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Central administration of porcine relaxin stimulates drinking behaviour in rats: an effect mediated by central angiotensin II

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Central injection of porcine relaxin into the lateral ventricle of water-replete rats caused a marked drinking response. Relaxin in 2 μL 0.9% saline caused a dose-dependent (range 10–50 ng), significant ($P<0.01$) dipsogenesis compared with saline-treated controls. There was no drinking response to <10 ng relaxin. At 10 ng relaxin ICV rats drank 4.2 ± 0.2 mL water within 15 min of injection. The amount of water taken increased with increasing dose and plateaued at 50 ng ICV (10.2 ± 1.3 mL) thereafter; increasing the dose of relaxin did not significantly increase the total volume of water consumed. In contrast, there was no significant increase in water consumed in rats treated with a deactivated form of porcine relaxin, or with insulin. Rats appeared to compensate for the period of hyperdipsia, as there was no significant difference in the water consumed in control (saline-injected) and relaxin-treated rats in the 23 h period after testing.

The effect of blocking the central action of angiotensin II on the dipsogenic effects of relaxin was tested by infusing of a specific angiotensin II receptor antagonist into the lateral ventricle before treatment with relaxin. Antagonism of the central angiotensin II system, confirmed by lack of a dipsogenic response to ICV exogenous angiotensin II (10 ng), completely blocked the dipsogenic response of relaxin (50 ng in 1 μL) in female rats.

These data demonstrate that exogenous porcine relaxin is dipsogenic in the rat and that the mechanism of action appears to be through the central angiotensin II system. It is possible that relaxin may affect water intake during pregnancy when relaxin levels are detectable in the plasma and the hormone may be implicated in the regulation of cardiovascular function in pregnancy.

Keywords: relaxin drinking; angiotensin salt-appetite

Introduction

The peptide hormone relaxin has been shown to affect the release of a number of pituitary peptides; exogenous relaxin suppresses the pulsatile release of luteinizing hormone in ovariectomized rats (Summerlee *et al.*, 1990), stimulates the release of prolactin (Bethea *et al.*, 1989; Sortino *et al.*, 1989) and stimulates vasopressin and oxytocin release (Mumford *et al.*, 1989; Way & Leng 1992; Geddes *et al.*, 1994; Summerlee *et al.*, 1995). The mechanism of action within the brain is not clear, but it has been proposed that the central angiotensin II system may be important in mediating the effects of relaxin on luteinizing hormone release (Summerlee *et al.*, 1990), and oxytocin and vasopressin release (Parry & Summerlee, 1991; Geddes *et al.*, 1994). If exogenous relaxin is acting on the brain by stimulating the central angiotensin II system, then it is possible that administration of exogenous relaxin may mimic the actions of exogenous angiotensin II i.e. induce dipsogenesis.

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The purpose of the present experiments was to examine the possible effects of relaxin dipsogenesis in the rat. Preliminary experiments were carried out by injecting porcine relaxin IV (Robertson *et al.*, 1991) and ICV injection (Robertson & Summerlee, 1991) in conscious rats. The data published in the current paper provides a full account of these experiments and investigates the possible involvement of the forebrain angiotensin system in mediating the pressor response to relaxin. Thornton & Fitzsimons (1989) have also published an abstract indicating that porcine relaxin increases water intake but does not affect salt appetite in rats.

Results

All data are expressed as mean ± SEM, and reference to statistical significance is at the 5% level.

Experiment 1. Effects of exogenous porcine relaxin on the drinking response in conscious rats.

Porcine relaxin ICV caused an almost immediate and significant drinking response in rats at doses greater than 5 ng (Figure 1) compared with saline-treated rats. There were no other behavioural effects of relaxin that were apparent. No drinking response was observed at 5 ng relaxin ICV. The latency to the onset of drinking (Figure 2) was 55 ± 17 s (range 17–125 s) but there was no significant difference in the latency to drinking between the relaxin treatment groups. At 10 ng relaxin ICV the rats consumed 4.2 ± 0.2 mL water. Increasing the dose to 50 ng, caused a dose-dependent increase in water consumed (10.2 ± 1.3 mL) but at doses greater than 50 ng relaxin there was no further increase in the amount of water consumed. Most of the drinking (up to 90% of the volume consumed in the first hour after treatment) occurred in the first 15 min (Figure 3) with the remainder being consumed in the next 45 min. The cumulative water intake over the next 23 h was not significantly different between any of the relaxin- or saline-treated rats (Table 1).

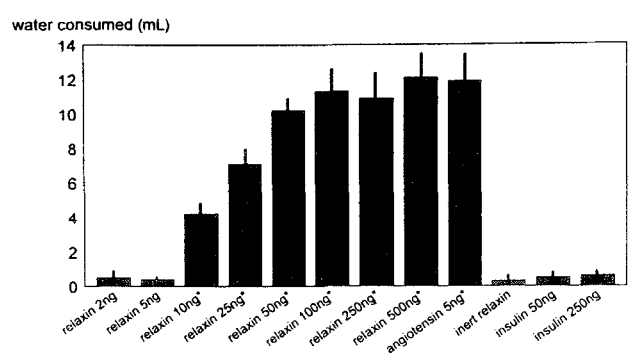

Figure 1 Mean (±SEM) volume of water consumed by female rats in 1 h after ICV injection of hormone. *$P<0.05$ compared with ICV injection of vehicle (0.9% NaCl)

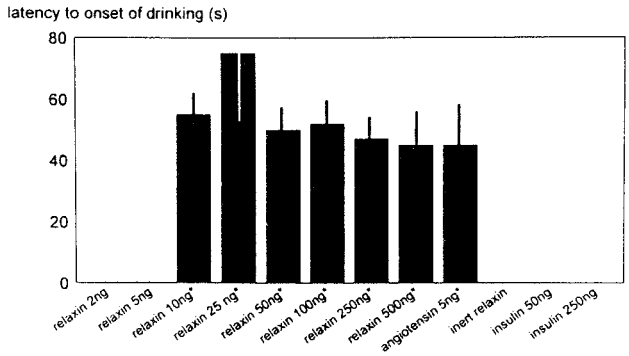

Figure 2 Mean (±SEM) latency to the onset of drinking in female rats after ICV injection of hormone. *$P < 0.05$ compared with ICV injection of vehicle (0.9% NaCl)

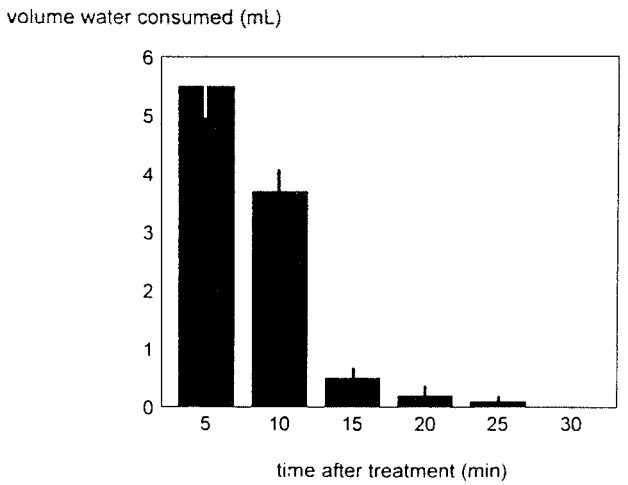

Figure 3 Mean (±SEM) volume of water consumed by female rats in 5 min time-bins after ICV injection of 50 ng porcine relaxin

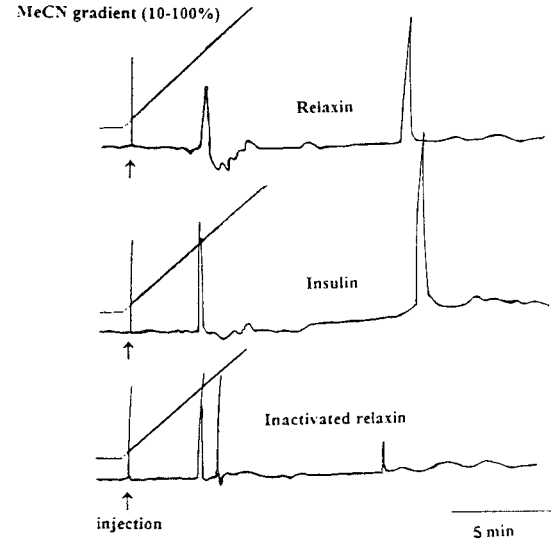

Figure 4 Absorbance profile of porcine relaxin, insulin and inactivated relaxin used in these experiments

Table 1 Water and food intake by female rats in the 24 h following ICV treatment with relaxin, insulin and angiotensin. Data are shown as mean±SEM and the numbers of animals used are shown in parentheses

ICV treatment	*Water intake* (mL)	*Food intake* (g)
0.9% saline ($n = 20$)	27.2 ± 3.2	23.1 ± 2.0
10 pmol angiotensin II ($n = 19$)	25.7 ± 3.7	24.5 ± 1.3
inert relaxin: 50 ng ($n = 20$)	25.5 ± 4.3	22.4 ± 2.2
10 ng relaxin ($n = 19$)	27.0 ± 3.2	21.7 ± 2.6
50 ng relaxin ($n = 22$)	28.4 ± 3.1	20.4 ± 2.7
500 ng relaxin ($n = 18$)	29.1 ± 3.6	21.7 ± 2.5
250 ng insulin ($n = 16$)	26.7 ± 4.3	28.2 ± 1.4*

*Significant ($P < 0.05$) compared with saline-treated rats

Comparisons were made between the dipsogenic effect of relaxin, angiotensin II, insulin and inert relaxin (Figure 1). Angiotensin II (5 ng) induced a drinking response that was comparable to 50 ng relaxin (latency 47 ± 15 s) but induced a significantly greater drinking response (21 ± 3 mL) at 10 ng (latency 45 ± 18 s). In contrast, neither insulin nor the inert relaxin induced a drinking response in any of the rats tested.

Experiment 2. Effects of antagonism of central angiotensin on the relaxin-induced dipsogenesis.

Infusion of saralasin in rats did not appear to affect behaviour. In angiotensin-antagonized rats, relaxin did not induce a drinking response but in all animals a second treatment with relaxin after the effect of the antagonist had worn off resulted in a significant drinking response (latency 63 ± 25 s; volume consumed 11.2 ± 1.0 mL).

Discussion

We set out to study the potential dipsogenic effects of relaxin in rats. Relaxin is essential for relaxation of the cervix (Downing & Sherwood, 1985a; Hwang *et al.*, 1989), and development of the nipples of the mammary glands (Hwang *et al.*, 1991) in rats in preparation for birth, and it may be important in the period of uterine quiescence in the prepartum period (Downing & Sherwood, 1985b). Relaxin has also been implicated in the cardiovascular changes in pregnancy including the reduction in blood pressure (St. Louis & Masicotte, 1985), and the decrease in plasma osmolality (Weisinger *et al.*, 1993) that occur during pregnancy, although there is now a considerable body of evidence to suggest that relaxin is hypertensive (Mumford *et al.*, 1989; Parry *et al.*, 1990, 1994; Geddes *et al.*, 1994). The present experiments suggest that relaxin could be involved in the changes in drinking behaviour that occur in pregnancy.

During pregnancy there is a gradual fall in blood pressure (Ahokas *et al.*, 1989) and approximately a 10 mosmol decrease in plasma osmolality (Durr *et al.*, 1981). The latter appears to be achieved by an increase in drinking, accompanied by a decrease in fluid excretion due to an increase in circulating concentrations of plasma vasopressin (Weisinger *et al.*, 1993): the result of these changes is a substantial increase in blood volume in pregnant animals. It has been suggested that these changes are mediated by re-setting central thresholds for angiotensin mechanisms involved in cardiovascular control but the driving force for these changes is not clear. Cardiovascular changes in the pregnant rat start on days 7–10 of gestation and develop over the second half of pregnancy. At the same time plasma relaxin levels become detectable in the plasma and the levels rise steadily throughout the second half of pregnancy (Sherwood *et al.*, 1980) and it is tempting to speculate that circulating relaxin may be important for promoting the changes in cardiovascular parameters in pregnancy. There is evidence to support this hypothesis: Weisinger *et al.* (1993) reported that infusion of

human relaxin in non-pregnant rats deprived of endogenous relaxin by ovariectomy show decreases in plasma osmolality and increases in circulating vasopressin similar to those observed in the second half of pregnancy. Relaxin has been shown to cause the release of vasopressin when injected IV (Geddes *et al.*, 1994) and ICV (Mumford *et al.*, 1989), although it has been shown that relaxin causes a marked pressor response when injected IV or ICV into anaesthetized rats (Mumford *et al.*, 1989; Parry *et al.*, 1990). Data in the present paper suggests that acute injection of relaxin ICV is dipsogenic.

Central actions of relaxin appear to be mediated by the central angiotensin system. Blockade of central angiotensin II receptors suppresses the inhibitory effect of porcine relaxin on the release of luteinizing hormone in conscious rats (Summerlee *et al.*, 1991) and blocks the relaxin-stimulated release of oxytocin and vasopressin (Parry & Summerlee, 1991; Geddes *et al.*, 1994). Moreover, lesion of the subfornical organ, a structure which is critically involved in the forebrain angiotensin system (Saavedra, 1992), including the dipsogenic response to central injection of angiotensin II (Saavedra, 1992), also blocks the effects of relaxin on blood pressure (Mumford *et al.*, 1989), oxytocin and vasopressin release (Geddes *et al.*, 1994), milk-ejection (Summerlee *et al.*, 1987), and birth (Summerlee & Wilson, 1994). Data in the present paper shows that the dipsogenic effect of relaxin may also be mediated by the central angiotensin system. However, the involvement of the angiotensin II system was not characterized further. No distinction was made between AT and AT receptor subtypes, nor was there an examination of the possible effects of relaxin on component of angiotensin II production within the brain. For example, it was important to know whether relaxin affects the synthesis of angiotensin II or affects the release of stored angiotensin II.

There is one other report in the literature that central injection of relaxin stimulates water intake. Thornton & Fitzsimons (1989) reported that porcine relaxin injected into the third ventricle of the rat brain induced an increase in water intake but did not stimulate sodium appetite. Data in their abstract is similar in some respects to the data published in the current paper: the overall volume of water consumed in response to the dose of relaxin used is similar but there is one major difference; Thornton & Fitzsimons reported that the latency to the onset of relaxin-induced drinking was greater than 15 min compared with the short latency to drinking observed in our laboratory. Thornton & Fitzsimons (1989) injected relaxin into the third ventricle but the exact site of injection is not given. Mumford *et al.* (1989) reported that two different pressor responses could be elicited to relaxin injection depending on the site of injection into the third ventricle; injection into the dorsal part of the third ventricle resulted in an almost immediate pressor response that was prolonged whereas injection into the lower portion of the third ventricle resulted in a delayed onset of the pressor response implying that the dorsal site is closer to the receptors (subsequently shown to be in the subfornical organ by Oscheroff and colleagues (Oscheroff *et al.*, 1990)). It is feasible that site of injection in the two sets of drinking experiments could have affected the latencies to the onset of the drinking response.

Insulin ICV had no effect on drinking behaviour. It is reported that systemic administration of insulin is dipsogenic by activating the peripheral renin-angiotensin system due to insulin-activated hypoglycemia (Denton, 1982). In contrast, systemic relaxin (IV) is dipsogenic (Robertson *et al.*, 1991) but here is no evidence that systemic administration activates the peripheral renin-angiotensin system. In fact, Parry (1992) showed that the pressor effect of systemically administered relaxin was not blocked by pretreatment with angiotensin-converting enzyme inhibitor systemically implying that, at least the pressor action of relaxin, is independent of the peripheral renin-angiotensin system. It has been suggested that both systemic and central relaxin acts where there is a high density of relaxin-binding sites (Oscheroff *et al.*, 1990). Moreover, lesion of the subfornical organ negates that actions of relaxin on blood pressure and vasopressin release (Mumford *et al.*, 1989), milk-ejection (Summerlee *et al.*, 1987) and birth (Summerlee & Wilson, 1994) and structures in the subfornical organ appear to be accessible to blood-borne, csf-borne and brain peptides (Bradbury, 1985).

The original hypothesis for the current work was that relaxin might act centrally by stimulating central angiotensin II release. We have shown that relaxin induces drinking, the classic' central action of angiotensin II and the actions of relaxin are inhibited by antagonism of angiotensin II receptors. It has also been shown that relaxin stimulates oxytocin and vasopressin release (Geddes *et al.*, 1994) which is similar to the effects of central administration of angiotensin. However, one of the actions of central angiotensin is not stimulated by relaxin: acute injection of relaxin does not stimulate sodium appetite (Fitzsimons & Thornton, 1992). There is a considerable natriorexigenic effect during pregnancy in the rat (Denton, 1982) which is temporally correlated with relaxin profiles (Sherwood *et al.*, 1980) so Denton (1982) suggested that relaxin might mediate this effect. It remains to be established whether or not lack of endogenous relaxin affects salt appetite in conscious, pregnant rats.

Data presented in this paper supports the growing body of evidence that relaxin acts on the brain and is involved in the regulation of cardiovascular function. As the greatest circulating levels of relaxin are observed during pregnancy which is the time of the greatest adjustments in cardiovascular control, it is tempting to suggest that relaxin may have a role in the physiology of pregnancy. However, critical evidence linking circulating levels of relaxin with such changes is still not available. It remains to be demonstrated that removal of endogenous relaxin blocks the hypotensive, hypo-osmolar and hyperdipsic changes that that occur in pregnancy.

Materials and methods

The experiments described were carried out using female, non-pregnant Harlan Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) weighing 270–320 g. The animals were housed in single cages and kept in the Central Animal Facilities of the University of Guelph, ON, CAN. They were kept in a 14 h light, 10 h dark lighting regimen with food and water available *ad libitum*. Animals were socialized to the laboratory, and to frequent handling for at least 2 weeks before experimentation. All experiments were carried out under the guidelines of the Canadian Council for Animal Care and approved by the Animal Care Committee of the University of Guelph. The operative procedures were carried out under strict aseptic conditions.

Implantation of chronic ventricular cannulae

Rats were premedicated with diazepam (Valium 10, Hoffman La Roche Ltd., Etobicoke, ON, CAN; 0.8 mg/kg IM) and anaesthetized with ketamine (Rogarsetic: Rogar STC Inc., London, ON, CAN 20 mg IP). The left saphenous vein was cannulated (polythene tubing, ID 0.28 mm, OD 0.61 mm: Portex Tubing Ltd., Arnold & Horwell, London, UK) for the administration of further anesthetic. Gradually, over a 15 min period, small incremental doses of barbiturate (sodium pentobarbitone: Somnotal, MTC Pharmaceutics, Cambridge, ON; 12 mg/mL IV) were administered IV to deepen the level of anaesthesia until the rat could be placed with its head in a stereotaxic frame (Narishige SR6, Tokyo, Japan). Atraumatic ear-bars were used to prevent rupture of the tympanic membranes. A longitudinal mid-line section was made in the scalp and the tissue reflected. A small hole (1 mm) was drilled in the skull vertically above the left lateral

ventricle of the brain using a sterile 1mm tungsten drill-bit and an ICV microcannula (220A; Kopf Instruments, San Francisco, CA), held in a micromanipulator (Narishige SM20) lowered into the hole so that the tip of the electrode was positioned in the lateral ventricle (co-ordinates used bregma, 2.5 mm lateral to midline, 3.0 mm ventral to the cortical surface: Pellegrino *et al.*, 1979). The shaft of the microcannula had been previously cut to the appropriate length for insertion into the ventricle and the cannula sterilized using ethylene oxide sterilization for 3 days. The microcannula was sealed in position using cold-curing acrylic and the skin sutured.

Post-operative analgesic (Mepiridine HCl: Demerol, 2.5 mg/rat IM every 4 h for 24 h) but no post-operative antibiotic was administered. The operations took 3–5 h to complete and rats were given 20–30 mL physiological saline SC to compensate for fluid loss during the experiments. Rats were nursed for 24 h post-operatively and socialization, in the laboratory, was continued for another 7 days to ensure that the rats were accommodated to their surroundings and to handling of the microcannula.

Relaxin

Highly purified relaxin (CMa' fraction; potency 3000 guinea pig units/mg) was used throughout the study. Relaxin was purified in the Department of Anatomy, University of Bristol, Bristol, UK, by extraction and purification from sow corpora lutea using previously described methodology (Sherwood & O'Byrne, 1974). The ovaries were supplied by the UK Meat and Livestock Commission. Purity of the relaxin was confirmed by SDS–PAGE and immunostaining with a relaxin-specific antibody (JJAB antibody kindly supplied by DG Porter, Biomedical Sciences, University of Guelph, ON, CAN). Relaxin was present as a single protein band at 6.2 kDaltons and there was no cross-reactivity of the relaxin-band with insulin-specific antibody (Parry, 1992).

Inert relaxin

To prepare the inactive relaxin, the hormone was reconstituted in saline and then microwaved for 30 s, followed by 30 s rest and a further 30 s of power. Destruction of the peptide was confirmed by three methods: (1) failure to react in the homologous porcine relaxin immunoassay, (2) change in the absorbance profile by reverse phase HPLC compared with the absorbance profile for standard relaxin (Figure 4), and (3) failure to induce a pressor response in anaesthetized non-pregnant rats (Parry *et al.*, 1990). Further confirmation of the lack of relaxin immunoactivity was carried out by subjecting fractions of the HPLC column to radioimmuoassay using an homologous radioimmunoassay developed for porcine relaxin (Taverne *et al.*, 1982). Briefly, the anitserum (JJAB5, 1:6,000 dilution) cross-reacted 100% with CMa' relaxin (18-28 AAE-CMa' kindly donated to Dr D.G. Porter by Dr B.G. Steinetz), 0.02% with porcine prolactin and $<0.01\%$ with porcine insulin (Sigma Chemicals, St. Louis, MI, USA). Sensitivity of the assay was between 3.5 and 33 pg/tube (mean 11.7 pg/tube) and the intra- and interassay coefficients of variation were 11% and 18% respectively ($n>12$).

Experiment 1. Effects of exogenous porcine relaxin on the drinking response in conscious rats

At least 7 days after implantation, a daily routine was established for testing the dipsogenic effects of porcine relaxin. Seven rats were treated with one dose per day of either: porcine relaxin (2, 5, 10, 25, 50, 100, 250 or 500 ng relaxin in 2 μL 0.9% saline), insulin (Insulin: Sigma Chemical Co., St. Louis, MO, USA; 50 or 250 ng in 2 μL 0.9% saline), inert relaxin (100 or 250 ng inactivated porcine relaxin in 2 μL 0.9% saline), angiotensin II (Angiotensin II: Sigma Chemical Co; 5 or 10 ng in 2 μL 0.9% saline), or 2 μL 0.9% saline alone. The study was blinded and randomized so each rat received a single treatment on a different day. In total, four repeats of each dose were tested on each animal throughout the test period.

Water replete rats were brought into the laboratory at least 30 min before testing and allowed free access to water. The animals were lifted from the cage, held lightly, and injected with the test solution through the neoprene seal of the microcannula using a Hamilton 10 μL microsyringe (701N: Hamilton Co., Reno, NV, USA) and replaced in the observation cage. The latency to the onset of drinking and the volume of water consumed every 5 min was recorded for 1 h. At the end of the hour, the animals were returned to the animal house and the cumulative intake of water and food monitored for the next 23 h.

Experiment 2. Effects of antagonism of central angiotensin on the relaxin-induced dipsogenesis

Experiments were done to test the effect of central relaxin treatment on the drinking behaviour of rats in which the central angiotensin II receptors had been blocked. A different group of seven chronically cannulated rats was used for this set of experiments. Rats were brought into the laboratory and settled as described above. A chronic infusion cannula was placed through the neoprene seal of the indwelling microcannula attached to a thin polythene tube (ID 0.28 mm, OD 0.61 mm) which was suspended above the animals cage by shirring elastic. This arrangement did not appear to affect the rats' behaviours. The polythene tube was filled with specific angiotensin II receptor antagonist (Sar^1-Ala^8-angiotensin II: Sigma Chemical Co., 10 mg/mL) and connected to a Sweden Infusion Pump (Model 11-1-900; Sweden Freezer Manufacturing Co., Seattle, WA, USA). The antagonist was infused for a least 1 h at a rate of 0.01 mL/h. Successful antagonism of the angiotensin receptors was confirmed by lack of a dipsogenic response to the injection of 10 ng angiotensin II ICV. Ten minutes after angiotensin treatment, rats were given an injection of either 50 ng or 250 ng porcine relaxin (in 2 μL 0.9% saline) and the effect on drinking observed. Thirty minutes after relaxin treatment, the infusion of the angiotensin II antagonist was terminated, and after a further 30 min, the effect of 50 ng relaxin ICV tested again.

Analysis of results

The mean values of each group were compared by one-way ANOVA and Neuman-Kreuls multiple comparison procedure to test for statistical differences between pairs of means. The Student's *t* test was used for comparison of means in experiments where independent groups of means were tested.

Confirmation of the sterility of the relaxin samples

Recent evidence indicates that the cytokine IL-1β, and possible endotoxin, induce the release of a variety of hypothalamic peptides (Naito *et al.*, 1991; Wilson & Summerlee, 1995) and affects body temperature (Rothwell, 1991) when injected ICV, therefore sterility of the samples tested in the present study were examined for the presence of bacterial growth. Separate batches of relaxin or control samples were made up in sterile eppendorffs and frozen until use. Each batch was used for a maximum of seven days and the possibility of bacterial contamination tested at the start, and at the end of the seven day period by plating on blood Agar plates (Veterinary Teaching Hospital, Clinical Microbiology Lab, Ontario Veterinary College, Guelph, ON, CAN). One sample was found to be contaminated the results from this batch were discarded in the analyses of data. Note: test were not run to ensure that samples were endotoxin-free, so previous contamination of the freeze-dried preparation of relaxin has

not be discounted by the saline used to dissolve the relaxin was endotoxin-free sterile saline.

Confirmation of the site of injection

At the end of the trials, rats were deeply anaesthetized with an overdose of barbiturate IP and 5 μL Indian Ink injected through the neoprene seal of the cannula before the animal stopped breathing. After death, the brain was removed and the presence of ink in the fourth ventricle and the central canal of the spinal cord were taken as proof of the site of injection into the ventricular system.

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References

Ahokas, R.A., Sibai, B.M. & Anderson, G.D. (1989). *Am. J. Obstet. Gynecol.*, **161,** 618–622.

Bethea, C.L., Cronin, M.J., Haluska, G.J. & Novy, M.J. (1989). *J. Clin. Endocrinol Metab.*, **69,** 956–962.

Bradbury, M.W.B. (1985). *Circ Res.*, **57,** 213–222.

Denton, D. (1982). *The Hunger for salt: an anthropological, physiological and medical analysis* Spinger-Verlag: Berlin. pp. 446–447.

Downing, S.J. & Sherwood, O.D. (1989a). *Endocrinology*, **116,** 1215–1220.

Downing, S.J. & Sherwood, O.D. (1989b). *Endocrinology*, **116,** 1200–1205.

Durr, J.A., Stamoutsis, B. & Lindheimer, M.D. (1981). *J. Clin. Invest.*, **68,** 337–346.

Fitzsimons, J.T. & Thornton, S.N. (1992). *J. Physiol.*, **446,** 457P.

Geddes, B.J., Parry, L.J. & Summerlee, A.J.S. (1994). *Endocrinology*, **134,** 1188–1192.

Hwang, J.-J., Shanks, R.D. & Sherwood, O.D. (1989). *Endocrinology*, **125,** 260–266.

Hwang, J.-J., Lee, A.B., Fields, P.A., Haab, L.M., Mojonnier, L.E. & Sherwood. (1991). *Endocrinology*, **129,** 3034–3042.

Mumford, A.D., Parry, L.J. & Summerlee, A.J.S. (1989). *J. Endocrinol.*, **122,** 747–755.

Naito, Y., Fukata, J., Shindo, K., Ebisui, O., Murakami, N., Tominaga, T., Nakai, Y., Mori, K., Kasting, N.W. & Imura, H. (1991). *Biochem. Biophys. Res. Comm.*, **174,** 1189–1195.

Oscheroff, P.L., Ling, V.T., Vandlen, R.L., Cronin, M.J. & Lofgren, J.A. (1990). *J. Biol. Chem.*, **265,** 9396–9401.

Parry, L.J. (1992). PhD Thesis, University of Guelph, ON, CAN

Parry, L.J. & Summerlee, A.J.S. (1991). *Endocrinology*, **129,** 47–52.

Parry, L.J., Poterski, R.S., Summerlee, A.J.S. & Jones, S.A. (1990). *J. Neuroendocrinol.*, **2,** 53–58.

Parry, L.J., Poterksi, R.S. & Summerlee, A.J.S. (1994). *Biol. Reprod.*, **50,** 622–628.

Pellegrino, L.T., Pellegrino, A.S. & Cashman, A.J. (1979). *A stereotaxic atlas of the rat brain* (2nd Edn). Plenum Press, New York.

Robertson, G.F. & Summerlee, A.J.S. (1991). *Proc. Soc. Endocrinology*, Abst 362.5

Robertson, G.F., Summerlee, A.J.S. & Chapman, H. (1991). *3rd IBRO World Congress of Neuroscience*, Montreal, CAN. Abst 219.

Rothwell, N. (1991). *Trends in Pharmacol. Sci.*, **12,** 430.

Saavedra, J.M. (1992). *Endocrin Rev.*, **13,** 329–380.

Sherwood, O.D. & O'Byrne, E.M. (1974). *Arch Biochem Biophys.*, **160,** 185–196.

Sherwood, O.D., Crnekovic, V.E., Gordon, W.L. & Rutherford, J.E. (1980). *Endocrinology*, **107,** 691–698.

Sortino, M.A., Cronin, M.J. & Wise, P.M. (1989). *Endocrinology*, **124,** 2013–2015.

St. Louis, J. & Massicotte, G. (1985). *Life Sci.*, **37,** 1351–1357.

Summerlee, A.J.S. & Wilson, B.C. (1994). *Endocrinology*, **134,** 2115–2120.

Summerlee, A.J.S., Mumford, A.D. & Smith, M.S. (1991). *J. Neuroendocrinol*, **3,** 1–7.

Summerlee, A.J.S., O'Byrne, K.T. & Jones, S.A. (1995). *J. Endocrinol.*, in press.

Summerlee, A.J.S., O'Byrne, K.T., Jones, S.A. & Eltringham, L. (1987). *J. Endocrinol.*, **115,** 347–353.

Taverne, M.A.M., Bevers, M., Bradshaw, J.M.C., Dickman, S.J., Willemse, A.H. & Porter, D.G. (1982). *J. Reprod. Fert.*, **65,** 85–96.

Thornton, S.N. & Fitzsimons, J.T. (1989). *Appetite*, **12,** 242.

Way, S. & Leng, G. (1992). *J. Endocrinol.*, **132,** 149–158.

Wilson, B.C. & Summerlee, A.J.S. (1995). *Biol. Reprod.* (in press).

Weisinger, R.S., Burns, P., Eddie, L.W. & Wintour, E.M. (1993). *J. Endocrinol.*, **137,** 505–510.