

Influences of Morphine on the Spontaneous and Evoked Excitatory Postsynaptic Currents in Lateral Amygdala of Rats

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Summary

Acute morphine exposure induces antinociceptive activity, but the underlying mechanisms in the central nervous system are unclear. Using whole-cell patch clamp recordings, we explore the role of morphine in the modulation of excitatory synaptic transmission in lateral amygdala neurons of rats. The results demonstrate that perfusion of 10 μ M of morphine to the lateral amygdala inhibits the discharge frequency significantly. We further find that there are no significant influences of morphine on the amplitude of spontaneous excitatory postsynaptic currents (sEPSCs). Interestingly, morphine shows no marked influence on the evoked excitatory postsynaptic currents (eEPSCs) in the lateral amygdala neurons. These results indicate that acute morphine treatment plays an important role in the modulation on the excitatory synaptic transmission in lateral amygdala neurons of rats.

Key words

Discharge frequency • Excitatory synaptic transmission • Evoked excitatory postsynaptic currents (eEPSCs) • Lateral amygdala • Morphine • Spontaneous excitatory postsynaptic currents (sEPSCs)

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Acute morphine exposure induced analgesia, while the underlying mechanism might be the rapid neural adaptations and the changes of synaptic transmission (Beckerman *et al.* 2013, Zheng *et al.* 2014). The amygdala, a forebrain structure that is positioned to influence pain-modulating circuits (Manning 1998), was involved in morphine analgesia, morphine-induced withdrawal, and morphine-induced conditioned place preference (Calvino *et al.* 1982, Freedman and Aghajanian 1985, Good and Westbrook 1995, Helmstetter *et al.* 1993, Lin *et al.* 2011, Stinus *et al.* 1990). Previous studies suggested that the glutamatergic and GABAergic transmission in amygdala were affected by acute morphine exposure (Beckerman *et al.* 2013, Rashvand *et al.* 2014). The amygdala is anatomically complex, consisting of the central amygdala (CeA), the basolateral amygdala (BLA) and the lateral amygdala (LA) (McDonald 1998). The BLA (Glass *et al.* 2005, He *et al.* 2011, Helmstetter *et al.* 1995) and CeA (Manning 1998, Manning and Mayer 1995a,b, Rezayof *et al.* 2011) were postulated to mediate morphine antinociception and rewarding effects, but whether LA plays important roles in acute morphine effects is not clear. Therefore, the present study was carried out to investigate the role of morphine in the modulation of excitatory synaptic transmission in rat LA neurons.

Juvenile Sprague-Dawley rats (P17-P30) were used. Rats were housed under a 12-h light/dark cycle with food and water *ad libitum*. All procedures were approved

by the Institutional Animal Care and Use Committee of Peking University.

Brain slices were prepared as described previously (Liu *et al.* 2011) with minor modifications. Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and decapitated. The brain was removed into ice-cold high sucrose cutting solution containing (in mM) 90 sucrose, 87 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 7 MgCl₂, 0.5 CaCl₂, 25 NaHCO₃, 15 glucose and cooled for 3 min. Then 300- μ m coronal slices were cut on a vibratome (Leica VT 1000 S, German). Immediately after cutting, slices were stored at 33 °C for 45 min in artificial cerebrospinal fluid (ACSF) containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 1 MgCl₂, 2 CaCl₂, 25 NaHCO₃ and 10 glucose and were equilibrated with 95 % O₂/5 % CO₂. Whole-cell recordings were done in a chamber superfused with carbogenated ACSF and were performed from visualized neurons located in the LA, with 10 μ M of bicuculline (Sigma, USA) in the superfusion ACSF to block GABA_A receptors. Glass microelectrodes (input resistance 2-4 M Ω) were filled with an internal solution containing (in mM):

122.5 Cs-gluconate, 17.5 CsCl, 10 HEPES, 0.5 EGTA, 2 MgCl₂, 4 ATP, pH 7.2-7.4, osmolarity 300-310 of mOsm. Neurons from LA were recorded using a Heka EPC10 amplifier (Heka, Germany). Synaptic currents were evoked at 0.067 Hz by stimulation of afferent fibers from the external capsule *via* a constant-voltage pulse (1 ms) delivered through a concentric electrode (FHC, USA). After 10 min baseline recording, according to the previous studies (Nicol *et al.* 1996, Yang *et al.* 2004), 10 μ M of morphine were applied for 15 min. As recovery from responses to morphine could not be obtained with washout up to 15 min (data not shown), the substance was applied only once to each slice. Morphine was added to the external ACSF.

sEPSCs were detected using Mini-Analysis (Jaejin software, Leonia, NJ, USA). Data from the electrophysiological recordings were analyzed with paired Student's t-test and one-way analysis of variance (ANOVA). Data are presented as mean \pm SEM. Differences were considered statistically significant at $P < 0.05$.

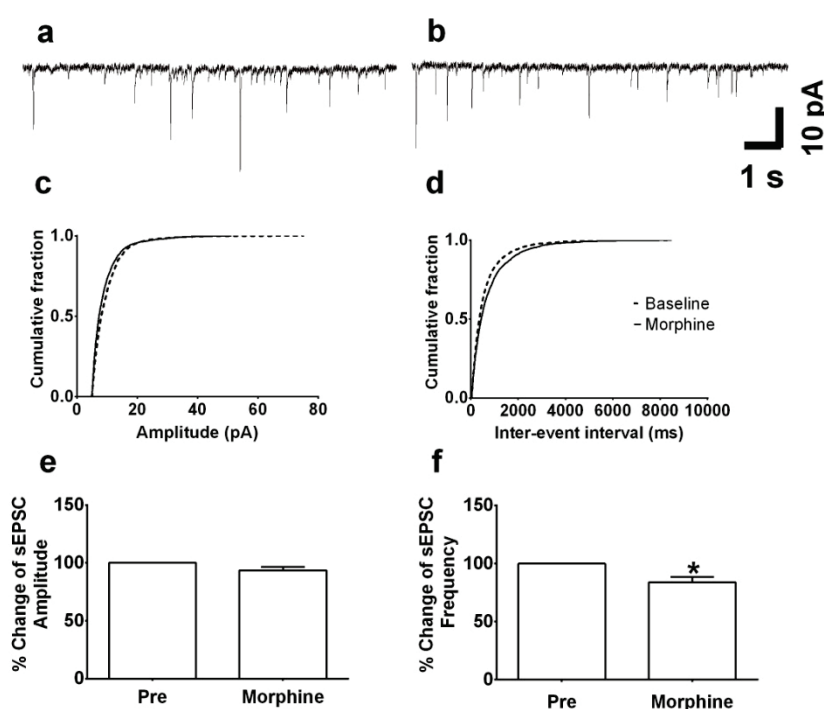


Fig. 1. Acute applying morphine induced decreases in the frequency of sEPSCs, but not amplitude of sEPSCs, of LA neurons in rats. **a:** Representative traces of sEPSCs from LA neurons under control conditions. **b:** Representative traces of sEPSCs during the presence of morphine. **c** and **d:** Morphine (10 μ M) applied acutely shifted the cumulative inter-event interval of sEPSCs to the right (suggesting a decrease), but it showed no influence on the amplitude of the sEPSCs ($n=7$). **e** and **f:** Normalized mean values for amplitude and frequency of the sEPSCs showed that the averaged sEPSC frequencies were decreased by acute application of morphine, whereas the averaged amplitudes of sEPSCs were unchanged. Statistical significance * $P < 0.05$.

First, we determined the effect of morphine on the activity of 7 LA neurons from 4 rats (Fig. 1). Significant decreases in frequency of spontaneous EPSCs (sEPSCs) were observed upon addition of 10 μ M morphine, while there were no significant changes in the

amplitude after morphine application. Typical current traces under control conditions (Fig. 1a) and during the presence of morphine (Fig. 1b), as well as cumulative amplitude (Fig. 1c) and inter-event interval (Fig. 1d), illustrate the lengthening of inter-event intervals but not

the amplitude upon addition of morphine. Normalized mean values for amplitude and frequency are shown in Figure 1e and Figure 1f (the last 5 min of baseline recording and morphine application). Here, the mean amplitude of the sEPSCs changed from 9.3 ± 0.4 pA in the absence to 8.7 ± 0.6 pA in the presence of morphine, showed no significant changes (Fig. 1e, $n=7$, $93.5 \pm 2.9\%$, $t=2.106$, $P>0.05$). By contrast, a significant decrease in average frequency of sEPSCs from 1.6 ± 0.3 Hz to 1.3 ± 0.2 Hz was detected, yielding a decrease to $83.8 \pm 4.8\%$ (Fig. 1f, $n=7$, $t=2.783$, $P<0.05$).

In the presence of bicuculline ($10 \mu\text{M}$), EPSCs evoked by stimulation of afferent fibers from the external capsule, containing cortical afferents were recorded in 6 LA neuron from 3 rats. Typical current traces under control conditions and during the presence of morphine were shown in Figure 2a. Bath application of morphine ($10 \mu\text{M}$) for 15 min showed no significant influences on the EPSC amplitude ($F_{19,119}=0.90$, $P>0.05$; $n=6$; Fig. 2b).

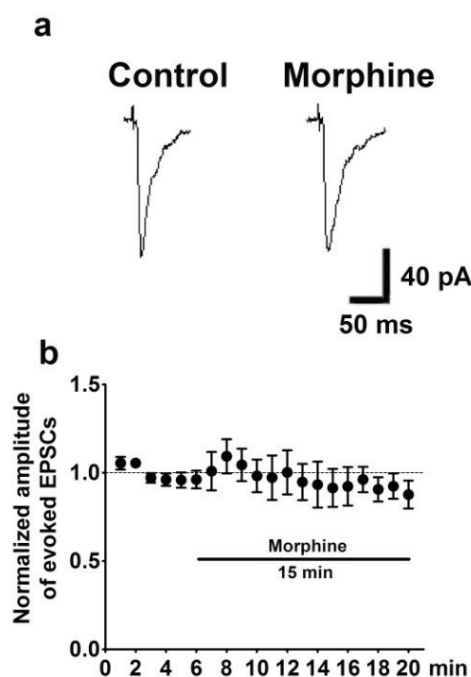


Fig. 2. Morphine showed no significant influences on eEPSCs in neurons of the LA. **a:** Representative eEPSCs in a LA neuron in control and morphine treatment. **b:** Time course of the morphine application on eEPSCs in LA neurons ($n=6$).

Acute morphine exposure induced analgesia, and studies focus on the pharmacological effect of morphine on the synaptic transmission in the central nervous system were necessary. Testing the sEPSCs and eEPSCs was widely used to study the effects of several drugs on the excitatory synaptic transmission in different brain

regions (Akaike *et al.* 2009, Kato and Shigetomi 2001, Kondratskaya *et al.* 2010, Kyung Park *et al.* 2002, Yamamoto *et al.* 2011). In the present study, morphine perfusion induced marked decreases in the sEPSCs frequency without influences on the amplitude of sEPSCs or EPSC in LA neurons. These results suggest that LA may be one of the earliest brain regions affected by morphine and need more concern. Studies also reported that LA was involved in morphine tolerance (Mitchell *et al.* 2000), so the present study may be helpful for understanding the effects of LA on morphine tolerance.

It has been reported that μ -opioid receptors (MORs) agonists presynaptically inhibit glutamatergic synaptic transmission in midbrain periaqueductal gray, cultured hippocampal neurons and the centrolateral nucleus (Hashimoto *et al.* 2009). Consistent with these findings, in the present study, evidence for a presynaptic locus of the morphine-induced inhibition on the glutamate transmission is based on the result that morphine reduced the frequency of sEPSCs, but not amplitude of sEPSCs. The effects of drugs on the sEPSCs were not always consistent with the effects on the eEPSCs. Previous studies found selective activation of presynaptic GABA_A receptors markedly facilitated sEPSCs frequency but inhibited eEPSCs amplitude (Yamamoto *et al.* 2011), and ATP significantly reduced the amplitude of eEPSCs but increased the frequency of sEPSCs (Kato and Shigetomi 2001). Because LA receives a great deal of inputs (Buffalari and See 2010), sEPSCs represent the sum of all the postsynaptic currents. In this study, synaptic currents were evoked by stimulation of afferent fibers from the external capsule, so eEPSCs only reflect part of the afferent projection. The frequency of sEPSCs might also reflect the number of excitatory synapses of the recorded neurons (Wang and Zheng 2001).

Glutamatergic inputs onto the LA primarily arise from sensory association cortices, prelimbic cortex, hippocampus and thalamus (Doron and Ledoux 2000, Kishi *et al.* 2006, McDonald 1998), which allows the LA to integrate information involving sensory inputs and past experiences. The present results suggest that morphine may inhibit glutamate release presynaptically in LA, while the neuronal circuit underlying the inhibition of morphine on the glutamatergic release remains to be determined.

In conclusion, the present study demonstrate that morphine inhibits the frequency of sEPSCs, but not the amplitude of sEPSCs, in the LA neurons. The results may provide a cellular basis for the function of LA in acute

morphine induced analgesia.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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