is unaffected by oxytocin receptor antagonism; nor was it accompanied by a change in the fractional excretion of potassium, suggesting that the tubular effect is located beyond the potassium secretory site, i.e. downstream of the cortical collecting tubule.

We conclude that circulating plasma concentrations of oxytocin during anaesthesia and moderate surgery are sufficient to enhance GFR and reduce fractional tubular sodium and water reabsorption. This has important implications for the interpretation of invasive studies such as micropuncture.

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Introduction

There is evidence that, in addition to its reproductive function, the neurohypophysial hormone oxytocin may play a role in controlling sodium excretion. Oxytocin release is known to be stimulated by dehydration (Windle *et al.* 1993, Huang *et al.* 1996), hypertonic saline loading (Balment *et al.* 1980, Huang *et al.* 1995) and partial nephrectomy (Huang *et al.* 1994), i.e. situations which all result in enhanced sodium excretion (in the latter case by the surviving nephrons). Moreover, infusion of oxytocin into experimental animals leads to a dose-dependent natriuresis (Balment *et al.* 1980, Conrad *et al.* 1986, Verbalis *et al.* 1991, Forsling *et al.* 1994). Relatively high doses of oxytocin can increase glomerular filtration rate (GFR), but lower doses, which are without effect on GFR, are nevertheless natriuretic (Conrad *et al.* 1986, Forsling *et al.* 1994), suggesting that the hormone inhibits sodium reabsorption somewhere along the nephron. The sole micropuncture study of oxytocin's action failed to demonstrate an effect of the hormone on proximal tubular reabsorption (Garland *et al.* 1983), although the latter was assessed using the stationary microperfusion ('split drop') technique which some authorities believe to be problematic (Cogan 1990). Therefore, identification of the nephron segments(s) responsible for oxytocin's natriuretic potency still awaits a systematic micropuncture investigation.

The actions of infused oxytocin on sodium excretion can be blocked by specific oxytocin antagonists (Verbalis *et al.* 1991, Huang *et al.* 1994, Windle *et al.* 1997). However, only fragmentary information is available concerning the effects of oxytocin antagonists in the absence of infused hormone. Huang *et al.* (1996) administered an antagonist by osmotic minipump to conscious rats and observed a small reduction in sodium excretion, but the significance of this was obscured by a comparable reduction in sodium intake (as a result of the minipump implantation procedure). Similarly, a study in anaesthetized animals showed a small (non-significant) reduction in sodium excretion, but the antagonist was administered for only 60 min before a confounding manoeuvre (unilateral nephrectomy) was superimposed (Huang *et al.* 1994). Time constraints also hampered

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interpretation of a more recent study in which three oxytocin antagonists were administered to conscious rats prior to neurohypophysial hormone infusion (Windle *et al.* 1997). In consequence, it is still unknown whether normal circulating levels of endogenous oxytocin influence sodium excretion.

In the present study, as a prelude to a micropuncture investigation of oxytocin's actions on the nephron, we administered a potent oxytocin receptor antagonist to rats which had been anaesthetized and subjected to surgery, but had not been infused with exogenous oxytocin, in order to determine the effects of endogenous oxytocin on overall renal function. In addition, as a means of assessing any changes in proximal tubular function, we measured the clearance of lithium, a semi-quantitative index of the volume of glomerular filtrate delivered to the end of the proximal tubules (Shirley & Walter 1996, Thomsen & Shirley 1997).

Materials and Methods

Surgical procedures

Adult male Sprague-Dawley rats (250–300 g) were anaesthetized with Intraval (May and Baker, Dagenham, Essex, UK; 110 mg kg^{-1}, i.p.) and prepared surgically for micropuncture experiments as described in previous studies from this laboratory (Walter *et al.* 1979, Shirley *et al.* 1990). First, the animals were placed on a servo-controlled heated operating table to maintain the rectal temperature at 37 °C. The right jugular vein was then cannulated, followed by a tracheotomy, cannulation of the right femoral artery, catherization of the bladder through a suprapubic incision and exposure of the left kidney via a flank incision. The kidney was cleared of perirenal fat, then placed in a Perspex cup and its ureter was catheterized. The kidney was bathed continuously in paraffin oil pre-warmed to 37 °C.

Experimental protocol

Rats were infused intravenously with isotonic saline containing 4·5 mM LiCl at a rate of 4 ml/h. One hour after the completion of surgery, [^{3}H]inulin (2 μCi primer; 2 μCi/h; Amersham International, Aylesbury, Bucks, UK) was included in the infusate. After a further hour had been allowed for equilibration, all rats underwent a 1 h clearance period (control period). This protocol was designed to establish baseline values for renal function prior to the administration of oxytocin antagonist or vehicle. The animals were then split into two groups. One group was infused with the oxytocin receptor antagonist d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Orn^8,Tyr (NH_2)^9]-vasotocin (Bachem, Saffron Walden, Essex, UK), given as a bolus (40 μg kg bw^{-1}) followed by an infusion at 40 μg h^{-1} kg bw^{-1} in the saline infusate; the second group received the vehicle alone (saline). These infusions were maintained for two subsequent 1 h periods (experimental periods). A 150 μl blood sample was taken for lithium and [^{3}H] inulin estimation at the start and end of each clearance period. Femoral arterial blood pressure was monitored throughout using a Druck (Groby, Leics, UK) transducer linked with a Maclab recording system (A. D. Instruments, Palo Alto, CA, USA). At the end of each experiment, a small (<1 ml) sample of arterial blood was taken for the measurement of haematocrit, plasma electrolyte concentrations and plasma osmolality, and a larger (<2 ml) sample was taken for the measurement of plasma oxytocin and vasopressin concentrations.

Analyses

Sodium and potassium concentrations in urine and plasma were measured by flame photometry and lithium concentrations were measured by atomic absorption spectroscopy. Haematocrit was measured using Hawksley micro-haematocrit tubes. [^{3}H]inulin activity in samples of urine and plasma, dispersed in Aquasol 2 scintillation cocktail (Canberra Packard, Pangbourne, Berks, UK), was measured by β-emission spectroscopy.

Radioimmunoassay of oxytocin and vasopressin

Oxytocin and vasopressin were extracted from plasma using C18 Sep-Pak columns (Waters Associates Ltd, Northwick Park, Middx, UK). Oxytocin was determined as described by Balment *et al.* (1986) using the Fourth International Standard for oxytocin (76/575). The lower limit of detection for the assay was 1·6 pmol/l, with intra- and inter-assay variations of 4·1% (n=10) and 9·0% (n=10) respectively for 10 pmol/l. Vasopressin was determined as described by Forsling & Peysner (1988) using the First International Standard for vasopressin (77/501). The lower limit of detection for the assay was 0·12 ± 0·02 pmol/l, with intra- and inter-assay variations of 7·7% (n=23) and 11·9% (n=10) respectively for 2·5 pmol/l.

Calculations and statistics

Although urine was collected from the two kidneys separately, no systematic differences in excretion rates or clearances between the two kidneys were observed. The values for the two kidneys were therefore pooled. Clearances were calculated using the usual formula:

$$C_x = U_x \times V/P_x$$

where U_x is the urine concentration of substance x, V is the urine flow rate and P_x is the plasma concentration of x. GFR was taken to be the clearance of [^{3}H] inulin. Fractional excretions of water, sodium, potassium and lithium were calculated as V/GFR, C_{Na}/GFR, C_K/GFR

and C_{Li}/GFR, respectively, where C_{Na}, C_K and C_{Li} are the clearances of sodium, potassium and lithium respectively.

Data are presented as means ± S.E.M. Statistical comparisons were made using one way analysis of variance with repeated measures, using the first time period (control period) as the co-variant (ANCOVA). Post-hoc comparisons between the two groups were made using Student's unpaired *t*-test. A difference was taken as statistically significant if $P<0{\cdot}05$.

Results

Body weights were similar in the two groups of animals (vehicle group: 267 ± 4 g, $n=8$; antagonist-treated group: 263 ± 3 g, $n=7$). Mean arterial pressure (MAP) tended to fall slightly during the course of the experiment (Fig. 1a), but at no time was there a statistically significant difference between the two groups. GFR was similar in the two groups during the control period (Fig. 1b), but, whilst it remained unchanged in the vehicle group, a small reduction was evident in the antagonist-treated animals ($P<0{\cdot}05$, ANCOVA).

Figure 2 shows Na^+ and water excretion, both in absolute terms and as a fraction of the filtered load. In the vehicle group, Na^+ excretion was stable throughout the experiment (Fig. 2a). In oxytocin antagonist-treated rats, however, there was an abrupt and sustained reduction in Na^+ excretion ($P<0{\cdot}01$, ANCOVA). A similar pattern was seen with respect to fractional Na^+ excretion (FE_{Na}; Fig. 2b), the reduction in antagonist-treated rats again being highly significant ($P<0{\cdot}01$, ANCOVA). The fall in Na^+ excretion was accompanied by an antidiuresis, significant reductions in both absolute urine flow rate and fractional water excretion being observed during treatment with the oxytocin antagonist ($P<0{\cdot}05$, ANCOVA, in each case).

In contrast to the observations on Na^+ and water excretion, infusion of the oxytocin antagonist had no significant effect on either absolute or fractional K^+ excretion (Fig. 3a, b). Lithium clearance (C_{Li}; used as an index of end-proximal fluid delivery) fell slightly, but not significantly, during treatment with the oxytocin antagonist (Fig. 3c). Fractional lithium excretion (FE_{Li}; C_{Li} expressed as a percentage of GFR) was almost identical in the two groups (Fig. 3d).

Blood samples taken at the end of the final clearance period yielded the data presented in Table 1 . There were no significant differences between the two groups of animals with respect to any of the variables measured.

Discussion

Infusion of the oxytocin receptor antagonist $d(CH_2)_5[Tyr(Me)^2,Thr^4,Orn^8,Tyr(NH_2)^9]$-vasotocin into anaesthetized rats which had been prepared surgically for micropuncture produced clear effects on sodium and water excretion. In an earlier study in conscious rats, this antagonist was shown to be a particularly effective blocker of oxytocin's actions (Windle *et al.* 1997). In the present investigation, we chose a dose approximately 10-fold higher than in the previous study, because in our experience sensitivity to infused peptides tends to be lowered during anaesthesia. The antagonist has negligible effects on vasopressin V_2 receptors (Manning *et al.* 1989) and, although it has been reported to partially block V_1 receptors (Manning *et al.* 1989), it had no effect on arterial pressure in the present study. (In any case, plasma vasopressin concentrations did not reach pressor levels.)

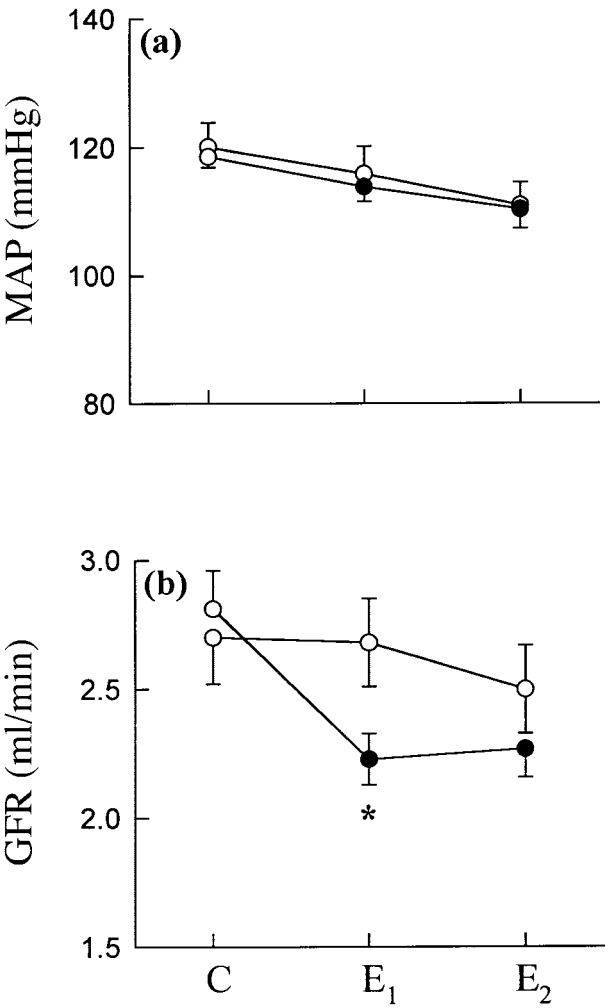

Figure 1 (a) Mean arterial pressure (MAP) and (b) glomerular filtration rate (GFR) during the control period (C) and during the two experimental periods (E_1, E_2) in rats treated with oxytocin antagonist (● $n=7$) or vehicle alone (○ $n=8$). Values are means ± S.E.M. *$P<0{\cdot}05$ compared with vehicle group (unpaired *t*-test).

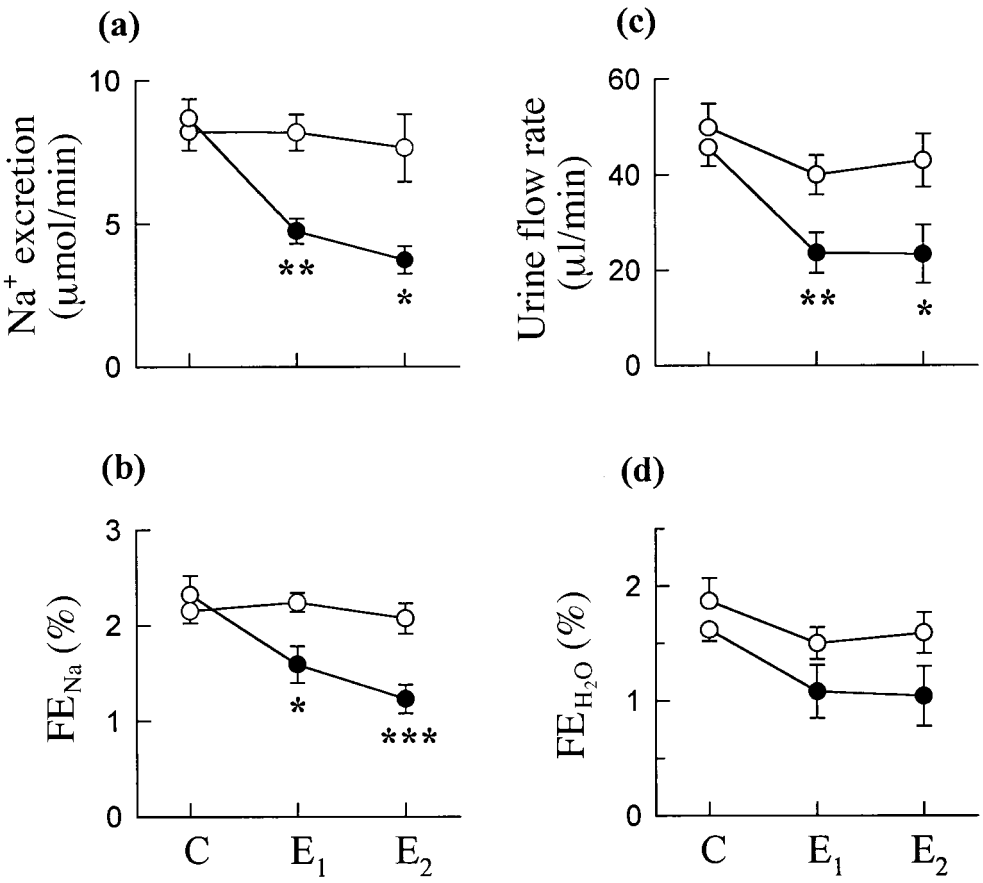

Figure 2 (a) Absolute Na^+ excretion, (b) fractional Na^+ excretion (FE_{Na}), (c) urine flow rate and (d) fractional water excretion (FE_{H_2O}) during the control period (C) and during the two experimental periods (E_1, E_2) in rats treated with oxytocin antagonist (● $n=7$) or vehicle alone (○ $n=8$). Values are means ± S.E.M. *$P<0{\cdot}05$, **$P<0{\cdot}02$, ***$P<0{\cdot}01$ compared with vehicle group (unpaired *t*-test).

Moreover, the earlier study found that this antagonist did not interfere with vasopressin's effects on sodium and water excretion (Windle *et al.* 1997).

We conclude that the antagonist's renal effects (reduced GFR and increased fractional reabsorption of sodium and water) resulted, not from blockade of vasopressin receptors, but from interference with the action of oxytocin on oxytocin receptors. The endogenous plasma concentrations of oxytocin in our anaesthetized rats were within the range achieved by doses of oxytocin previously shown to increase GFR (Forsling *et al.* 1994, Windle *et al.* 1997). Receptors for oxytocin have been located in the glomerulus (Stoeckel *et al.* 1987) and the macula densa region of the tubule (Stoeckel & Freund-Mercier 1989, Arpin-Bott *et al.* 1997), and it seems reasonable to propose that these might have mediated the observed change in GFR (the macula densa being intimately involved in the tubulo-glomerular feedback control of GFR; Briggs & Schnermann 1987).

Although the reduction in GFR will undoubtedly have contributed to the observed falls in sodium and water excretion during oxytocin antagonist infusion, the marked increase in fractional sodium reabsorption, and lesser increase in fractional water reabsorption, strongly suggest a tubular effect. In the absence of a systematic micropuncture investigation, the precise nephron site(s) involved is unknown. However, by including lithium clearance (C_{Li}) data, the present study was able to provide information on the contribution of the proximal tubule. On the basis that filtered lithium ions are reabsorbed exclusively in the proximal tubule, in proportion to the reabsorption of sodium and water, C_{Li} is widely used as a measure of end-proximal fluid delivery (Thomsen 1990). Although it is now known that neither of these premises is absolutely correct, the errors involved are small, and C_{Li} can still provide an adequate, semi-quantitative estimate of changes in end-proximal delivery (Thomsen & Shirley 1997). In the event, C_{Li} fell slightly (but not significantly)

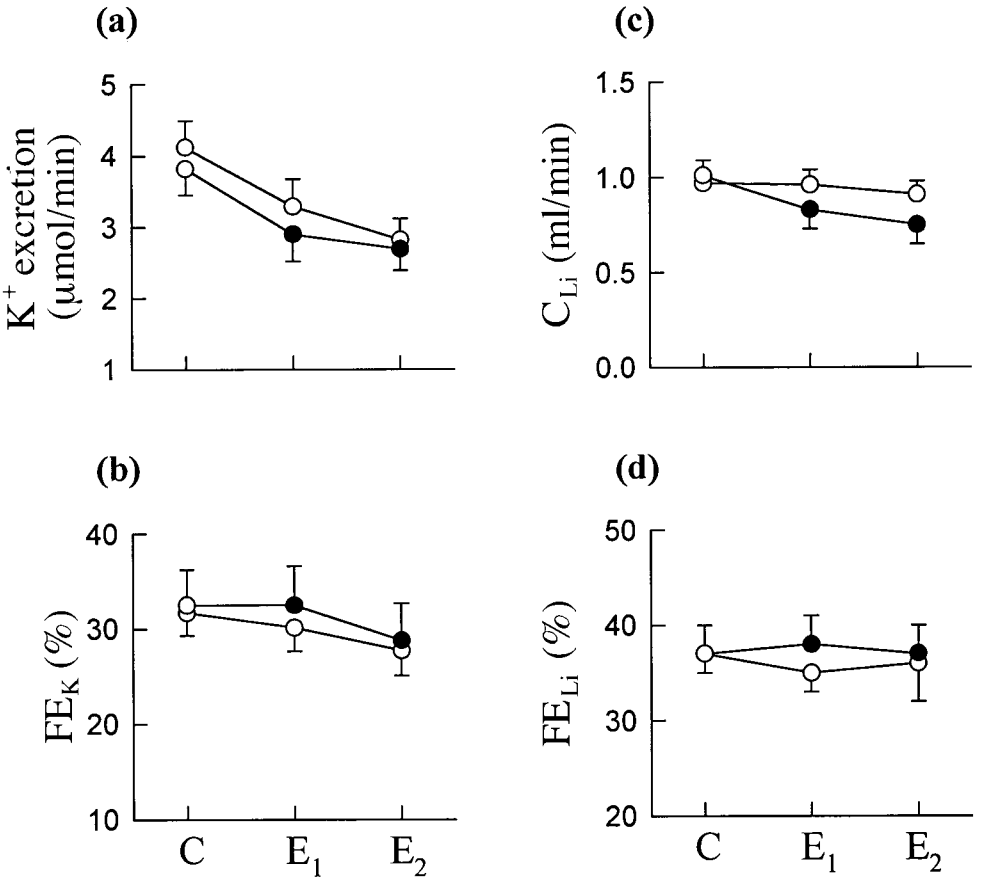

Figure 3 (a) Absolute K^+ excretion, (b) fractional K^+ excretion (FE_K), (c) lithium clearance (C_{Li}) and (d) fractional lithium excretion (FE_{Li}) during the control period (C) and during the two experimental periods (E_1, E_2) in rats treated with oxytocin antagonist (● $n=7$) or vehicle alone (○ $n=8$). Values are means ± S.E.M.

during treatment with oxytocin antagonist, very much in line with the fall in GFR. Consequently, FE_{Li} was unchanged. The results from this study, the first in which the effect of oxytocin on C_{Li} has been assessed, indicate that fractional proximal tubular reabsorption is unchanged by a blockade of oxytocin receptors. The data therefore substantiate the earlier split-drop micropuncture study of Garland *et al.* (1983).

Table 1 Haematocrit and plasma data in rats treated with oxytocin antagonist or with vehicle alone

	Vehicle group ($n=8$)	**Oxytocin antagonist-treated group** ($n=7$)
Haematocrit (%)	41·7 ± 0·4	41·1 ± 0·3
Plasma Na^+ (mmol/l)	138 ± 2	137 ± 2
Plasma K^+ (mmol/l)	4·1 ± 0·2	4·1 ± 0·2
Plasma osmolality (mosmol/kg H_2O)	288 ± 3	287 ± 3
Plasma oxytocin (pmol/l)	19·6 ± 2·8	20·5 ± 2·4
Plasma vasopressin (pmol/l)	12·1 ± 3·9	10·3 ± 2·9

There were no significant differences between the two groups with respect to any variable.

Thus, the site(s) of increased fractional reabsorption is/are likely to be beyond the proximal tubule. In this context, the lack of effect of the oxytocin antagonist on potassium excretion may be relevant. Any increase in sodium (and water) reabsorption upstream of the sites of tubular potassium secretion (late distal tubule and cortical collecting tubule) would reduce sodium delivery to, and reabsorption in, the potassium secreting sites, which in turn would be expected to result in reduced potassium secretion (and therefore excretion) (Giebisch 1998). The absence of a change in potassium excretion therefore suggests that the site of increased sodium and water reabsorption is downstream of the potassium-secreting site, i.e. the medullary collecting duct. Although this conclusion is based only on circumstantial evidence, it is consistent with reports of oxytocin receptors in the inner

medullary collecting duct (Stoeckel *et al.* 1987, Wargent *et al.* 1999). Somewhat contradictory to this, there is some evidence for an inhibitory effect of oxytocin on amiloride-sensitive sodium transport across the luminal membrane of rabbit cortical collecting duct segments perfused *in vitro* (Inoue *et al.* 1993). However, in the latter study no time-control measurements were made; moreover, the concentrations of oxytocin required in the bathing solution in order to elicit a response exceeded by several orders of magnitude those normally circulating in the plasma.

Finally, it should be stressed that the effects we have described resulted from blockade of the action of fairly high endogenous oxytocin concentrations (~20 pmol/l) which are comparable to those found during dehydration and hypernatraemia (Windle *et al.* 1993, Huang *et al.* 1995) but which exceed normal circulating levels in conscious male rats (2–8 pmol/l; Conrad *et al.* 1986, Verbalis *et al.* 1991, Forsling *et al.* 1994). In order to determine the day-to-day role of the hormone, it would be necessary to administer the antagonist to undisturbed conscious animals kept in sodium and water balance. However, the present study has demonstrated that in the anaesthetized rat prepared for micropuncture (the standard means of investigating segmental tubular function), the plasma levels of oxytocin are sufficient to contribute significantly to sodium and water excretion. This has major implications for future (and past) invasive studies of renal function.

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References

Arpin-Bott MP, Waltisperger E, Freund-Mercier MJ & Stoeckel ME 1997 Two oxytocin-binding site subtypes in rat kidney: pharmacological characterization, ontogeny and localization by *in vitro* and *in vivo* autoradiography. *Journal of Endocrinology* **153** 49–59.

Balment RJ, Brimble MJ & Forsling ML 1980 Release of oxytocin induced by salt loading and its influence on renal excretion in the male rat. *Journal of Physiology* **308** 439–449.

Balment RJ, Brimble MJ, Forsling ML & Musabayane CT 1986 The influence of neurohypophysial hormones on renal function in the acutely hypophysectomized rat. *Journal of Physiology* **381** 439–452.

Briggs JP & Schnermann J 1987 The tubulo-glomerular feedback mechanism: functional and biochemical aspects. *Annual Reviews of Physiology* **49** 251–273.

Cogan MG 1990 Atrial natriuretic peptide. *Kidney International* **37** 1148–1160.

Conrad KP, Gellai M, North WG & Valtin H 1986 Influence of oxytocin on renal hemodynamics and electrolyte and water excretion. *American Journal of Physiology* **251** F290–F296.

Forsling ML & Peysner K 1988 Pituitary and plasma vasopressin concentrations and fluid balance throughout the oestrous cycle of the rat. *Journal of Endocrinology* **117** 397–402.

Forsling ML, Judah JM & Windle RJ 1994 The effect of vasopressin and oxytocin on glomerular filtration rate in the conscious rat: contribution to the natriuretic response. *Journal of Endocrinology* **141** 59–67.

Garland HO, Balment RJ & Brimble MJ 1983 Oxytocin and renal function in the rat; an investigation of a possible proximal site of action. *Acta Endocrinologica* **102** 517–520.

Giebisch G 1988 Renal potassium transport: mechanisms and regulation. *American Journal of Physiology* **274** F817–F833.

Huang W, Lee S-L & Sjöquist M 1994 Effect of neurohypophyseal antagonists in postnephrectomy natriuresis in male rats. *Kidney International* **45** 692–699.

Huang W, Lee S-L & Sjöquist M 1995 Natriuretic role of endogenous oxytocin in male rats infused with hypertonic NaCl. *American Journal of Physiology* **268** R634–R640.

Huang W, Lee S-L, Arnason SS & Sjöquist M 1996 Dehydration natriuresis in male rats is mediated by oxytocin. *American Journal of Physiology* **270** R427–R433.

Inoue T, Naruse M, Nakayama M, Kurokawa K & Sato T 1993 Oxytocin affects apical sodium conductance in rabbit cortical collecting duct. *American Journal of Physiology* **265** F487–F503.

Manning M, Kruszynski M, Bankowski K, Olma A, Lammek B, Cheng LL, Klis WA, Seto J, Halder J & Sawyer WH 1989 Solid-phase synthesis of 16 potent (selective and non selective) *in vivo* antagonists of oxytocin. *Journal of Medicinal Chemistry* **32** 382–391.

Shirley DG & Walter SJ 1996 Is lithium a useful tool in studies on renal sodium handling? *Journal of Nephrology* **9** 126–132.

Shirley DG, Zewde T & Walter SJ 1990 Renal function in normal and potassium-depleted rats before and after preparation for micropuncture experimentation. *Pflügers Archiv* **416** 74–79.

Stoeckel ME & Freund-Mercier MJ 1989 Autoradiographic demonstration of oxytocin-binding sites in the macula densa. *American Journal of Physiology* **257** F310–F314.

Stoeckel ME, Freund-Mercier MJ, Palacios JM, Richard PM & Porte A 1987 Autoradiographic localizations of binding sites for oxytocin and vasopressin in the rat kidney. *Journal of Endocrinology* **113** 179–182.

Thomsen K 1990 Lithium clearance as a measure of sodium and water delivery from the proximal tubules. *Kidney International* **37** (Suppl 28) S10–S16.

Thomsen K & Shirley DG 1997 The validity of lithium clearance as an index of sodium and water delivery from the proximal tubules. *Nephron* **77** 125–138.

Verbalis JG, Mangione MP & Stricker EM 1991 Oxytocin produces natriuresis in rats at physiological plasma concentrations. *Endocrinology* **128** 1317–1322.

Walter SJ, Laycock JF & Shirley DG 1979 A micropuncture study of proximal tubular function after acute hydrochlorothiazide administration to Brattleboro rats with diabetes insipidus. *Clinical Science* **57** 427–434.

Wargent ET, Burgess WJ, Laycock JF & Balment RJ 1999 Separate receptors mediate oxytocin and vasopressin stimulation of cAMP in rat inner medullary collecting duct cells. *Experimental Physiology* **84** 17–25.

Windle RJ, Forsling ML, Smith CP & Balment RJ 1993 Patterns of neurohypophysial hormone release during dehydration in the rat. *Journal of Endocrinology* **137** 311–319.

Windle RJ, Judah JM & Forsling ML 1997 Effect of oxytocin receptor antagonists on the renal actions of oxytocin and vasopressin in the rat. *Journal of Endocrinology* **152** 257–264.

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