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Acute morphine administration and withdrawal from chronic morphine increase afterdepolarization amplitude in rat supraoptic nucleus neurons in hypothalamic explants

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ABSTRACT

Supraoptic nucleus (SON) neurons secrete either oxytocin or vasopressin into the bloodstream from their axon terminals in the posterior pituitary gland. SON neurons are powerfully inhibited by the classical μ-opioid receptor agonist, morphine. Oxytocin neurons develop morphine dependence when chronically exposed to this opiate, and undergo robust withdrawal excitation when morphine is subsequently acutely antagonized by naloxone. Morphine withdrawal excitation is evident as an increased firing rate and is associated with an increased post-spike excitability that is consistent with the expression of an enhanced post-spike afterdepolarization (ADP) during withdrawal. Here, we used sharp electrode recording from SON neurons in hypothalamic explants from morphine naïve and morphine treated rats to determine the effects of morphine on the ADP, and to test the hypothesis that morphine withdrawal increases ADP amplitude in SON neurons. Acute morphine administration (0.05–5.0 μM) caused a dose-dependent hyperpolarization of SON neurons that was reversed by concomitant administration of 10 μM naloxone, or by washout of morphine; counter-intuitively, acute exposure to 5 μM morphine increased ADP amplitude by 78 ± 11% (mean ± SEM). Naloxone-precipitated morphine withdrawal did not alter baseline membrane potential in SON neurons from morphine treated rats, but increased ADP amplitude by 48 ± 11%; this represents a hyper-activation of ADPs because the basal amplitude of the ADP was similar in SON neurons recorded from explants prepared from morphine naïve and morphine treated rats. Hence, an enhanced ADP might contribute to morphine withdrawal excitation of oxytocin neurons.
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1. Introduction

Morphine is the classical agonist of μ-opioid receptors, which are widely expressed within the central nervous system (Mansour et al., 1995). However, relatively few neuronal phenotypes develop dependence when chronically exposed to morphine; these include hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) magnocellular oxytocin neurons (Brown and Russell, 2004; Brown et al., 2000) that project to the posterior pituitary gland where they secrete oxytocin into the systemic circulation.

Morphine acutely inhibits oxytocin neurons *in vivo* (Ludwig et al., 1997), but during chronic intracerebroventricular (ICV) administration, oxytocin neurons develop dependence on morphine. Dependence is revealed by a marked and sustained increase in firing rate of oxytocin neurons (and a consequent large increase in oxytocin secretion) upon morphine withdrawal *in vivo* (Bicknell et al., 1988; Blackburn-Munro et al., 2000; Brown et al., 1996, 1998; Bull et al., 2003) that is accompanied by increased Fos protein and oxytocin heteronuclear RNA expression in oxytocin neurons (Johnstone et al., 2000), as well as increased oxytocin release into the brain from oxytocin neuron somata and dendrites (Brown et al., 1997; Russell et al., 1992). Withdrawal excitation occurs in oxytocin neurons without a marked change in the activation of their major afferent inputs (Murphy et al., 1997). Moreover, oxytocin neurons are excited by direct administration of the opioid receptor antagonist, naloxone, into the SON of morphine treated rats (Johnstone et al., 2000; Ludwig et al., 1997). Thus, oxytocin neurons evidently develop dependence at a cellular level and provide a robust model that is amenable to detailed analysis of the cellular mechanisms of morphine dependence (Brown and Russell, 2004).

By contrast to oxytocin neurons, SON vasopressin neurons do not show morphine dependence; after chronic morphine treatment, naloxone administration excites some vasopressin neurons

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and inhibits others (Bicknell et al., 1988; Brown et al., 2005), so the overall effect is to induce only a modest increase in plasma vasopressin concentrations (Bicknell et al., 1988).

Excitability of SON neurons is strongly influenced by non-synaptic post-spike potentials (Brown, 2004; Brown and Bourque, 2006), including an afterdepolarization (ADP) and a medium afterhyperpolarization (mAHP). mAHP amplitude is reduced in SON neurons during morphine withdrawal (Brown et al., 2005), indicating that a reduced mAHP might contribute to the increased firing rate of oxytocin neurons evident during morphine withdrawal. However, the changes in post-spike excitability that accompany the withdrawal-induced increase in firing rate *in vivo* indicate that morphine withdrawal might also expose an ADP in oxytocin neurons (Brown et al., 2005), which could drive withdrawal excitation.

Here, we used sharp electrode recording from SON neurons in hypothalamic explants from morphine naïve and morphine treated rats to determine the effects of chronic morphine on the ADP and to test the hypothesis that morphine withdrawal increases ADP amplitude in SON neurons.

2. Material and methods

2.1. Ethical approval

All experimental procedures were approved by the University of Otago Animal Ethics Committee and were carried out in accordance with the recommendations of the Australian and New Zealand Council for the Care of Animals in Research and Teaching.

2.2. Electrophysiology

Female Sprague-Dawley rats (200–350 g) were restrained in a soft plastic cone (5–10 s) and decapitated. The brains were rapidly removed and a block of tissue 8 × 8 × 2 mm (dorso-ventral) containing the basal hypothalamus was excised using razor blades and pinned, ventral surface up, to the silicone elastomer base of a superfusion chamber. Within 2–3 min, the excised hypothalamic explant was superfused (0.5–1.0 ml min^{-1}, 32–33 °C) with carbogenated (95% oxygen and 5% carbon dioxide) artificial CSF (aCSF; see below) delivered via tubing placed over the medial tuberal region. The arachnoid membrane overlying the SON was removed using fine forceps, and a tissue paper wick was placed at the rostral tip of the explant to facilitate aCSF drainage. The aCSF (pH 7.4; 295 ± 3 mOsmol kg^{-1}) consisted of (in mM): 120 NaCl, 3 KCl, 1.2 $MgCl_2$, 26 $NaHCO_2$, 2.5 $CaCl_2$, and 10 glucose (Sigma).

Intracellular recordings were made using sharp micropipettes prepared from glass capillaries (1.5 mm O.D. 0.86 mm I.D.) pulled on a P-97 Flaming–Brown puller (Sutter Instruments, Novato, CA). Micropipettes were filled with 2 M potassium acetate to yield DC resistances of 70–160 MΩ to a Ag–AgCl wire electrode immersed in aCSF. Voltage recordings were obtained using an Axoclamp 2B amplifier (Molecular Devices, Foster City, CA) in continuous current-clamp ('bridge') mode. Digitized signals (10 kHz; DigiData 1320 Interface, Molecular Devices) were stored on a personal computer running pClamp9.2 (Molecular Devices) and analyzed offline.

Recordings were made from SON neurons impaled with sharp electrodes in superfused hypothalamic explants. The cells had resting membrane potentials more negative than −50 mV, input resistances of >150 MΩ, and spike amplitudes of >60 mV when measured from baseline. Each cell displayed frequency-dependent spike broadening and transient outward rectification when depolarized from initial membrane potentials more negative than −75 mV (characteristics specific to SON neurons (Renaud and Bourque, 1991)). To determine the effects of acute morphine, and withdrawal from chronic morphine, on SON neuron membrane potential, recordings were made from an initial baseline membrane potential 5–10 mV below spike threshold (maintained by injection of hyperpolarizing current if necessary, with a constant current injected throughout the recording from each neuron).

Depolarizing current injection (80 ms) was applied to elicit a train of 4–5 action potentials (the number of spikes in the trains was kept constant for each cell). Subthreshold ADPs were recorded from cells maintained at a baseline membrane potential approximately 5–10 mV below the spike threshold whereas spontaneous action potential afterdischarge during suprathreshold ADPs was measured from cells held approximately 2 mV below threshold. To determine the effects of the drugs on ADP and mAHP amplitude, as well as afterdischarge, measurements in the presence of drug were made from a baseline membrane potential between 1 mV more positive and 2 mV more negative than that used for pre-drug measurements.

2.3. Induction of morphine dependence

Morphine dependence was induced as previously described (Rayner et al., 1988). Briefly, virgin female Sprague-Dawley rats (bodyweight 200–350 g) were anaesthetized with halothane (≤5% in O_2). An Alzet model 2001 mini-osmotic pump (Charles River Ltd.) was placed subcutaneously and connected via polythene tubing to a 21 gauge stainless steel cannula implanted 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). The pump and tubing were filled with morphine sulfate (Sigma) in sterile pyrogen-free water to deliver increasing doses (10 μg h^{-1}, 20 μg h^{-1} for 40 h each and 50 μg h^{-1} for the remainder at 1 μl h^{-1}) over 5 days. The cannula was secured using dental acrylic bonded to stainless steel screws inserted in the skull. Following surgery rats were housed individually with food and water available *ad libitum*.

For electrophysiological recording (the sixth day following minipump implantation for morphine treated rats) in explants from morphine treated rats, the aCSF contained 5 μM morphine.

2.4. Statistics

All averaged data are expressed as the mean ± the standard error of the mean (SEM). All differences within groups were evaluated with SigmaPlot software (SPSS Science, Chicago, IL, USA) using Student's *t*-tests, paired *t*-tests or one-way repeated measures (RM) ANOVA, where appropriate. Where the *F*-ratio was significant, *post-hoc* comparisons were completed as described in the figure legends.

3. Results

3.1. Effects of acute morphine and naloxone-precipitated withdrawal from chronic morphine on SON neuron membrane potential

To determine the effects of acute morphine administration, membrane potential was measured during superfusion of 50 nM, 500 nM and 5 μM morphine followed by 10 μM naloxone (in the continued presence of morphine). Morphine caused a small,

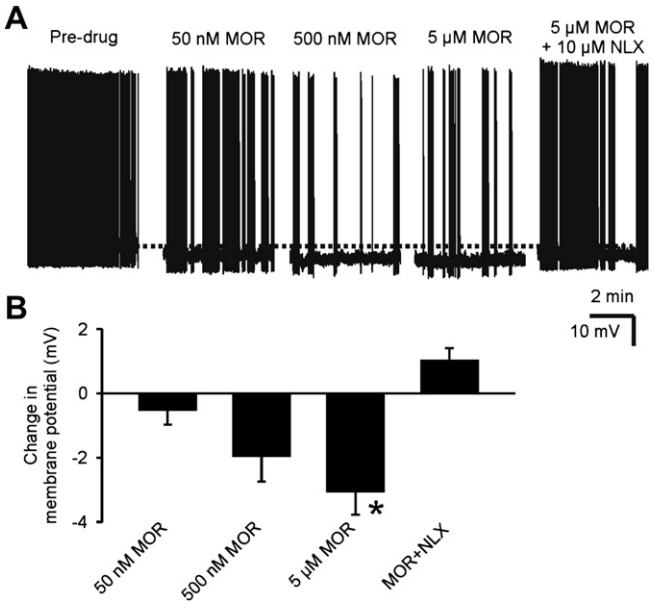

Fig. 1. Acute morphine hyperpolarizes SON neurons *in vitro*. A, Example of the reversible, dose-dependent effects of morphine on SON neuron membrane potential, typical of all five neurons tested. The segments of recording shown were all made during injection of 25 pA of hyperpolarizing current and show the steady-state effects of each treatment on baseline membrane potential (relative to control: dashed line after >5 min superfusion of drug); continuous gap free recordings were interrupted to permit measurement of post-spike potentials. B, Change in membrane potential (mean ± SEM) of five SON neurons during superfusion of morphine (MOR) and morphine + 10 μM naloxone (MOR + NLX), showing a dose-dependent, naloxone-reversible hyperpolarization of SON neurons by morphine ($P = 0.012$, one-way RM ANOVA). *$P < 0.05$ versus pre-drug and MOR + NLX, Student–Newman–Keuls *post-hoc* tests.

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