# Activation of oxytocin neurones by systemic cholecystokinin is unchanged by morphine dependence or withdrawal excitation in the rat

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1. Morphine inhibits supraoptic nucleus oxytocin neurones directly and presynaptically via inhibition of afferent noradrenergic endings.

2. We studied whether morphine tolerance/dependence (induced by intracerebroventricular (I.C.V.) morphine infusion) alters the responsiveness of oxytocin neurones to systemic cholecystokinin (CCK), a stimulus which activates oxytocin neurones via the release of noradrenaline.

3. CCK (20 $\mu$g kg$^{-1}$, I.V.) increased plasma oxytocin concentrations similarly in urethane-anaesthetized morphine-naive and -dependent rats. In naive rats, I.C.V. (10 $\mu$g) and I.V. morphine (0·5 mg kg$^{-1}$) reduced CCK-induced oxytocin secretion by 95 ± 4 and 49 ± 10%, respectively. In dependent rats, I.V. morphine reduced CCK-induced release by only 8 ± 9%, indicating tolerance.

4. In urethane-anaesthetized rats, I.V. CCK increased the firing rates of oxytocin neurones similarly in morphine-naive and -dependent rats (by 1·2 ± 0·2 and 1·4 ± 0·3 spikes s$^{-1}$ maximum, respectively, over 5 min). Naloxone did not alter spontaneous or CCK-induced activity in naive rats but increased activity in dependent rats (by 3·4 ± 0·5 spikes s$^{-1}$), indicative of withdrawal excitation; however, the response to CCK remained unchanged after naloxone.

5. Systemic CCK did not trigger withdrawal, nor did it have a greater excitatory effect in dependent rats. Thus, morphine withdrawal excitation of oxytocin neurones does not involve supersensitivity to the noradrenergic input, or hypersensitivity of this input to I.V. CCK. Tolerance apparently occurs both at the cell bodies of oxytocin neurones in the supraoptic nucleus and in their noradrenergic input. However, dependence is apparent only at the cell bodies.

Magnocellular neurones of the supraoptic and paraventricular nuclei in the hypothalamus secrete oxytocin or vasopressin from their nerve endings in the posterior pituitary gland. The oxytocin neurones are potently inhibited by the $\mu$-opioid agonist morphine and they develop both tolerance to, and dependence upon morphine when chronically exposed to this drug (Russell, Leng & Bicknell, 1995). Intracerebroventricular (I.C.V.) administration of morphine acutely inhibits oxytocin neurones, but during continuous I.C.V. infusion of morphine for 5 days, near-normal activity of the neurones returns as tolerance is established. After 5 days, tolerance is apparent as a large increase in the threshold dose of intravenous (I.V.) morphine required to inhibit the firing rate of oxytocin neurones *in vivo* (Pumford, Russell & Leng, 1991). At this time, withdrawal of morphine, elicited by I.V. injection of the opiate antagonist naloxone, evokes a prompt 60- to 100-fold prolonged increase in plasma oxytocin concentrations, resulting in large part from a prolonged and marked elevation in the firing rate of oxytocin neurones and in part from antagonism of endogenous opioid actions at the posterior pituitary (Bicknell, Leng & Russell, 1988). This withdrawal hyperexcitation demonstrates dependence upon morphine. A major outstanding question is whether oxytocin neurones themselves develop morphine dependence, or whether they are excited during naloxone-precipitated withdrawal by passively following an excitatory input that is excited.

The supraoptic nucleus contains $\mu$-opioid receptors and the density of these receptors in the nucleus is reduced following chronic morphine treatment (Sumner, Coombes, Pumford & Russell, 1990). Some of the receptors are present on the oxytocin neurones themselves, but others are located on

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afferent nerve endings. In particular, noradrenergic nerve endings innervating the oxytocin neurones bear functional $\mu$-receptors (Bicknell, Leng & Russell, 1987). Thus in the supraoptic nucleus, morphine acts upon both the oxytocin neurones and upon afferent noradrenergic endings (Onaka, Luckman, Antonijevic, Palmer & Leng, 1995*b*). Does tolerance to morphine, and does dependence upon morphine, develop at one or other of these sites, or at both of them? The present study was carried out to determine whether the hyperexcitation of oxytocin neurones that accompanies morphine withdrawal in morphine-dependent rats is the consequence of withdrawal-induced hyperactivity of the noradrenergic inputs to the supraoptic nucleus, or of hyperexcitability of supraoptic neurones in response to this input. The noradrenergic projection from the A2 cell group (Cunningham & Sawchenko, 1991) is known to mediate the stimulatory effects of systemically administered cholecystokinin (CCK) upon oxytocin release (Onaka, Luckman, Antonijevic, Palmer & Leng, 1995*a*). Here, we compared the effects of I.V. CCK upon oxytocin neuronal activity in morphine-naive rats and in rats treated chronically with I.C.V. morphine to induce tolerance and dependence, before and after morphine withdrawal precipitated by naloxone.

## METHODS

### Induction of morphine dependence

Female Sprague–Dawley rats (*ca* 250 g body weight) were anaesthetized with halothane (5% in an oxygen–nitrous oxide mixture) and placed in a stereotaxic frame. A cannula was placed into the right lateral cerebral ventricle (3·0 mm caudal, 2·0 mm lateral to bregma and 4·5 mm below the surface of the skull) connected to a subcutaneously implanted osmotic minipump (Alzet 2001; Alza Corporation, Palo Alto, CA, USA) via polythene tubing set to deliver increasing doses of morphine sulphate over 6 days (10 $\mu$g h$^{-1}$ for 40 h, 20 $\mu$g h$^{-1}$ for 40 h and 50 $\mu$g h$^{-1}$ for the remainder, at 1 $\mu$l h$^{-1}$). This treatment is effective at inducing morphine tolerance and dependence in mechanisms regulating activity of oxytocin neurones (Pumford *et al.* 1991).

### Blood sampling

To determine the acute effects of central morphine on the responses of oxytocin neurones to systemic CCK, twelve rats were anaesthetized with urethane (ethyl carbamate, 1·25 g kg$^{-1}$, I.P.) and the left femoral artery and vein cannulated for blood sampling for oxytocin radioimmunoassay and replacement of blood cells resuspended in 0·9% saline, respectively. The right jugular vein was also cannulated for administration of drugs. The left lateral cerebral ventricle was cannulated (0·6 mm caudal and 1·6 mm lateral to bregma and 4·1 mm below the surface of the skull) for the administration of morphine. CCK (20 $\mu$g kg$^{-1}$, I.V.) was administered immediately following the first blood sample ($t = 0$ min). Further blood samples were withdrawn 5 and 25 min later. Morphine (10 $\mu$g, I.C.V.; $n = 7$) or vehicle (5 $\mu$l 0·9% saline; $n = 5$) was infused at 30 min. At 35 min, a blood sample was withdrawn and this was immediately followed by a second injection of CCK. Two final blood samples were withdrawn 5 and 25 min after the second CCK injection.

The dose dependence of morphine inhibition of oxytocin release evoked by systemic CCK was investigated in eleven virgin female rats. The animals were cannulated as above but without an I.C.V. cannula. CCK (20 $\mu$g kg$^{-1}$, I.V.) was administered at 35 min intervals and blood samples withdrawn immediately prior to, and 5 and 25 min following, each CCK injection. Morphine (0·5 and 5·0 mg kg$^{-1}$, I.V.; $n = 6$) or vehicle (0·9% saline; $n = 5$) was administered 5 min before the second and third CCK injections.

To determine whether the excitatory effect of CCK on oxytocin neurones develops tolerance to inhibition by morphine, seven animals were made morphine dependent as described above and eight control animals were implanted with minipumps containing vehicle (sterile, pyrogen-free water). On the sixth day following minipump implantation, the femoral artery and vein and jugular vein were cannulated for blood sampling, infusion of resuspended blood cells and drug injections, respectively. CCK (20 $\mu$g kg$^{-1}$, I.V.) was administered at 0, 35 and 70 min. Morphine (0·5 and 5·0 mg kg$^{-1}$, I.V.) was administered 5 min prior to the second and third CCK injections, respectively. Blood samples were withdrawn immediately before and 5 and 25 min after each CCK injection. At the end of the experiment the rats were killed by an overdose of anaesthetic.

All blood samples were 0·3 ml in volume and were immediately replaced with an equal volume of red blood cells resuspended in isotonic saline. Plasma was removed after brief centrifugation, and stored at −20 °C until radioimmunoassay.

### Radioimmunoassay for oxytocin

Plasma oxytocin concentrations were measured in duplicate aliquots by specific radioimmunoassay as previously described (Leng *et al.* 1988) using antiserum kindly provided by Dr T. Higuchi (Higuchi, Honda, Fukuoka, Negoro & Wakabayashi, 1985). The assay sensitivity was $< 2{\cdot}4$ pg ml$^{-1}$ and the intra- and interassay coefficients of variation were 11·0 and 10·6%, respectively. The oxytocin concentrations in all plasma samples from each experiment were determined in a single assay.

### *In vivo* electrophysiology

To investigate whether tolerance to the inhibitory actions of morphine on CCK excitation of oxytocin neurones develops centrally, eighteen morphine-dependent and ten morphine-naive rats were prepared for extracellular recording of supraoptic putative oxytocin neurones. Briefly, the rats were anaesthetized with urethane (1·25 g kg$^{-1}$, I.P.) and placed supine in a stereotaxic frame. The ventral surface of the brain was exposed over the supraoptic nucleus and the infundibular stalk. A stimulating electrode (SNEX-100, Clarke Electromedical) was placed on the neural stalk of the pituitary and set to deliver single matched biphasic pulses (1 ms duration, < 1 mA peak to peak). A glass recording microelectrode (20–40 MΩ) was placed in the supraoptic nucleus for extracellular recording of firing rate. Supraoptic neurones that were identified antidromically as projecting to the neural stalk, and whose activity increased following CCK (20 $\mu$g kg$^{-1}$, I.V.) injection were used in the study since an excitatory response to I.V. CCK reliably identifies oxytocin neurones (Renaud, Tang, McCann, Stricker & Verbalis, 1987). The first injection of CCK was made after recording basal activity for at least 10 min. Naloxone (5 mg kg$^{-1}$, I.V.) was administered 10 min after the CCK injection, and a further 5–10 min later, a second CCK injection was given. In some experiments, further injections of CCK were given at intervals of 20–30 min. All rats were killed at the end of the experiment, as above.

### Firing-rate analysis

The firing rates of putative oxytocin cells were recorded using the Spike2 software package (Cambridge Electronic Design, Cambridge, UK) and interface to a personal computer. The mean firing rate of each cell was calculated for the 5 min immediately prior to each

CCK injection. The mean firing rates were then calculated for each 1 min period following CCK injection and these were normalized by subtraction of the control (pre-CCK) firing rate. Mean normalized responses were then compared as described below.

### Drugs

Morphine sulphate was obtained from the Royal Infirmary of Edinburgh Dispensary (Edinburgh, UK). Naloxone hydrochloride was purchased from Sigma and the biologically active, sulphated octapeptide fragment of CCK from Bachem (UK) Ltd (Saffron Walden, Essex, UK).

### Statistics

All blood sampling and electrophysiological data were analysed by two-way repeated-measures analysis of variance (ANOVA) using SigmaStat statistical software (Jandel Scientific GmbH, 40699 Erkrath, Germany). Where the $F$ ratio was significant, this was followed by *post hoc* analysis using the Student–Newman–Keuls method. The CCK response ratios were analysed by Student's unpaired $t$ test or, where appropriate, by one-way ANOVA followed by the Student–Newman–Keuls test where the $F$ ratio was significant.

## RESULTS

### Acute I.C.V. morphine actions on stimulation of oxytocin neurones by systemic CCK

To establish whether acute I.C.V. administration of morphine would suppress CCK-evoked oxytocin secretion at concentrations similar to those achieved during chronic I.C.V. infusion, plasma concentrations of oxytocin were measured following two I.V. injections of CCK separated by an I.C.V. injection of either morphine or vehicle. Basal plasma oxytocin concentrations were not significantly different between the two groups, and the first injection of CCK (CCK1) significantly increased the plasma oxytocin concentration, measured 5 min after injection, from 32·3 ± 8·0 to 132·8 ± 33·9 pg ml$^{-1}$, ($P < 0{\cdot}05$; $n = 12$). Oxytocin concentrations returned to baseline levels within a further 20 min. In rats given an I.C.V. injection of vehicle, a second injection of CCK (CCK2) resulted in a similar release of oxytocin; the ratio of oxytocin released in response to the second and first CCK injections was close to unity (Fig. 1). By contrast, in rats given an I.C.V. injection of morphine, the CCK2 : CCK1 ratio was reduced by 94% ($P < 0{\cdot}001$) indicating a marked inhibition of CCK-evoked oxytocin release (Fig. 1).

### Dose dependence of I.V. morphine inhibition of systemic CCK excitation of oxytocin secretion

To establish the acute effects of I.V. morphine upon CCK-induced oxytocin release, plasma oxytocin concentrations were measured in response to repeated injections of CCK separated by increasing doses of morphine or vehicle. Basal oxytocin concentrations were similar in the two groups of rats (39·9 ± 13·9 and 49·5 ± 14·6 pg ml$^{-1}$, respectively), as was the response to the initial injection of CCK (124·0 ± 28·7 and 133·8 ± 67·8 pg ml$^{-1}$ increase, respectively). In vehicle-injected rats, the second and third CCK responses were of a similar magnitude to the initial response to CCK. However, in morphine-injected rats the response to CCK was reduced by ~55% following 0·5 mg kg$^{-1}$ morphine and by ~80% following 5 mg kg$^{-1}$ morphine (Fig. 2).

### Systemic CCK excitation of oxytocin release following I.V. morphine in morphine-tolerant/dependent and -naive rats

To establish the existence or otherwise of tolerance to morphine in the responsiveness of oxytocin neurones to CCK following chronic morphine administration, plasma oxytocin concentrations were measured in morphine-dependent and -naive rats. In this study, the basal plasma oxytocin concentrations were similar in morphine-dependent and -naive rats (17·0 ± 2·6 pg ml$^{-1}$ ($n = 8$) and 23·0 ± 7·3 pg ml$^{-1}$ ($n = 7$), respectively) as were the levels reached following an initial injection of CCK (concentrations increased to 45·5 ± 9·3 and 50·4 ± 10·0 pg ml$^{-1}$, respectively; both $P < 0{\cdot}05$). The normal magnitude of the response to CCK in dependent rats is itself indicative of tolerance, but to test this directly, rats were subsequently injected with increasing doses of I.V. morphine before further injections of CCK. In naive rats, I.V. morphine again inhibited the oxytocin secretion evoked by CCK; the oxytocin secretory response to CCK following

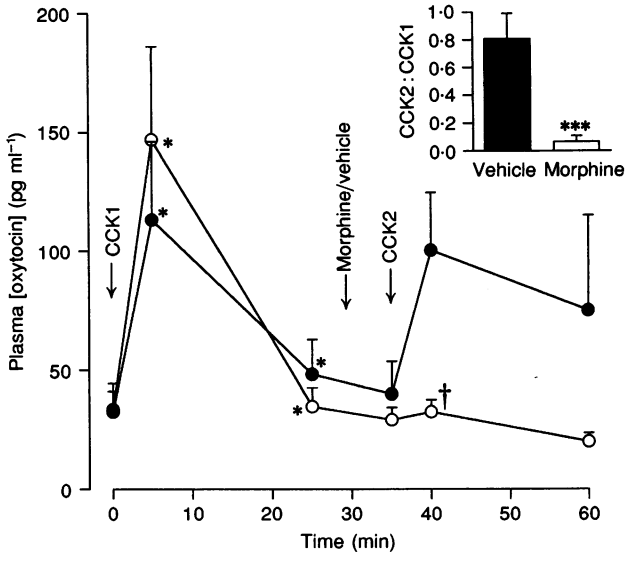


**Figure 1. I.C.V. morphine attenuates oxytocin secretion in response to injection of CCK**

Plasma oxytocin concentrations (+ S.E.M.) in rats administered CCK (20 μg kg$^{-1}$, I.V.) at 0 and 35 min and morphine (10 μg in 5 μl, I.C.V., ○) or vehicle (5 μl 0·9% saline, ●) at 30 min. * $P < 0{\cdot}05$ compared with the preceding sample; † $P < 0{\cdot}05$ compared with the first CCK response (5 min sample). Inset: ratios of the increase in plasma oxytocin concentrations following the second CCK injection to that following the first after injection of vehicle (■) or morphine (□; *** $P < 0{\cdot}001$, unpaired $t$ test).

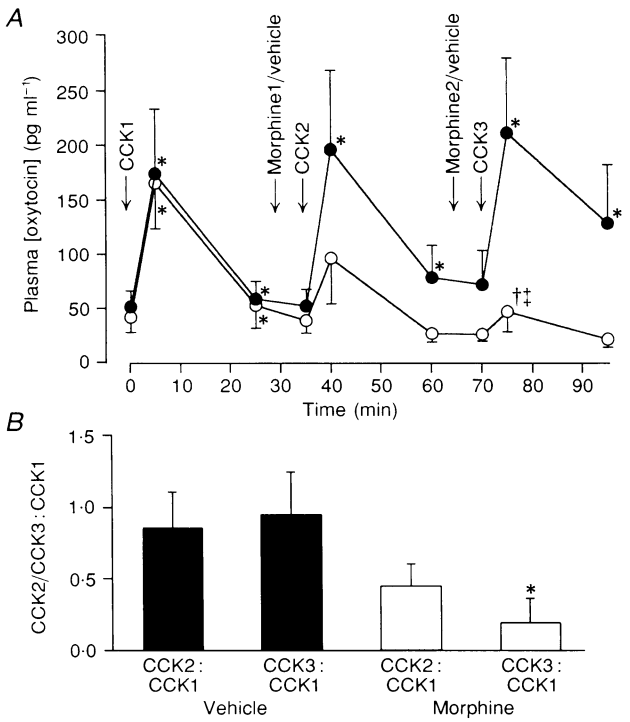


**Figure 2. Systemic administration of morphine produces a dose-dependent inhibition of CCK-induced oxytocin release**

*A*, plasma oxytocin concentrations (± S.E.M.) in rats injected with CCK (20 $\mu$g kg$^{-1}$, I.V.) at 0, 35 and 70 min and with 0·5 mg kg$^{-1}$ I.V. morphine at 30 min and 5·0 mg kg$^{-1}$ I.V. morphine at 65 min (○) or vehicle (0·9% saline) at 30 and 65 min (●); * $P < 0·05$ compared with the preceding sample; † $P < 0·05$ compared with the first CCK response (5 min sample); ‡ $P < 0·05$ compared with the time-matched, vehicle-treated control. *B*, ratios of the increase in plasma oxytocin concentrations following the second and third injections of CCK to that following the first after administration of vehicle (■) or morphine (□; * $P < 0·05$ compared with CCK3 : CCK1 in vehicle-treated rats; one-way ANOVA followed by the Student–Newman–Keuls test).

both doses of morphine was significantly reduced ($P < 0·05$) compared with the initial response to CCK. By contrast, in morphine-dependent rats, 0·5 mg kg$^{-1}$ morphine (I.V.) did not prevent the rise in plasma oxytocin concentrations resulting from CCK administration. The oxytocin concentrations found in morphine-dependent rats 5 min after CCK injection subsequent to injection of 5·0 mg kg$^{-1}$ morphine (I.V.) were significantly lower than those 5 min after the initial CCK injection and not significantly different from those of naive rats after the same morphine treatment (Fig. 3).

## Systemic CCK excitation of oxytocin neurone firing rate in morphine-dependent and -naive rats before and after naloxone administration

Stable, continuously active neurones antidromically identified as projecting to the neural lobe were selected for test in response to injections of CCK. The basal firing rates of the oxytocin neurones tested in morphine-dependent and -naive rats were similar (2·4 ± 0·6 spikes s$^{-1}$ ($n = 18$) and 3·6 ± 1·1 spikes s$^{-1}$ ($n = 10$), respectively). In morphine-naive rats, CCK injections evoked a prompt increase in firing rate,

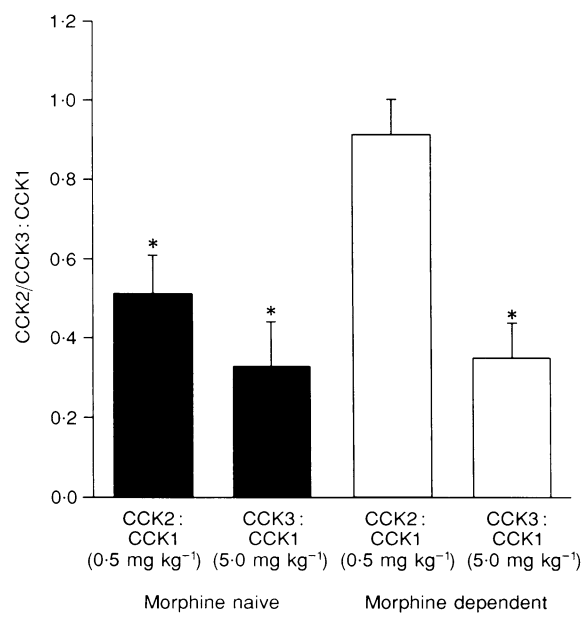


**Figure 3. Chronic I.C.V. morphine treatment results in tolerance to the inhibitory effects of morphine upon CCK-induced oxytocin release**

Ratios of the increase in plasma oxytocin concentrations following CCK injection (20 $\mu$g kg$^{-1}$, I.V.) after 0·5 and 5·0 mg kg$^{-1}$ morphine (I.V.) to that following CCK alone in morphine-naive (■) and -dependent rats (□): * $P < 0·05$ compared with CCK2 : CCK1 in morphine-dependent rats; one-way ANOVA followed by the Student–Newman–Keuls test).

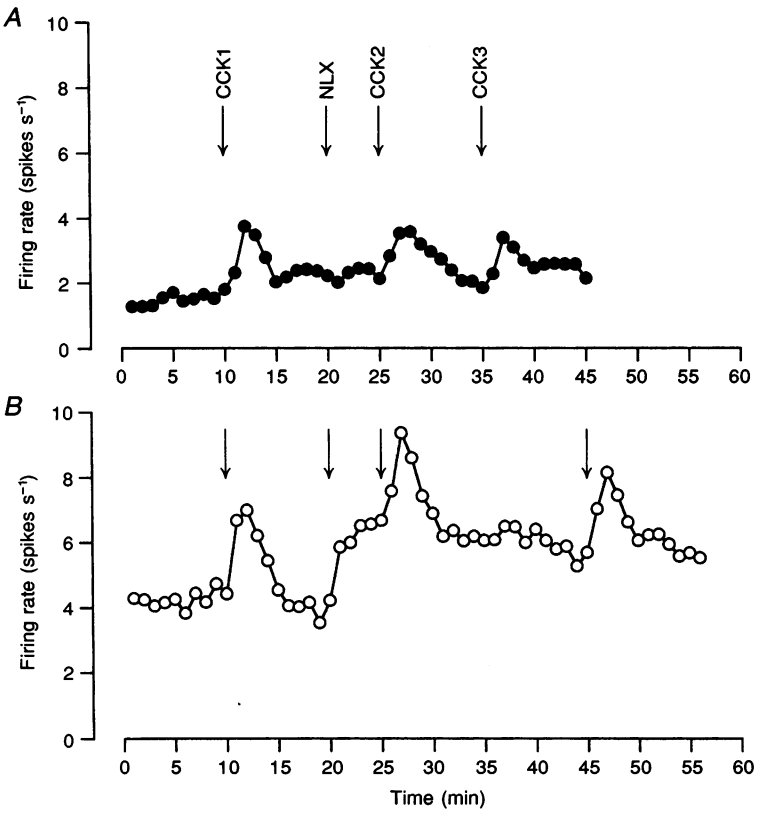


**Figure 4. CCK-induced excitation of oxytocin cells in morphine-dependent and morphine-naive rats**

Example recordings of the firing rates of antidromically identified putative oxytocin neurones from the supraoptic nucleus of a morphine-naive (*A*) and morphine-dependent (*B*) rat. The spontaneous firing rate and the magnitude of the increase in firing rate following CCK (CCK1; 20 $\mu$g kg$^{-1}$, I.V.) at 10 min were similar between animals. Naloxone (NLX; 5 mg kg$^{-1}$, I.V.) at 20 min increased the firing rate only in the morphine-dependent rat (withdrawal excitation), but did not alter the increase in firing rate induced by CCK at 25 min (CCK2) or subsequently (CCK3) in either the morphine-treated or the morphine-naive rat.

reaching a maximum at 1–2 min after injection and returning to control levels within 15 min. The magnitude of the response to CCK was not correlated to the initial spontaneous firing rate of the cells tested ($P > 0{\cdot}05$, $n = 52$, Spearman rank-order correlation). Oxytocin cells in morphine-dependent rats showed a similar response to CCK as observed in naive rats both in magnitude and time course (Figs 4 and 5).

In morphine-naive rats injection of naloxone produced no significant change in basal firing rate (mean change $+0{\cdot}9 \pm 0{\cdot}4$ spikes s$^{-1}$), and had no significant effect upon the neuronal response to CCK; the ratio of the response to the two injections of CCK was close to unity (CCK2 : CCK1 during 5 min was $0{\cdot}93 \pm 0{\cdot}26$). By contrast, in morphine-dependent rats, naloxone injection produced, as expected, a large and prolonged increase in electrical activity (mean change over the first 10 min, $+3{\cdot}4 \pm 0{\cdot}5$ spikes s$^{-1}$; $P < 0{\cdot}05$). However, the neuronal response to CCK, superimposed upon this elevated background activity, was remarkably unchanged in both magnitude and time course (Figs 4 and 5). Thus, while naloxone increased the spontaneous firing of oxytocin neurones in morphine-dependent but not morphine-naive rats, the profiles of the CCK responses in these two groups of animals were unaltered.

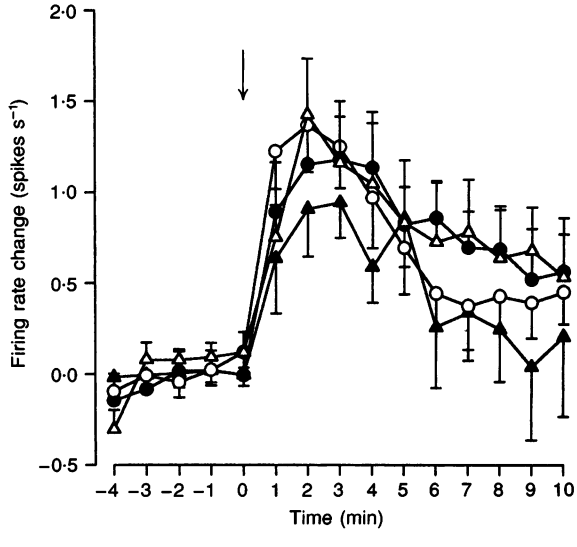


**Figure 5. CCK-induced excitation of oxytocin cells in morphine-dependent and morphine-naive rats**

The change in firing rate (± S.E.M.) of putative oxytocin neurones in the supraoptic nucleus of urethane-anaesthetized rats following administration of CCK (20 $\mu$g kg$^{-1}$, I.V. at time zero; arrow) in morphine-dependent and -naive rats before and after injection of naloxone (5 mg kg$^{-1}$, I.V.). ●, morphine-naive, pre-naloxone ($n = 10$); ▲, morphine-naive, post-naloxone ($n = 10$); ○, morphine-dependent, pre-naloxone ($n = 18$); △, morphine-dependent, post-naloxone ($n = 18$).

## DISCUSSION

In morphine-naive rats the magnocellular oxytocin neurones are highly sensitive to the inhibitory actions of opiates; systemic injections of 1–10 $\mu$g kg$^{-1}$ morphine I.V. will significantly reduce their electrical activity (Pumford *et al.* 1991). Yet in the sustained presence of very high concentrations of morphine (50 $\mu$g h$^{-1}$, I.C.V.) the oxytocin cells recover to near normal levels of spontaneous activity within 4–5 days (Bicknell *et al.* 1988), at which time they are relatively insensitive to systemic administration of morphine (Pumford, Russell & Leng, 1993). The present study demonstrates that this profile applies not only to spontaneous activity, but also to activity in response to a defined, noradrenergic afferent pathway. We have shown that CCK-induced oxytocin release can be blocked in rats following acute I.C.V. morphine, yet is normal following several days of continuous I.C.V. administration of morphine, at which point CCK-induced oxytocin release is relatively insensitive to inhibition by systemically administered morphine. Thus, oxytocin neurones display tolerance to morphine, and in particular their response to CCK injection displays tolerance.

The noradrenergic projection to the oxytocin neurones derives mainly from the A2 cell group within the nucleus tractus solitarii (NTS) (Cunningham & Sawchenko, 1991). This excitatory projection mediates the stimulatory effect of systemically administered cholecystokinin (CCK) upon oxytocin secretion. Systemically administered CCK acts upon $CCK_A$ receptors located on gastric vagal afferents (Luckman, Hamamura, Antonijevic, Dye & Leng, 1993), which relay in the brainstem. Following injection of CCK there is an extensive induction of Fos (the protein product of the immediate early gene, c-*fos*) in the NTS (Luckman, 1992). In particular, most of the A2 neurones which project to the supraoptic nucleus express Fos in response to CCK, as shown by retrograde labelling, coupled with double labelling for Fos protein and tyrosine hydroxylase immunoreactivity (Onaka *et al.* 1995*a*). In the dorsal, oxytocin-rich part of the supraoptic nucleus there is an increase in noradrenaline release following systemic CCK (Kendrick, Leng & Higuchi, 1991). Pharmacological antagonism of noradrenergic activity blocks the excitatory effect of CCK upon oxytocin neurones (Ueta, Kannan, Higuchi, Negoro & Yamashita, 1993), and destruction of the hypothalamic noradrenergic innervation by a selective neurotoxin eliminates the stimulatory effect of CCK upon oxytocin release (Onaka *et al.* 1995*a*).

CCK administration also results in Fos expression in the oxytocin neurones of the supraoptic nucleus (Verbalis, Stricker, Robinson & Hoffman, 1991). Systemically administered morphine blocks the CCK-induced Fos expression in the supraoptic nucleus, but not that in the NTS (Onaka *et al.* 1995*b*). However, morphine blocks the noradrenaline release that occurs in the supraoptic nucleus following CCK, and it does so whether given systemically or directly into the supraoptic nucleus (Onaka *et al.* 1995*b*). Thus the inhibitory effects of morphine upon CCK-induced noradrenaline release in the supraoptic nucleus appear to be attributable to an exclusively presynaptic action at the endings within the supraoptic nucleus. For tolerance to develop in the mechanisms leading to CCK-evoked oxytocin release, tolerance must be present at both the level of the oxytocin neurone and at the level of the noradrenergic input.

At the noradrenergic terminals, tolerance to opiates may consist of a downregulation of $\mu$-receptors (Sumner *et al.* 1990), or alternatively, of a compensatory increase in the releasability of noradrenaline, while at the level of the oxytocin neurones tolerance might involve a compensatory hyperexcitability in response to noradrenaline. However, morphine withdrawal revealed neither hyperexcitability of the oxytocin neurones in response to noradrenergic inputs, nor hyperexcitability of the noradrenergic inputs in response to afferent stimulation evoked by CCK, since either would be manifest as an increased response of oxytocin cells to CCK after morphine withdrawal.

In morphine-dependent rats, although oxytocin neurones are relatively insensitive to morphine, they are acutely sensitive to the opiate antagonist naloxone, indicating that functionally coupled opioid receptors are still present. When naloxone is administered to provoke acute morphine withdrawal, there is a prompt and sustained increase in the firing rate of oxytocin cells leading to a massive secretion of oxytocin (Bicknell *et al.* 1988). This indicates that, in addition to tolerance, the oxytocin system exhibits dependence upon morphine; that is, there is an amplified reversal of the initial effect of morphine upon antagonism with naloxone. In the present study we tested whether the withdrawal excitation of oxytocin neurones is a consequence of dependence developing in their noradrenergic input.

In clinical studies, the $\alpha_2$-adrenergic agonist clonidine acutely relieves the behavioural symptoms of morphine withdrawal (Gold, Redmond & Kleber, 1978) presumably by reducing noradrenaline release via actions at presynaptic autoinhibitory $\alpha_2$ adrenoceptors. Increased noradrenaline release occurs in several brain areas during morphine withdrawal, including the hippocampus (Done, Silverstone & Sharp, 1992; Silverstone, Done & Sharp, 1993), where clonidine attenuates this release, the prefrontal cortex (Rossetti, Longu, Mercuro & Gessa, 1993) and the supraoptic nucleus (Murphy, Onaka, Kendrick & Leng, 1995). *In vivo* studies have shown that the noradrenaline content of the whole hypothalamus is reduced after naloxone-precipitated morphine withdrawal (Martinezpinero, Milanes, & Vargas, 1994), and this reduction can be prevented by clonidine (Gonzalvez, Milanes, Martinezpinero, Marin & Vargas, 1994). The activation of noradrenaline release appears to follow increased electrical

activity of noradrenergic neurones (Aghajanian, 1978; Stornetta, Norton & Guyenet, 1993).

The present results demonstrate that activation of endogenous noradrenaline release (evoked by CCK) in morphine-dependent rats does not trigger withdrawal excitation of oxytocin neurones. The amplitude and profile of oxytocin cell activation and oxytocin release following CCK were no different from those observed in morphine-naive rats. Moreover, in morphine-dependent rats, the magnitude and profile of the oxytocin neurone response to CCK before and after naloxone were identical, although the post-naloxone response was superimposed on a greatly raised baseline of electrical activity. From this we conclude that the noradrenergic input is not maximally activated following withdrawal, since the oxytocin response to CCK was not occluded. Equally, as observed above, the oxytocin neurones were not hypersensitive to noradrenergic activation following morphine withdrawal and the noradrenergic input was not hypersensitive to activation by CCK; we observed nothing analogous to the hyperalgesia that typically accompanies morphine withdrawal (Rasmussen, Beitner-Johnson, Krystal, Aghajanian & Nestler, 1990). The simplest explanation would be that chronic administration of morphine leads to a loss of opioid receptors on the noradrenergic nerve terminals, leading to tolerance but not dependence at this site, while at the oxytocin cell bodies, both tolerance and dependence develop.

It is possible that we observed unaltered neuronal responses to CCK as a consequence of a fortuitous balance between partial occlusion and partial hypersensitivity. However, we note that the most parsimonious explanation of an observation of no change is that there has been no change. Thus in the supraoptic nucleus, chronic morphine treatment results in tolerance to the inhibitory effects of morphine both at the level of the oxytocin neurones themselves, and at the presynaptic nerve endings of afferent noradrenergic neurones. Dependence upon morphine appears to be a feature of the former but not of the latter.


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