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Opiate activation of potassium conductance inhibits calcium action potentials in rat locus coeruleus neurones

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Opiates act on μ-receptors to increase the potassium conductance of rat locus coeruleus neurones. Opiates also depress the rate of rise and peak amplitude of calcium action potentials in these cells. The action of opiates on calcium action potentials was prevented by two procedures which blocked the opiate-induced potassium current, intracellular caesium and extracellular barium. This indicates that the opiate reduction in calcium entry is secondary to an increased potassium current.

Introduction Opiates and opioid peptides inhibit neurotransmitter release from a variety of sites in the peripheral and central nervous system (Kosterlitz & Waterfield, 1975; Yaksh, Jessell, Gamse, Mudge & Leeman, 1980). One of these is the noradrenergic terminals of neurones with cell bodies in the nucleus locus coeruleus (LC) (Montel, Starke & Webber, 1974). This might result from a failure of action potential propagation (Morita & North, 1981), a reduction in inward calcium current (Mudge, Leeman & Fischbach, 1979) or an impairment of the ability of intracellular calcium to initiate release. The second of these possibilities can be tested directly by intracellular recording from the cell bodies of LC neurones if one assumes that calcium action potentials measured in the cell body are similar to those at the transmitter release sites.

Methods Intracellular recordings were made from more than 100 LC neurones in slices cut from rat pons (Henderson, Pepper & Shefner, 1982; Williams, Egan & North, 1982). The slices were submerged in continuously flowing, heated (37°C) solution containing (mM): NaCl 126, KCl 2.5, $CaCl_2$ 2.4, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $NaHCO_3$ 25, glucose 11, gassed with 5% CO_2 + 95% O_2. Recording electrodes contained 2 M KCl or 1 M CsCl (d.c. resistance 50–100 MΩ) and were incorporated into a bridge circuit so that currents could be passed across the cell membrane (Egan, Henderson, North & Williams, 1983). Membrane potentials were amplified and displayed on a chart recorder (Gould 2400). Action potentials were stored, averaged and then displayed (Dagan 4800). Drugs used were tetrodotoxin (Sankyo), morphine (Mallinckrodt), normorphine (NIDA), naloxone (Endo), D-Ala^2-NMePhe^4-Met(O)^5-enkephalin (FK33824) (Peninsula) and D-Ala^2-D-Leu^5-enkephalin (DADLE) (Peninsula). The compounds were applied both by superfusion and by pressure ejection. For pressure ejection the drug was contained in a pipette, the tip of which (10–15 μm diameter) was positioned above the brain slice but beneath the surface of the superfusing solution.

Results *Opiate hyperpolarizations* Normorphine and DADLE inhibited the spontaneous firing and hyperpolarized the membrane of all LC neurones, as we have described previously (Williams *et al.*, 1982) (e.g. Figure 1d). This effect is dose-dependent in the range of normorphine concentration 100 nM–10 μM, and results from interaction with a μ-receptor leading to opening of membrane potassium channels (Williams *et al.*, 1982; North & Williams, 1983). Addition of barium (100 μM–2 mM) to the superfusing solution caused a reversible block of opiate hyperpolarizations. When recordings were made with CsCl filled electrodes opiate hyperpolarizations decreased rapidly with time after impalement; by 30 min opiates caused only small hyperpolarizations even when applied in concentrations of 10 μM or more (Figure 1e). By contrast, when KCl electrodes were used opiates hyperpolarized all LC neurones, and this action was repeatable during the course of impalements lasting up to 10 h.

Opiate inhibition of calcium action potentials Opiates hyperpolarized all LC neurones and stopped spontaneous firing; therefore small depolarizing currents were passed through the recording electrode so that the firing rate returned to its control value (usually 1–2 Hz). The action potential configuration in these conditions was compared before and after opiates,

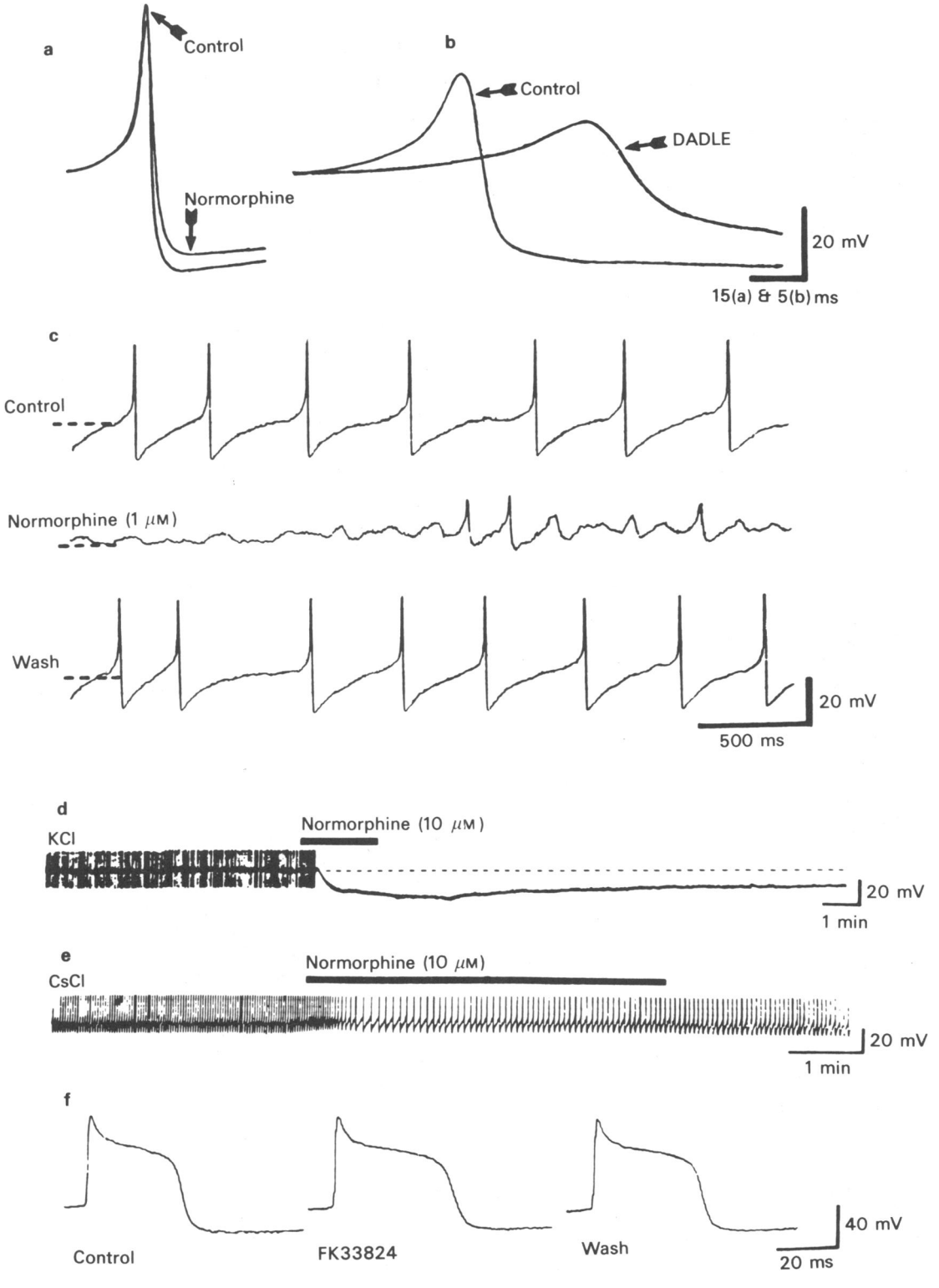
a
Control
Normorphine
b
Control
DADLE
20 mV
15(a) & 5(b) ms
c
Control
Normorphine (1 μM)
Wash
20 mV
500 ms
d
KCl
Normorphine (10 μM)
20 mV
1 min
e
CsCl
Normorphine (10 μM)
20 mV
1 min
f
Control
FK33824
Wash
40 mV
20 ms

Figure 1 The effect of opiates on calcium action potentials in rat locus coeruleus (LC) neurones. In (a), (b) and (c) all recordings were made in the presence of tetrodotoxin (TTX, 1 μM) and records in the presence of opiates were made after resetting the membrane potential to its control level. (a) and (b) show typical opiate effects on two different neurones. In (a) normorphine (1 μM) was applied by superfusion. In (b) DADLE was applied by pressure (70 kPa, 100 ms), The more rapidly rising action potentials were less depressed by normorphine. Among nine cells there was significant negative correlation ($P < 0.01$) between the maximum rate of rise of the action potential and the percentage depression by normorphine. In (c) a chart recording of a similar experiment is shown. In this cell, the calcium spike rose very slowly (12 Vs^{-1}) and was fully reproduced by the pen recorder. The calcium spike was almost completely abolished by normorphine (1 μM). The dotted line indicates − 57 mV. In (d), (e) and (f) opiates did not depress calcium action potentials when potassium currents were blocked. In (d) typical hyperpolarization caused by normorphine (10 μM) when recording with a KCl filled electrode is shown; (e), very small effect of normorphine when applied 30 min after impalement with a CsCl electrode; (f), the prolonged action potential recorded with a CsCl filled electrode was unaffected by FK 33824 pressure ejection (35 kPa, 50 ms), even though a small hyperpolarization still occurred. Spike was recorded after resetting membrane potential to original level. This action potential is recorded in the absence of TTX; addition of cobalt reduced the duration of such action potentials to about 2 ms (not shown).

and it was found that the 'shoulder' on the falling phase was somewhat depressed. A similar depression of the shoulder was caused by cobalt (2 mM). This finding suggested that opiates may reduce a calcium component to the action potential. Therefore, the LC neurones were studied in the presence of tetrodotoxin (300 nM–10 μM) (TTX). Spontaneous firing continued in the presence of TTX, but the rate of rise and amplitude of the action potentials were reduced. (Rate of rise: control 131 ± 9 Vs^{-1} (15), in TTX 20.9 ± 1.8 Vs^{-1} (17) and amplitude: control 82.5 ± 1.0 mV (6), in TTX 45.9 ± 4.0 (12); results expressed as mean ± s.e. mean for number (in parentheses) of cells). The TTX-resistant spikes were reversibly abolished in calcium-free solutions, or by addition of cobalt chloride (1–2 mM). Opiates reduced the amplitude and rate of rise of these calcium spikes and depressed the amplitude of the afterhyperpolarization which followed them. This effect, like the membrane hyperpolarization, was observed in all cells tested; it was maintained throughout the period of opiate application and reversed rapidly with washing. This action of opiates was completely reversed by naloxone applied either by pressure ejection or by perfusion (100 nM). The magnitude of the depression of the calcium spike varied considerably from cell to cell, but more slowly rising and lower amplitude calcium spikes were more depressed by opiates than those with fast rise times (Figure 1a, b). The inhibition of the TTX-resistant spike was observed only with concentrations of opiates (1–10 μM) that caused at least 15 mV hyperpolarization (prior to resetting the membrane potential). Within this range, the depression of the TTX-resistant spike was dependent on the concentration of opiate applied.

Opiate inhibition of calcium action potential results from potassium activation Since a reduction in the calcium spike was never observed without a concomitant increase in potassium conductance, it was likely that the spike was reduced not because of a direct effect on calcium channels but as a consequence of the increased potassium conductance. Barium (30–2 mM) reversibly prevented the inhibition of the calcium spike by normorphine and DADLE ($n = 15$). This action of barium occurred at concentrations which themselves did not significantly alter spike duration (30 μM). Higher concentrations increased the amplitude and duration of the action potential, but blocked opiate effects.

After a neurone was impaled with CsCl filled electrode the spike duration began to increase and the afterhyperpolarization following the spike decreased. The increase in spike duration was progressive with time until, after an hour or more, most spikes lasted from 20–100 ms. Application of TTX reduced the rate of rise of the spike to values similar to those observed with KCl filled electrodes, although the peak amplitude of the spike was only slightly depressed by TTX. This suggests that the amplitude and duration of the calcium spike are partly determined by the opening of potassium channels which do not easily pass caesium. Opiates were applied repeatedly at increasing times after impalement with a caesium filled electrode. The hyperpolarizations became progressively smaller, until after 30 min of impalement hyperpolarizations could be observed only with normorphine concentrations of 10 μM or more (Figure 1e). Normorphine, DADLE and FK 33,824 had no effect on the TTX-resistant spike after 30 min of recording with a caesium filled electrode (Figure 1), even in those cells in which a small hyperpolarization persisted.

Discussion The results indicate that opiates indirectly modulate calcium entry into central neurones by shortening the action potential duration, as a result of an increased potassium conductance. The shortening of the action potential was observed only with opiate concentrations (> 1 μM) which caused

large (15–20 mV) hyperpolarizations. In immature chick sensory ganglion cells (Mudge *et al.*, 1979) enkephalin directly reduces calcium entry without increasing potassium conductance (experiments were done in high barium). Similar results have been reported in mouse sensory cells (Werz & Macdonald, 1982) and frog spinal cord cells (Bixby & Spitzer, 1983). It seems that the direct opiate action on calcium channels is a feature of a stage of development, which disappears in the adult (Williams & Zieglgansberger, 1982). Or the action on calcium channels could result from occupation of a different type of receptor; in those experiments in which high agonist concentrations were used naloxone K_is were not determined.

The present experiments do not allow one to conclude that the indirect modulation of calcium entry is the most important way in which opiates inhibit transmitter release. A decrease in excitability of the nerve fibres, perhaps by a hyperpolarization, may be much more significant by blocking action potential propagation (Nakamura, Tepper, Young, Ling & Groves, 1982).

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