Effects of exogenous relaxin on oxytocin and vasopressin release and the intramammary pressure response to central hyperosmotic challenge

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Abstract

Experiments were done to study the effects of porcine relaxin on osmotically evoked changes in intramammary pressure and the release of oxytocin and vasopressin in anaesthetized rats. Injections (1 μl) of hypertonic (0.75 M) NaCl into the left lateral cerebral ventricle were used to induce consistent rises in intramammary pressure and the release of oxytocin and vasopressin. Plasma hormone concentration was determined by radioimmunoassay. Relaxin (5 μg i.v.) significantly (P<0.05) suppressed the intramammary pressure response to osmotic challenge 5 and 10 min after treatment. However, pretreatment with a specific vasopressin V1 receptor antagonist completely negated the effect of relaxin on intramammary pressure.

Baseline levels of oxytocin and vasopressin in unstimulated rats were 41 ± 1.6 and 36 ± 1.1 pmol/l respectively. Osmotic challenge induced significant (P<0.05) rises in plasma levels of both hormones (62.8 ± 1.1 and 67.9 ± 1.2 pmol/l respectively) which were further augmented by relaxin (81.3 ± 1.8 and 117.1 ± 2.4 pmol/l respectively; P<0.05). The data confirm that central osmotic challenge provokes the release of oxytocin and vasopressin but the effects of oxytocin at the level of the mammary gland may be obscured by the action of vasopressin affecting blood flow to the gland.

Journal of Endocrinology (1994) 141, 75-80

Introduction

Relaxin is a polypeptide hormone produced in the rat by the corpora lutea of pregnancy (for review see Sherwood 1994). It has been shown that relaxin causes the release of vasopressin and a rise in blood pressure (Mumford et al. 1989, Parry et al. 1990). As well, relaxin affects oxytocin release; decreasing plasma oxytocin levels at parturition (Jones & Summerlee 1986) and inhibiting reflex milk ejection in the anaesthetized lactating rat (Summerlee et al. 1984). Recently, Way & Leng (1992) re-examined the effect of relaxin on oxytocin release in the lactating rat. They found that relaxin reduced the number of milk ejections observed yet plasma oxytocin levels were enhanced. Thus, the effect of relaxin on oxytocin release is unclear.

To date, studies have focused on the effect of relaxin on suckling-stimulated oxytocin release but other stimuli are known to cause the release of both magnocellular peptides. For example, Clarke & Merrick (1982) reported that injection of hypertonic NaCl into the ventricular system of the rat brain caused a consistent and prolonged rise in intramammary pressure concurrent with elevations in plasma oxytocin and vasopressin. The present set of experiments was therefore carried out to investigate the effect of relaxin on oxytocin and vasopressin release and the rise in intramammary pressure stimulated by central osmotic challenge. A preliminary report of these data is published elsewhere (Wilson & Summerlee 1991).

Materials and Methods

Lactating female Sprague-Dawley rats (280–330 g; n=66) used in these studies were housed in the Central Animal Facilities of the University of Guelph. They were maintained on a 14 h light:10 hours darkness photoperiod and food and water were available ad libitum. Litters were standardized to contain ten pups on day 1 of lactation. On the evening of day 8 of lactation, all pups but one were separated from each female to promote mammary engorgement (Lincoln et al. 1973). The following morning rats were anaesthetized with urethane (ethyl carbamate; Sigma Chemical Co., St Louis, MO, USA; 1·25 g/kg i.p.) and rompun (xylazine; BAYVET, Chemagro Ltd, Etobicoke, Ontario, Canada; 1 mg/kg i.m.). The left saphenous vein and the main galactophore of a caudal mammary gland were cannulated for the administration of drugs and the measurement of intramammary pressure respectively. Intramammary pressure was recorded on
a calibrated Grass polygraph (Grass Polygraph Model 5C; Grass Instrumentation Co., Quincy, MA, USA) connected to the cannula via a pressure transducer (Bell and Howell, Elcomatic Ltd, Glasgow, UK). Each rat was placed in a stereotaxic frame (Narishige SR6; Narishige, Tokyo, Japan) and a 10 µl Hamilton microsyringe (Hamilton model 7D; Hamilton Co., Reno, NV, USA), filled with a solution of 0.75 M NaCl, was placed with the tip of the needle in the left lateral cerebral ventricle (co-ordinates: 0.9 mm caudal to bregma; 2.2-2.5 mm lateral to midline; 3.0 mm ventral to the cortical surface).

Relaxin

Highly purified porcine relaxin (CMa fraction; carboxymethylcellulose α'; see Sherwood (1994); potency 3000 guinea-pig units/mg; 5 µg in 0.1 ml saline i.v.) was used in each experiment. Relaxin was prepared in the Department of Anatomy, University of Bristol, Bristol, UK by extraction and purification from sow corpora lutea using the method of Sherwood & O’Byrne (1974). The ovaries were supplied by the UK Meat and Livestock Commission.

Experiment 1: Effect of i.v. relaxin on the intramammary pressure response to osmotic stimulation

This experiment was designed to determine the effect of i.v. relaxin on the intramammary pressure response to osmotic challenge. Anaesthetized rats were prepared as described above and pretreated with 1 µl 0.75 M NaCl i.c.v. to obtain a baseline intramammary pressure response. Following this, rats received relaxin (5 µg in 0.1 ml saline i.v.; n=8) or saline alone (0.1 ml i.v.; n=8). Subsequently, each rat received 1 µl 0.75 M NaCl i.c.v. at 5, 10, 15, 20 and 30 min after treatment and the intramammary pressure responses were recorded.

Experiment 2: Plasma oxytocin and vasopressin concentrations following osmotic stimulation and i.v. relaxin

This experiment was done to determine plasma oxytocin and vasopressin concentrations following central osmotic stimulation and i.v. relaxin. Animals were anaesthetized and prepared as described in Experiment 1. Following treatment with 1 µl 0.75 M NaCl i.c.v., the latency to onset of the intramammary pressure response was determined. This time was used to time the collection of trunk blood. Five minutes after treatment with relaxin (5 µg in 0.1 ml saline i.v.; n=8) or saline (0.1 ml i.v.; n=8), rats were given a second dose of hypertonic NaCl i.c.v. and trunk blood was collected following decapitation. Blood was collected from additional rats with i.c.v. and intramammary cannulae as described above, to establish baseline levels of oxytocin and vasopressin in untreated rats (n=8) and to establish oxytocin and vasopressin levels in rats treated with relaxin alone (n=8). Plasma was separated by centrifugation (10 000 r.p.m. for 5 min) and frozen at -25 °C until assay.

Oxytocin and vasopressin radioimmunoassay

Plasma concentrations of oxytocin were measured by specific radioimmunoassay (Fourth International Standard for Oxytocin 76/578) on unextracted plasma. Rabbit anti-porcine antiserum (85/F2) was prepared by Dr S Birkett, Department of Anatomy, University of Bristol, Bristol, UK. The lower limit of detection was 5.2 pmol/l and the inter- and intra-assay coefficients of variation were 7-1% and 3-7% respectively at 10 pmol/l. Cross-reactivity was 12.5% for mesotocin, 0.03% with lysine vasopressin and <0.02% for arginine vasopressin, the neurophysins and a variety of other hypothalamic peptides.

Plasma vasopressin concentrations were also measured by specific radioimmunoassay (First International Standard 77/501) following petroleum–ether extraction. Antiserum AS84–1 was prepared by Dr S Birkett. The lower limit of detection was 7.8 pmol/l and the inter- and intra-assay coefficients of variation were 4-9% and 2.4% respectively at 10 pmol/l. Cross-reactivity was <0.02% for oxytocin, neurophysins to oxytocin and vasopressin and a variety of other hypothalamic peptides.

Experiment 3: The effect of i.v. relaxin on the intramammary pressure response to osmotic stimulation after vasopressin antagonism

Intravenous relaxin induces the release of vasopressin (Parry et al. 1990). Therefore, experiment 1 was repeated in order to determine the effect of relaxin on the intramammary pressure response to central osmotic challenge after antagonism of endogenous vasopressin. Eight animals were pretreated with a specific vasopressin V1 receptor antagonist ([(β-mercapto-β-γ-cyclopentamethylene-proprionyl1, O-Me-Tyr2, Arg3]-vasopressin; Sigma Chemical Co.; 8.3 µg/kg in 0.1 ml saline i.v.; Kruszynski et al. 1980) 2 min before treatment with relaxin. This protocol has been shown to antagonize the pressor response to exogenous 5 µU vasopressin completely (Parry et al. 1990). Additional experiments were done to control for the non-specific effects of the vasopressin V1 receptor antagonist on the intramammary pressure response to oxytocin. Five animals were anaesthetized and prepared as described. Following the intramammary pressure response to pretreatment with oxytocin (Sigma Chemical Co.; 0.5 µU in 0.1 ml saline i.v.), each rat received the vasopressin V1 receptor antagonist (8.3 µg/kg in 0.1 ml saline i.v.). Three minutes later, rats were given a second identical treatment with oxytocin and the intramammary pressure response was recorded. In a second group of animals, the intramammary pressure response to central osmotic challenge was compared before and after
treatment with the vasopressin V1 receptor antagonist. Five animals were anaesthetized and prepared as described. After establishing the intramammary pressure response to hypertonic NaCl (1 μl i.c.v.), rats were given the vasopressin V1 receptor antagonist (8.3 μg/kg in 0.1 ml saline i.v.) followed 2 min later with saline (0.1 ml i.v.). Three minutes later, rats were given a second injection of hypertonic NaCl (1 μl i.c.v.) and the resultant intramammary pressure response was recorded.

**Analysis of data**

Three parameters of the intramammary pressure responses were considered: the latency to onset of the intramammary pressure response (in seconds), the initial peak rise in intramammary pressure (mmHg) and the duration of the intramammary pressure response (in seconds). These three measurements of intramammary pressure responses at each time-interval, as well as plasma hormone concentrations, were compared between rats using one-way analysis of variance (ANOVA) and mean values for each experimental group compared using Scheffe's test. Differences between means were considered significant at P<0.05.

**Results**

Standard doses of hypertonic NaCl (1 μl i.c.v.) consistently caused a prolonged increase in intramammary pressure in all rats tested. The mean latency to onset of intramammary pressure responses was 35±3±2.0 s, the mean initial peak rise was 6.3±0.6 mmHg and the mean duration of responses was 282±7±20.9 s (n=24; mean±s.e.m.).

**Experiment 1**

Treatment with saline (0.1 ml i.v.) had no significant effect on the intramammary pressure response to central osmotic challenge (Figs 1 and 2). Relaxin (5 μg in 0.1 ml saline i.v.) did not significantly affect latency to onset of intramammary pressure responses (Fig. 1). However, the hormone did cause a significant decrease in the initial peak rise of intramammary pressure responses 5 and 10 min after treatment (Figs 1 and 2a). Duration of intramammary pressure responses was also depressed significantly at 5 min after treatment (Figs 1 and 2b). Relaxin itself evoked a short-term rise in intramammary pressure in seven out of eight rats tested (mean latency: 20.8±3.9 s; mean peak rise: 1.2±0.4 mmHg; mean duration: 89.4±16.6 s; Fig. 1).

**Experiment 2**

Basal oxytocin and vasopressin concentrations in control plasma were 41±1.6 and 36±1.1 pmol/l respectively (means±s.e.m.; n=8). Intravenous relaxin stimulated release of both peptides resulting in significant elevations in plasma oxytocin 1 min after treatment (Fig. 3). Hypertonic NaCl i.c.v. evoked the release of oxytocin and vasopressin significantly elevating plasma concentrations of both hormones (Fig. 3). Treatment of osmotically stimulated animals with relaxin significantly enhanced this release causing mean concentrations of plasma oxytocin and vasopressin to increase by 29.5% and 72.5% respectively.

**Experiment 3**

Pretreatment of rats with the vasopressin V1 receptor antagonist completely negated relaxin suppression of the intramammary pressure response to central osmotic challenge seen in experiment 1 (Figs 1 and 2). There was no reduction in the latency to onset, initial peak rise or duration of intramammary pressure responses to central osmotic challenge following relaxin treatment in these animals (Figs 1 and 2). Relaxin per se still induced a small rise in intramammary pressure in animals with a compromised vasopressin system (Fig. 1). The vasopressin V1 receptor antagonist itself had no significant effect on mammary responsiveness to exogenous oxytocin. Furthermore, the antagonist had no significant effect on the latency to onset, initial peak rise or duration of intramammary pressure responses to i.c.v. hypertonic NaCl.

**Discussion**

These experiments were performed to investigate the effects of exogenous relaxin on osmotically evoked release of oxytocin and vasopressin. Standard doses of hypertonic
sodium chloride applied to the cerebrospinal fluid caused consistent increases in intramammary pressure and the release of oxytocin and vasopressin. Intravenous relaxin decreased the osmotically evoked rise in intramammary pressure but significantly augmented the release of both oxytocin and vasopressin. Pretreatment of rats with a vasopressin antagonist specific for V1 receptors blocked the inhibitory effect of relaxin on intramammary pressure responses to osmotic challenge. These data indicate that relaxin stimulates release of oxytocin and vasopressin above that induced by osmotic stimulation alone, but the effect of osmotically evoked oxytocin release on the mammary gland is attenuated following relaxin treatment. Osmotic stimulation at 5-min intervals did not affect mammary sensitivity to circulating oxytocin as intramammary pressure responses to successive i.c.v. treatments with hypertonic NaCl were not significantly different in control animals. Furthermore, relaxin does not decrease mammary sensitivity to oxytocin as the intramammary pressure response to 0.5 mU oxytocin i.v. administered 5 min after i.v. relaxin (5 µg) is not significantly different from that observed before relaxin treatment (B C Wilson, unpublished observations). The effect of oxytocin at the mammary gland may be masked by changes in blood flow to the gland following i.v. relaxin treatment.

Data from previous studies are contradictory with regard to the effect of relaxin on oxytocin release. Summerlee et al. (1984) demonstrated that relaxin suppressed the milk-ejection reflex in anaesthetized rats implying that
oxytocin release was inhibited. However, there was no direct evidence to support this contention. In an *in vitro* study of isolated neurohypophysial nerve terminals, Dayanithi et al. (1987) reported that relaxin inhibited basal release of oxytocin yet enhanced oxytocin release evoked by potassium-induced depolarization. However, O’Byrne et al. (1986) found that i.v. relaxin reduced the amount of oxytocin released on electrical stimulation of the neurohypophysis in the anaesthetized lactating rat. Recently, Way & Leng (1992) confirmed the finding of Summerlee et al. (1984) that relaxin suppressed reflex milk ejection in the anaesthetized rat. However, they found that relaxin enhanced basal oxytocin release (Way & Leng 1992). An interesting observation from our experiments is that injections of relaxin *per se* caused a slight increase in intramammary pressure which is in line with data of Way & Leng (1992) and Dayanithi et al. (1987).

There is a strong body of evidence to support relaxin’s stimulatory action on vasopressin release. Dayanithi et al. (1987) reported that relaxin enhances vasopressin release from the stimulated neural lobe *in vitro* and relaxin causes vasopressin release *in vivo*: Mumford et al. (1989) showed that i.c.v. relaxin caused a significant increase in circulating vasopressin 3 min after treatment and Way & Leng (1992) reported that relaxin stimulated vasopressin release at 15 min after injection. The data in the present paper do not show that relaxin induces the release of vasopressin, but it should be emphasized that the results reported were plasma vasopressin levels at 45–60 s after injection.

Until recently, vasopressin was thought to have minor cardiovascular effects in intact organisms at plasma concentrations less than 20 pmol/l. However, evidence suggests that much lower plasma levels of vasopressin have profound influences on water excretion and blood flow (Weitzman & Fisher 1978). Moreover, the vasoconstrictive effects of vasopressin are different in different vascular beds (Liard 1985): vasopressin is most potent as a vasoconstrictor in blood vessels of the skin, skeletal muscle, thyroid gland, colon and fat, while it is least effective in kidney and liver vasculature. There are no data on the effect of vasopressin on vasculature in the mammary gland. Nevertheless, plasma concentrations of vasopressin in excess of 50 pmol/l were observed in the present study. So it is possible to speculate that vasopressin might cause vasoconstriction of vessels to mammary parenchyma and thereby affect blood flow and access of oxytocin to the myoepithelial cells. The substantial elevation in plasma vasopressin following relaxin and osmotic stimulation (100 pmol/l) could quite plausibly limit the supply of oxytocin to the mammary gland; an effect that would be reversed by pretreatment with the competitive vasopressin V1 receptor antagonist. Alternatively, other mechanisms could be involved. For example, vasopressin appears to have both facilitatory and inhibitory effects on adrenocorticotrophin release: enhancing release through actions on corticotropes in the anterior pituitary and inhibiting release when injected into the ventricular system (Plotzky 1985). Furthermore, vasopressin facilitates noradrenaline release (Plotzky 1985). However, pretreatment of rabbits with an α1-adrenergic receptor antagonist does not affect the blood pressure response to i.c.v. injections of hypertonic saline (A J S Summerlee, unpublished observations). It is possible that vasopressin *per se* acts to stimulate an increase in intramammary pressure. This action would presumably not involve V1 receptors and, if oxytocin receptors are involved, might account for greater intramammary pressure responses as plasma vasopressin levels increased following relaxin treatment.

If vasopressin is affecting blood supply to the mammary gland and i.c.v. hypertonic NaCl stimulates oxytocin and vasopressin secretion, treatment with the vasopressin V1 receptor antagonist might have been expected to enhance the intramammary pressure response to hypertonic NaCl. The antagonist alone did not enhance the intramammary pressure response. A possible explanation for this observation is that circulating levels of vasopressin (50 pmol/l) seen after hypertonic saline alone, may not be sufficient to affect blood supply to the mammary gland. In contrast, higher circulating levels of vasopressin (approximately 120 pmol/l) seen after hypertonic saline and relaxin treatment may be sufficient to affect blood flow to the mammary gland. Based on this suggestion, it would be interesting to know whether the V1 receptor antagonist affects blood pressure changes in response to i.c.v. hypertonic saline, with or without relaxin treatment.

It is possible that the previously reported effects of relaxin on reflex milk ejection (Summerlee et al. 1984, Way & Leng 1992) might be due to vasopressin affecting blood supply to the mammary gland. However, it has been shown that pretreatment with a vasopressin V1 receptor antagonist does not significantly affect relaxin suppression of reflex milk ejection in anaesthetized rats (Wilson et al. 1991). This finding suggests that the action of relaxin on sucking and osmotically induced release of oxytocin may be different. During nursing, afferent somatosensory pathways relay information regarding mechanical stimulation of the mammary glands via the spinal cord and brain stem to impinge on the final common pathway, oxytocin neurones in the supraoptic and paraventricular nuclei (for review, see Wakerley et al. 1988). In contrast, osmotic stimuli applied within the ventricular system are reported to act on neurones in the subformical organ and structures within the region anterior and ventral to the third ventricle (AV3V) region (Blackburn et al. 1987) which relay information to the magnocellular nuclei, and it has been shown that relaxin binds specifically to the subformical organ and organum vasculosum of the lamina terminalis (Osheroff & Phillips 1991). It is, therefore, possible that relaxin may have different actions on the afferent components of sucking-induced and osmotically evoked release of oxytocin.
From this study we conclude that relaxin further augments osmotically evoked release of oxytocin and vasopressin in anaesthetized rats. The relaxin-induced vasopressin release masks the effects of elevated plasma oxytocin levels on the mammary gland, thus causing the attenuation of osmotically evoked intramammary pressure responses. However, whether or not relaxin has a role in osmoregulation remains to be elucidated.

Acknowledgements

The authors wish to thank Fred Black and the members of the Central Animal Facilities for their excellent care of the experimental animals, Dr D G Porter for the supplies of porcine relaxin, David Jones for carrying out oxytocin and vasopressin radioimmunoassays and Dr S Birkett for donations of antisera.

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Received 16 July 1993
Accepted 8 February 1994