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MORPHINE ACTIONS ON SUPRAOPTIC OXYTOCIN NEURONES IN ANAESTHETIZED RATS: TOLERANCE AFTER I.C.V. MORPHINE INFUSION

BY K. M. PUMFORD, G. LENG* AND J. A. RUSSELL†

*From the Department of Physiology, University Medical School, Teviot Place, Edinburgh EH8 9AG and the *Department of Neuroendocrinology, AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge CB2 4AT*

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SUMMARY

1. The effects of acute I.V. administration of morphine on putative oxytocin neurones of the supraoptic nucleus were studied in urethane-anaesthetized female rats which had been exposed to I.C.V. infusion of morphine (up to 50 $\mu g\ h^{-1}$) or vehicle for 5 days.

2. In vehicle-infused rats, I.V. morphine inhibited the spontaneous activity of six out of seven putative oxytocin neurones. Increasing doses of morphine were given, from 1 $\mu g\ kg^{-1}$ to 5 $mg\ kg^{-1}$. The median cumulative threshold dose to produce significant inhibition was 20 $\mu g\ kg^{-1}$ (seven cells in six rats); six out of seven cells were inhibited at 161 $\mu g\ kg^{-1}$. The highest doses tested inhibited by $\sim 90\%$ (excluding one unaffected cell). Inhibition was fully reversed by I.V. naloxone without overshoot, indicating a lack of acute dependence.

3. Injection of morphine I.C.V. inhibited firing at doses that were ineffective by I.V. injection and the effects of I.C.V. morphine were reversed by I.V. naloxone.

4. Acute morphine (500 $\mu g\ kg^{-1}$ I.V.) reduced the plasma concentration of oxytocin, measured after 15 min by specific radioimmunoassay, by 34% ($n = 14$).

5. In lactating rats I.C.V. injection of morphine (1–2 μg) inhibited the activity of supraoptic neurones identified as oxytocinergic by their responses to suckling.

6. In seventeen rats infused with I.C.V. morphine the initial firing rate of twenty-eight spontaneously active, non-phasic neurones was significantly less, by 24%, than thirty-four similar cells in control rats, indicating incomplete tolerance to I.C.V. morphine. Morphine (up to 161 $\mu g\ kg^{-1}$ given I.V.) inhibited none of nine active non-phasic neurones ($P < 0.01$ compared to control rats), but at higher doses inhibited four of nine cells; the overall median threshold cumulative dose (1660 $\mu g\ kg^{-1}$) was significantly greater than in vehicle-infused controls, indicating tolerance to I.V. morphine. In contrast with control rats, some cells (5/9) were modestly excited by low doses of morphine. Naloxone (5 $mg\ kg^{-1}$ I.V.) produced withdrawal excitation: the firing rate of putative oxytocin neurones increased to $\sim 260\%$ of the pre-I.V. morphine value, indicating dependence in mechanisms regulating the firing rate of these neurones.

† To whom correspondence should be sent.

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7. In morphine-infused rats, the basal firing rate of nineteen phasically active, putative vasopressin supraoptic neurones was not different in nineteen phasic cells in controls ($6{\cdot}4 \pm 0{\cdot}7$ *vs.* $4{\cdot}2 \pm 0{\cdot}6$ Hz).

8. Thus morphine potently inhibits the firing of magnocellular oxytocin neurones in the female rat, inhibiting oxytocin secretion. Morphine tolerance and dependence develop during I.C.V. infusion of morphine for 5 days. Similar tolerance to and dependence upon endogenous opioids during pregnancy may be important in the preparation of oxytocin neurones for parturition.

INTRODUCTION

Given acutely by peripheral or intracerebroventricular (I.C.V.) injection morphine inhibits oxytocin secretion in the rat (Evans & Olley, 1988; Grell, Christensen & Fjalland, 1988; Russell, Gosden, Humphreys, Cutting, Fitzsimons, Johnston, Liddle, Scott & Stirland, 1989). The effects of opiates are powerful: 2–4 μg of I.C.V. morphine will inhibit the milk ejection reflex and 18 μg slows established parturition for several hours, in both cases by inhibiting oxytocin secretion (Clarke, Wood, Merrick & Lincoln, 1979; Russell *et al.* 1989). Given peripherally, μ-type opioid agonists inhibit the milk ejection reflex with a potency order similar to their analgesic actions (Clarke & Wright, 1984).

In urethane-anaesthetized lactating rats, oxytocin neurones respond to suckling by firing high-frequency bursts of action potentials at intervals of several minutes, each burst signalling the secretion of a bolus of oxytocin (Lincoln & Wakerley, 1974). Between bursts, and in non-suckled rats, oxytocin neurones fire continuously, distinct from the phasic firing pattern of vasopressin-secreting neurones (Poulain & Wakerley, 1982). It has been reported previously that acute I.C.V. injection of morphine affects neither the background firing rate nor the milk-ejection bursts of oxytocin neurones in suckled lactating rats, although the milk ejection reflex is inhibited (Clarke *et al.* 1979), suggesting that morphine acts at the level of the neurohypophysis to inhibit oxytocin secretion. However, while agonists selective for κ-receptors act at the level of the neurohypophysis to inhibit electrically stimulated oxytocin secretion, we have not found morphine to act at this site (Coombes & Russell, 1988), and μ-agonists other than morphine are also inactive at the neurohypophysis *in vitro* (Zhao, Chapman & Bicknell, 1988).

Chronic morphine treatment typically leads to two related phenomena: tolerance and dependence. Tolerance is the loss of the initial effect of the opiate or a requirement for a larger dose to produce the same effect (Jaffe, 1985). Dependence reflects adaptation of the system affected so that on opiate withdrawal, frequently induced experimentally by naloxone, there is not only reversal of the initial opiate effect but also 'overshoot'; thus, if the initial opiate action is inhibitory, then naloxone produces withdrawal excitation. Dependence develops in the mechanisms that regulate the electrical activity of oxytocin neurones since naloxone-induced withdrawal from chronic morphine treatment results in a large increase in their firing rate, and in consequence a large increase in oxytocin secretion (Bicknell, Leng, Lincoln & Russell, 1988*a*). The development of tolerance during chronic I.C.V. infusion of morphine has been presumed from the resumption of the milk ejection reflex, and from normal circulating oxytocin levels in lactating rats (Bicknell *et al.*

1988*a*; Rayner, Robinson & Russell, 1988). However, it was apparent in our previous study that tolerance to morphine was not absolute, since I.V. injection of morphine (5 mg kg^{-1}) could still inhibit the milk ejection reflex (Rayner *et al.* 1988). In the present study we have sought to confirm and quantify tolerance to I.V. morphine in virgin female rats treated chronically with I.C.V. morphine by comparing the potency of morphine in these rats and in morphine-naive controls. Some of the results in the present paper have previously been published in abstract form (Leng, Pumford & Russell, 1990).

METHODS

Electrophysiological studies in vehicle- and morphine-infused rats

Virgin female Sprague–Dawley rats (body weight ~ 270 g), kept in our standard conditions, were anaesthetized with ether and a stainless-steel cannula was inserted into a lateral cerebral ventricle and connected via polythene tubing to a subcutaneously implanted osmotic minipump (Alzet 2001) to deliver vehicle (sterile, pyrogen-free distilled water, 1 $\mu l\ h^{-1}$) or morphine sulphate (10 $\mu g\ h^{-1}$ for 40 h, 20 $\mu g\ h^{-1}$ for 40 h, finally 50 $\mu g\ h^{-1}$ for 40 h) (see Rayner *et al.* (1988) for full details). On the sixth day of infusion, the rats were anaesthetized with urethane (ethyl carbamate, 1·25 g kg^{-1} I.P.), a tracheal cannula was inserted, and a jugular vein was cannulated. The right supraoptic nucleus and the neurohypophysial stalk were exposed by ventral surgery. A bipolar stimulating electrode (Rhodes Medical Instruments, SNE-100) was placed on the neurohypophysial stalk, and extracellular recordings were made with a glass micropipette (20–40 MΩ, filled with 0·9% saline) from supraoptic neurones antidromically identified by stimulating the neurohypophysial stalk (Bicknell *et al.* 1988*a*). Antidromically identified neurones that showed alternating periods of activity at > 3 Hz and quiescence for > 5 s were classified as phasically firing and thus as vasopressin neurones (Poulain & Wakerley, 1982). For active non-phasic neurones, the firing rate was recorded for at least 10 min, then morphine sulphate was given I.V. (as solution in 500 $\mu l\ kg^{-1}$ 0·9% NaCl) in increasing doses at 10 min intervals (1, 10, 50, 100, 500 and 1000 $\mu g\ kg^{-1}$, and 5 mg kg^{-1}). If, after a dose of morphine, a unit failed to fire spontaneously for at least 10 min or the upper limit of the test dose range was reached, then naloxone was administered (up to 5 mg kg^{-1} I.V.). The mean firing rate for the 10 min following each injection was calculated and a significant effect was defined as separation of the 95% confidence bands for the post-drug mean from those for the control period mean. If a cell was lost, data from another cell from the same rat were included only if the next two doses of morphine were ineffective, indicating that the threshold dose had not been reached (one of seven cells in vehicle-infused and three of nine cells in I.C.V. morphine-infused rats).

To study actions of acute I.C.V. morphine, virgin female Wistar rats (body weight ~ 300 g) were anaesthetized with urethane (1·25 g kg^{-1} I.P.). A guide cannula was inserted into the right lateral cerebral ventricle (Rayner *et al.* 1988) and oxytocin neurones were recorded as above. An injection cannula, connected via a polythene tube to a microsyringe filled with morphine sulphate in 0·9% saline (0·1–0·5 $\mu g\ \mu l^{-1}$), was inserted via the guide cannula. After recording the activity of a neurone for 10 min, morphine was injected I.C.V. in doses ranging from 0·1 to 1·0 μg in a volume of 1 μl. For some neurones, excitation by I.V. cholecystokinin (CCK 8S (26–33), Peninsula Laboratories, 20 $\mu g\ kg^{-1}$) and absence of inhibition by I.V. phenylephrine (L-phenylephrine HCl, Sigma; 10 μg in 0·1 ml 0·9% NaCl) were used to confirm their identity, since CCK excites oxytocin neurones and not vasopressin neurones and phenylephrine inhibits vasopressin neurones but not oxytocin neurones (Harris, 1979; Renaud, Tang, McCann, Stricker & Verbalis, 1987).

Electrophysiological studies on lactating rats

Wistar dams, separated from all but one pup overnight on day 8 post-partum, were anaesthetized with urethane (1·1 g kg^{-1} I.P.). An inguinal mammary gland was cannulated to record intramammary pressure (see Rayner *et al.* 1988), a femoral vein and jugular vein were cannulated to inject oxytocin (Syntocinon, Sandoz Pharmaceuticals, 10 mU ml^{-1} 0·9% saline), and naloxone, respectively. Two 21 g cannulae (as used for chronic I.C.V. infusion) were inserted through the dorsal surface of the skull 1·6 mm lateral and 0·6 mm caudal, and 2·0 mm lateral and 3·0 mm caudal to bregma respectively, so their tips lay in the lateral ventricle. Each cannula was connected to a microsyringe via polythene tubing filled with morphine sulphate (1 $\mu g\ \mu l^{-1}$ 0·9% NaCl) for the

rostral cannula, or oxytocin (1 mU μl^{-1}) for the caudal cannula. A stimulating electrode (Rhodes Medical, SNEX 100) was placed stereotaxically upon the neurohypophysial stalk and a microelectrode lowered stereotaxically to record the electrical activity of antidromically identified supraoptic neurones (see Dyball & Leng, 1986). Oxytocin neurones were identified by their firing a high-frequency burst of action potentials about 12 s before each milk ejection, evident as a sharp rise in intramammary pressure (Lincoln & Wakerley, 1974). Propranolol was injected (250 μg kg^{-1}, I.V., in 0·9% saline) to facilitate the milk ejection reflex (Juss & Wakerley, 1981), and eight to 10 pups were applied to the nipples to suckle. If milk ejections did not begin within 15 min, 1 mU oxytocin (2 ng) was injected I.C.V. (in 1 μl) to induce the reflex (Freund-Mercier & Richard, 1984).

Blood sampling for radioimmunoassay of oxytocin in peripheral plasma

To measure effects of I.V. morphine on oxytocin secretion, twenty-eight untreated virgin female Sprague–Dawley rats (body weight ~ 250 g) were anaesthetized with urethane (1·25 g kg^{-1} I.P.). A femoral artery and vein were cannulated for blood sampling and replacement, and a jugular vein for drug injection. Two hours after surgery a 0·3 ml blood sample was withdrawn and replaced with 0·9% saline; a second sample was withdrawn 10 min later and replaced with the cells from the previous sample resuspended in 0·9% saline; 5 min later either morphine (500 μg kg^{-1}, 1 mg ml^{-1} in 0·9% saline) or an equivalent volume of vehicle was injected. Further blood samples were taken, and replaced with resuspended blood cells, 5 and 15 min after the I.V. injection. Plasma samples were separated and stored at −20 °C, prior to radioimmunoassay for oxytocin in a single assay following a method described previously (Higuchi, Honda, Fukuoka, Negoro & Wakabayashi, 1985; Leng, Mansfield, Bicknell, Blackburn, Brown, Chapman, Dyer, Hollingsworth, Shibuki, Yates & Way, 1988). The detection limit was 4·4 pg ml^{-1}.

RESULTS

Electrophysiological properties of supraoptic neurones in vehicle- and morphine-infused rats

Control period recordings were obtained from fifty-nine cells in seventeen vehicle-infused rats and from seventy-two cells in seventeen morphine-infused rats. In morphine-infused rats, the proportions of the different types of neurone (phasic, continuous, silent) were not significantly different from those in the control group (Table 1). The mean basal firing rate of active non-phasic cells recorded before any I.V. injection of morphine was $4·55 \pm 0·61$ Hz ($n = 30$ cells in fourteen rats) in the vehicle-infused rats, and $3·47 \pm 0·72$ Hz in the morphine-infused rats ($n = 28$ cells in seventeen rats), $P < 0·05$, Wilcoxon test for independent samples. This difference was a result of reduced firing rate of active cells firing at < 2 Hz (Table 1). Compiling the activity of continuous, slow irregular and silent neurones (in correct proportion), the mean firing rate of putative oxytocin neurones in the morphine-infused rats was $2·49 \pm 0·54$ Hz ($n = 39$) and in the vehicle-infused rats was $4·02 \pm 0·59$ Hz ($n = 34$), $P < 0·01$ (Wilcoxon test for independent samples).

For active non-phasic neurones there were no significant differences in the antidromic activation latency or threshold between the vehicle- and morphine-infused rats (Table 1). For phasic neurones, basal firing rate was greater in the I.C.V. morphine group (by 52%; Table 1), and their antidromic activation latency was less (Table 1), but antidromic activation threshold was similar in vehicle- and morphine-infused rats.

Actions of I.V. morphine in vehicle-infused rats

Morphine injections at low doses were followed by significant sustained inhibition of six out of seven continuously active neurones (from seven different rats). The median cumulative threshold dose to produce significant inhibition was 20 μg kg^{-1};

TABLE 1. Electrophysiological characteristics of antidromically identified supraoptic neurones in I.C.V. vehicle- or I.C.V. morphine-infused rats

Neurone type (firing pattern) ...	Phasic		Continuous (> 2 Hz)		Slow irregular (< 2 Hz)		Silent	
I.C.V. treatment	Vehicle	Morphine	Vehicle	Morphine	Vehicle	Morphine	Vehicle	Morphine
Percentage of neurones	35·6	37·5	42·4	29·2	15·3	18·1	6·8	15·3
n	21	27	25	21	9	13	4	11
Basal firing rate (Hz)	4·2 ± 0·6	6·4 ± 0·7	5·5 ± 0·7	5·7 ± 1·0	1·4 ± 0·1	0·9 ± 0·1**	0	0
n	19	19	23	15	7	13	4	11
			Continuous + slow-irregular					
			Vehicle			Morphine		
Antidromic activation latency (ms)	14·6 ± 0·9	11·7 ± 0·5*	13·7 ± 0·8			13·0 ± 1·0	18·8	13·0 ± 1·2
n	12	22	24			23	1	10
Antidromic activation threshold voltage (V)	27·8 ± 4·1	26·7 ± 5·6	27·8 ± 5·7			38·1 ± 5·9	28·0	15·0 ± 4·2
n	9	17	12			11	1	4

Firing rate was measured in 30 s bins over 10 min periods. Activity pattern was determined from continuous records of action potential discharge; phasic neurones were alternately active at > 3 Hz and quiescent for periods of > 5 s. Other properties were determined before I.V. injection of drugs; continuous and slow-irregular neurones were combined since both populations were subsequently tested with drugs. Values are means ± S.E.M. *n* is the number of cells. Statistical comparisons: *, $P < 0.05$, **, $P < 0.01$ *vs.* I.C.V. vehicle group (Wilcoxon).

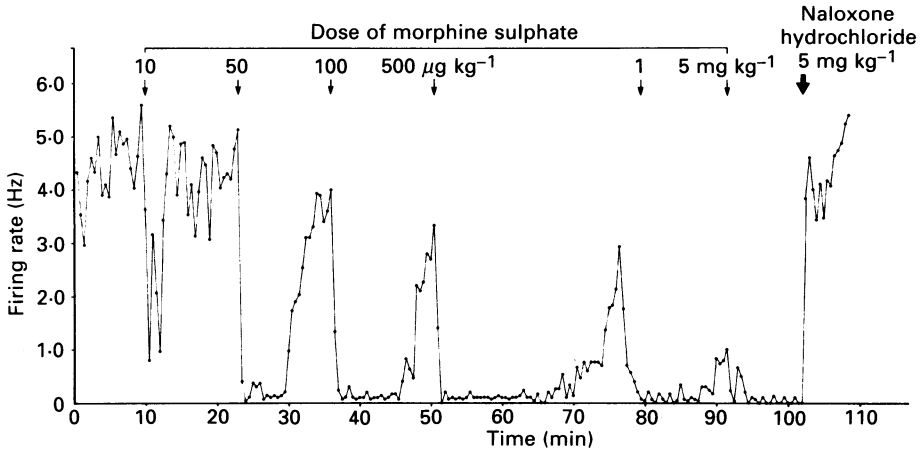

Fig. 1. Extracellular recording of the firing rate of a continuously active putative oxytocin neurone in the supraoptic nucleus of a morphine-naive urethane-anaesthetized virgin female rat. The rat had received I.C.V. vehicle infusion for 5 days. The neurone was antidromically identified by electrical stimulation of the neurohypophysial stalk. Firing rate was measured in 30 s bins. Sensitivity to morphine was tested by giving increasing I.V. doses of morphine sulphate. The lowest dose (10 μg kg^{-1}), decreased the firing rate of the neurone, but only for 2 min; a further dose of 50 μg kg^{-1} markedly depressed firing rate for 6 min; further increase in dose prolonged the maximal inhibitory effect. The cumulated effects of the I.V. morphine were fully reversed by naloxone (5 mg kg^{-1} I.V.), which promptly restored the firing rate to the pre-morphine value.

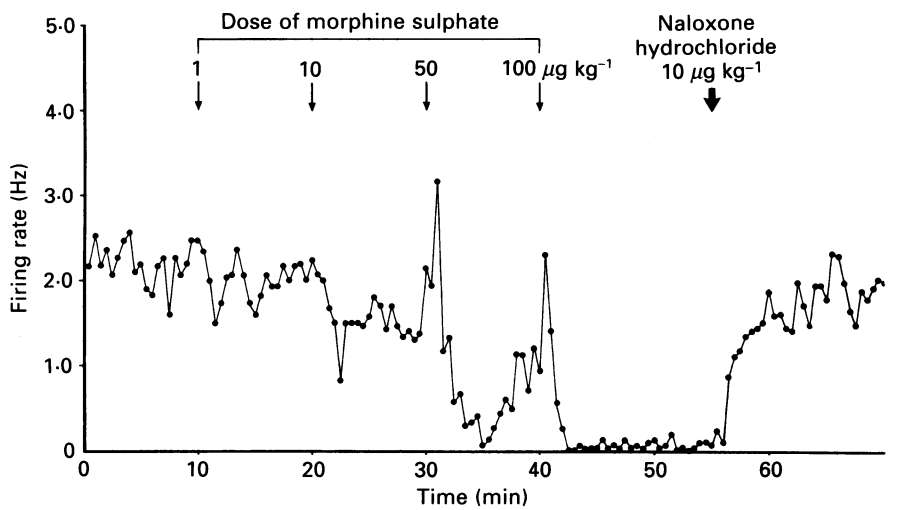

Fig. 2. Extracellular recording of the firing rate of a putative oxytocin neurone in the supraoptic nucleus of a morphine-naive urethane-anaesthetized virgin female rat. Details as in Fig. 1. Although this neurone was transiently excited immediately after two of the morphine injections (50 and 100 μg kg^{-1}) it was then strongly inhibited. The threshold cumulative inhibitory dose was 61 μg kg^{-1}, and it was silenced only after a further 100 μg kg^{-1}; essentially complete inhibition was then sustained for at least 12 min. A low dose of naloxone (10 μg kg^{-1} I.V.) fully reversed the inhibition by morphine, without overshoot.

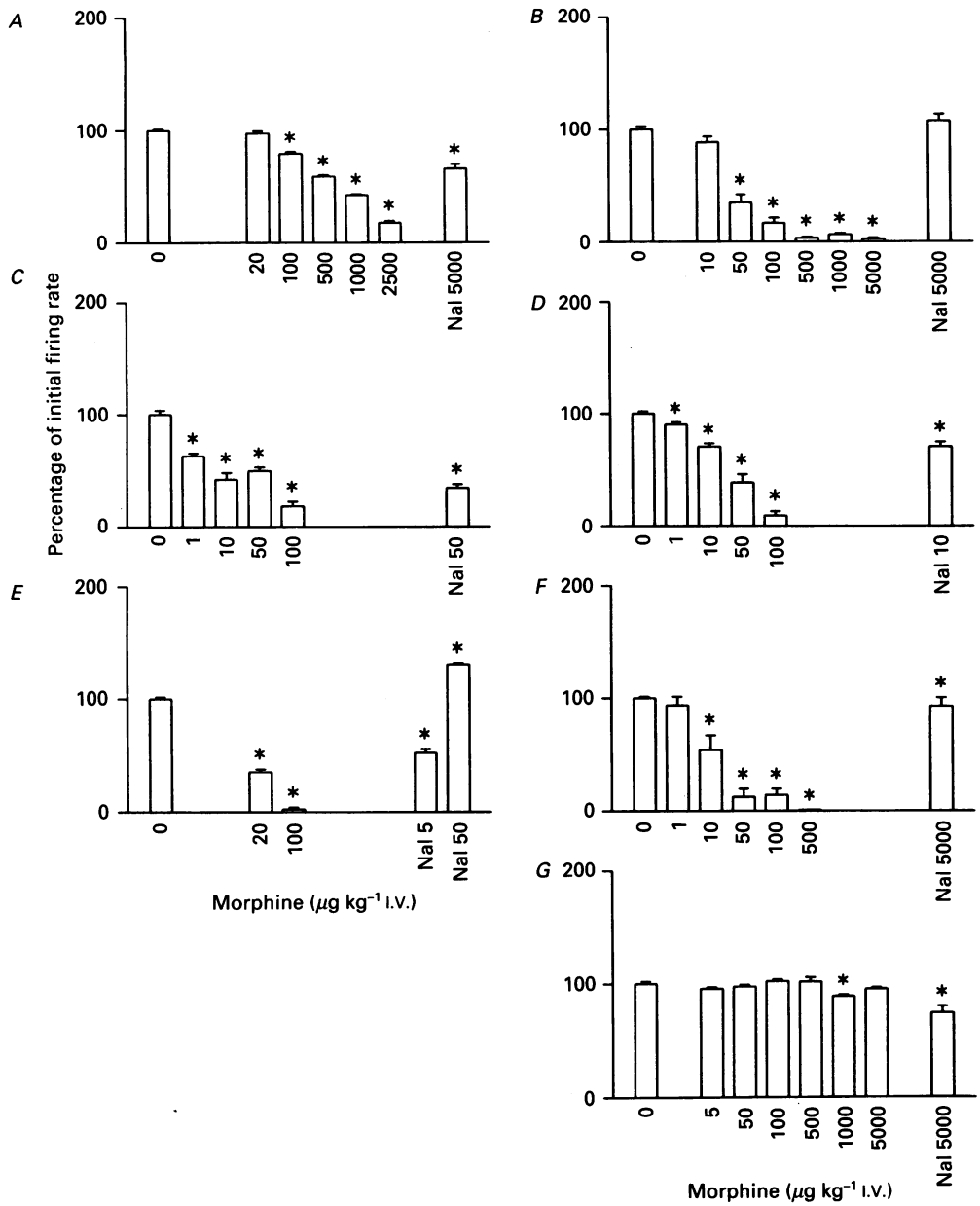

Fig. 3. Summary of effects of I.V. morphine on putative oxytocin neurones in the supraoptic nucleus of morphine-naive urethane-anaesthetized virgin female rats. The rats had received I.C.V. vehicle infusion for 5 days. Increasing doses of morphine were given I.V., 10 min apart. Each histogram represents one neurone, each from one rat. Mean ± S.E.M. firing rate during each 10 min was calculated from 30 s bins and expressed as percentage of the initial firing rate. Significant changes (separation of 95% confidence bands) are indicated by asterisks, and significant drug effects were inferred from dose-related responses. Naloxone, (Nal, dose in micrograms) reversibility was tested at the end of the series of morphine injections. All neurones except one (*G*) were significantly inhibited by 161 $\mu g\ kg^{-1}$ morphine, or less. Initial firing rates were: *A*, 9·30 Hz; *B*, 4·40 Hz; *C*, 2·34 Hz; *D* 2·21 Hz; *E*, 3·79 Hz; *F*, 4·55 Hz; *G*, 14·04 Hz. Neurone *G* may have been a fast-firing vasopressin or oxytocin neurone.

the six responding cells were all markedly inhibited by a cumulative dose of 161 μg kg^{-1} (Fig. 3; Table 2); the initial mean firing rate of the non-responding neurone was 14·04 Hz (fast enough possibly to be an excited vasopressin neurone). The effect of I.V. morphine was usually evident within 30 s (Fig. 1); with the highest doses used firing rate was reduced by a mean of 91·6 ± 3·3 % over the 10 min following

TABLE 2. Responses of non-phasic active supraoptic neurones to I.V. morphine and naloxone in I.C.V. vehicle- or I.C.V. morphine-infused rats

Chronic treatment group	I.C.V. vehicle ($n = 6$)	I.C.V. morphine ($n = 8$)
Initial firing rate (Hz)	4·43 ± 1·06[a] ($n = 6$)	3·2 ± 1·18 ($n = 9$)
Effects of I.V. morphine:		
Proportion of neurones inhibited	6/7	4/9[b]
Median threshold inhibitory dose (μg kg^{-1}) (range)	20[a](1–162)	1660**(661–> 6661)[c]
Maximum inhibition of firing rate (%)	91·6 ± 3·3[d] ($n = 6$)	54·2 ± 10·8[e]* ($n = 5$)
Effects of I.V. naloxone (5 mg kg^{-1}):		
Post-naloxone firing rate (% of basal)	97·7 ± 12·4 ($n = 4$)	260·4 ± 31·3† ($n = 4$)

Supraoptic neurones were antidromically identified and firing rate was measured in 30 s bins over 10 min periods; phasically active neurones were excluded. Increasing I.V. doses of morphine were given at 10 min intervals, followed finally by I.V. naloxone. Data for cells shown in Figs 3 and 7.

[a] Excluding one non-responding cell firing at 14·04 Hz.

[b] Unaffected cells were tested with 161 (one cell), 661 (two cells), 6661 (one cell) and 9100 μg kg^{-1} (one cell).

[c] For the inhibited cells, the threshold inhibitory doses were 661 (one cell), 1661 (one cell), 6600 (one cell) and 6661 μg kg^{-1} (one cell).

[d] Maximum doses used were 161 (three cells), 661 (one cell) and 6661 μg kg^{-1} (two cells).

[e] Maximum dose used was 6661 μg kg^{-1} in all five cells.

Values given are means ± S.E.M. n is the number of rats. Statistical comparisons: *, $P < 0·02$, ** $P < 0·01$ *vs.* I.C.V. vehicle group (Wilcoxon); † $P < 0·01$ *vs.* I.C.V. vehicle group (Student's t test) and *vs.* basal value (paired t test).

injection ($n = 6$ cells). The effects of morphine were fully reversed by I.V. naloxone (5 mg kg^{-1}) (Figs 1, 2 and 3; Table 2). Overall there was no evidence of enhanced excitation on antagonism of morphine with naloxone.

Actions of acute I.C.V. morphine

Given the potency of I.V. morphine in suppressing oxytocin neurone activity, previous reports of the ineffectiveness of I.C.V. morphine on spontaneous activity appeared paradoxical (see Introduction). We therefore reinvestigated the effects of I.C.V. morphine in virgin animals.

In each of five experiments on virgin female rats, the spontaneous activity of a continuously active, putative oxytocin neurone in the supraoptic nucleus was significantly inhibited, to less than 50% of the initial firing rate, following I.C.V. injection of 0·1–0·5 μg morphine. The effects were in each case completely reversed by I.V. injection of 100–250 μg kg^{-1} naloxone (Fig. 4). In three lactating rats, six supraoptic neurones, identified as oxytocin neurones by their bursting behaviour associated with reflex milk ejection, were recorded during I.C.V. injections of 1–2 μg

morphine. The spontaneous activity of each cell was significantly reduced for at least 5 min following the injection, and for five of the six cells the spontaneous activity was eliminated. In three out of six cells, bursting activity in response to suckling persisted despite inhibition of their continuous activity by morphine, although the

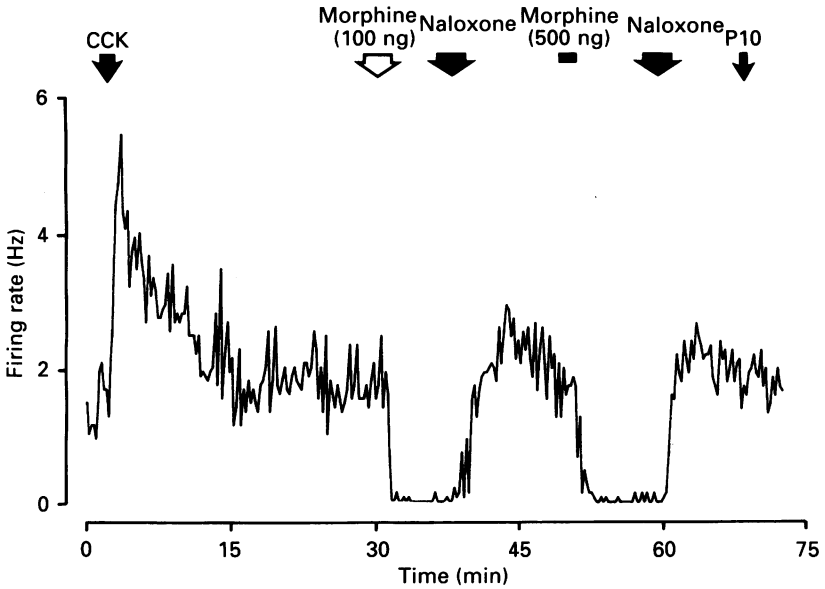

Fig. 4. Firing rate of a continuously active, putative oxytocin neurone in the supraoptic nucleus, recorded extracellularly, in a urethane-anaesthetized virgin female rat. Firing rate was averaged in 30 s bins. This neurone, firing initially at $1{\cdot}36 \pm 0{\cdot}15$ Hz, was identified as oxytocinergic by its excitatory response to I.V. injection of cholecystokinin (CCK, 20 μg kg^{-1}) and by its lack of response to I.V. phenylephrine (P10, 10 μg), injected at the end of the experiment. Morphine sulphate solution was injected via an implanted cannula into a lateral cerebral ventricle. Morphine (100 ng) almost completely inhibited firing of the neurone; the inhibition was fully reversed, without overshoot, by injection of naloxone (100 μg kg^{-1}, I.V.). Further slow I.C.V. injection of 500 ng morphine decreased firing rate stepwise to the same level as before; the effects of the cumulative dose of I.C.V. morphine were completely and rapidly reversed by naloxone (100 μg kg^{-1}, I.V.).

number of spikes per burst was markedly reduced in one cell (Fig. 5). Naloxone administered I.V. at doses of 250–1000 μg kg^{-1} restored firing rate to normal in each of four cells thus tested.

Effects of I.V. morphine on supraoptic neurones in morphine-infused rats

Only four out of nine non-phasic neurones were significantly inhibited by I.V. morphine (Figs 6 and 7; Table 2). The overall median threshold inhibitory dose was 1660 μg kg^{-1} ($n = 9$ cells), significantly greater ($P = 0{\cdot}01$, Wilcoxon) than in vehicle-infused rats; the proportions of continuous cells in the two groups inhibited by < 161 μg kg^{-1} morphine were significantly different (6/7 *vs.* 0/9, $p < 0{\cdot}01$). At the highest I.V. morphine doses used, mean firing rate was reduced by only $54{\cdot}2 \pm 10{\cdot}8$ %, significantly less than the maximal inhibition in vehicle-infused rats (Table 2).

At low doses of I.V. morphine, five continuous neurones in morphine-infused rats

were excited (maximally to 128·6 ± 8·9% of initial firing rate at 11, 61, 161, 500 and 661 μg kg^{-1} $n = 5$), but at higher doses of I.V. morphine these neurones, where tested (3/3), were also inhibited (Figs 6 and 7).

Naloxone (5 mg kg^{-1} I.V.) produced withdrawal excitation (Figs 6 and 7):

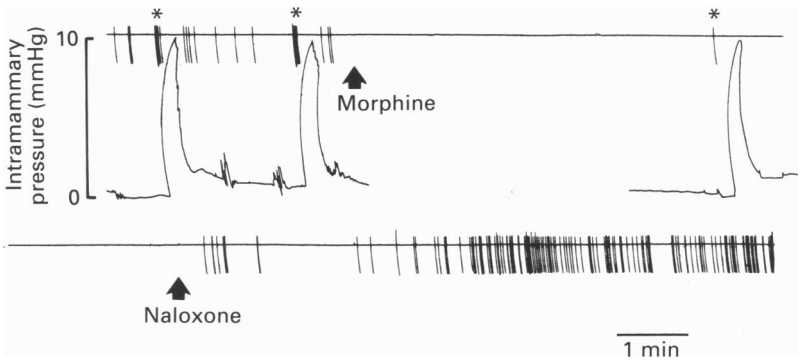

Fig. 5. Action potentials of an oxytocin neurone in the supraoptic nucleus of a urethane-anaesthetized lactating rat. Simultaneous extracellular recording of action potentials (top record) and intramammary pressure (middle record) during suckling by eight to ten pups. The neurone was antidromically identified by electrical stimulation of the neurohypophysial stalk. Prior to the segments of recording shown, oxytocin (2 ng) was injected into a lateral cerebral ventricle to promote the milk ejection reflex. The upper left-hand part of the record shows two high-frequency burst discharges of the neurone, ~ 2 min apart (asterisks); the sharp increases in intramammary pressure ~ 12 s after each burst indicate secretion of a pulse of oxytocin as a result of synchronous bursting of magnocellular oxytocin neurones, effecting a reflex milk ejection. Injection into a lateral cerebral ventricle (I.C.V.) of morphine (2 μg in 2 μl 0·9% NaCl) completely inhibited the background continuous firing of this neurone; a further sharp increase in intramammary pressure (right-hand side of record) indicates reflex secretion of a further pulse of oxytocin. Immediately prior to this increase in intramammary pressure the recorded neurone fired only a single action potential (asterisk); thus morphine had inhibited the burst firing of this neurone during suckling, but the burst firing of other oxytocin neurones must have been relatively unaffected, as found previously (Clarke *et al.* 1979), to secrete sufficient oxytocin to effect a reflex milk ejection. The background activity of the recorded neurone was fully restored by injection of naloxone HCl, (0·5 mg kg^{-1} I.V.).

compared with the pre-I.V. morphine control period, the firing rate was markedly increased after naloxone, in contrast to the simple return of firing rate after naloxone to the initial value in the vehicle-infused rats (Table 2).

Radioimmunoassay data: morphine and oxytocin secretion

The averaged basal plasma concentration of oxytocin in urethane-anaesthetized rats was 11·8 ± 2·3 pg ml^{-1} (mean ± S.E.M.; $n = 14$ rats); after I.V. injection of 500 μg kg^{-1} morphine, the concentration was unchanged at 5 min (10·8 ± 2·3 pg ml^{-1}), but was decreased at 15 min after morphine to 7·8 ± 2·0 pg ml^{-1} ($P < 0·03$, one-tailed paired t test). The mean change 15 min after morphine was −4·0 ± 1·9 pg ml^{-1}. In controls (not given I.V. morphine) concentrations of oxytocin in plasma at times corresponding to those in the I.V. morphine group did not change; these values were

$17{\cdot}5 \pm 4{\cdot}4$, $15{\cdot}5 \pm 3{\cdot}9$ and $18{\cdot}3 \pm 4{\cdot}8$ pg ml^{-1} ($n = 14$ rats); the mean change 15 min after I.V. vehicle was $+0{\cdot}8 \pm 2{\cdot}3$ pg ml^{-1}.

DISCUSSION

The present results show that I.V. morphine inhibits oxytocin secretion and, at low doses, inhibits the firing rate of putative oxytocin neurones in the supraoptic nucleus

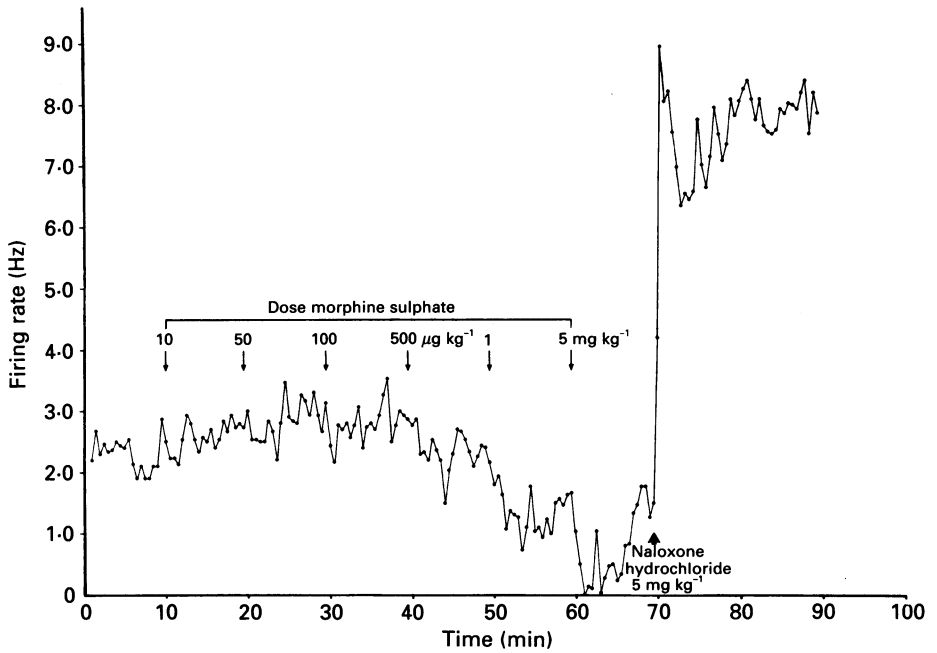

Fig. 6. Firing rate of a continuously active, putative oxytocin neurone in the supraoptic nucleus of a urethane-anaesthetized virgin female rat after I.C.V. morphine infusion for 5 days. Details as in Fig. 1. Firing rate was significantly greater after morphine injection (50 and 100 μg kg^{-1}) than in the initial control period. Further doses of I.V. morphine up to a total cumulative dose of 660 μg kg^{-1} did not significantly affect firing rate; after a further 1 mg kg^{-1} morphine firing rate was inhibited, and after a further 5 mg kg^{-1} the neurone was almost silenced for a few minutes. Naloxone (5 mg kg^{-1} I.V.) rapidly produced withdrawal hyperexcitation; firing rate increased to $\sim 350\%$ of the firing rate before I.V. morphine.

of virgin female rats. The dose required to inhibit firing rate compares satisfactorily with the peripheral dose in lactating rats required to inhibit the milk ejection reflex (Clarke & Wright, 1984; Russell & Spears, 1984). Since I.V. morphine, even at high doses (up to 5 mg kg^{-1}), does not inhibit oxytocin secretion elicited by electrical stimulation of the neurohypophysial stalk in lactating rats (Coombes & Russell, 1988), the inhibitory effect of I.V. morphine appears to be mainly the result of inhibition of the firing rate of oxytocin neurones (in contradiction to earlier suggestions; Clarke *et al.* 1979). Morphine is highly selective for μ-type opioid receptors (Magnan, Paterson, Tavani & Kosterlitz, 1982), and its effectiveness at low

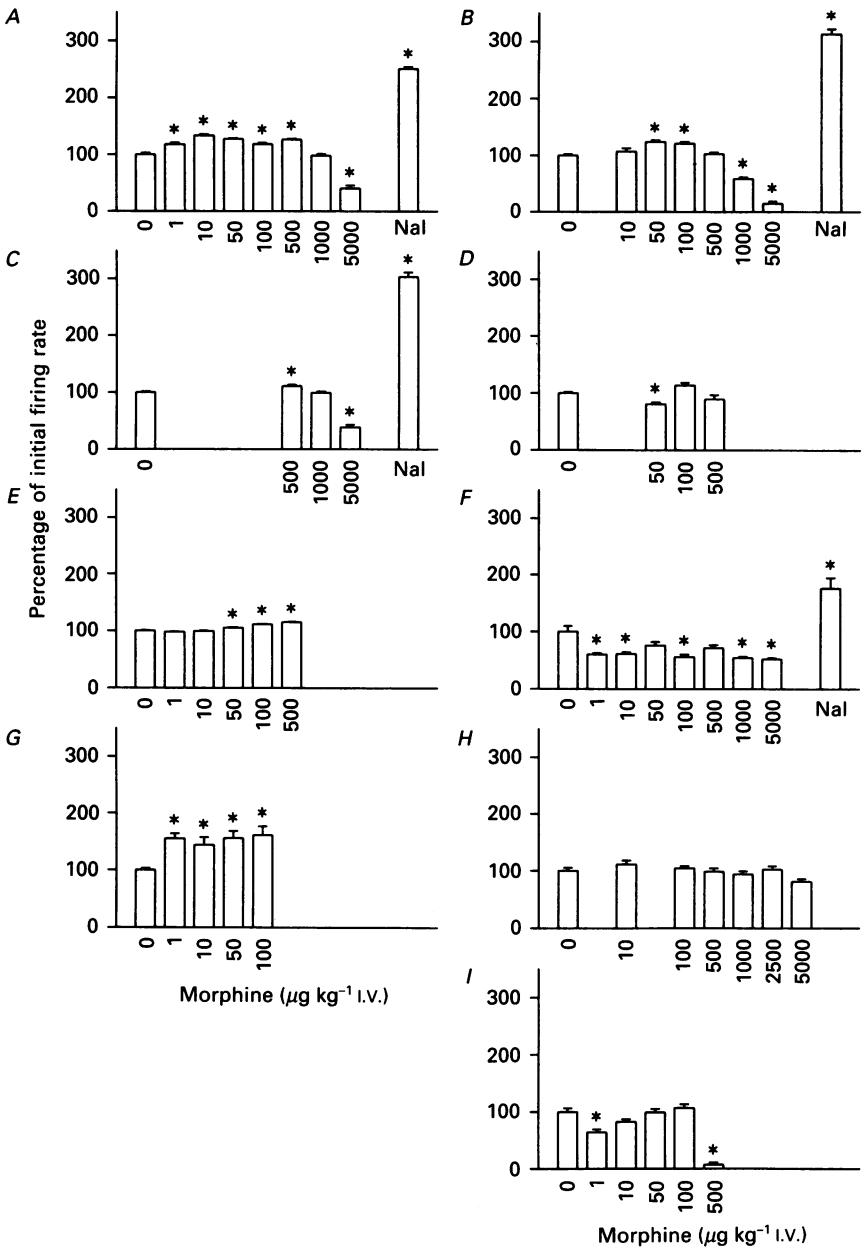

Fig. 7. Summary of effects of I.V. morphine on putative oxytocin neurones in the supraoptic nucleus of urethane-anaesthetized virgin female rats after I.C.V. morphine infusion for 5 days. As in Fig. 3, increasing doses of morphine were given I.V., 10 min apart. Each histogram represents one neurone, each from one rat. Mean ± S.E.M. firing rate during each 10 min was calculated from 30 s bins and expressed as percentage of the initial firing rate. Significant changes (separation of 95% confidence bands) are indicated by asterisks, and significant drug effects inferred from dose-related responses. Naloxone (Nal, 5 mg kg^{-1} I.V.) reversibility was tested at the end of the series of morphine injections. No neurones were inhibited by 161 μg kg^{-1} morphine, or less, in contrast with

concentrations (11–61 μg kg^{-1}) is consistent with its acting on μ-receptors. Similarly, naloxone is 10 and 15 times more avid for μ-receptors than for κ- or δ-receptors, respectively (Kosterlitz, 1985), and its effectiveness in antagonizing, at low concentrations (e.g. 10 μg kg^{-1}), inhibition produced by much larger doses of morphine (Fig. 2) is consistent with its acting to displace morphine from μ-receptors (Kosterlitz, 1985). The inhibitory actions of opioids on oxytocin secretion at the level of the neurohypophysis are mediated via κ-receptors (Herkenham, Rice, Jacobson & Rothman, 1986; Coombes & Russell, 1988; Zhao *et al.* 1988), and the increase in oxytocin secretion after naloxone administration to morphine-naive rats shown in previous studies (Bicknell, Grossmann, Leng & Russell, 1986; Bicknell *et al.* 1988*a*) is explained by antagonism of endogenous opioid action, probably that of dynorphins, co-released with vasopressin from terminals adjacent to those releasing oxytocin (Watson, Akil, Fischli, Goldstein, Zimmerman, Nilaver & van Wimersma Greidanus, 1982; Whitnall, Gainer, Cox & Molineaux, 1983; Summy-Long, Miller, Rosella-Dampman, Hartman & Emmert, 1984; Zhao *et al.* 1988). In contrast, circumstances have not yet been identified in which endogenous opioid pathways inhibit the firing rate of oxytocin neurones: naloxone alone has no effect on the electrical activity of supraoptic oxytocin neurones in lactating rats (Bicknell *et al.* 1988*a*) or in osmotically stimulated rats (Shibuki, Leng & Way, 1988).

Morphine could act at several sites to influence the firing rate of oxytocin neurones. The supraoptic nucleus contains μ-receptors (Sumner, Coombes, Pumford & Russell, 1990), as well as κ-receptors (Mansour, Khachaturian, Lewis, Akil & Watson, 1988), but other possible target sites include the subfornical organ and the region anterior and ventral to the third ventricle (AV3V area) which contain opioid receptors and neurones which can be affected by morphine (Buranarugsa & Hubbard, 1979; Sharif & Hughes, 1989); these regions provide an excitatory input essential for the tonic activity of supraoptic oxytocin neurones (Russell, Blackburn & Leng, 1988; Leng, Blackburn, Dyball & Russell, 1989*a*). Part of the withdrawal excitation of oxytocin neurones in morphine-infused rats is through a site outside the blood–brain barrier (Leng, Russell & Grossmann, 1989*b*). However, a direct effect of morphine within the supraoptic nucleus is indicated by inhibition of supraoptic neurones *in vitro* (Wakerley, Noble & Clarke, 1983; Pumford, Hunter & Russell, 1987). Morphine given by acute I.C.V. injection in suckled lactating rats also strongly inhibited the basal activity of oxytocin neurones. The present results are not consistent with those in a previous report stating that I.C.V. morphine in doses similar to or larger than those we used did not affect the firing rate of supraoptic oxytocin neurones (Clarke *et al.* 1979).

In chronic morphine-infused rats the initial firing rate of non-phasic neurones was

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control rats (Fig. 3); and five cells (*A*, *B*, *C*, *E* and *G*) were excited by low doses of morphine. Naloxone (5 mg kg^{-1}) reversed acute inhibitory effects of morphine but increased firing rate significantly above the initial value. Initial firing rates were: *A*, 3·14 Hz; *B*, 2·30 Hz; *C*, 3·58 Hz; *D*, 3·52 Hz; *E*, 12·14 Hz; *F*, 1·46 Hz; *G*, 0·85 Hz; *H*, 0·81 Hz; *I*, 0·98 Hz. Neurones *A–E* firing continuously at > 2 Hz are putative oxytocin neurones; neurone *F*, although firing at < 2 Hz, is identified as oxytocinergic by its withdrawal response after naloxone; neurones *G–I*, firing at < 2 Hz, could be slow-firing oxytocin or vasopressin neurones.

lower (for those firing at < 2 Hz) than in vehicle-infused rats. Since the proportion of phasic (vasopressin) neurones was not altered by chronic I.C.V. morphine, these changes are consistent with inhibition of oxytocin neurones, and indicate incomplete tolerance to the I.C.V. infusion of 1 μg morphine each minute, an amount more than sufficient as a single I.C.V. dose to powerfully inhibit oxytocin neurones in control rats (Fig. 4). In contrast, phasic neurones fired faster, and had shorter antidromic latencies in morphine-infused rats than in controls, indicating increased excitability of these neurones; however, vasopressin secretion is not increased in morphine-infused rats (Bicknell *et al.* 1988*a*). In previous studies on fewer cells we have not found significant differences in oxytocin neurone firing rates between vehicle- and morphine-infused rats, and we have found normal, or only slightly depressed, plasma oxytocin levels in morphine-infused rats (Bicknell *et al.* 1988*a*; Sumner *et al.* 1990). The normal, or near-normal, secretion of oxytocin in morphine-infused rats is not a result of increased synthesis or releasability of oxytocin since the oxytocin mRNA content of these neurones is unaltered (Sumner, Kawata & Russell, 1989), as is their content of oxytocin in lactating rats (Rayner *et al.* 1988), and the fraction of stored oxytocin released from the neurohypophysis *in vitro* per electrical stimulus pulse is also unaltered (Bicknell, Chapman, Leng & Russell, 1985; Russell, 1989). To test for tolerance in the central mechanisms controlling oxytocin cell activity, we compared the effectiveness of increasing doses of I.V. morphine. In morphine-infused rats there was a large shift to the right in the dose–response relationship for the action of I.V. morphine on the firing rate of oxytocin neurones and a significant decrease in the maximum inhibition obtained with I.V. morphine. Comparable changes have also been reported for other central neurones in morphine tolerance (e.g. Christie, Williams & North, 1987). Possible mechanisms for morphine tolerance are: the neurones or their inputs lose opioid receptors; the affinity of the receptors is altered; the coupling of receptors to the intracellular effector mechanism is altered; or endogenous opioid production is decreased or an endogenous antagonist is produced (Mocchetti & Costa, 1986; Christie *et al.* 1987; Giagnoni, Casiraghi, Basilico, Pecora, Mennuni, Parolaro, Colleoni & Gori, 1988; Loh, Tao & Smith, 1988; Takahashi, Portoghese & Takemori, 1988; Werling, McMahon & Cox, 1989). We have found a selective decrease in the density of μ-receptors in the supraoptic nuclei of rats given I.C.V. morphine for 5 days (Sumner *et al.* 1990), but whether this decrease is sufficient to explain the large shift in sensitivity of oxytocin neurones to morphine depends on the affinity of the opiate receptors and on the initial size of the receptor pool relative to the number required to be activated for effect (the pool of 'spare' receptors; Stephenson, 1956). Changes in receptor affinity have not been found in some studies on other systems (Tempel, Habas, Paredes & Barr, 1988), but a decreased number of μ-receptors in the high affinity state has been reported (Werling *et al.* 1989); increased receptor number has also been found (Brady, Herkenham, Long & Rothman, 1989). Since sufficiently large doses of I.V. morphine given to morphine-infused rats were able to inhibit some putative oxytocin neurones, sufficient μ-receptors may remain available for activation even in morphine-infused rats and the available receptors appear to be coupled to the effector systems. In addition, since withdrawal excitation of oxytocin neurones provoked by low doses of naloxone is mediated by μ-receptors (Leng *et al.* 1989*b*), there must be sufficient receptors in

morphine-infused rats to allow this marked excitation to be expressed. However, the mechanism of withdrawal excitation, and thus dependence, need not otherwise be closely related to the mechanism of tolerance or to the normal mechanism of acute inhibition by opioids (Seidl & Schulz, 1983; Johnson & Duggan, 1984). Hypersecretion of oxytocin in morphine-infused rats given naloxone confirms that naloxone-induced withdrawal excitation of oxytocin neurones is not simply the reversal of acute inhibition by morphine (Bicknell *et al.* 1986, 1988*a*). Furthermore, dependence is not a consequence of acute exposure to morphine since naloxone simply restored firing rate to pre-morphine values in control rats. The excitatory effect of I.V. morphine on a few continuous supraoptic neurones, but only in morphine-infused rats, was unexpected. The excitation could reflect an inhibitory action of morphine on inhibitory input to these neurones or even a direct excitatory effect like that recently described for dorsal root ganglion neurones (Crain & Shen, 1990). We have found such an excitatory effect of morphine and of a κ-agonist on oxytocin neurones in other control rats (Leng *et al.* 1990).

The present study shows the powerful inhibitory effect of relatively low doses of morphine on the electrical activity of oxytocin neurones of the rat supraoptic nucleus, and explains the inhibition of oxytocin secretion by morphine. The sensitivity of these neurones to morphine decreases markedly during chronic morphine treatment. Whether this plasticity in opioid responsiveness extends to changes in physiological circumstances remains to be established, but it could be important in the long-term regulation of the activity of oxytocin neurones. Thus during sustained endogenous opioid action, oxytocin neurones would be first inhibited, then become tolerant and resume normal activity, and finally increase activity on cessation of opioid action. Such a sequence has been postulated to take place during pregnancy, in preparation for parturition (Bicknell, Leng, Russell, Dyer, Mansfield & Zhao, 1988*b*; Leng & Russell, 1989).

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REFERENCES

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., GROSSMANN, R., LENG, G. & RUSSELL, J. A. (1986). Hypersensitivity to naloxone in magnocellular neurones of virgin female rats treated chronically with intracerebroventricular (I.C.V.) morphine. *Journal of Physiology* **382**, 151*P*.

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 opioid mechanisms controlling oxytocin neurones during parturition. *Brain Research Bulletin* **20**, 803–810.

BRADY, L. S., HERKENHAM, M., LONG, B. & ROTHMAN, R. B. (1989). Chronic morphine increases μ-opiate receptor binding in rat brain: a quantitative autoradiographic study. *Brain Research* **477**, 382–386.

BURANARUGSA, P. & HUBBARD, J. I. (1979). The neuronal organization of the subfornical organ *in vitro* and a test of the osmo- and morphine-receptor hypotheses. *Journal of Physiology* **291**, 101–116.

CHRISTIE, M. J., WILLIAMS, J. T. & NORTH, R. A. (1987). Cellular mechanisms of opioid tolerance: studies in single brain neurons. *Molecular Pharmacology* **32**, 633–638.

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. (1984). A comparison of analgesia and suppression of oxytocin release by opiates. *British Journal of Pharmacology* **83**, 799–806.

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*.

CRAIN, S. M. & SHEN, K.-F. (1990). Opioids can evoke direct receptor-mediated excitatory effects on sensory neurons. *Trends in Pharmacological Sciences* **11**, 77–81.

DYBALL, R. E. J. & LENG, G. (1986). Regulation of the milk ejection reflex in the rat. *Journal of Physiology* **380**, 239–256.

EVANS, R. G. & OLLEY, J. E. (1988). Comparison of the oxytocin response to water-deprivation, hyperosmolarity and administration of morphine or naltrexone in lactating and virgin female rats. *Neuroscience Letters* **94**, 177–181.

FREUND-MERCIER, M. J. & RICHARD, PH. (1984). Electrophysiological evidence for facilitatory control of oxytocin neurones by oxytocin during suckling in the rat. *Journal of Physiology* **352**, 447–466.

GIAGNONI, G., CASIRAGHI, L., BASILICO, L., PECORA, N., MENNUNI, L., PAROLARO, D., COLLEONI, M. & GORI, E. (1988). Cerebral extract from morphine-tolerant rats shows antiopiate properties in guinea pig ileum bioassay. *Pharmacological Research Communications* **20**, 307–322.

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

HARRIS, M. C. (1979). Effects of chemoreceptor and baroreceptor stimulation on the discharge of hypothalamic supraoptic neurones in rats. *Journal of Endocrinology* **82**, 115–125.

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.

JAFFE, J. H. (1985). Drug addiction and drug abuse. In *The Pharmacological Basis of Therapeutics*, 7th edn, ed. GILMAN, A. G., GOODMAN, L. S., RALL, T. W. & MURAD, F., pp. 532–581. Macmillan, New York.

JOHNSON, S. M. & DUGGAN, A. W. (1984). Dependence in the absence of tolerance to morphine. *European Journal of Pharmacology* **97**, 305–308.

JUSS, T. S. & WAKERLEY, J. B. (1981). Mesencephalic areas controlling pulsatile oxytocin release in the suckled rat. *Journal of Endocrinology* **91**, 233–244.

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

LENG, G., BLACKBURN, R. E., DYBALL, R. E. J. & RUSSELL, J. A. (1989*a*). Role of anterior peri-third ventricular structures in the regulation of supraoptic neuronal activity and neurohypophysial hormone secretion in the rat. *Journal of Neuroendocrinology* **1**, 35–46.

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**, 345–346.

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. (1989). Opioids, oxytocin and parturition. In *Brain Opioid Systems in Reproduction*, ed. DYER, R. G. & BICKNELL, R. J., chap. 15, pp. 231–256. Oxford University Press, Oxford.

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

LINCOLN, D. W. & WAKERLEY, J. B. (1974). Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *Journal of Physiology* **242**, 533–554.

LOH, H. H., TAO, P.-L. & SMITH, A. P. (1988). Role of receptor regulation in opioid tolerance mechanisms. *Synapse* **2**, 457–462.

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.

MANSOUR, A., KHACHATURIAN, H., LEWIS, M. E., AKIL, H. & WATSON, S. J. (1988). Anatomy of CNS opioid receptors. *Trends in Neurosciences* **11**, 308–314.

MOCCHETTI, I. & COSTA, E. (1986). Down regulation of hypothalamic proopiomelanocortin system during morphine tolerance. *Clinical Neuropharmacology* **9**, suppl. 4, 125–127.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neuroscience* **7**, 773–808.

PUMFORD, K., HUNTER, M. J. & RUSSELL, J. A. (1987). Paradoxical effects of naloxone and morphine on the electrical activity of magnocellular supraoptic neurones in rat hypothalamic slices. *Neuroscience Letters Supplement* **29**, S44.

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.

RENAUD, L. P., TANG, M., MCCANN, M. J., STRICKER, E. M. & VERBALIS, J. G. (1987). Cholecystokinin and gastric distension activate oxytocinergic cells in rat hypothalamus. *American Journal of Physiology* **253**, R661–665.

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

RUSSELL, J. A., BLACKBURN, R. E. & LENG, G. (1988). The role of the AV3V region in the control of magnocellular oxytocin neurons. *Brain Research Bulletin* **20**, 803–810.

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

RUSSELL, J. A. & SPEARS, N. (1984). Morphine inhibits suckling-induced oxytocin secretion in conscious lactating rats, but also disrupts maternal behaviour. *Journal of Physiology* **346**, 133*P*.

SEIDL, E. & SCHULZ, R. (1983). Selective opiate tolerance in the guinea-pig ileum is not associated with selective dependence. *Life Sciences* **33**, suppl. I, 357–360.

SHARIF, N. A. & HUGHES, J. (1989). Discrete mapping of brain mu and delta opioid receptors using selective peptides: quantitative autoradiography, species differences and comparison with kappa receptors. *Peptides* **10**, 499–522.

SHIBUKI, K., LENG, G. & WAY, S. (1988). Effects of naloxone and of intraperitoneal hypertonic saline upon oxytocin release and upon supraoptic neuronal activity. *Neuroscience Letters* **88**, 75–80.

STEPHENSON, R. P. (1956). A modification of receptor theory. *British Journal of Pharmacology* **11**, 379–393.

SUMMY-LONG, J. Y., MILLER, D. S., ROSELLA-DAMPMAN, L. M., HARTMAN, R. H. & EMMERT, S. E. (1984). A functional role for opioid peptides in the differential secretion of vasopressin and oxytocin. *Brain Research* **309**, 363–366.

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.

SUMNER, B. E. H., KAWATA, M. & RUSSELL, J. A. (1989). Does acute, intense stimulation of oxytocin neurones in the supraoptic nucleus increase their content of oxytocin mRNA? *Brain Research* **489**, 283–290.

TAKAHASHI, M., PORTOGHESE, P. S. & TAKEMORI, A. E. (1988). Irreversible binding of [^{3}H]beta-funaltrexamine to brain slices of morphine-tolerant and -dependent mice. *European Journal of Pharmacology* **149**, 205–212.

TEMPEL, A., HABAS, J., PAREDES, W. & BARR, G. A. (1988). Morphine-induced downregulation of mu-opioid receptors in neonatal rat brain. *Brain Research* **469**, 129–133.

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.

WATSON, S. J., AKIL, H., FISCHLI, W., GOLDSTEIN, A., ZIMMERMAN, E. A., NILAVER, G. & VAN WIMERSMA GREIDANUS, TJ. B. (1982). Dynorphin and vasopressin: common localization in magnocellular neurons. *Science* **216**, 85–89.

WERLING, L. L., MCMAHON, P. N. & COX, B. M. (1989). Selective changes in μ opioid receptor properties induced by chronic morphine exposure. *Proceedings of the National Academy of Sciences of the USA* **86**, 6393–6397.

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.

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