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# MORPHINE TOLERANCE AND INHIBITION OF OXYTOCIN SECRETION BY κ-OPIOIDS ACTING ON THE RAT NEUROHYPOPHYSIS

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SUMMARY

1. The present study investigated the mechanisms by which endogenous opioids regulate oxytocin secretion at the level of the posterior pituitary gland. Effects of the selective κ-agonist U50,488 on oxytocin secretion were studied in urethane-anaesthetized lactating rats. Oxytocin secretion in response to electrical stimulation (0·5 mA, matched biphasic 1 ms pulses, 50 Hz, 60–180 pulses) of the neurohypophysial stalk was bioassayed on-line by measuring increases in intramammary pressure, calibrated with exogenous oxytocin. Intravenous (I.V.) U50,488 inhibited electrically stimulated oxytocin secretion, without affecting mammary gland sensitivity to oxytocin. The inhibition was dose related, with an $ID_{50}$ of 441 (+194, −136) μg/kg and was naloxone reversible. Antagonism of endogenous β-adrenoceptor activation by propranolol (1 mg/kg) reduced the potency of U50,488. The selective μ-agonist morphine (up to 5 mg/kg), had no effect on electrically stimulated oxytocin secretion, but depressed the mammary response to oxytocin.

2. In lactating rats given intracerebroventricular (I.C.V.) morphine infusion for 5 days to induce tolerance and dependence, I.V. U50,488 still inhibited electrically stimulated oxytocin secretion, but the $ID_{50}$ was reduced to 170 (+78, −54) μg/kg; thus at the posterior pituitary the sensitivity of κ-receptors is enhanced rather than reduced in morphine-tolerant rats, indicating the absence of cross-tolerance. In these rats, naloxone produced a large, sustained, fluctuating increase in intramammary pressure indicating morphine-withdrawal excitation of oxytocin secretion; I.V. U50,488 diminished this response, confirmed by radioimmunoassay, demonstrating the independence of μ- and κ-receptors regulating oxytocin secretion.

3. In pregnant rats, I.C.V. infusion of morphine from day 17–18 of pregnancy delayed the start of parturition by 4 h, but did not significantly affect the progress of parturition once established, indicating tolerance to the inhibitory actions of morphine on oxytocin secretion in parturition, and lack of cross-tolerance to endogenous opioids restraining oxytocin in parturition.

4. Neurointermediate lobes from control and I.C.V. morphine-infused virgin rats were impaled on electrodes and perifused *in vitro*. Vasopressin and oxytocin release from the glands was measured by radioimmunoassay. Each gland was exposed to two periods of electrical stimulation (13 Hz, for 3 min). Naloxone ($5 \times 10^{-6}$ M) was

added before the second stimulation; half the lobes from each I.C.V. treatment were exposed to $5 \times 10^{-5}$ M morphine throughout. Oxytocin secretion in response to the first stimulation was similar in the four groups, indicating no acute effect of morphine, and no cross-tolerance from morphine to endogenous opioids released by electrical stimulation. In the presence of naloxone, stimulated oxytocin secretion was potentiated in all four treatment groups, again indicating lack of cross-tolerance to the endogenous opioids that restrain oxytocin secretion. Naloxone was more effective in potentiating oxytocin release from neurointermediate lobes of morphine-infused rats, consistent with increased endogenous $\kappa$-opioid actions. For vasopressin, no significant actions of either morphine treatments or of endogenous neurohypophysial opioids were seen.

5. Thus two distinct opioid mechanisms regulate oxytocin secretion. Both $\mu$- and $\kappa$-agonists act centrally to inhibit the activity of oxytocin neurones but only $\kappa$-agonists (and not $\mu$-agonists) act at the secretory terminals. A decrease in the sensitivity to the central effects of $\mu$-agonists can occur without affecting the terminal effects of $\kappa$-agonists.

## INTRODUCTION

Endogenous opioids restrain oxytocin secretion by an action on the terminals of oxytocin neurones in the posterior pituitary gland (Bicknell & Leng, 1982; Bicknell & Zhao, 1989). The three families of endogenous opioids act with different affinities at three principal types of opioid receptor (Kosterlitz, 1985). The posterior pituitary contains $\kappa$-opioid receptors but lacks classical $\mu$- and $\delta$-opioid receptors (Herkenham, Rice, Jacobson & Rothman, 1986; Sumner, Coombes, Pumford & Russell, 1990). *In vitro*, agonists selective for the $\kappa$-receptor inhibit electrically stimulated oxytocin release whereas other opioids have little effect (Zhao, Chapman & Bicknell, 1988*b*). However, the first and highly influential report of opioid actions at the posterior pituitary implied that morphine, a $\mu$-agonist, acts at this site in lactating rats to suppress suckling-induced oxytocin release (Clarke, Wood, Merrick & Lincoln, 1979). *In vivo*, both $\mu$- and $\kappa$-agonists inhibit oxytocin secretion (Grell, Christensen & Fjalland, 1988; Evans, Olley, Rice & Abrahams, 1989*b*; Russell *et al.* 1989*a*) but both can inhibit the firing of oxytocin neurones (Leng, Pumford & Russell, 1990; Pumford, Leng & Russell, 1991), so we have now re-examined, in lactating rats, whether the posterior pituitary is a site of action of $\mu$-selective opioids, as previously reported (Clarke *et al.* 1979; Clarke & Patrick, 1983). Oxytocin secretion following electrical stimulation of the neurohypophysial stalk was monitored by measuring intramammary pressure, and we studied the effects of I.V. morphine or U50,488, selective $\mu$- and $\kappa$-opioid agonists respectively (Magnan, Paterson, Tavani & Kosterlitz, 1982; Kosterlitz, 1985; Clark & Pasternak, 1988). Inhibition of the milk-ejection reflex by U50,488 is reversed by the $\beta$-adrenoceptor antagonist propranolol, which may nullify adrenomedullary activation by the $\kappa$-agonist (Clarke & Wright, 1987), while conversely *in vitro*, $\beta$-receptors in the posterior pituitary mediate stimulatory effects of noradrenaline on oxytocin release (Zhao, Chapman, Brown & Bicknell, 1988*c*; Bicknell & Zhao, 1989), so we studied interactions of propranolol with U50,488 on electrically stimulated oxytocin release *in vivo* to try to clarify sites of propranolol interactions with the $\kappa$-agonist.

During chronic I.C.V. morphine infusion oxytocin release in lactating rats is at first suppressed, but within 4 days near normal activity of the magnocellular oxytocin system is restored, indicating tolerance (Bicknell, Leng, Lincoln & Russell, 1988*a*; Rayner, Robinson & Russell, 1988). Dependence also develops in this system since naloxone provokes a withdrawal excitation of oxytocin secretion (Bicknell *et al.* 1988*a*; Rayner *et al.* 1988). We have now investigated whether cross-tolerance develops in the neurohypophysis to the endogenous opioids released at this site, by examining the effects of naloxone *in vitro*, and the effects of $\kappa$-agonists *in vivo* on electrically stimulated oxytocin release. These experiments have also allowed us to seek acute effects of morphine *in vitro* and to test whether dependence develops in the posterior pituitary.

Given acutely, morphine interrupts parturition by inhibiting oxytocin secretion (Russell *et al.* 1989*a*), whereas naloxone increases oxytocin secretion and accelerates parturition, thus indicating endogenous opioid restraint (Bicknell *et al.* 1988*b*). We have investigated whether morphine tolerance extends to parturition and whether parturition is faster in morphine-tolerant rats, which would indicate cross-tolerance to the endogenous opioid restraining oxytocin secretion in parturition. Some of the results in this paper have previously been briefly presented (Bicknell, Chapman, Leng & Russell, 1985; Coombes & Russell, 1988; Coombes & Russell, 1991).

## METHODS

### *Animals*

Albino rats were kept at an ambient temperature of 21–23 °C, with lights on for 13 h, off for 11 h for pregnant or lactating rats or on for 12 h, off for 12 h, and fed pelleted food and water *ad libitum*. Sprague–Dawley rats in their first lactation, weighing *ca* 400 g, were used in terminal experiments under urethane anaesthesia on days 7–14 postpartum, with litters adjusted to ten pups on day 2 postpartum, or were implanted with an I.C.V. infusion system on days 2–3 postpartum prior to the terminal experiment 5 days later; litter size was adjusted to ten pups at the time of I.C.V. cannulation. In the study on pregnant rats, virgin Sprague–Dawley females were caged singly with a male and separated on the day of finding a vaginal plug (day 1 of pregnancy), and implanted with an I.C.V. infusion system on days 17–18, and observed throughout parturition.

### *Recovery procedures*

*Intracerebroventricular infusion.* Under ether anaesthesia (Sprague–Dawley rats), or pentobarbitone anaesthesia (Wistar rats, Sagatal, May & Baker, UK, 36 mg/kg I.P.), a stainless-steel cannula, connected via a coil of polythene tubing to a subcutaneous osmotic minipump (Alzet 2001, 1 $\mu$l/h), was inserted into the right cerebral ventricle as previously described (Rayner, Robinson & Russell, 1988). The I.C.V. infusion system was filled with either: (*a*) sterile pyrogen-free distilled water (vehicle) to provide control rats, or (*b*) 20 $\mu$l vehicle followed by 20 $\mu$l morphine solution (25 $\mu$g/$\mu$l) then morphine solution (50 $\mu$g/$\mu$l) in the minipump (virgin Wistar rats), or (*c*) 40 $\mu$l morphine solution (10 $\mu$g/$\mu$l), a further 40 $\mu$l morphine solution (20 $\mu$g/$\mu$l), then morphine solution (50 $\mu$g/$\mu$l) in the minipump (pregnant or lactating rats). Air bubbles of 1 $\mu$l were introduced to separate the solutions in the tubing.

### *Monitoring of rats receiving I.C.V. infusion*

*Lactating rats.* After implantation of the I.C.V. cannula, litter weight gain and indices of maternal behaviour were recorded each day (Rayner *et al.* 1988) and only rats still lactating were used in the terminal experiment.

*Pregnant rats.* These rats were weighed daily and observed continually from the early morning of day 22 of gestation during the lights-on phase to record the time of birth of the first and subsequent pups in each litter (Russell *et al.* 1989*a*).

*Virgin rats: I.C.V. morphine infusion.* These rats were weighed daily and rectal temperature was recorded to confirm, from the temperature rise, the effectiveness of the I.C.V. morphine infusion (Rayner *et al.* 1988).

*Non-recovery experiments*

*Lactating rats.* After overnight separation from all but one pup in each litter, the dams (either untreated or given I.C.V. infusion) were anaesthetized with urethane (ethyl carbamate, 25% w/v, 1·25 g/kg, I.P.) and cannulae (polythene, o.d. 0·8 mm) filled with sterile 0·9% saline were inserted into three abdominal milk ducts to record intramammary pressure via transducers and a chart recorder (Rayner *et al.* 1988); cannulae filled with heparinized sterile 0·9% saline were inserted into a femoral vein and a jugular vein for injection of calibration doses of oxytocin and drugs (see below) respectively. The trachea was cannulated. Body temperature was regulated via a thermostatically controlled electric blanket (Rayner *et al.* 1988). With the rat's head held in a stereotaxic frame, the pituitary stalk was exposed by a ventral surgical approach (Leng, 1980). A concentric bipolar stimulating electrode (SNEX-50 mm, Clarke-Electromedical), was lowered onto the exposed pituitary stalk 1 h after completion of surgery. Matched biphasic square-wave pulses (0·5 mA peak-to-peak, 1 ms duration, 50 Hz) were delivered in trains of 60–240 pulses, adjusted to initially produce a mammary gland response per train equivalent to *ca* 0·8 mU oxytocin; trains were at least 3 min apart.

*Autobioassay of oxytocin secretion*

The amount of oxytocin released following each stimulus was estimated by measuring the resulting rise in intramammary pressure and by extrapolation from a dose–response curve for oxytocin constructed from the responses to bolus I.V. injections delivered from a repeating 1 ml glass microsyringe (Hamilton PB600 dispenser); doses of 0·1, 0·25, 0·5, 0·75 and 1·0 mU (Syntocinon, Sandoz Products Ltd, UK, 5 mU/ml 0·9% saline) were injected in random order 3 min apart before or after each stimulus, and after each drug injection to compensate for effects of drug on mammary gland sensitivity to oxytocin. The stimulus was tested twice just before and after I.V. drug injection, with at least 3 min between tests. The percentage change in oxytocin release for a fixed stimulus was calculated for each drug treatment, and means were calculated at each dose for each treatment group.

*Drugs*

Morphine sulphate (BP) was diluted in sterile pyrogen-free distilled water for I.C.V. infusion. Morphine sulphate, U50,488 (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide methanesulphonate; Sigma, UK), naloxone HCl (Sigma, UK) and propranolol (Inderal, ICI, UK 1 mg/ml) were diluted in 0·9% saline for I.V. injection (0·5 ml/kg).

*In vitro experiments*

Around 09.00 h on day 6 of I.C.V. morphine infusion, virgin rats were decapitated and the pituitary glands rapidly removed; the neurointermediate lobes were separated and impaled on electrodes and placed singly in perifusion chambers through which a modified Krebs solution was pumped at 150 $\mu$l/min (Bicknell & Leng, 1982). After a stabilization period of 60 min, perifusates were collected every 3 min for radioimmunoassay of oxytocin and vasopressin content. After 15 min each neurointermediate lobe was stimulated ($S_1$) for 3 min (13 Hz, 5 mA matched biphasic square-wave, 2 ms pulse width), then 30 min after the start of $S_1$, the stimulus was applied again ($S_2$) for 3 min but in the presence of naloxone ($5 \times 10^{-6}$ M) added to the perifusate 15 min after the start of $S_1$. The amount of oxytocin or vasopressin released in each 3 min collection was expressed as a percentage of the total released during all twenty 3 min intervals in the whole experiment; hormone release was also summed during each 15 min poststimulation period and the summed release in the respective preceding equivalent basal period ($B_1$ or $B_2$) was subtracted to give stimulated release. The stimulated release ratio with and without naloxone was calculated ($S_2/S_1$).

There were four experimental groups, consisting of: neurointermediate lobes from I.C.V. vehicle- or morphine-infused rats with no additions to the perifusate, other than naloxone as above; or with morphine ($5 \times 10^{-5}$ M) in the perifusate throughout (this concentration of morphine was previously found in the neurointermediate lobe *in vivo* following I.C.V. [$^3$H]morphine infusion (Rayner *et al.* 1988)).

*Radioimmunoassay*

Heparinized blood samples (0·3 ml) for oxytocin measurement were centrifuged, and plasma frozen and stored at −20 °C prior to assay in unextracted samples (Higuchi, Honda, Fukuoka, Negoro & Wakabayashi, 1985). Oxytocin and vasopressin content in the *in vitro* perifusates were measured as described previously (Sheldrick & Flint, 1981; Bicknell & Leng, 1982).

*Statistical analysis*

Parametric or non-parametric analyses were used as appropriate. In the experiments on lactating rats, the effects of different drug treatments on electrically stimulated oxytocin release from the posterior pituitary were compared by estimating the $ID_{50} \pm$ confidence limits for each treatment ($ID_{50}$ is the dose required to inhibit oxytocin release by 50%). The raw data of log dose against response were used to obtain a linear regression line, from which the $ID_{50}$ was calculated:

$$x_{50} = \frac{50-a}{b} (a = y \text{ intercept}, b = \text{gradient}).$$

The confidence limits were calculated as follows:

$$\frac{\text{Var}(a)}{(50-a)^2} + \frac{\text{Var}(b)}{b^2} + \frac{2\,\text{Cov}(a,b)}{b(50-a)},$$

where Var $(a)$ = variance $a$, Var $(b)$ = variance $b$ and Cov $(a,b)$ = covariance $(a,b)$.

## RESULTS

### *Opioids and oxytocin secretion in lactating rats*

*Effects of morphine*

Stimulation of the neural stalk with 60–120 pulses reproducibly evoked increases in intramammary pressure similar to those produced by I.V. injection of 0·5–1·0 mU oxytocin (Fig. 1). Morphine administration resulted consistently in a reduction in the magnitude of the intramammary pressure rise following stimulation (Fig. 1*A*). However, morphine decreased the sensitivity of the mammary gland to oxytocin (Figs 1*A* and 2*A*), and this was reversed by I.V. naloxone (1 mg/kg). When this action of morphine on the mammary gland sensitivity to oxytocin was taken into account by using the postmorphine dose–response curve for oxytocin and intramammary pressure (Fig. 2*A*), it was evident that morphine given to eleven rats at doses between 0·1 and 5 mg/kg I.V., had no detectable effect on electrically stimulated oxytocin secretion (Fig. 3).

*Effects of U50,488*

U50,488 administration markedly reduced the increase in intramammary pressure following neural stalk stimulation (Fig. 1*B*), but U50,488 had little effect on the sensitivity of the mammary gland to injected oxytocin (Figs 1*B* and 2*B*). Consequently, measured from changes in intramammary pressure, oxytocin secretion following electrical stimulation of the neurohypophysial stalk in each of nine rats was inhibited by I.V. U50,488 in a dose-related manner between 0·1 and 5 mg/kg (Fig. 3). At the highest dose tested, stimulated oxytocin secretion was almost completely suppressed (Fig. 3); linear regression analysis allowed estimation of the $ID_{50}$ dose as

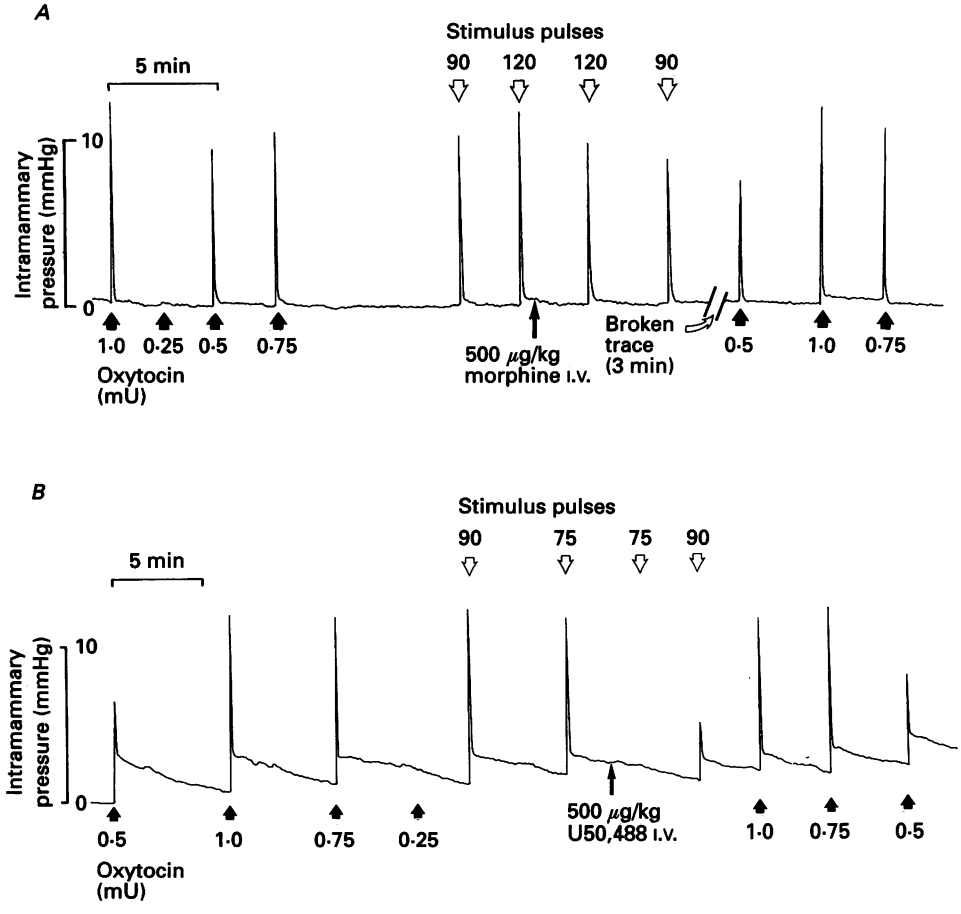


Fig. 1. Effects of I.V. morphine or U50,488 on oxytocin secretion evoked by electrical stimulation of the neurohypophysial stalk in lactating rats. Typical intramammary pressure recordings, urethane-anaesthetized rats. Increases in intramammary pressure after stalk stimulation at 50 Hz (90 or 120 pulses, 0·5 mA peak-to-peak, 1 ms; open arrows), were compared with responses to bolus I.V. injection of 0·25, 0·5, 0·75 or 1 mU oxytocin (broad filled arrows) from which dose–response curves were constructed, and the amounts of oxytocin released by stimulation estimated. Effects of I.V. morphine or U50,488 were tested on the response to a stimulus train that evoked the release of *ca* 0·8 mU oxytocin and on the responses to exogenous oxytocin. *A*, effects of I.V. morphine, 500 μg/kg (narrow filled arrow). Morphine slightly reduced the mammary gland response to electrical stimulation of the neural stalk, but also reduced responses to exogenous oxytocin. The response to 90 pulses was equivalent to between 0·5 and 0·75 mU oxytocin before and after morphine, so stimulated oxytocin secretion was not inhibited. *B*, effects of I.V. U50,488 (500 μg/kg, narrow filled arrows). U50,488 slightly reduced the responses to exogenous oxytocin but abolished the response to 75 pulse stimulation of the neural stalk and substantially reduced the response to 90 pulses. The response to 90 pulses was equivalent to 1 mU oxytocin before U50,488 but only to less than 0·5 mU after U50,488, so stimulated oxytocin secretion was inhibited.

441 + 194, −136 μg/kg. Naloxone (1 mg/kg) given after the last, highest dose of U50,488 fully reversed the inhibitory effect of the agonist (Fig. 3).

*Effects of propranolol on actions of U50,488*

U50,488 may activate adreno-medullary secretion, and possibly affect oxytocin secretion indirectly; so propranolol (1 mg/kg) was injected into six rats 20 min prior

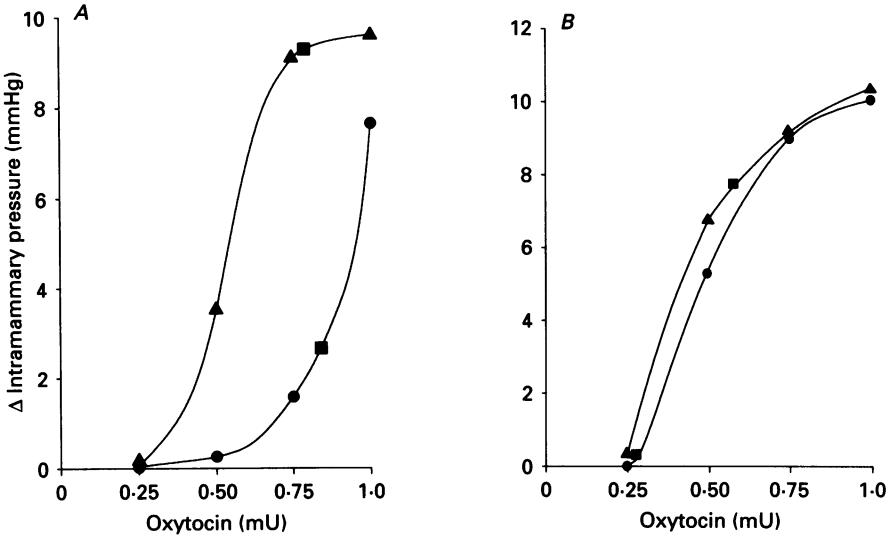


Fig. 2. Measurement of the effects of I.V. morphine or U50,488 on oxytocin secretion evoked by neurohypophysial stalk stimulation. Typical intramammary pressure dose–response curves from individual rats for effects of exogenous oxytocin before and after opioid, with superimposition of typical responses to stalk stimulation (50 Hz, 0·5 mA peak-to-peak, 1 ms pulses). ▲, response to exogenous oxytocin before I.V. opioid; ●, response to exogenous oxytocin after I.V. opioid; ■, response to the same electrical stimulus applied to the neural stalk before and after I.V. opioid. *A*, effects of I.V. morphine (500 μg/kg). Morphine depressed intramammary pressure responses especially to the lower doses of oxytocin. Although the intramammary pressure response to 90 pulse stalk stimulation was reduced, because of the right-shift in the dose–response curves the estimated amount of oxytocin release was not altered (see Fig. 3). *B*, effects of I.V. U50,488 (500 μg/kg). U50,488 depressed the intramammary pressure response to 60 pulse stalk stimulation with little effect on responses to exogenous oxytocin, so U50,488 substantially reduced the stimulated secretion of oxytocin.

to U50,488 administration to block β-adrenoceptors. Propranolol alone increased mammary gland sensitivity to exogenous oxytocin (Fig. 4*A*). None the less, following propranolol pretreatment, the lowest dose of U50,488 used (100 μg/kg) slightly increased the amount of oxytocin released by stalk stimulation (Fig. 3) and this was significantly different from the small inhibitory effect of U50,488 alone at this dose (Fig. 3). With further increase in dose, U50,488 was inhibitory, and linear regression analysis showed a dose-related effect, with $ID_{50}$ of 1583 (+603, −437) μg/kg, significantly greater than U50,488 alone ($P < 0{\cdot}05$, by separation of confidence limits).

Naloxone (1 mg/kg I.V.) reversed the inhibitory effect of U50,488 after propranolol pretreatment, without enhancing the response to stimulation above the initial value (Fig. 3).

*Effects of U50,488 in morphine-tolerant/dependent rats*

U50,488 inhibited electrically stimulated oxytocin secretion in a dose-related manner (Fig. 5), without affecting the sensitivity of the mammary gland to

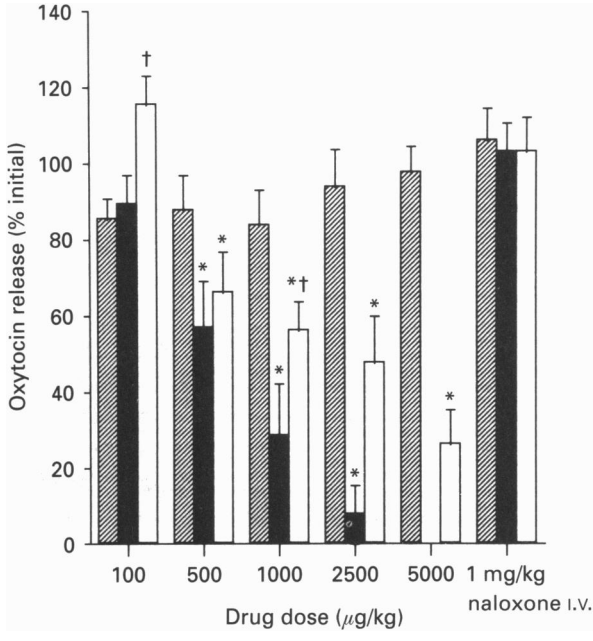


Fig. 3. Effects of I.V. morphine or U50,488 without and with propranolol pretreatment on electrically stimulated oxytocin secretion in lactating rats measured as shown in Figs 1 and 2. Values are estimates of electrically stimulated oxytocin secretion calculated as mean percentage ± S.E.M. of release before increasing I.V. doses of either morphine or U50,488; in each rat the number of stimulus pulses was the same throughout and was that which initially released *ca* 0·8 mU oxytocin. Hatched bars, I.V. morphine, $n = 11$; filled bars, I.V. U50,488, $n = 9$; open bars, I.V. propranolol (1 mg/kg) before testing with U50,488, $n = 6$. Naloxone (1 mg/kg I.V.) was given before the last stimulus. Morphine had no effect. U50,488 inhibited oxytocin secretion in a dose-related manner. $ID_{50}$ was 441 (+194, −136) μg/kg. Naloxone reversed the effect of U50,488. Propranolol significantly reduced the inhibitory action of U50,488. The $ID_{50}$ in the propranolol-treated group was 1583 (+603, −437) μg/kg, $P < 0{\cdot}05$ *vs.* $ID_{50}$ for U50,488 alone. †$P < 0{\cdot}05$ *vs.* U50,488 alone at this dose (unpaired *t* test); *$P < 0{\cdot}05 > 0{\cdot}01$ *vs.* pre-U50,488 (paired *t* test).

exogenous oxytocin (Fig. 4*B*). Linear regression analysis showed that the dose–response relationship was shifted significantly to the left compared with that in control rats (Fig. 5); in the morphine-infused rats the $ID_{50}$ for U50,488 was 170 (+78, −54) μg/kg, significantly less than in control rats, where the $ID_{50}$ for U50,488 was 441 (+194, −136) μg/kg ($P < 0{\cdot}05$, by separation of confidence limits).

Thus sensitivity to U50,488 was enhanced rather than reduced in morphine-infused rats. In these rats, after a sequence of I.V. injections of U50,488, naloxone (1–5 mg/kg) produced a rapid, large increase in intramammary pressure (peak

7·7 ± 0·6 mmHg, $n = 5$, Fig. 6*A*), sustained for at least 20 min. This morphine-withdrawal response reflects activation of oxytocin secretion, and was not seen after naloxone in morphine-naive rats acutely given U50,488 or, as previously found, morphine (Rayner *et al.* 1988). In three rats we induced this withdrawal response

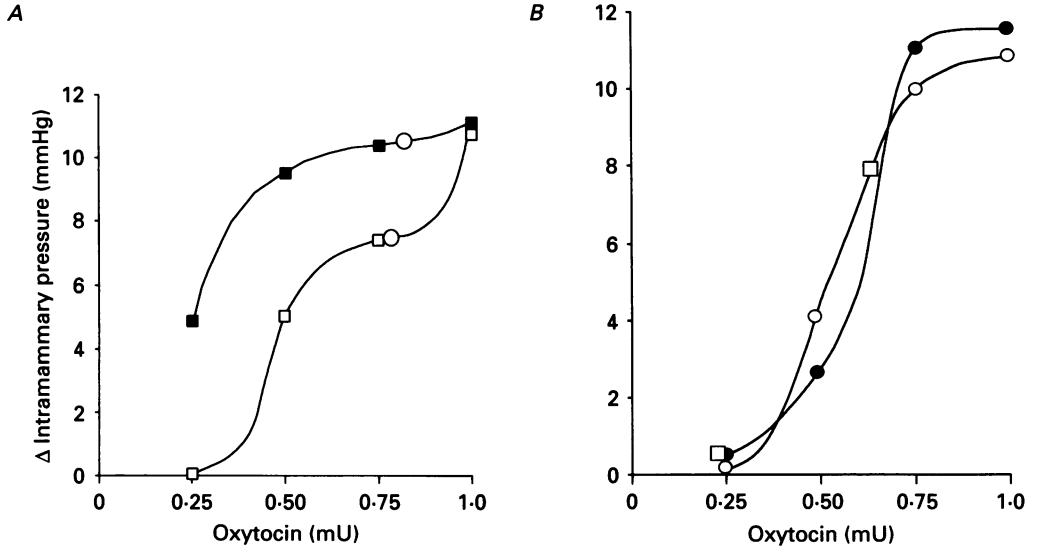


Fig. 4. Measurement of the effects of I.V. propranolol in control rats and U50,488 in morphine-dependent rats on oxytocin secretion evoked by neurohypophysial stalk stimulation. Typical intramammary pressure dose–response curves as in Fig. 2. *A*: □, ■, responses to exogenous oxytocin before and after I.V. propranolol (1 mg/kg), respectively; ○, 90 pulse stimulation of neurohypophysial stalk showing essentially no change in response despite increased mammary gland sensitivity after propranolol. *B*: ○, ●, responses to exogenous oxytocin before and after I.V. U50,488 (500 μg/kg) in a morphine-dependent rat, respectively; □, 120 pulse stimulation of neurohypophysial stalk showing decreased response after U50,488 (see Fig. 2*B*).

with 1 mg/kg naloxone (Fig. 6*B*); the mean peak response was 8·8 ± 0·3 mmHg, and the mean time to peak was 6·1 ± 0·4 min, similar to the morphine-tolerant rats given 5 mg/kg naloxone. At the peak, U50,488 was injected, and this quickly reduced intramammary pressure, with a mean ± S.E.M. time to half-peak of 1·8 ± 0·7 min ($n = 3$). Intramammary pressure in these rats reached a nadir just above baseline about 5 min after U50,488. In these rats, a further and larger injection of naloxone (5 mg/kg) rapidly increased intramammary pressure again to a value similar to that in tolerant rats at an equivalent time after a single naloxone injection (Fig. 6*B*).

In two rats blood samples were withdrawn for oxytocin radioimmunoassay just before the first injection of naloxone (1 mg/kg), 5 min after naloxone, 8 min after injection of U50,488 and 5 min after the second naloxone injection (5 mg/kg). In the first rat given U50,488 (5 mg/kg) these values were 52, 126, 100 and 219 pg/ml, and in the second rat given 10 mg/kg these values were 34, 227, 78 and 298 pg/ml, consistent with inhibition by U50,488 of naloxone-induced withdrawal stimulation of oxytocin secretion indicated by the intramammary pressure records (Fig. 6*B*).

In three morphine-tolerant/dependent rats an electrolytic lesion of the neural stalk was made (1·5 mA D.C., 10 s, Nichrome electrode; Russell, Pumford & Bicknell, 1992) proximal to the stimulating electrode about 5 min before the stimulation protocol. In these rats electrical stimulation evoked oxytocin secretion normally, but I.V. naloxone failed to produce a withdrawal response (Fig. 7).

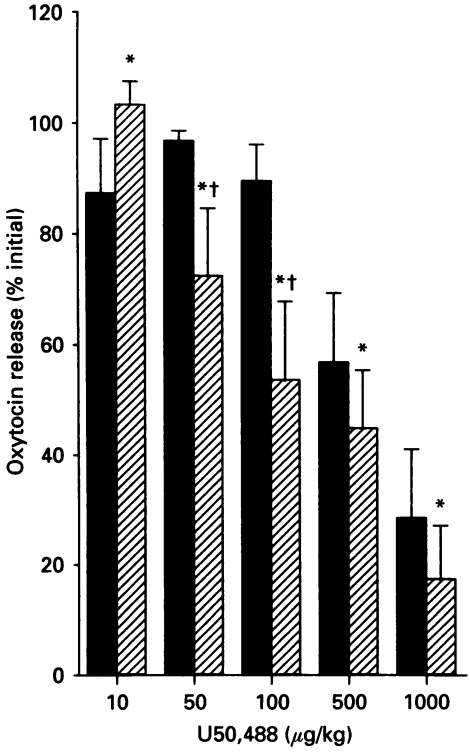


Fig. 5. Inhibitory effects of I.V. U50,488 on electrically stimulated oxytocin secretion in lactating rats given I.C.V. morphine or vehicle infusion for 5 days. Oxytocin secretion was measured as shown in Fig. 4. Values are mean ± S.E.M. percentage estimates of electrically stimulated oxytocin secretion after increasing doses of I.V. U50,488, as in Fig. 3. Filled columns: morphine-naive, $n = 9$; striped columns: I.C.V. morphine, $n = 6$. $ID_{50}$ for U50,488 was 170 (+78, −54) μg/kg in I.C.V. morphine-infused rats ($P < 0·05$ *vs.* morphine-naive animals, by calculation of confidence limits). †$P < 0·05$ *vs.* morphine-naive at this dose (unpaired *t* test); *$P < 0·01$ *vs.* preU50,488 (paired *t* test).

*Pregnant rats*

Daily weight gain of the pregnant rats was not different between the I.C.V. vehicle and I.C.V. morphine groups, e.g. on day 2 following surgery mean weight gain ± S.E.M. was 16·0 ± 1·2 and 14·4 ± 2·7 g respectively ($n = 9$ and 8); in both groups weight gain fell at the end of pregnancy, on day 5 of I.C.V. infusion to 5·0 ± 2·8 and −3·0 ± 4·8 g respectively.

Parturition began in the light phase of day 22 in 1/9 I.C.V. vehicle-infused rats, and in 0/8 I.C.V. morphine rats, during the lights-out phase of days 22–23 in one rat from each group, and during the light phase of day 23 in the remainder, except for one I.C.V. morphine-infused rat that began delivery after lights-out on day 23. The

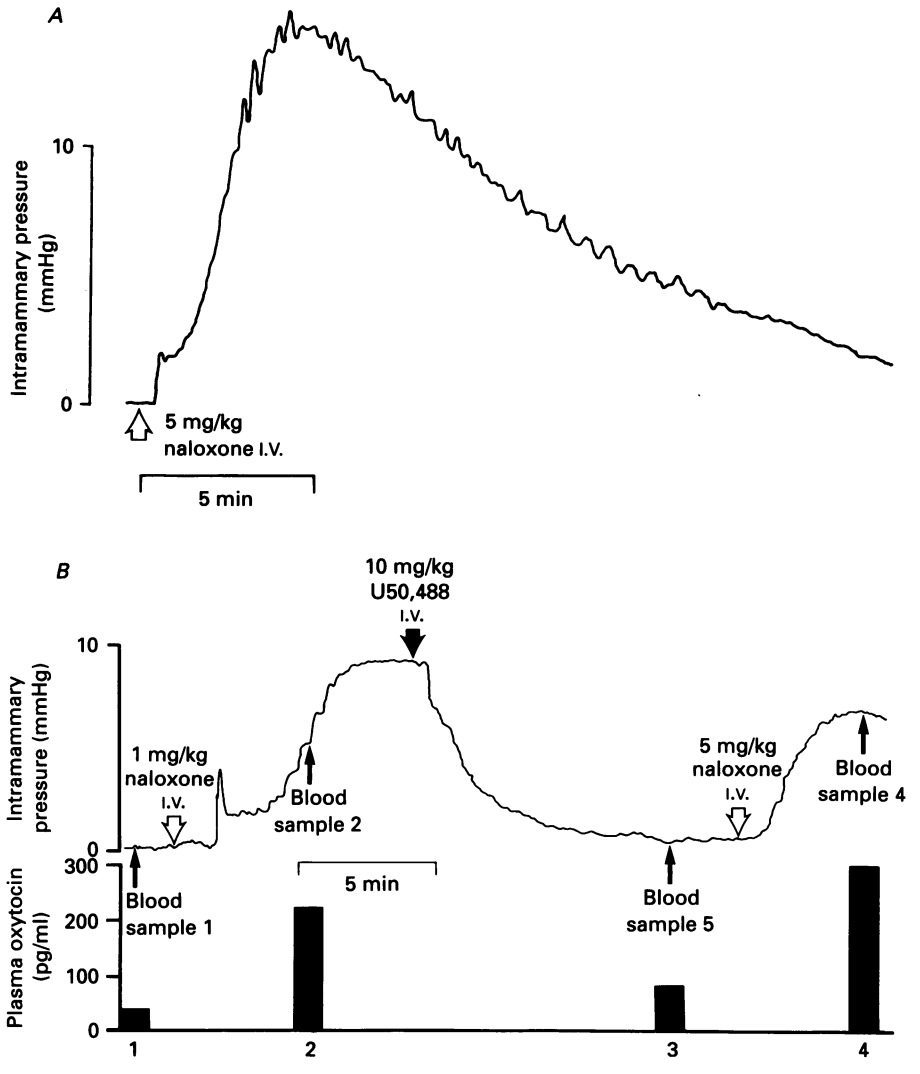


Fig. 6. Effects of I.V. U50,488 on naloxone-induced excitation of oxytocin secretion in lactating rats given I.C.V. infusion of morphine for 5 days. Typical intramammary pressure recordings from urethane-anaesthetized rats, showing segments of recording after determination of effects of U50,488 on electrically stimulated oxytocin secretion. *A*, effects of naloxone (5 mg/kg I.V.) alone. Naloxone rapidly produced a large increase in intramammary pressure, peaking within 5 min, and slowly but steadily declining. This typical withdrawal response reflects a large sustained increase in oxytocin secretion (e.g. see Rayner *et al.* 1988; Bicknell *et al.* 1988*a*). *B*, effects of U50,488 on responses to naloxone. Naloxone (1 mg/kg I.V.; broad open arrow), produced a large increase in intramammary pressure peaking within 5 min; I.V. injection of U50,488 (10 mg/kg; broad filled arrow) was followed by a rapid fall in intramammary pressure to baseline, indicating suppression of oxytocin secretion; I.V. injection of a higher dose of naloxone (5 mg/kg), produced a large increase in intramammary pressure. Similar results were obtained in a further two rats tested. Narrow filled arrows indicate when femoral arterial blood samples were taken, and the plasma oxytocin concentrations measured by radioimmunoassay are shown in the lower panel (filled columns); the changes in radioimmunoassayed plasma oxytocin concentration confirm the changes inferred from the intramammary pressure record.

median time of parturition onset was *ca* 12.00 h on day 23 in the control rats and *ca* 16.00 h on day 23 in the I.C.V. morphine-infused rats ($P < 0{\cdot}02$, two-sample sign test).

The progress of parturition, once initiated, between the groups was similar. The mean cumulative interval between pups was not significantly different (Fig. 8) in the

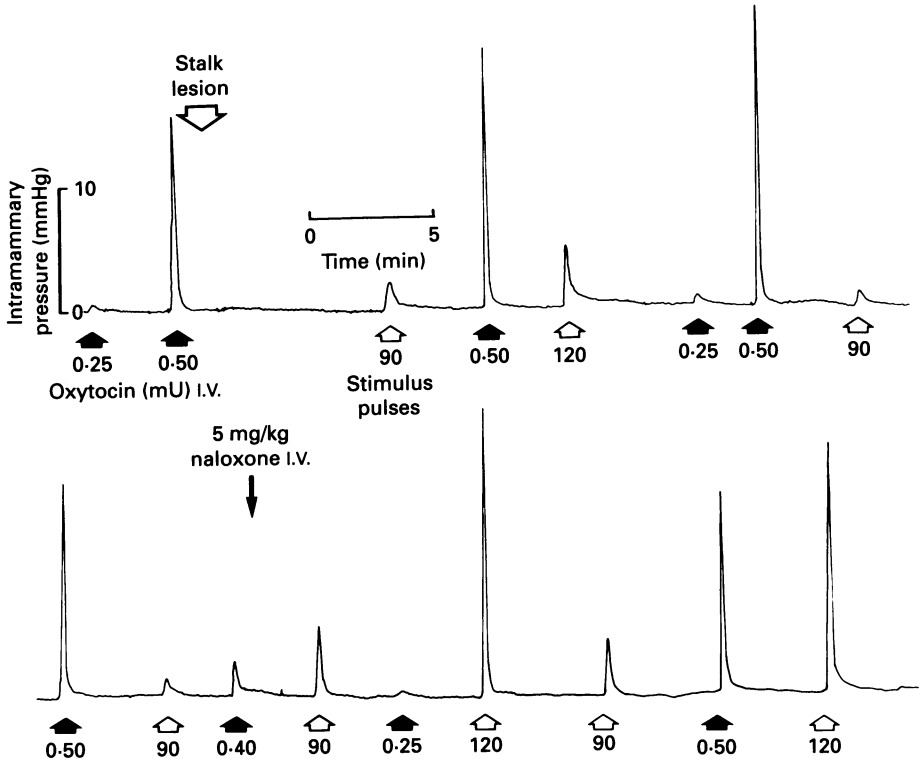


Fig. 7. In some I.C.V. morphine-infused lactating rats, an electrolytic lesion (1·5 mA, 10 s; downward open arrow) of the exposed pituitary stalk was made prior to I.V. naloxone injection. Electrical stimulation (50 Hz, 0·5 mA, 1 ms pulses; open arrows) of the stalk distal to the lesion still effected oxytocin release, and I.V. injection of naloxone (5 mg/kg) facilitated stimulated oxytocin release, but there was no detectable increase in intramammary pressure between stimuli, in contrast to the effects of naloxone in intact I.C.V. morphine-infused rats (see Fig. 6). Sensitivity to calibration doses of I.V. oxytocin (filled broad arrows) was not increased after naloxone.

two groups, and there were no significant differences between the means of individual interbirth intervals between the groups, nor for the interbirth interval cumulative frequency distribution (Kolmogorov-Smirnov, using 4 min bins); one rat in the I.C.V. morphine group had a protracted parturition, with long intervals between the first four and last two pups (38, 52, 51 and 91 min respectively), largely accounting for the greater variance of mean cumulative time in the I.C.V. morphine group (Fig. 8).

Mean litter size in the two groups was similar ($11{\cdot}4 \pm 1{\cdot}1$ and $11{\cdot}8 \pm 0{\cdot}8$ pups in the I.C.V. morphine and vehicle groups, respectively), and the immediate postpartum (< 1 h) pup viability rate was also similar in the two groups (100% in the I.C.V.

morphine group and 99±2% in the I.C.V. vehicle group; on the day following birth, additional viability was 91·4±4·6 and 99·2±0·8%, respectively).

*Oxytocin and vasopressin release from electrically stimulated neurointermediate lobes in vitro*

*Oxytocin release*

Without morphine addition *in vitro*, there was no significant difference between lobes from control and I.C.V. morphine-infused rats in the amount of oxytocin

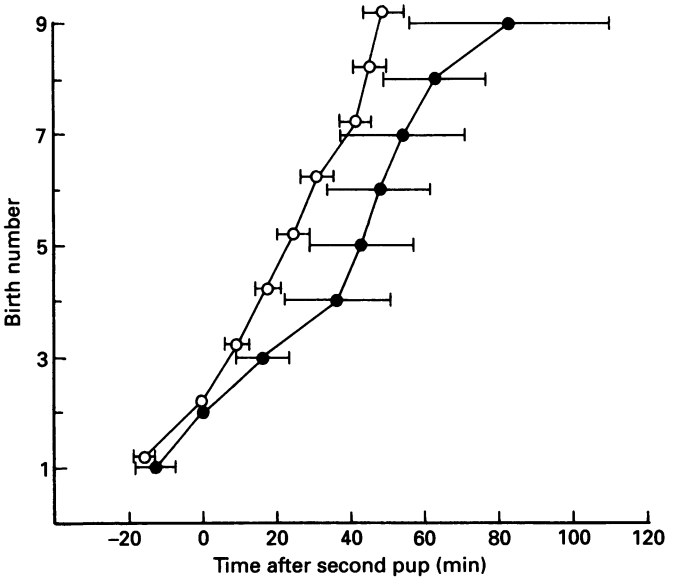


Fig. 8. Effects of I.C.V. morphine in pregnancy on cumulative birth times in parturition. Morphine or vehicle was infused for the preceding 5 days and continued through parturition. Values are mean±S.E.M. times from the birth of the second pup (taken as reference zero since the first interbirth interval is usually the most variable). ○, I.C.V. vehicle infusion, $n = 6$; ●, I.C.V. morphine infusion, $n = 6$. No significant differences.

released as a result of the first electrical stimulation ($S_1$; Fig. 9; Table 1); continuous presence of morphine had no effect on oxytocin release during $S_1$ (Fig. 9*C*; Table 1). Oxytocin release following $S_2$, in the presence of naloxone, was greater than $S_1$ in all groups (i.e. $S_2/S_1$ ratio was $> 1$, Fig. 9*A* and *C*; Table 1). The $S_2/S_1$ oxytocin ratio was not significantly different for the lobes from I.C.V. morphine-infused rats with or without morphine *in vitro*, compared either with each other or with the lobes from control rats without morphine added *in vitro* (Fig. 9*A* and *C*, Table 1). The $S_2/S_1$ oxytocin ratio for lobes from control rats was not affected by addition of morphine *in vitro* (Fig. 9*A* and *C*; Table 1). This ratio was less for lobes from control rats with morphine present throughout compared with lobes from morphine-infused rats kept in morphine (Fig. 9*C*; Table 1) and the proportion of total oxytocin released in $S_2$ for the lobes from morphine-infused rats kept in morphine was about 20% greater than for the lobes from vehicle-infused rats (Fig. 9*A* and *C*, $P < 0{\cdot}05$, Duncan's test ANOVA). However, this difference may possibly be accounted for by

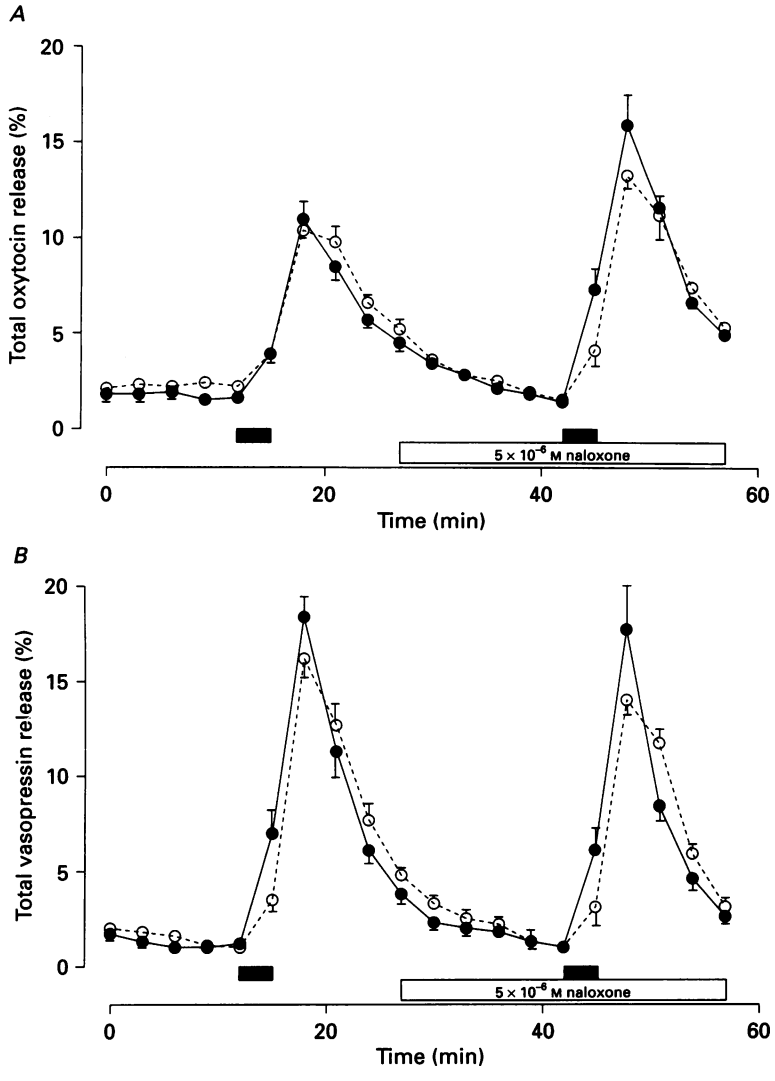


Fig. 9 *A*, *B*. For legend see facing page.

the greater oxytocin release in the second basal period ($B_2$) in the control lobes kept in morphine (data not shown; $P < 0{\cdot}005$ ANOVA, $P < 0{\cdot}05$, Duncan's test *vs.* other groups), including a greater proportion of total oxytocin released in $B_2$ (but not in $B_1$ or $S_1$) in this group (Fig. 9*C*).

None the less, the amount of oxytocin released in $S_2$ was not different between groups and the ratio of the proportion of total oxytocin released in $S_2$ to that in $S_1$ was not different between the groups (ANOVA; data from Fig. 9*A* and *C*).

*Vasopressin release*

Without morphine addition *in vitro*, there was no significant difference between lobes from control and I.C.V. morphine-infused rats, in the release in response to the first stimulation, $S_1$ (Fig. 9*B*). The $S_2/S_1$ ratios for vasopressin release were not above

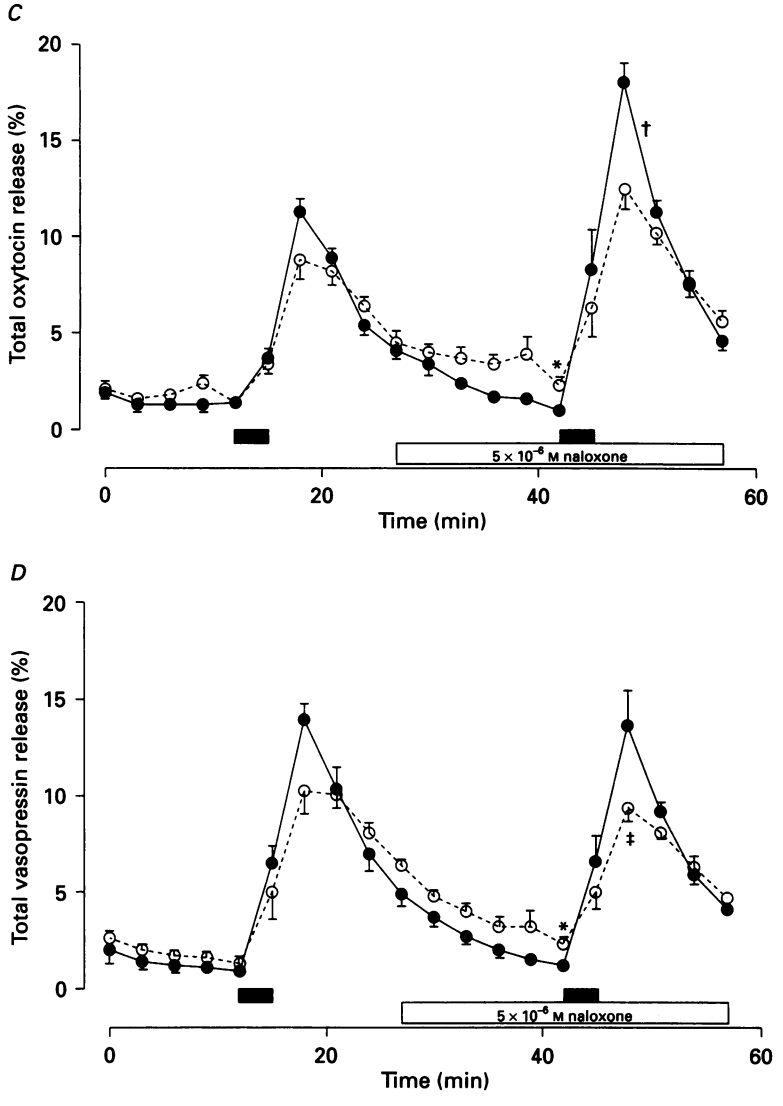


Fig. 9. Release of oxytocin and vasopressin from isolated neurointermediate lobes superfused *in vitro*. Oxytocin or vasopressin released in each consecutive 3 min sample is expressed as a percentage of the total released by each lobe in the experiment (60 mins); values are means ± S.E.M. Values for hormone release (ng) are shown in Table 1. There were two periods of electrical stimulation, with 13 Hz, 5 mA, 2 mS biphasic pulses for 3 min ($S_1$, $S_2$, filled bars); naloxone ($5 \times 10^{-6}$ M) was added to the superfusate after $S_1$ (open bar). ○, *in vivo* I.C.V. vehicle pretreatment; ●, *in vivo* I.C.V. morphine pretreatment, $n = 6$ per group. *A*, oxytocin release, no morphine added *in vitro*; *B*, vasopressin release, no morphine added *in vitro*; *C*, oxytocin release, morphine $5 \times 10^{-5}$ M present throughout; *D*, vasopressin release, morphine $5 \times 10^{-5}$ M present throughout. Stimulated release of oxytocin, but not vasopressin, was greater in the presence of naloxone (see Table 1). In the I.C.V. vehicle group with morphine *in vitro*, basal release of both oxytocin and vasopressin was increased in the presence of naloxone (*$P < 0.05$ *vs.* other groups). The percentage of vasopressin released during the second stimulation was reduced in this group (‡$P < 0.05$, ANOVA, Duncan's test). The percentage of oxytocin released after naloxone was greater in the I.C.V. morphine/morphine *in vitro* group than in the I.C.V. vehicle groups (†$P < 0.05$).

unity in any group (Fig. 9*B* and *D*, Table 1), and there were no significant differences in this ratio between groups (ANOVA, $P = 0{\cdot}2$).

TABLE 1. Electrically stimulated release of oxytocin and vasopressin *in vitro* from neurointermediate lobes from control and I.C.V. morphine-infused rats

| I.C.V./*in vitro* treatment group | Oxytocin release: Stimulus 1 $S_1$(ng/15 min) | Release ratio: $S_2$ naloxone:$S_1$ control | |
|---|---|---|---|
| | | Oxytocin | Vasopressin |
| Vehicle/no morphine | 3·29±0·68 | 1·38±0·11‡ | 0·84±0·06 |
| Morphine/no morphine | 3·25±0·75 | 1·65±0·20‡ | 0·85±0·08 |
| Vehicle/morphine ($5 \times 10^{-5}$ M) | 3·81±0·92 | 1·25±0·09‡ | 0·66±0·04 |
| Morphine/morphine ($5 \times 10^{-5}$ M) | 4·48±0·66 | 1·69±0·08*‡ | 0·91±0·13 |

Values are means±S.E.M., $n = 6$ per group. Statistical comparisons: *$P < 0{\cdot}05$ *vs.* vehicle/morphine group, Dunn's test/Kruskal–Wallis; ‡$S_2 > S_1$, $P < 0{\cdot}05$, Wilcoxon.

## DISCUSSION

Morphine potently inhibits oxytocin secretion, and strongly depresses the firing of supraoptic oxytocin neurones (Grell, Christensen & Fjalland, 1988; Evans *et al.* 1989*b*; Pumford *et al.* 1991). It has previously been reported that morphine given by I.C.V. injection to lactating rats inhibits oxytocin secretion evoked by electrical stimulation of the neural stalk, indicating an action at the posterior pituitary; morphine was also reported to act at this site to inhibit oxytocin secretion during suckling (Clarke *et al.* 1979). However, in the present studies, we found that morphine depressed the sensitivity of the mammary gland to oxytocin; taking this reduced sensitivity into account, we found no effect of morphine on oxytocin secretion evoked by electrical stimulation of the neural stalk at a frequency similar to that at which oxytocin neurones fire periodically during suckling (Lincoln & Wakerley, 1975). These results indicate that such $\mu$-receptors as may be present in the posterior pituitary (Sumner *et al.* 1990) are not coupled to mechanisms regulating oxytocin release. The inhibitory effect of morphine on oxytocin secretion would thus appear to be confined to inhibition of the firing rate of oxytocin neurones (Wakerley, Noble & Clarke, 1983; Pumford *et al.* 1991).

In contrast, the selective $\kappa$-opioid agonist U50,488 potently inhibited oxytocin secretion evoked by electrical stimulation of the neural stalk; in the present study the $ID_{50}$ was 441 $\mu$g/kg, and nearly full inhibition was seen with doses of 2·5 mg/kg. The posterior pituitary contains predominantly $\kappa$-type opioid receptors and U50,488 inhibits both electrically stimulated oxytocin secretion from the posterior pituitary *in vitro* (Zhao *et al.* 1988*b*), and $K^+$-stimulated oxytocin release from neurosecretosomes (Zhao *et al.* 1988*a*). Naloxone reversed the inhibitory action of U50,488 on electrically stimulated oxytocin secretion *in vivo*, confirming that U50,488 acts via opioid receptors. Inhibition by U50,488 of oxytocin secretion stimulated by suckling (Clarke & Wright, 1989), during parturition (Douglas *et al.* 1990) or by hyperosmotic stimulation (Evans *et al.* 1989*b*) is thus likely to involve

actions at the level of the posterior pituitary as well as inhibition of the firing rate of oxytocin neurones (Russell, Pumford & Leng, 1989*b*; Leng *et al.* 1990).

The $ID_{50}$ for U50,488 was increased after pretreatment with the $\beta$-adrenergic antagonist propranolol indicating the involvement of an inhibitory action of adrenaline on oxytocin secretion (Clarke & Wright, 1987); however, U50,488 could still fully inhibit oxytocin secretion after $\beta$-adrenergic receptor blockade. Within the posterior pituitary, $\beta$-adrenergic receptor activation facilitates oxytocin secretion by an indirect mechanism involving pituicytes (Zhao *et al.* 1988*c*; Bicknell & Zhao, 1989); the present results indicate that this mechanism is not important in opposing the actions of U50,488 on oxytocin secretion.

In lactating rats given I.C.V. morphine infusion for 5 days the milk-ejection reflex operates normally, and the near-normal oxytocin concentrations in peripheral blood and near-normal firing rate of supraoptic oxytocin neurones in these and in similarly treated virgin rats indicates tolerance to morphine (Bicknell *et al.* 1988*a*; Rayner *et al.* 1988; Leng, Russell & Grossmann, 1989; Russell, 1989). Indeed, the dose of I.V. morphine required to inhibit the firing rate of supraoptic oxytocin neurones is greatly increased in rats given I.C.V. morphine infusion (Pumford *et al.* 1991), whereas the sensitivity of the firing rate of oxytocin neurones to I.V. U50,488 is not altered (Leng *et al.* 1990), indicating lack of cross-tolerance between $\mu$-type and $\kappa$-type opioid receptors. The density of $\mu$-receptors is reduced in the supraoptic nucleus in morphine-tolerant rats, but the density of $\kappa$-receptors is not altered in either the supraoptic nucleus or posterior pituitary (Sumner *et al.* 1990). The present results show that rats made tolerant to morphine do not display cross-tolerance to $\kappa$-opioids at the posterior pituitary since the potency of U50,488 on electrically stimulated oxytocin secretion *in vivo* was not decreased, but rather was significantly increased. Previous studies on interactions of U50,488 with analgesic actions of morphine have indicated increased sensitivity in morphine-tolerant rats (Yamamoto, Ohno & Ueki, 1988), while studies on opioid actions on the hypothalamo–pituitary adrenal axis have similarly shown lack of cross-tolerance between different opioid receptor types and their selective ligands (Iyengar, Kim & Wood, 1987).

Thus $\kappa$-mechanisms can still inhibit oxytocin secretion from the posterior pituitary while central $\mu$-receptor mechanisms are desensitized. Further evidence of this was provided by the studies during morphine withdrawal; naloxone injection in morphine-tolerant rats produced the expected large increase in oxytocin secretion, this withdrawal activation of oxytocin secretion indicating morphine-dependence (Rayner *et al.* 1988). None the less I.V. U50,488 given in an appropriately large dose to compete with naloxone at $\kappa$-receptors (for which naloxone has a relatively low affinity; Kosterlitz, 1985) was still able to rapidly suppress oxytocin secretion. Electrophysiological studies show that in these circumstances U50,488 is still effective in reducing the firing rate of supraoptic oxytocin neurones during morphine-withdrawal excitation (G. Leng, K. M. Pumford & J. A. Russell). These findings reinforce the distinctness of the mechanisms involved in $\mu$- and $\kappa$-opioid control of oxytocin neurones.

*In vitro*, neurointermediate lobes from control rats released more oxytocin during stimulation in the presence of naloxone, as previously found (Bicknell & Leng, 1982). The explanation for this effect of naloxone is considered to be that it antagonizes the

restraining actions upon oxytocin secretion of endogenous opioids released during electrical stimulation (Gerstberger & Barden, 1986; Bondy, Gainer & Russell, 1988; Bicknell & Zhao, 1989). Morphine did not reduce electrically stimulated oxytocin release *in vitro*, confirming that $\mu$-receptors in the posterior pituitary are either not functionally coupled on oxytocin terminals or are absent (Herkenham *et al.* 1986; Zhao *et al.* 1988*b*; Sumner *et al.* 1990). Neurointermediate lobes from I.C.V. morphine-infused rats, whether or not morphine was present in the perifusion medium, released oxytocin following the first stimulation in similar amounts to the lobes from control rats (without morphine), again indicating lack of cross-tolerance from morphine to the endogenous opioid acting on oxytocin terminals in the posterior pituitary. Lack of cross-tolerance is also indicated by the potentiation in the presence of naloxone of electrically stimulated oxytocin release, since tolerance to endogenous opioids would be expected to lead to a reduced effect of naloxone. Indeed, the effect of naloxone was greater on lobes from morphine-tolerant/dependent rats. Given our findings that morphine does not act on the posterior pituitary, this enhanced effect of naloxone probably reflects increased action of endogenous opioid mechanisms restraining oxytocin terminals in rats made morphine tolerant. This interpretation is consistent with the increased sensitivity of oxytocin terminals to inhibition by U50,488 in morphine-tolerant rats (see above), and suggests that in these rats the sensitivity of $\kappa$-receptor mechanisms on oxytocin terminals is increased. This could possibly be mediated by increased synthesis of prodynorphin peptides during initial morphine administration (Lightman & Young, 1988) in neighbouring vasopressin neurones (Whitnall, Gainer, Cox & Molyneaux, 1983) or in the oxytocin neurones themselves (Meister, Villar, Ceccatelli & Hökfelt, 1990).

The absence of naloxone-induced hypersecretion of oxytocin in morphine-dependent rats following acute lesions of the neural stalk supports previous findings (Sheward, Coombes, Bicknell, Fink & Russell, 1989) and confirms that the posterior pituitary is not a site of morphine dependence. As reported previously, the greatly increased oxytocin secretion provoked by naloxone in I.C.V. morphine-infused rats is explained by withdrawal excitation of the electrical activity of oxytocin neurones, amplified by the facilitatory effect of antagonizing endogenous opioid actions at the posterior pituitary (Bicknell *et al.* 1988*a*).

In contrast to its actions on oxytocin secretion, naloxone did not facilitate stimulated vasopressin secretion in any of the groups, confirming previous observations (Bicknell & Leng, 1982; Bicknell & Zhao, 1989) although minor effects can be discerned under different stimulation conditions (Zhao *et al.* 1988*b*). The failure of naloxone to enhance stimulated vasopressin release in any of the morphine-treated groups indicates lack of morphine actions and *in vivo*, vasopressin secretion is minimally affected by naloxone-precipitated morphine withdrawal (Bicknell *et al.* 1988*a*; Coombes, Robinson, Antoni & Russell, 1991).

The effects on parturition of I.C.V. morphine infusion for the last few days of pregnancy were modest, a delay in the onset by *ca* 4 h being the only detectable effect. Oxytocin secretion is increased at the end of pregnancy, but this is not the critical factor in initiating parturition (Higuchi, Tadakoro, Honda & Negoro, 1986; Russell *et al.* 1991) although oxytocin secretion is essential for the normal progress of established parturition. Since we have not measured blood oxytocin levels we cannot

tell whether oxytocin secretion was normal on the day of expected parturition but the normal progress of parturition, once established, is consistent with normal oxytocin secretion. A similar delay in the onset of parturition has been reported in rats treated systemically for 2–3 days with a $\mu$-/$\kappa$-agonist, and in these studies no effects on maternal oxytocin secretion were reported, although fetal pituitary oxytocin content was increased (Evans, Rice & Olley, 1988; Evans, Olley, Rice & Abrahams, 1989*a*). In normal rats, the secretion of oxytocin and the pace of parturition are both increased by injection of naloxone, indicating that endogenous opioids restrain oxytocin secretion during parturition (Leng *et al.* 1988). The present results, showing that the rate of birth of pups once parturition was established was no faster than normal, indicate lack of cross-tolerance between morphine and the endogenous opioid that inhibits oxytocin secretion in parturition. This endogenous mechanism could be central or in the posterior pituitary.

In conclusion, the study shows that a $\kappa$-, but not a $\mu$-opioid agonist, acts at the posterior pituitary gland to inhibit oxytocin secretion. The probable source of a $\kappa$-selective endogenous opioid acting in the posterior pituitary has been considered to be the terminals of vasopressin neurones which release dynorphins (Anhut & Knepel, 1982; Whitnall, Gainer, Cox & Molineaux, 1983); however, oxytocin neurones also contain dynorphins (Meister *et al.* 1990) as well as pro-enkephalin peptides, and the C-terminally extended Met-enkephalins are active at $\kappa$-receptors (Adachi, Hisano & Daikoku, 1985; Castanas *et al.* 1985; Gaymann & Martin, 1987). It therefore remains an open question whether oxytocin terminals are subject to auto- or cross-inhibition by opioids from oxytocin or from vasopressin terminals respectively (Summy-Long, 1989; Leng, Dyball & Way, 1992). Chronic exposure to morphine leads to morphine tolerance and dependence in relation to the electrical activity of the cell bodies of oxytocin neurones (Bicknell *et al.* 1988*a*; Pumford *et al.* 1991), and at this site the sensitivity to $\kappa$-agonists is not decreased (Leng *et al.* 1990). In the posterior pituitary there seems to be up-regulation of $\kappa$-receptor mechanisms following the depression of the magnocellular oxytocin system during chronic morphine treatment. Clearly the magnocellular oxytocin system can show altered sensitivity to $\mu$-opioids with undiminished or increased sensitivity to $\kappa$-opioids. This may be important in parturition, in relation to which the present results indicate a role for $\mu$-opioids in its onset.

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## REFERENCES

ADACHI, T., HISANO, S. & DAIKOKU, S. (1985). Intragranular colocalisation of immunoreactive methionine-enkephalin and oxytocin within the nerve terminals in the posterior pituitary. *Journal of Histochemistry and Cytochemistry* **33**, 891–899.

ANHUT, H. & KNEPEL, W. (1982). Release of dynorphin-like immunoreactivity of rat neurohypophysis in comparison to vasopressin after various stimuli in vitro and in vivo. *Neuroscience Letters* **31**, 159–164.

BICKNELL, R. J., CHAPMAN, C., LENG, G. & RUSSELL, J. A. (1985). Chronic morphine exposure and oxytocin neurones in rats: lack of both morphine dependence and cross tolerance to endogenous opioids in the neurohypophysis. *Journal of Physiology* **361**, 32*P*.

BICKNELL, R. J. & LENG, G. (1982). Endogenous opiates regulate oxytocin but not vasopressin secretion from the neurohypophysis. *Nature* **298**, 161–162.

BICKNELL, R. J., LENG, G., LINCOLN, D. W. & RUSSELL, J. A. (1988*a*). Naloxone excites oxytocin neurones in the supraoptic nucleus of lactating rats after chronic morphine treatment. *Journal of Physiology* **396**, 297–317.

BICKNELL, R. J., LENG, G., RUSSELL, J. A., DYER, R. G., MANSFIELD, S. & ZHAO, B.-G. (1988*b*). Hypothalamic mechanisms controlling oxytocin neurones during parturition. *Brain Research Bulletin* **20**, 743–749.

BICKNELL, R. J. & ZHAO, B.-G. (1989). Secretory terminals of oxytocin neurones as a site of opioid modulation. In *Brain Opioid Systems in Reproduction*, ed. DYER, R. G. & BICKNELL, R. J., pp. 288–306. Oxford University Press, Oxford.

BONDY, C. A., GAINER, H. & RUSSELL, J. T. (1988). Dynorphin A inhibits and naloxone increases the electrically-stimulated release of oxytocin but not vasopressin from the terminals of the neural lobe. *Endocrinology* **122**, 1321–1327.

CASTANAS, E., BOURHIM, N., GIRAUD, P., BOUDOURESQUE, F., CANTAU, P. & OLIVER, C. (1985). Interaction of opiates with opioid binding sites in the bovine adrenal medulla: II. Interaction with $\kappa$ sites. *Journal of Neurochemistry* **45**, 688–699.

CLARK, J. A. & PASTERNAK, G. W. (1988). U50,488: a kappa-selective agent with poor affinity for $mu_1$ opiate binding sites. *Neuropharmacology* **27**, 331–332.

CLARKE, G. & PATRICK, G. (1983). Differential inhibitory action by morphine on the release of oxytocin and vasopressin from the isolated neural lobe. *Neuroscience Letters* **39**, 175–180.

CLARKE, G., WOOD, P., MERRICK, L. & LINCOLN, D. W. (1979). Opiate inhibition of peptide release from the neurohumoral terminals of hypothalamic neurones. *Nature* **282**, 746–748.

CLARKE, G. & WRIGHT, D. M. (1987). Opioids, catecholamines and the secretion of oxytocin. *Neuroscience Letters Supplement* **29**, S40.

CLARKE, G. & WRIGHT, D. M. (1989). Opioid inhibition of milk yield in conscious lactating rats. In *Progress in Opioid Research, Advances in Biosciences* vol. 75, ed. CROS, J., MEUNIER, J. CL. & HAMON, M., pp. 607–610. Pergamon Press, Oxford.

COOMBES, J. E., ROBINSON, I. C. A. F., ANTONI, F. C. & RUSSELL, J. A. (1991). Release of oxytocin into blood and into cerebrospinal fluid induced by naloxone in anaesthetized morphine-dependent rats: the role of the paraventricular nucleus. *Journal of Neuroendocrinology* **3**, 551–561.

COOMBES, J. E. & RUSSELL, J. A. (1988). Inhibition by a kappa-opioid receptor agonist (U50,488H) of electrically-stimulated oxytocin secretion from the neurohypophysis in anaesthetized lactating rats. *Journal of Physiology* **401**, 34*P*.

COOMBES, J. E. & RUSSELL, J. A. (1991). Lack of cross-tolerance in the posterior pituitary to the inhibitory actions of U50,488H on oxytocin secretion in morphine-dependent, anaesthetized lactating rats. *Journal of Physiology* **434**, 88*P*.

DOUGLAS, A. J., CLARKE, G., MACMILLAN, S. J., NEUMANN, I., BULL, P. & RUSSELL, J. A. (1990). A kappa-opioid agonist, U50,488H, reduces oxytocin secretion and slows parturition in rats. *Journal of Reproduction and Fertility* series 6, 8 (abstract).

EVANS, R. G., RICE, G. E. & OLLEY, J. E. (1988). Studies of the effects of subacute treatment with N-(cyclopronylmethyl)-19-isopentylnororvinol (M320) on timing of parturition in the rat. *British Journal of Pharmacology* **95**, 777–782.

EVANS, R. G., OLLEY, J. E., RICE, G. E. & ABRAHAMS, J. M. (1989*a*). Effects of subacute opioid administration during late pregnancy in the rat on the initiation, duration and outcome of parturition and maternal levels of oxytocin and arginine vasopressin. *Clinical and Experimental Pharmacology and Physiology* **16**, 169–178.

EVANS, R. G., OLLEY, J. E., RICE, G. E. & ABRAHAMS, J. M. (1989*b*). $\mu$- and $\kappa$-opiate receptor agonists reduce plasma neurohypophysial hormone concentrations in water-deprived and normally-hydrated rats. *Clinical and Experimental Pharmacology and Physiology* **16**, 191–197.

GAYMANN, W. & MARTIN, R. (1987). A re-examination of the localisation of immunoreactive $dynorphin_{1-8}$, Leu-enkephalin and Met-enkephalin in the rat neurohypophysis. *Neuroscience* **20**, 1069–1080.

GERSTBERGER, R. & BARDEN, N. (1986). Dynorphin$_{1-8}$ binds to opiate kappa receptors in the neurohypophysis. *Neuroendocrinology* **42**, 376–382.

GRELL, S., CHRISTENSEN, J. D. & FJALLAND, B. (1988). The influence of morphine and naloxone on plasma oxytocin. *Pharmacology and Toxicology* **63**, 274–276.

HERKENHAM, M., RICE, K. C., JACOBSON, A. E. & ROTHMAN, R. B. (1986). Opiate receptors in rat pituitary are confined to the neural lobe and are exclusively kappa. *Brain Research* **382**, 365–371.

HIGUCHI, T., HONDA, K., FUKUOKA, T., NEGORO, H. & WAKABAYASHI, K. (1985). Release of oxytocin during suckling and parturition in the rat. *Journal of Endocrinology* **105**, 339–346.

HIGUCHI, T., TADOKORO, Y., HONDA, K. & NEGORO, H. (1986). Detailed analysis of blood oxytocin levels during suckling and parturition in the rat. *Journal of Endocrinology* **110**, 251–256.

IYENGAR, S., KIM, H. S. & WOOD, P. L. (1987). $\mu$-, $\delta$-, $\kappa$- and $\epsilon$-opioid receptor modulation of the hypothalamic–pituitary–adrenocortical (HPA) axis: subchronic tolerance studies of endogenous opioid peptides. *Brain Research* **435**, 220–226.

KOSTERLITZ, H. W. (1985). Opioid peptides and their receptors. *Proceedings of the Royal Society* B **225**, 27–40.

LENG, G. (1980). Rat supraoptic neurones: the effects of locally applied hypertonic saline. *Journal of Physiology* **304**, 405–414.

LENG, G., DYBALL, R. E. J. & WAY, S. (1992). Naloxone potentiates the release of oxytocin induced by systemic administration of cholecystokinin without enhancing the electrical activity of supraoptic oxytocin neurones. *Experimental Brain Research* **88**, 321–325.

LENG, G., MANSFIELD, S., BICKNELL, R. J., BLACKBURN, R. E., BROWN, D., CHAPMAN, C., DYER, R. G., HOLLINGSWORTH, S., SHIBUKI, S., YATES, J. O. & WAY, S. (1988). Endogenous opioid actions and effects of environmental disturbance on parturition and oxytocin secretion in rats. *Journal of Reproduction and Fertility* **84**, 345–356.

LENG, G., PUMFORD, K. & RUSSELL, J. A. (1990). Actions of the $\kappa$-type opioid receptor agonist U50,488H on oxytocin neurones in the supraoptic nucleus (SON) in normal and morphine-tolerant urethane-anaesthetized rats. *Journal of Physiology* **420**, 71*P*.

LENG, G., RUSSELL, J. A. & GROSSMANN, R. (1989). Sensitivity of magnocellular oxytocin neurones to opioid antagonists in rats treated chronically with intracerebroventricular (i.c.v.) morphine. *Brain Research* **484**, 290–296.

LIGHTMAN, S. L. & YOUNG, W. S. III. (1988). Corticotrophin-releasing factor, vasopressin and pro-opiomelanocortin mRNA responses to stress and opiates in the rat. *Journal of Physiology* **403**, 511–523.

LINCOLN, D. W. & WAKERLEY, J. B. (1975). Factors governing the periodic activation of supraoptic and paraventricular neurosecretory cells during suckling in the rat. *Journal of Physiology* **250**, 443–461.

MAGNAN, J., PATERSON, S. J., TAVANI, A. & KOSTERLITZ, H. W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn-Schmiedeberg's Archives of Pharmacology* **319**, 197–205.

MEISTER, B., VILLAR, M. J., CECCATELLI, S. & HÖKFELT, T. (1990). Localization of chemical messengers in magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei: an immunohistochemical study using experimental manipulations. *Neuroscience* **37**, 603–633.

PUMFORD, K., LENG, G. & RUSSELL, J. A. (1991). Morphine actions on supraoptic oxytocin neurones in anaesthetized rats: tolerance after I.C.V. morphine infusion. *Journal of Physiology* **440**, 437–454.

RAYNER, V. C., ROBINSON, I. C. A. F. & RUSSELL, J. A. (1988). Chronic intracerebroventricular morphine and lactation in rats: dependence and tolerance in relation to oxytocin neurones. *Journal of Physiology* **396**, 319–347.

RUSSELL, J. A. (1989). Opiate dependence and tolerance in oxytocinergic neurones. *Biomedical Research* **10**, suppl. 3, 95–106.

RUSSELL, J. A., GOSDEN, R. G., HUMPHREYS, E. M., CUTTING, R., FITZSIMONS, N., JOHNSTON, V., LIDDLE, S., SCOTT, S. & STIRLAND, J. A. (1989*a*). Interruption of parturition in rats by morphine: a result of inhibition of oxytocin secretion. *Journal of Endocrinology* **121**, 521–536.

RUSSELL, J. A., LENG, G., COOMBES, J., CROCKETT, S. A., DOUGLAS, A. J., MURRAY, I. & WAY, S. (1991). Pethidine (Meperidine) inhibition of oxytocin secretion and action in parturient rats. *American Journal of Physiology* **261**, R358–369.

RUSSELL, J. A., PUMFORD, K. M. & BICKNELL, R. J. (1992). The region anterior and ventral to the third ventricle (AV3V) and naloxone-provoked morphine withdrawal excitation of oxytocin secretion in urethane-anaesthetized rats. *Neuroendocrinology* **55**, 183–192.

RUSSELL, J. A., PUMFORD, K. M. & LENG, G. (1989*b*). Opiate dependence in a neuroendocrine neurone system. In *Progress in Opioid Research*, vol. 75, *Advances in Biosciences*, ed. CROS, J., MEUNIER, J.-CL. & HAMON, M., pp. 767–770. Pergamon Press, Oxford.

SHELDRICK, E. L. & FLINT, A. P. F. (1981). Circulating concentrations of oxytocin during the estrous cycle and early pregnancy in sheep. *Prostaglandins* **22**, 631–636.

SHEWARD, W. J., COOMBES, J. E., BICKNELL, R. J., FINK, G. & RUSSELL, J. A. (1989). Release of oxytocin but not corticotrophin-releasing factor-41 into rat hypophysial portal vessel blood can be made opiate dependent. *Journal of Endocrinology* **124**, 141–150.

SUMMY-LONG, J. Y. (1989). Cross-inhibition of oxytocin neurones during activation of the vasopressin system. In *Brain Opioid Systems in Reproduction*, ed. DYER, R. G. & BICKNELL, R. J., pp. 271–287. Oxford University Press, Oxford.

SUMNER, B. E. H., COOMBES, J. E., PUMFORD, K. M. & RUSSELL, J. A. (1990). Opioid receptor subtypes in the supraoptic nucleus and posterior pituitary gland of morphine-tolerant rats. *Neuroscience* **37**, 635–645.

WAKERLEY, J. B., NOBLE, R. & CLARKE, G. (1983). Effects of morphine and D-Ala-D-Leu enkephalin on the electrical activity of supraoptic neurosecretory cells *in vitro*. *Neuroscience* **10**, 73–81.

WHITNALL, M. H., GAINER, H., COX, B. M. & MOLINEAUX, C. J. (1983). Dynorphin-A-(1–8) is contained within vasopressin neurosecretory vesicles in rat pituitary. *Science* **222**, 1137–1139.

YAMAMOTO, T., OHNO, M., UEKI, S. (1988). A selective $\kappa$-opioid agonist, U-50,488H, blocks the development of tolerance to morphine analgesia in rats. *European Journal of Pharmacology* **156**, 173–176.

ZHAO, B.-G., CHAPMAN, C. & BICKNELL, R. J. (1988*a*). Functional $\kappa$-opioid receptors on oxytocin and vasopressin nerve terminals isolated from the rat neurohypophysis. *Brain Research* **462**, 62–66.

ZHAO, B.-G., CHAPMAN, C. & BICKNELL, R. J. (1988*b*). Opioid-noradrenergic interactions in the neurohypophysis I. Differential opioid receptor regulation of oxytocin, vasopressin and noradrenaline release. *Neuroendocrinology* **48**, 16–24.

ZHAO, B.-G., CHAPMAN, C., BROWN, D. & BICKNELL, R. J. (1988*c*). Opioid-noradrenergic interactions in the neurohypophysis II. Does noradrenaline mediate the action of endogenous opioids on oxytocin and vasopressin release? *Neuroendocrinology* **48**, 25–31.