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SHORT COMMUNICATION
Metabotropic glutamate receptor 2/3-dependent long-term depression in the nucleus accumbens is blocked in morphine withdrawn mice

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Abstract

The nucleus accumbens (NAc) plays a crucial role in addiction. We have recently shown that activation of presynaptic metabotropic glutamate 2/3 receptors (mGlu2/3) induces long-term depression (LTD) at glutamatergic synapses in the mouse nucleus accumbens (NAc) through the long lasting inhibition of P/Q-type Ca^{2+} channels and the cAMP/protein kinase A (PKA) pathway. Because presynaptic mGlu2/3 functions are augmented in the ventral tegmental area of morphine-withdrawn rats, we have evaluated the consequences of opiate treatment on mGlu2/3 LTD at prelimbic NAc glutamatergic synapses. Here we report that mGlu2/3 LTD is abolished after 1 week of withdrawal from chronic morphine treatment; in the morphine-withdrawn group LTD measured 5.99 $\pm$ 4.84% ($P < 0.05$) compared with 21.13 $\pm$ 5.42% in the sham group. In contrast, chronic morphine treatment did not alter the mechanisms normally underlying mGlu2/3 LTD, such as the cAMP/PKA pathway or P/Q-type Ca^{2+} channels. This study shows that one long-term consequence of morphine treatment is an alteration of synaptic plasticity at glutamatergic synapses in the NAc. Considering that mGlu2/3 agonists (e.g. LY-354740 used in the present study to induce LTD) reduce behavioural symptoms of morphine withdrawal, these findings could be important in the understanding of the cellular events underlying the dependence-inducing properties of opiates.

Introduction

The nucleus accumbens (NAc) is implicated in behaviours associated with natural rewards, such as feeding or reproduction (Kelley & Berridge, 2002) and plays a crucial role in addiction to most drugs of abuse (De Vries & Shippenberg, 2002; Everitt & Wolf, 2002; White, 2002). In the NAc, pharmacological inhibition of glutamatergic transmission alters the addictive property of drugs such as cocaine or ethanol (Pulvirenti *et al*., 1992; Rassnick *et al*., 1992; Cornish *et al*., 1999; Cornish & Kalivas, 2000), suggesting that glutamate receptors participate to the development of addiction. Our recent work shows that at the glutamatergic synapses between prelimbic cortex afferents and medium spiny neurons of the NAc, activation of presynaptic mGlu2/3 receptors can induce LTD (Robbe *et al*. 2002). A current hypothesis is that addiction is associated with long-term alterations of synaptic functions (Williams *et al*. 2001). Because it has been shown previously that: (i) presynaptic mGlu2/3 functions are augmented in the ventral tegmental area (VTA) of morphine-withdrawn rats (Manzoni & Williams, 1999); and (ii), mGlu2/3 receptors modulation reduce behavioural signs of morphine withdrawal (Fundytus & Coderre, 1997; Fundytus *et al*., 1997; Vandergriff & Rasmussen, 1999), we decided to evaluate the consequences of morphine withdrawal on mGlu2/3 LTD at prelimbic NAc synapses.

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Materials and methods

Mice withdrawal protocol and morphine preparation

Mice (C57B1/6 strain; 4 weeks old) were group housed and acclimatized to the laboratory conditions (12 h light : darkness cycle) 1 week before the experiment with access to food and water *ad libitum*. Animals were injected intraperitoneally twice daily between 08.00 h and 09.00 h and between 18.00 h and 19.00 h for 7 days with morphine sulphate (20 mg/kg) or vehicle. On days 8–14, mice received no injection. On day 15 mice were anaesthetised and killed by decapitation and slices of NAc were prepared as described below. Morphine sulphate was prepared in a solution of 0.9% NaCl at a concentration of 4 mg/mL. A total volume of 100 µL was injected for mice weighing 20 g.

Slice preparation and electrophysiology

Extracellular field excitatory postsynaptic potential (fEPSP) recordings were made from medium spiny neurons in parasagittal slices of mouse NAc as described previously (Robbe *et al*., 2002). To evoke synaptic potential, stimuli (150 µs duration) were delivered at 0.033 Hz through bipolar tungsten electrodes placed at the prefrontal

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cortex–accumbens border. Input–output curves were performed at the beginning of each experiment by measuring fEPSP evoked by a range of increasing intensities of stimulation. The intensity inducing the third of the maximum response was used as the test stimulation during the entire experiment. Recordings were made in the rostral–medial dorsal accumbens close to the anterior commissure.

Data analysis and drugs

All values are given as mean ± SEM. Statistical analyses were carried out with the Mann–Whitney *U*-test ($P < 0.05$ was taken as indicating statistical significance) using Statview (Abacus Concepts Inc., USA). Drugs used were picrotoxin, forskolin, DPCPX (Sigma); 2-amino-2-(2-carboxycyclopropan-1-yl)-3-(dibenzopyran-4-yl) propanoic acid (LY-341495) (Tocris, France); ω-agatoxin IVA (Alexis, France); (+)-2-aminobicyclo-(3.1.0) hexane-2,6-dicarboxylic acid (LY-354740) was a gift of Drs A. Schoepp and Monn at Eli Lilly (Indianapolis, IN, USA). Other chemicals were from the highest commercial grade available.

Results

mGlu2/3 LTD was induced by a 10 min application of the selective mGlu2/3 agonist, LY-354740 (200 nM). In the sham group LY-354740 induced an initial fEPSP inhibition of 57.49 ± 5.75%, followed 60 min after washout by a LTD of 21.13 ± 5.42% (representative experiment in Fig. 1B and averaged data in Fig. 1D). These results reproduced those obtained previously (Robbe *et al*., 2002) and show that saline treatment did not alter mGlu2/3 LTD. In slices from morphine-withdrawn mice, LY-354740 induced an initial fEPSP inhibition of 58.52 ± 3.67%, similar to that observed in the sham group ($P > 0.05$ vs. sham). Strikingly, LTD, measured 60 min after washout of LY-354740, was reduced greatly compared with LTD observed in sham experiments (representative experiment in Fig. 1C and averaged data in Fig. 1D). In the morphine-withdrawn group LTD measured 5.99 ± 4.84% ($P < 0.05$ vs. 21.13 ± 5.42% in the sham group).

We next investigated how morphine withdrawal alters mGlu2/3 LTD. Input–output curves obtained in measuring fEPSP amplitudes evoked by an increasing range of stimulation intensity were similar between the two groups (Fig. 2A), suggesting that in the NAc, the efficacy of synaptic transmission is unchanged in slices of morphine-withdrawn mice. In the NAc, mGlu2/3 LTD involves long-term inhibition of P/Q-type Ca^{2+} channels (Robbe *et al*., 2002). Application of the selective P/Q-type Ca^{2+} channel blocker, ω-agatoxin IVA decreases fEPSP in a similar way in slices prepared from sham and morphine-withdrawn mice (Fig. 2B). This strongly suggests that mGlu2/3 LTD reduction does not result from changes in

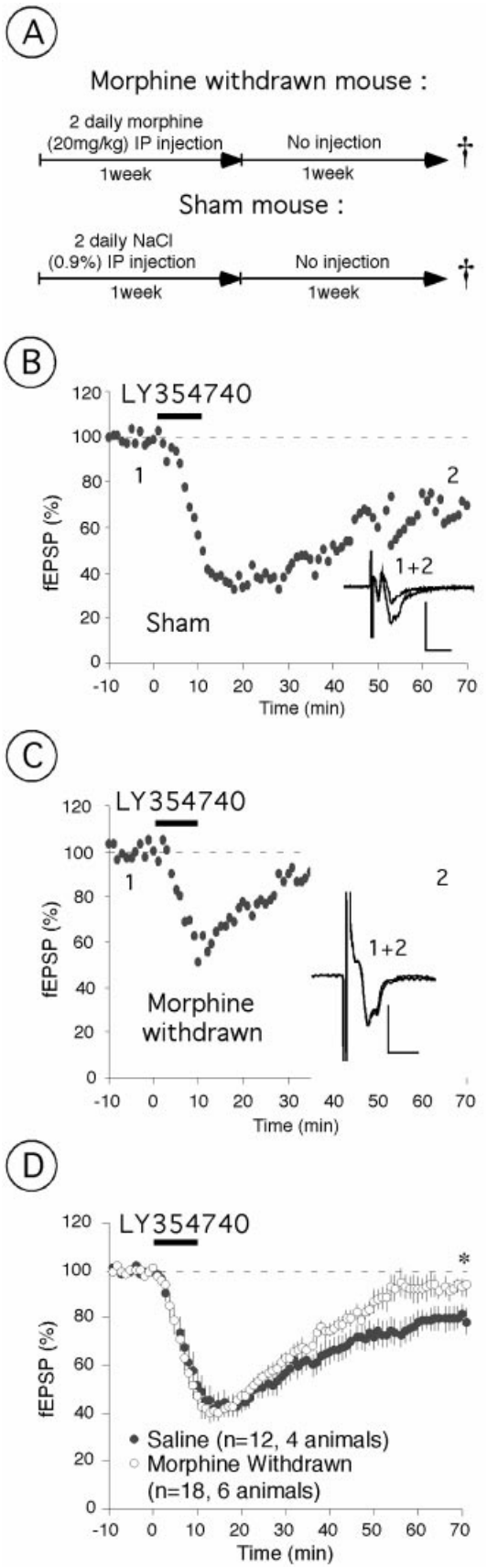

FIG. 1. mGlu2/3 LTD is suppressed in morphine-withdrawn mice. (A) Drug treatment for the two groups of mice. (B) Typical fEPSP experiment showing LTD induced by LY-354740 in a slice from a sham mouse. Inset shows traces (average of 10 consecutive eEPSCs) taken at points indicated on graph. Calibration bars, 0.3 mV, 20 ms. (C) Typical fEPSP experiment showing that LY-354740 failed to induce LTD in a slice from a morphine-withdrawn mouse. Inset shows traces (average of 10 consecutive eEPSCs) taken at points indicated on graph. Calibration bars, 0.3 mV, 20 ms. (D) Summarized data showing that application of LY-354740 (200 nM) results in an initial depression which is followed by LTD. LTD was greatly reduced in slices from morphine-withdrawn animals.

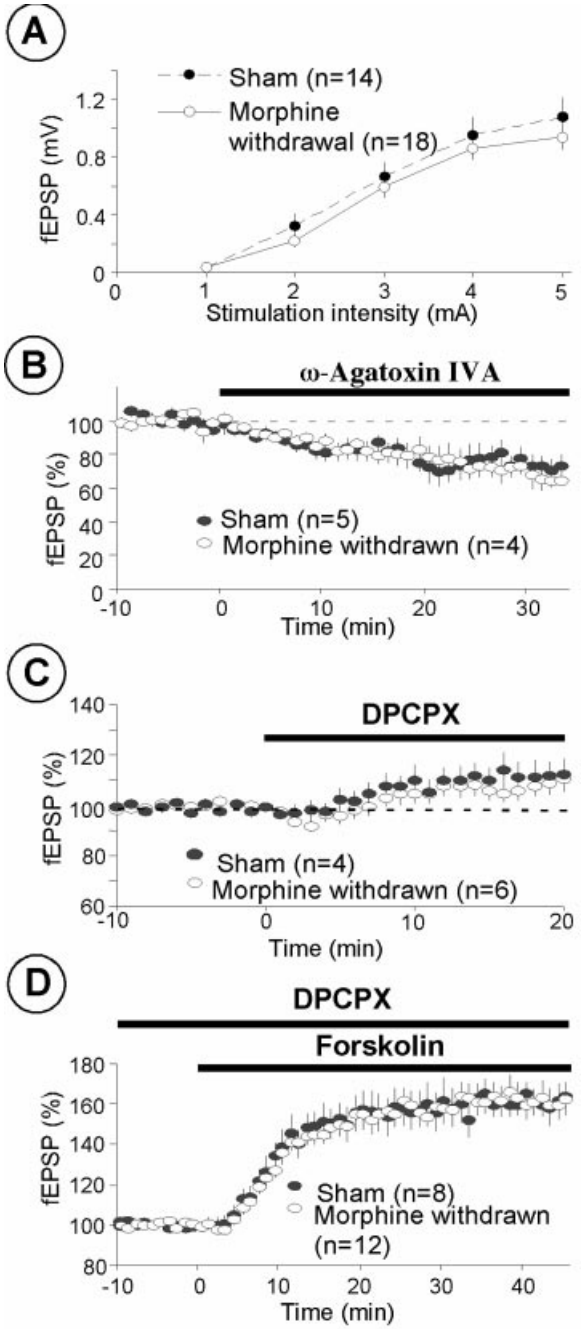

FIG. 2. Efficacy of NAc glutamatergic synaptic transmission and main molecular components of mGlu2/3 LTD are not affected by morphine withdrawal. (A) Input–output curves for slices of sham and morphine-withdrawn mice are similar. (B) Summarized data showing that application of ω-agatoxin IVA (200 nM) inhibits fEPSP with the same magnitude in the two groups of experiments. (C) DPCPX (500 nM) induces a small increase of fEPSP of the same magnitude in slices from morphine-withdrawn and sham mice. (D) Application of forskolin (10 μM) in presence of DPCPX (500 nM) induces a large increase of the fEPSP. This increase is similar in slices from morphine-withdrawn or sham mice.

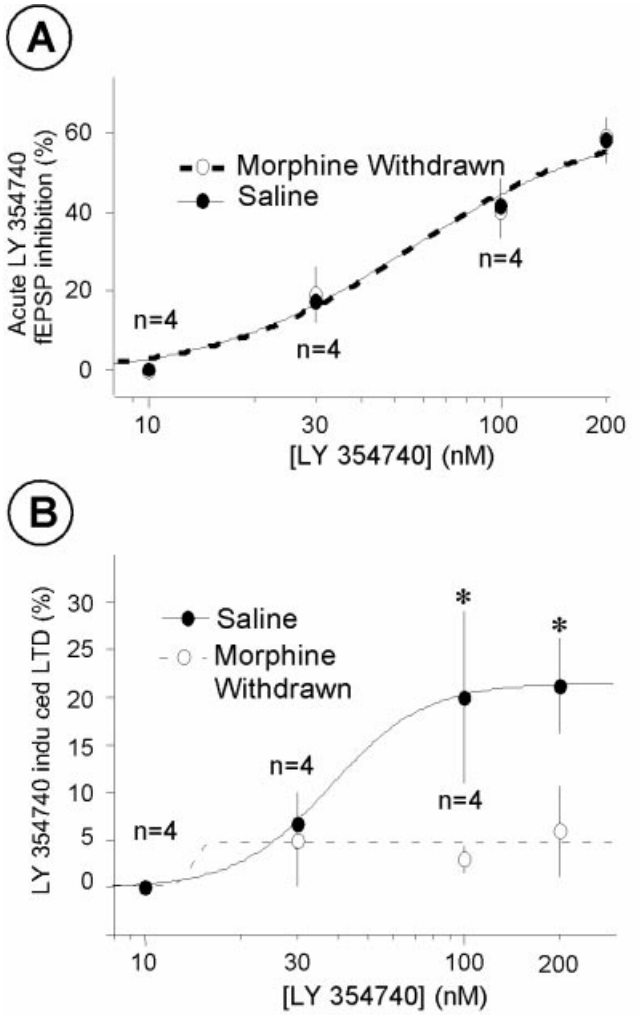

FIG. 3. Morphine withdrawal selectively reduced mGlu2/3 LTD but not mGlu2/3-induced initial fEPSP inhibition. (A) Dose–response curves for the LY-354740-induced initial inhibition of the fEPSP are similar in sham and morphine-withdrawn mice. (B) Dose–response curves for the LY-354740-induced LTD. In slices from morphine-withdrawn mice, LTD was abolished at the two doses that induced LTD in sham mice.

the contribution of P/Q-type Ca^{2+} channels to evoked synaptic transmission in response to morphine withdrawal. Because mGlu2/3 LTD in the NAc depends on the cAMP/PKA cascade (Robbe *et al*., 2002), a transduction pathway altered by chronic morphine treatment (Nestler, 2001; Williams *et al*., 2001), we next evaluated if adenylate cyclase activity was modified in slices prepared from the morphine-withdrawn group. Cyclic AMP is metabolized rapidly to adenosine, which inhibits synaptic transmission through A1 receptors (Rosenberg *et al*., 1994; Bonci & Williams, 1996; Brundege *et al*., 1997). A change in adenylate cyclase activity could therefore be reflected by a change in endogenous activation of A1 receptor by adenosine (Bonci & Williams, 1996). To test this hypothesis, slices were treated with the A1 receptor antagonist DPCPX (500 nM). DPCPX induces a small increase of fEPSP indicating the existence of a tonic fEPSP inhibition by adenosine at the prelimbic NAc synapses. No difference was observed between slices of morphine-withdrawn and sham mice (Fig. 2C). This result is in perfect agreement with another study showing that tonic adenosine inhibition of glutamatergic transmission in rat NAc is not affected by chronic morphine treatment (Brundege & Williams, 2002). The effect of an activator of adenylate cyclase, forskolin (10 μM) was also tested, in the presence of DPCPX. Forskolin increased fEPSP with the same magnitude in the two groups of animals (Fig. 2D). These results suggest that the cAMP/PKA pathway is not altered during morphine withdrawal at the prelimbic NAc synapses.

Another possible explanation for the absence of mGlu2/3-LTD in morphine-withdrawn mice is that morphine treatment caused a reduction in the number of presynaptic mGlu2/3 receptors or in their efficacy to inhibit glutamate release. LY-354740 induces an initial fEPSP inhibition in a dose-dependent manner (Robbe *et al*., 2002). A change in number or function of mGlu2/3 should be reflected in the dose–response curve of LY-354740-induced initial fEPSP inhibition. Figure 3A shows that initial fEPSP inhibition by LY-354740 is unchanged at all the concentrations tested. In marked contrast, Fig. 3B shows that in morphine-withdrawn slices, LTD is reduced at the concentrations that induced LTD in the sham experiments. These results show that morphine withdrawal is accompanied by a selective decrease in the transduction pathway leading to LTD but leaves intact mGlu2/3-induced initial fEPSP inhibition.

Discussion

This study shows the first evidence that morphine treatment affects a presynaptic form of synaptic plasticity in the brain; presynaptic mGlu2/3 LTD was absent in the NAc of morphine-withdrawn mice. Surprisingly, chronic morphine treatment did not alter the pathways normally mediating mGlu2/3 LTD. As we have shown recently (Robbe *et al*., 2002) mGlu2/3 LTD at prelimbic cortex–NAc synapses depends on P/Q-type Ca^{2+} channels and a cAMP/PKA pathway. Similar fEPSP inhibition by ω-agatoxin IVA and fEPSP enhancement by DPCPX and forskolin were found in both sham and morphine-withdrawn groups, suggesting that morphine withdrawal reduces mGlu2/3 LTD but not two of its key molecular components. Thus an important conclusion of this work, based on the observation that morphine withdrawal selectively abolishes the long-term effect of mGlu2/3 activation, is that mGlu2/3 LTD and mGlu2/3 initial fEPSP inhibition are distinct processes triggered by the same receptor (Robbe *et al*., 2002). At γ-aminobutyric acid (GABA)ergic synapses of NAc interneurons, both forskolin- and DPCPX-induced increases in GABAergic transmission were augmented in slices of morphine-withdrawn animals (Chieng & Williams, 1998). Such alterations have not been observed at the glutamatergic synapses between prelimbic cortex and NAc (present report and Brundege & Williams, 2002) indicating that adaptation of the cAMP/PKA pathway after chronic drug treatment is synapse-specific (Williams *et al*., 2001).

Other explanations of the absence of mGlu2/3 LTD in morphine-withdrawn mice were sought without result. For instance, we observed similar dose–responses curves for the LY354740-induced initial fEPSP inhibition in the morphine-withdrawn and sham groups, arguing against the idea that morphine withdrawal leads to a decrease of mGlu2/3 receptor number. It is noteworthy that our observation is reminiscent of another recent study showing that a single *in vivo* injection of cocaine altered synaptic plasticity in the VTA 'without changing the total expression of AMPA receptors '(Ungless *et al*., 2001; see also Thomas *et al*., 2001). Other possibilities could not be tested because of limitations inherent to our model. First, LY354740-induced mGlu2/3 LTD does not depend on presynaptic activity (Robbe *et al*. 2002) hampering the investigation of the effects of morphine withdrawal on presynaptic release of glutamate. Second, in the periaqueductal grey area of morphine-withdrawn rats, enhanced opioid inhibition of synaptic transmission results from an alteration of the signal transduction pathway linking μ-opioid receptors to presynaptic potassium channels (Ingram *et al*., 1998). Such an alteration in the coupling between mGlu2/3 and P/Q-type Ca^{2+} channels could be responsible for the decreased mGlu2/3 LTD by morphine withdrawal but because mGlu2/3 LTD was nearly completely abolished in our study, this interesting hypothesis could not be tested.

Although the mechanisms underlying the selective decrease of mGlu2/3 LTD during morphine withdrawal remain elusive, the fact that mGlu2/3 LTD is reduced by morphine withdrawal could prove important for an understanding of the synaptic consequences of addictive drug abuse. First, pharmacological agents acting at mGlu2/3 receptors (such as LY-354740, used here to induce LTD) have proven remarkably effective in reducing the behaviours associated not only with morphine (Fundytus & Coderre, 1997; Fundytus *et al*., 1997) or nicotine (Helton *et al*., 1997) withdrawal but also with psychostimulant and phencyclidine use (Cartmell *et al*., 1999, 2000). Second, in the VTA, mGlu2/3 presynaptic function is increased in morphine-withdrawn rats (Manzoni & Williams, 1999) which, together with the present study, strongly suggests that regulation of mGlu2/3 synaptic function (specifically synaptic plasticity) in the mesolimbic system could be a key cellular event in the development of addiction to opiates. Finally, together with other data showing alterations in both LTD (Thomas *et al*., 2001) and long-term potentiation (Ungless *et al*., 2001; Harrison *et al*., 2002) in response to various regimens of treatment with addictive drugs (cocaine and morphine) the present results reinforce the emerging idea that alterations of synaptic plasticity in the central nervous system participate in the development of addiction.

Abbreviations

fEPSP, extracellular field excitatory postsynaptic potential; GABA, γ-aminobutyric acid; LTD, long-term depression; NAc, nucleus accumbens; PKA, protein kinase A.

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