Local opioid inhibition and morphine dependence of supraoptic nucleus oxytocin neurones in the rat *in vivo*

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1. Single neurones of the rat supraoptic nucleus were recorded during microdialysis of naloxone onto the ventral surface of the nucleus in anaesthetized rats. We used this combination of techniques to test whether the acute or chronic effects of systemically or centrally applied opioids upon oxytocin cell activity were due to actions of the opioids within the nucleus itself.

2. Supraoptic nucleus oxytocin neurones were identified antidromically and by an excitatory response to intravenously injected cholecystokinin. Acute intravenous injection of the κ-agonist U50488H or the μ-agonist morphine (1–5 mg kg^{-1}) reduced the firing rate of identified oxytocin neurones by 97·7 ± 4·8% ($n = 6$) and 94·1 ± 4·1% ($n = 7$), respectively. The inhibition by each of these opioids was completely reversed after administration by microdialysis (retrodialysis) of the opioid antagonist naloxone (0·1–1·0 μg μl^{-1} at 2 μl min^{-1}) onto the exposed ventral surface of the supraoptic nucleus.

3. Retrodialysis of naloxone (0·1–10·0 μg μl^{-1}) onto the supraoptic nucleus of rats made dependent by intracerebroventricular morphine infusion for 5 days increased the firing rate of oxytocin neurones from 0·9 ± 0·4 to 3·1 ± 0·7 spikes s^{-1} ($P < 0·05$, $n = 6$). This increase in firing rate from basal was 58·5 ± 15·1% of that following subsequent intravenously injected naloxone (5 mg kg^{-1}).

4. Thus, the acute inhibition of supraoptic nucleus oxytocin neurones which results from systemic administration of opioid agonists primarily occurs within the supraoptic nucleus itself, since the antagonist naloxone was effective when given into the supraoptic nucleus. Furthermore, oxytocin neurones develop morphine dependence by a mechanism which is distinct from an action on their distant afferent inputs. Nevertheless, withdrawal excitation of these afferent inputs may enhance the magnitude of oxytocin neurone withdrawal excitation.

The peptides oxytocin and vasopressin are synthesized predominantly within the magnocellular neurones of the hypothalamic supraoptic and paraventricular nuclei and are secreted from the axon terminals of these neurones in the posterior pituitary gland. The oxytocin system is highly susceptible to inhibition by opioid peptides: opioid agonists inhibit the electrical activity of magnocellular oxytocin neurones, and hence oxytocin secretion, interrupting both the milk-ejection reflex and parturition (Russell, Leng & Bicknell, 1995). As both μ- and κ-opioid receptors are present in the supraoptic nucleus (Sumner, Coombes, Pumford & Russell, 1990), it is possible that opioids inhibit oxytocin neurones by a direct action on the oxytocin neurones themselves. However, since oxytocin neurones receive an afferent input from the A2 noradrenergic cell group in the nucleus tractus solitarii (Raby & Renaud, 1989) and μ-agonists reduce noradrenaline release into the supraoptic nucleus in response to systemic cholecystokinin (Onaka, Luckman, Guevara-Guzman, Ueta, Kendrick & Leng, 1995), it is also possible that a proportion of the morphine inhibition of oxytocin neurones results from presynaptic inhibition of excitatory inputs to these neurones. Furthermore, opioid receptors have been localized in many of the brain regions which project to the supraoptic nucleus, such as the median preoptic nucleus, the subfornical organ and organum vasculosum of the lamina terminalis (Mansour, Khachaturian, Lewis, Akil & Watson, 1988; Sharif & Hughes, 1989), and the locus coeruleus and nucleus tractus solitarii in the brainstem (Beaudet, Tremeau, Menez & Droz, 1979; Mansour *et al.* 1988), indicating that these inputs may themselves be sensitive to opioid inhibition. It is thus currently unclear whether the inhibition of oxytocin neurones following systemic administration of opioid agonists results principally from a reduction in the activity of excitatory afferent neurones, from presynaptic actions on the axon terminals of these inputs, from a direct action of the opioids on the oxytocin neurones themselves, or from a combination of these three possibilities.

Prolonged exposure of oxytocin neurones to morphine results in the development of both tolerance and dependence.

Tolerance is seen as a reduction in the magnitude of the inhibition which results from a given dose of morphine. Dependence is revealed by acute antagonism of the opioid agonist by naloxone which results in an immediate and long-lasting morphine-withdrawal hyperexcitation of the oxytocin system; this is seen as marked increases in the firing rate of oxytocin neurones *in vivo*, oxytocin heteronuclear RNA and immediate early gene expression in the supraoptic and paraventricular nuclei, intra-supraoptic nucleus release of oxytocin from dendrites and secretion of oxytocin into the systemic circulation (Russell *et al.* 1995).

The A2 cell group which projects to the supraoptic nucleus (Raby & Renaud, 1989) also develops morphine dependence and undergoes withdrawal excitation (Stornetta, Norton & Guyenet, 1993). Thus, morphine withdrawal excitation of oxytocin neurones may result from excitation of afferent inputs such as that from the A2 cell group at this time.

To study whether μ- and κ-opioid agonists act within the supraoptic nucleus to inhibit the activity of oxytocin neurones and whether morphine dependence of the oxytocin system develops at the level of the supraoptic nucleus, we combined microdialysis, for local drug administration (retrodialysis) to the ventral surface of the nucleus, with extracellular recording of identified oxytocin neurones.

METHODS

Induction of morphine tolerance/dependence

Virgin female Sprague–Dawley rats (220–350 g) were anaesthetized by inhalation of 5% halothane in a mixture of O_2 and N_2O (both flow rates at ca. 500 ml min^{-1}), and a 28 gauge stainless-steel cannula was stereotaxically implanted into the right lateral cerebral ventricle (3·0 mm caudal and 2·0 mm lateral to bregma and 4·5 mm below the upper surface of the skull, Paxinos & Watson, 1986). The cannula was attached via polythene tubing to a subcutaneous osmotic minipump (Alzet 2001; Charles River Ltd, Margate, Kent, UK). The pump and tubing contained morphine dissolved in sterile pyrogen-free water to deliver increasing doses over 5 days (10 and 20 μg h^{-1} for 40 h each and 50 μg h^{-1} for the remaining 40 h at 1 μl h^{-1}; Rayner, Robinson & Russell, 1988). Following surgery animals were housed individually with free access to food and water.

Electrophysiology and retrodialysis

The pituitary stalk and right supraoptic nucleus were exposed by the transpharyngeal approach under urethane anaesthesia (ethyl carbamate, 1·25 g kg^{-1} I.P.), and a dialysis probe was used for local drug administration (Ludwig & Leng, 1997). An in-house designed U-shaped microdialysis probe (total membrane length, 2·0 mm; Spectra/Por RC Hollow Fibers®, Spectrum Med. Inc., Houston, TX, USA) was bent to position the loop of the membrane flat onto the exposed ventral surface of the brain on the ventral glial lamina of the supraoptic nucleus after removal of the meninges. The supraoptic nucleus was dialysed with artificial cerebrospinal fluid (ACSF; pH 7·2, composition (mM): NaCl, 138; KCl, 3·36; $NaHCO_3$, 9·52; Na_2HPO_4, 0·49; urea, 2·16; $CaCl_2$, 1·26; $MgCl_2$, 1·18) at a flow rate of 2 μl min^{-1}. The glass recording microelectrode (filled with 0·9% saline, 20–40 MΩ resistance) was placed through the centre of the loop of the dialysis membrane. A stimulating electrode (SNEX-200X, Clarke Electromedical) was placed on the neural stalk of the pituitary gland and set to deliver single matched biphasic pulses (1 ms duration, <1 mA peak to peak) for antidromic identification of supraoptic nucleus neurones. A femoral vein catheter was inserted for systemic drug administration. Oxytocin neurones were distinguished from vasopressin neurones by their firing pattern (oxytocin neurones being non-phasic) and by their differing responses to intravenously injected cholecystokinin (I.V. CCK, 20 μg kg^{-1}), i.e. transient excitation of oxytocin neurones (at least 1 spike s^{-1} increase in firing rate over 1 min within 4 min of injection) and no effect or short-term inhibition for vasopressin neurones (Renaud, Tang, McCann, Stricker & Verbalis, 1987).

Drug administration

U50488H (a selective κ-agonist) or morphine (a selective μ-agonist) was injected I.V. (both 1·0–5·0 mg kg^{-1} in an injection volume of 0·5 ml kg^{-1}) until the firing rate of the cell being recorded was inhibited to less than 0·2 spikes s^{-1}. Naloxone was retrodialysed onto the supraoptic nucleus (0·7 μg μl^{-1} after U50488H and 0·1–10·0 μg μl^{-1} in 10-fold increments after morphine, at 2 μl min^{-1}) for periods of 10 or 30 min. Since we were looking for inhibitory actions, cells which were silent or showed a very low firing rate were not studied in this experiment. At the end of each experiment the rats were killed by overdose of pentobarbitone anaesthetic (60 mg kg^{-1}, I.V.).

Firing rate analysis

The firing rates of identified oxytocin cells were downloaded onto a personal computer using the Spike2 software package (Cambridge Electronic Design, Cambridge, UK). The mean firing rate of each cell was calculated for the 5 min period immediately before each treatment and for successive 5 min periods after treatment. Statistical analyses were completed using the SigmaStat® software package (Jandel Scientific GmbH, Erkrath, Germany). All responses to drug administration were analysed by one-way repeated measures (RM) analysis of variance (ANOVA). Where the F ratio was significant this was followed by *post hoc* analyses using the Student–Newman–Keuls test. All values are expressed as means ± S.E.M. and differences were considered statistically significant if $P < 0·05$. n, indicates the number of neurones.

Drugs

Morphine sulphate was supplied by The Royal Infirmary of Edinburgh (Edinburgh, UK); U50488H (trans-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide) and naloxone hydrochloride were purchased from Sigma; and cholecystokinin-(26–33)-sulphated (CCK) was from Bachem (Bachem Ltd, Saffron Walden, Essex, UK).

RESULTS

Effects of retrodialysis of naloxone onto the supraoptic nucleus on electrical activity of oxytocin neurones after acute U50488H or morphine inhibition

A single identified magnocellular oxytocin neurone was recorded from the supraoptic nucleus of thirteen rats. The mean spontaneous firing rate of the thirteen supraoptic nucleus neurones was 3·4 ± 0·5 spikes s^{-1}; these cells were characterized as oxytocin neurones by their transient increase in firing rate following I.V. CCK (1·4 ± 0·3 spikes s^{-1} increase averaged over 5 min). This firing rate and response to systemic CCK are similar to those previously reported for

oxytocin neurones in both morphine-naive and -dependent rats (Brown, Munro, Murphy, Leng & Russell, 1996).

Six rats from which supraoptic nucleus neurones were recorded were injected I.V. with U50488H in doses of 1–5 mg kg^{-1} until cessation of neuronal activity, or until no further inhibition of the neurones resulted (97·7 ± 4·8% maximum inhibition, $P < 0{\cdot}05$, $n = 6$, Figs 1*A* and 2*A*). One neurone was recorded for a further 55 min after U50488H injection during which time no recovery from the U50488H-induced inhibition was evident, confirming the long duration of inhibition following these doses of U50488H, as seen in previous studies (Pumford, Russell & Leng, 1993). In the other five rats, retrodialysis of 0·7 $\mu g\ \mu l^{-1}$ (2 mM) naloxone onto the supraoptic nucleus for 10–30 min completely reversed U50488H-induced inhibition of oxytocin neurones. Subsequent I.V. injection of 5 mg kg^{-1} U50488H within 15 min of the termination of retrodialysis of naloxone did not alter the firing rate of these neurones.

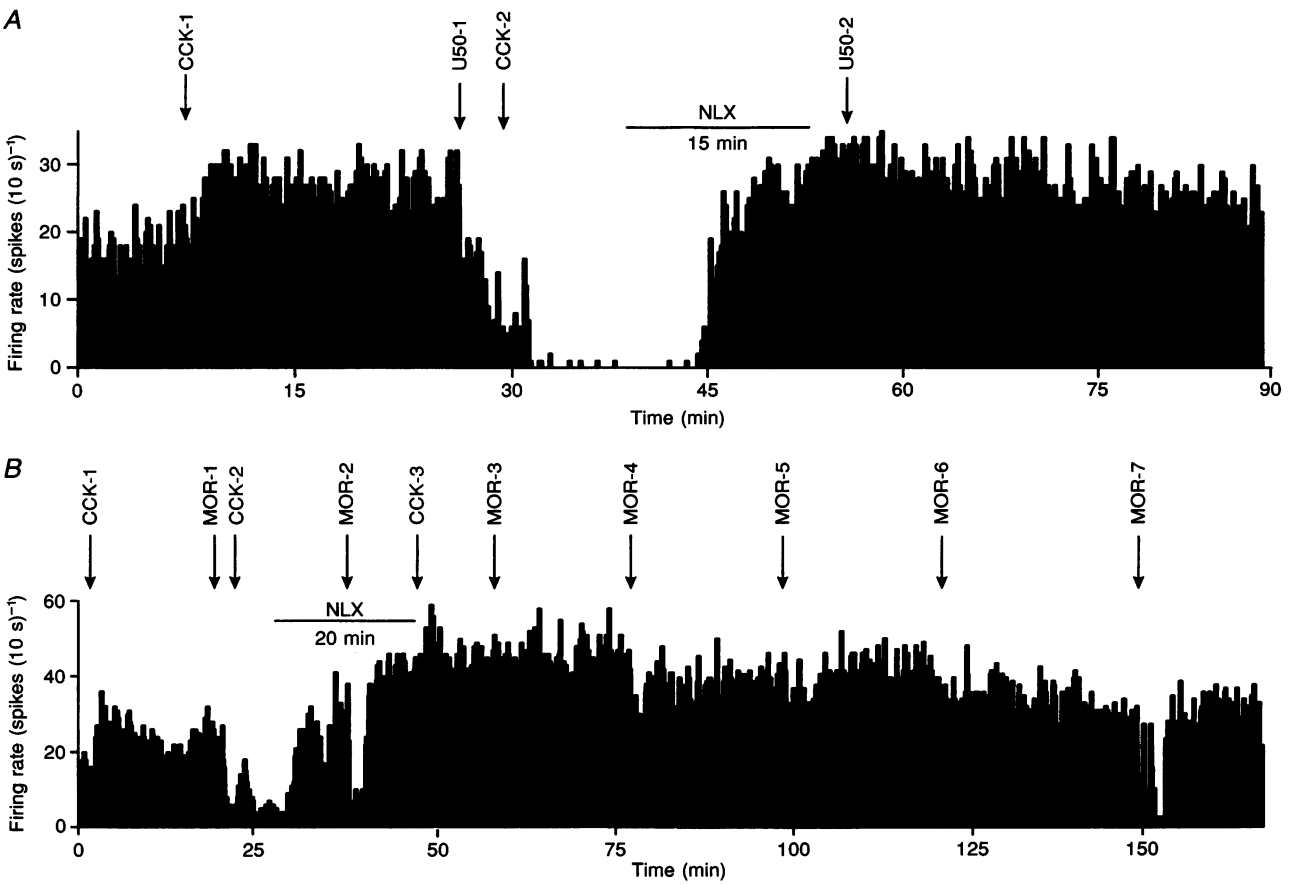

Figure 1. Retrodialysis of naloxone onto the supraoptic nucleus reverses opioid inhibition of oxytocin neurones

The panels show the spontaneous firing rates (in 10 s bins) of two supraoptic nucleus oxytocin neurones identified by their transient increase in firing rate following I.V. injection of 20 $\mu g\ kg^{-1}$ CCK (CCK-1). *A*, I.V. injection of 1 mg kg^{-1} U50488H (U50-1) eliminated the spontaneous activity of this neurone and reduced the magnitude of its response to a second injection of 20 $\mu g\ kg^{-1}$ CCK (CCK-2). Retrodialysis of naloxone (NLX, 0·7 $\mu g\ \mu l^{-1}$) onto the supraoptic nucleus over 15 min restored the spontaneous activity of the neurone and prevented further inhibition by a second injection of 5 mg kg^{-1} U50488H (U50-2). *B*, I.V. injection of 1 mg kg^{-1} morphine (MOR-1) after 20 $\mu g\ kg^{-1}$ I.V. CCK (CCK-1) markedly reduced the spontaneous activity and the CCK-responsiveness (CCK-2, 20 $\mu g\ kg^{-1}$) of this neurone. Retrodialysis of naloxone (NLX, 0·7 $\mu g\ \mu l^{-1}$) onto the supraoptic nucleus over 20 min restored the spontaneous activity of this neurone. This recording further indicates the temporal features of antagonism of systemically applied morphine by retrodialysed naloxone. Retrodialysis of naloxone reduced the inhibitory effect of morphine given during retrodialysis (MOR-2) and abolished the effect of morphine given 15 min after retrodialysis (MOR-3). Following termination of naloxone retrodialysis, a third injection of 20 $\mu g\ kg^{-1}$ CCK (CCK-3) again produced a transient increase in spontaneous activity of this neurone. Subsequent repeated injection of 1 mg kg^{-1} morphine resulted in progressively greater inhibition of the spontaneous firing rate (MOR-4–7), presumably resulting from elimination of naloxone from the local environment.

In seven rats, morphine was injected I.V. at doses of 1–5 mg kg^{-1}, resulting in a 94·1 ± 4·1% inhibition of the firing rate of the neurones recorded ($P < 0·05$, $n = 7$, Figs 1*B* and 2*B*). In five of these seven neurones, naloxone retrodialysed at concentrations of 0·1–1·0 μg μl^{-1} onto the supraoptic nucleus completely reversed the morphine-induced inhibition, and subsequent I.V. injection of 1 or 5 mg kg^{-1} morphine within 15 min of the termination of naloxone retrodialysis did not reduce the firing rate of these neurones.

Recordings from two of the cells were lost approximately 35 and 50 min after injection of the 5 mg kg^{-1} dose of morphine which silenced the cells; these cells were still maximally inhibited at the time that the recordings were lost.

Effects of retrodialysis of naloxone onto the supraoptic nucleus on electrical activity of oxytocin neurones in morphine-dependent rats

As in untreated rats, oxytocin neurones in morphine-dependent/tolerant rats are excited by systemic injection of CCK (Brown *et al.* 1996). Here, oxytocin neurones in morphine-dependent rats were characterized by their transient increase in firing rate following I.V. CCK (1·1 ± 0·5 spikes s^{-1} increase averaged over 5 min, $n = 6$). Naloxone was retrodialysed onto the supraoptic nucleus in 10-fold increments over the following ranges: 0·1–10·0 μg μl^{-1} for 10 min at each dose (from 10 ng μl^{-1} in one case; $n = 4$) and 1·0–10·0 μg μl^{-1} for 30 min at each dose ($n = 2$). Naloxone (0·1–10·0 μg μl^{-1}) dose dependently increased the firing rate of the six neurones recorded from 0·9 ± 0·4 to 3·1 ± 0·7 spikes s^{-1} ($P < 0·05$). The overall increase in firing rate over basal after retrodialysis of the 10·0 μg μl^{-1} dose of naloxone was 58·5 ± 15·1% of that following subsequent I.V. administration of 5 mg kg^{-1} naloxone, at which time the firing rate of the neurones was 3·9 ± 0·3 spikes s^{-1} (Fig. 3).

DISCUSSION

The present results demonstrate that inhibition of supraoptic nucleus oxytocin neurones following systemic administration of μ- and κ-opioid agonists is predominantly attributable to opioid actions within the supraoptic nucleus, since the inhibition was fully reversed by administration of the opioid antagonist naloxone directly onto the nucleus. The present results cannot differentiate between post-synaptic actions on the oxytocin neurones themselves and presynaptic inhibition at axon terminals impinging on the oxytocin neurones. The A2 cell group projection to the supraoptic nucleus is subject to presynaptic inhibition by morphine (Onaka *et al.* 1995), while the effects of opioids at the axon terminals of other projections to the supraoptic nucleus are unknown. U50488H hyperpolarizes both oxytocin and vasopressin neurones *in vitro*, and the κ-agonist dynorphin reduces the magnitude of excitatory postsynaptic potentials evoked by focal stimulation in supraoptic nucleus neurones (Inenaga, Nagatomo, Nakao, Yanaihara & Yamashita, 1994). Morphine also inhibits both oxytocin and vasopressin neurones in hypothalamic slices

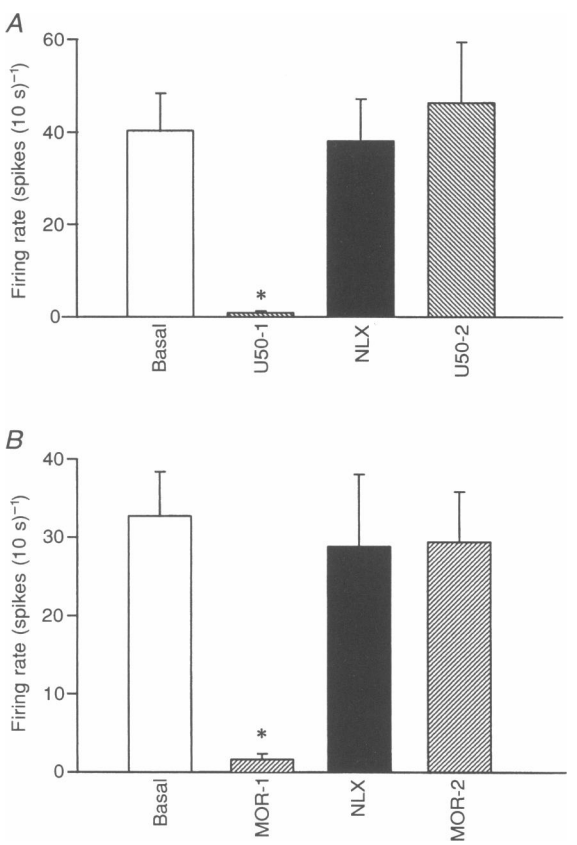

Figure 2. Summary of the effects of retrodialysis of naloxone onto the supraoptic nucleus after inhibition of oxytocin neurones by U50488H or morphine

The firing rates of identified supraoptic nucleus oxytocin neurones, means + S.E.M. (indicated by columns and bars) averaged over 5 min before (Basal) and after inhibition by 1–5 mg kg^{-1} I.V. U50488H (U50-1, *A*) and 1–5 mg kg^{-1} I.V. morphine (MOR-1, *B*). After retrodialysis of 0·7 μg μl^{-1} naloxone (NLX) the spontaneous activity was restored in both *A* and *B* and subsequent injection of 1–5 mg kg^{-1} I.V. U50488H (U50-2, *A*) or 1–5 mg kg^{-1} I.V. morphine (MOR-2, *B*) within 15 min of the termination of naloxone retrodialysis did not then inhibit the activity of the neurones. * $P < 0·001$ (one-way RM ANOVA); $n = 5$ in both *A* and *B*.

(Pittman, Hatton & Bloom, 1980; Wakerley, Noble & Clarke, 1983). Both μ- and κ-agonists inhibit the spontaneous activity of oxytocin neurones *in vivo* (Pumford *et al.* 1993), and also the responses of these neurones to all stimuli against which these opioids have been tested, including parturition, suckling and increased osmolality (Russell *et al.* 1995). While it is possible that all of the afferent inputs to oxytocin neurones possess both μ- and κ-receptors, the simplest explanation is that the predominant inhibitory actions of opioids are those exerted directly upon the oxytocin neurones themselves.

Retrodialysis of naloxone onto the supraoptic nucleus of morphine-dependent rats elicited a dose-dependent excitation of oxytocin neurones. Thus, morphine withdrawal excitation of oxytocin neurones occurs at the level of the supraoptic nucleus. Again it is possible that this is manifested at the level of the oxytocin neurones themselves or presynaptically on their afferent inputs or some combination of the two.

In the present study, following retrodialysis of naloxone onto the supraoptic nucleus, systemic administration of naloxone further increased the firing rate of oxytocin neurones in the morphine-dependent rats. As administration of naloxone onto the supraoptic nucleus completely reversed the acute inhibition of oxytocin neurones by morphine or U50488H, it appears unlikely that naloxone at similar doses failed to saturate supraoptic nucleus μ-receptors in the

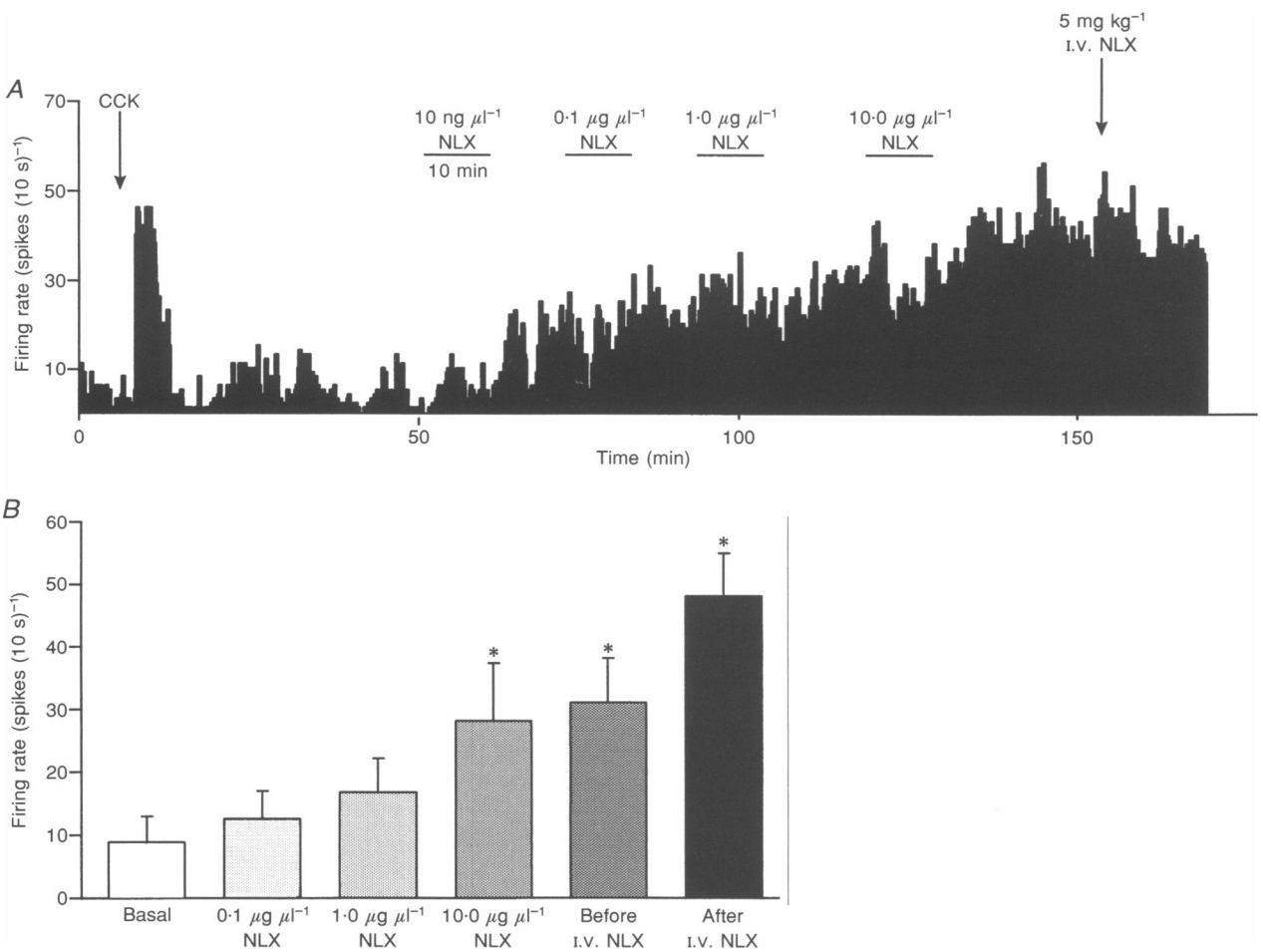

Figure 3. Local application of naloxone induces withdrawal excitation of supraoptic oxytocin neurones in morphine-dependent rats

A, the spontaneous firing rate (in 10 s bins) of a supraoptic nucleus oxytocin neurone in a morphine-dependent rat identified by its transient increase in firing rate following 20 μg kg^{-1} I.V. CCK (CCK). Retrodialysis of increasing concentrations (10 ng μl^{-1} to 10 μg μl^{-1}) of naloxone (NLX) onto the supraoptic nucleus over 10 min each induced a dose-dependent increase in the firing rate of this neurone. Subsequent I.V. injection of 5 mg kg^{-1} naloxone (I.V. NLX) induced a further small rise in the firing rate of the neurone. *B*, the firing rates (averaged over 5 min; means + S.E.M.) of identified supraoptic nucleus oxytocin neurones recorded from morphine-dependent rats ($n = 6$) before (Basal) and after retrodialysis of increasing concentrations (0·1 μg μl^{-1} to 10 μg μl^{-1}) of naloxone (NLX) and before and after I.V. injection of 5 mg kg^{-1} naloxone (NLX). *$P < 0·05$ (one-way RM ANOVA).

morphine-dependent rats. Thus, a more likely explanation for the further increased activity following systemic naloxone is that some excitatory inputs to the supraoptic nucleus also undergo morphine-withdrawal excitation.

Although μ-receptors are widely distributed in the CNS (Mansour, Fox, Burke, Akil & Watson, 1995), few of the areas where μ-receptors are located display dependence after chronic morphine treatment (Nye & Nestler, 1996). Following naloxone-precipitated morphine withdrawal, there is intense induction of Fos protein expression in the supraoptic and paraventricular nuclei reflecting the withdrawal activation of oxytocin neurones at these sites, but strikingly there is little or no similar induction of Fos elsewhere in the hypothalamus, and in particular none in regions adjacent to the supraoptic nucleus (Russell *et al.* 1995; Jhamandas, Harris, Petrov & Jhamandas, 1996). However, neurones of the A2 and A6 noradrenergic cell groups also exhibit morphine dependence (Aghajanian, 1978; Stornetta *et al.* 1993) and these areas project to the supraoptic nucleus (Cunningham & Sawchenko, 1991). Other afferent neurones which have been studied, notably those in the lamina terminalis, do not appear to be activated during morphine withdrawal (Murphy, Onaka, Brown & Leng, 1997), but it is possible that withdrawal excitation of oxytocin neurones in part reflects withdrawal excitation of noradrenergic afferents.

During morphine withdrawal noradrenaline is released in various brain regions, such as the hippocampus (Done, Silverstone & Sharp, 1992), and this release is prevented by prior administration of the α_2-adrenergic agonist clonidine (Silverstone, Done & Sharp, 1992). Clonidine also reduces the behavioural signs of opiate withdrawal in humans (Gold, Redmond & Kleber, 1978) and in rats (Taylor, Elsworth, Garcia, Grant, Roth & Redmond, 1988). Thus, it has been suggested that noradrenaline may play a pivotal role in morphine withdrawal excitation in behavioural systems. However, the noradrenaline release into the supraoptic nucleus following morphine withdrawal is rather modest (Murphy *et al.* 1997), being less than that which results from systemic administration of cholecystokinin (Onaka *et al.* 1995), a stimulus which increases the activity of oxytocin neurones to a much lesser extent than morphine withdrawal (Brown *et al.* 1996).

Perhaps the most extensively studied region in which morphine dependence develops is the locus coeruleus, where morphine treatment increases G-protein subunit, adenylate cyclase, cyclic AMP-dependent protein kinase and tyrosine hydroxylase levels (Nestler, Alreja & Aghajanian, 1994). However, an increase in glutamate release into the locus coeruleus during morphine withdrawal also contributes to the withdrawal-induced activation (Aghajanian, Kogan & Moghaddam, 1994). Thus, in the locus coeruleus it appears that withdrawal excitation is generated by an interplay between intracellular mechanisms and extrinsic inputs.

Withdrawal excitation of oxytocin neurones is unchanged by central catecholaminergic lesion (Murphy, Brown, Leng & Russell, 1995) and is reduced, but not eliminated, by lesion of the region anterior and ventral to the third ventricle (AV3V) (Russell, Pumford & Bicknell, 1992). The projection from the AV3V region to the supraoptic nucleus contains a glutamatergic component (Yang, Senatorov & Renaud, 1994) but few of the neurones that project from the AV3V region to the supraoptic nucleus are activated by morphine withdrawal (Murphy *et al.* 1997). Nevertheless, as in the locus coeruleus, it seems that withdrawal excitation of supraoptic nucleus oxytocin neurones results from a combination of intrinsic and extrinsic factors, including inputs from glutamatergic and/or noradrenergic neurones.

In any experiment involving focal administration of a drug, the concentration at its site of action is subject to uncertainty. Inhibition of oxytocin cells by 1 mg kg^{-1} I.V. U50488H is fully antagonized only by an I.V. dose of naloxone of 5 mg kg^{-1} or higher (Pumford *et al.* 1993). Assuming conservatively that this dose of naloxone is distributed in a volume of 10 ml per 100 g body weight gives an estimated effective concentration of 50 μg ml^{-1} at its site of action. This is lower by a factor of about 15 than the concentration of naloxone in the dialysate itself. However, the tissue concentrations reached during dialysis are certainly much lower than the concentration in the dialysate. When dialysis is used conventionally to measure the concentrations of substances in the extracellular fluid, typical recoveries are of the order of 2–10% of the extracellular concentration (Benveniste & Hüttemeier, 1990), but the more appropriate calculation is based on estimates of efflux from dialysis probes. Most of the efflux from the probes *in situ* will not enter the supraoptic nucleus (since the probe is apposed to the ventral surface without penetrating, only part of the membrane is in contact with brain tissue), and most of what enters will be cleared by the vasculature, which is fully intact in this preparation (blood flow through the supraoptic nucleus is amongst the highest of any brain region, and the vasculature occupies a high proportion of the volume of the supraoptic nucleus; Gross, Sposito, Pettersen & Fenstermacher, 1986). When Fluorogold was administered through dialysis probes apposed to the ventral surface of the supraoptic nucleus, subsequent histological analysis indicated little penetration of tissue beyond about 200 μm of the surface (Ludwig & Leng, 1997) – little more in fact than the ventral glial lamina of the supraoptic nucleus, which contains only the dendrites of supraoptic neurones and not their cell bodies (Armstrong, Scholer & McNeill, 1982). The inference that penetration is limited is strongly supported by results obtained using tetrodotoxin. Tetrodotoxin blocks action potentials in oxytocin neurones at a concentration of 10^{-6} M and below, but dialysis of 10^{-4} M tetrodotoxin, though it consistently blocks spontaneous activity in supraoptic neurones, does not generally block antidromically evoked action potentials,

implying that the concentration achieved at the somata (rather than the dendrites) of supraoptic neurones following even 30 min of dialysis remains at least two orders of magnitude below the dialysate concentration (Ludwig & Leng, 1997). Thus, while naloxone is more lipophilic than Fluorogold and tetrodotoxin, it is likely that the diffusion of naloxone away from the dialysis probe is also highly restricted. Furthermore, there is a high density of both μ- and κ-opioid receptors throughout the entire supraoptic nucleus but not in the immediate vicinity surrounding the supraoptic nucleus (Sumner *et al.* 1990). Therefore, it is probable that the effects of naloxone observed in the present study result from actions within the supraoptic nucleus itself.

Given the potency of directly applied naloxone in antagonizing the actions of systemically applied U50488H, it was surprising that higher concentrations were needed to evoke morphine withdrawal, since naloxone is approximately 50-fold more potent at μ-receptors than at the κ-receptors through which U50488H acts. However, although very low doses of systemically administered naloxone will excite supraoptic oxytocin neurones in morphine-dependent rats (Leng, Russell & Grossmann, 1989), full withdrawal again requires a systemic dose of 5 mg kg^{-1}, and an inferred maximal effective tissue concentration of 50 μg ml^{-1}. The highest dialysate concentration used in the present study was 10 mg ml^{-1}, over two orders of magnitude greater than the maximum effective systemic dose; this dialysis concentration appears to be appropriate for delivering an effective concentration of naloxone to the supraoptic nucleus, perhaps with a requirement to penetrate beyond the dendritic layer to reach the cell bodies of the neurones to elicit morphine withdrawal excitation.

In conclusion, the acute inhibition of oxytocin neurones by μ- and κ-opioid agonists are mediated within the supraoptic nucleus, probably directly upon the oxytocin neurones themselves but possibly also by presynaptic inhibition. Furthermore, oxytocin neurones express morphine withdrawal excitation, and presumably morphine dependence, separately from their distant afferent inputs.

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