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Excitatory amino acid receptors are involved in morphine-induced synchronous oscillatory discharges in the locus coeruleus of rats

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Abstract

Our previous studies demonstrated that morphine not only decreases the firing rate of locus coeruleus neurons, but that it induces synchronous oscillatory discharges in the locus coeruleus. In the present study, we examined the role of excitatory amino acid input in the mechanisms of the morphine-induced synchronous oscillation in the locus coeruleus. Using a multiple-electrode recording technique, locus coeruleus neuronal activities were recorded under halothane anesthesia in adult Sprague–Dawley rats. Among 175 locus coeruleus neurons recorded after intracerebroventricular (i.c.v.) injection of morphine (26 nmol), 88 of them exhibited both decreased firing rates and synchronous oscillatory discharges. The morphine-induced oscillation and synchrony were reversed by i.c.v. injection of the non-selective excitatory amino acid receptor antagonist kynurenic acid, the selective NMDA receptor antagonist DL-2-Amino-5-phosphonopentanoic acid (AP-5), or the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), but not saline vehicle. These results suggest that excitatory amino acid input contributes to the morphine-induced synchronous oscillatory activity in the locus coeruleus. The results also provide us a pharmacology tool to study the influence of blockade of the locus coeruleus synchrony on neurotransmitter release and synaptic plasticity in the locus coeruleus target areas.

Keywords: Locus coeruleus; Morphine; Synchronous activity; Single unit recording; Excitatory amino acid

1. Introduction

The noradrenergic locus coeruleus is enriched with opioid receptors (Temple and Zukin, 1987; Arvidsson et al., 1995; Van Bockstaele et al., 1996) and is a useful model to study the neuronal mechanism of opioid addiction (for review see Nestler et al., 1994; Nestler and Aghajanian, 1997; Zhu et al., 1998). Alteration in locus coeruleus neuronal activity has been suggested to contribute to the development of opioid tolerance and dependence. Acutely, systemic or intracoerulear administration of morphine, a typical μ-opioid receptor agonist, inhibits the spontaneous firing rate of locus coeruleus neurons (Korf et al., 1974; Bird and Kuhar, 1977; Aghajanian, 1978; Valentino and Wehby, 1988). With chronic morphine treatment, locus coeruleus neurons develop tolerance to the acute inhibitory actions of morphine, as their neuronal firing rates gradually recover from the acute inhibitory effect. During withdrawal from chronic morphine treatment, locus coeruleus neurons exhibit a marked increase in their neuronal activity (Aghajanian, 1978; Rasmussen et al., 1990). However, these earlier electrophysiological studies only investigated the effects of opioids on the activity of individual locus coeruleus neurons. Until recently, the effect of opioids on the temporal relationships among the activities of multiple locus coeruleus neurons has only been assumed. Using a multiple-electrode recording technique that allows several locus coeruleus neurons to be recorded simultaneously, we found a novel effect of morphine on the firing pattern of locus coeruleus neurons. In addition to its well-known inhibitory action, our data show that a single dose of morphine induces long-lasting synchronous oscillatory burst activities in the locus coeruleus (Zhu and Zhou, 2001). The synchronized locus coeruleus firing may have significant influence on the locus coeruleus target areas. As a result of temporal and spatial summation, the morphine-induced synchronous activity could facilitate the release of neurotransmitter norepinephrine in the

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locus coeruleus target areas. Norepinephrine is an important neuromodulator that has been shown to induce and facilitate synaptic plasticity in several brain regions (Neuman and Harley, 1983; Lacaille and Harley, 1985; Hopkins and Johnston, 1984, 1988, for review see Bailey et al., 2000). The morphine-induced synchronous activity in the locus coeruleus could be an important neuronal signal that induces synaptic plasticity leading to opioid addiction. Understanding the neural mechanisms underlying the opioid-induced locus coeruleus synchrony will improve our understanding of the mechanisms of opioid addiction.

One possible mechanism is that the morphine-induced synchronous activity in the locus coeruleus is driven by its excitatory input. Excitatory amino acid is the major excitatory input to the locus coeruleus neurons (Aston-Jones et al., 1986) and has been associated with opioid withdrawal-induced hyperactivity of locus coeruleus neurons (Rasmussen et al., 1990; Akaoka and Aston-Jones, 1991). In the present study, we examined whether blockade of excitatory amino acid transmission has any effect on the morphine-induced synchronous activity in the locus coeruleus.

2. Materials and methods

2.1. Animals and surgery

Male Sprague–Dawley rats weighing 300–350 g (Harlan Sprague–Dawley, Indianapolis, IN) were used in this study. All procedures were approved by the Institutional Animal Care and Use Committee at University of Mississippi Medical Center. For chronic implantation of electrode, a bundle of eight microwires (NB LABS, Dennison, TX, USA) was stereotaxically implanted into the locus coeruleus under sodium pentobarbital (50 mg/kg, i.p.) anesthesia. A 23-gauge guide cannula (Plastic One, Roanoke, VA, USA) was implanted into the lateral cerebral ventricle for drug injection. The microwire bundle and guide cannula were secured in place with 4 stainless steel screws trepanned through the skull and adhered with dental acrylic. Before recording, rats were given at least a week to recover following the surgery.

2.2. Electrophysiological recordings

Locus coeruleus neuronal activity was recorded under halothane anesthesia (1.25%, mixed with oxygen) as described before (Zhu and Zhou, 2001). Body temperature was maintained at 37 °C with a heating pad. After the baseline recording, a single dose of morphine sulfate (26 nmol, 5μl, in saline) was injected intracerebroventricularly (i.c.v.) through a Hamilton syringe. Non-selective glutamate receptor antagonist kynurenic acid (4-hydroxyquinoline-2-carboxylic acid, 500 nmol), selective NMDA receptor antagonist AP-5 (DL-2-Amino-5-phosphonopentanoic acid, 127 nmol), non-NMDA receptor antagonist CNQX (6-cyano-7-nitroquinoxaline-2,3-dione disodium salt, 45 nmol) or saline vehicle (5 μl) was injected intraventricularly about 10–20 min after morphine administration. All of the chemicals were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). Our previous studies showed that only a subpopulation of locus coeruleus neurons exhibited both decreases in firing rates and synchronous oscillations. Another subpopulation of locus coeruleus neurons exhibited sustained decreases in firing rates, but neither oscillatory discharges nor synchrony after morphine injection (Zhu and Zhou, 2001). Since these two subpopulations of locus coeruleus neurons were indistinguishable in terms of mean firing rates before and after morphine administration, the excitatory amino acid antagonists were given after the onset of the morphine-induced synchronous oscillation.

Online isolation and discrimination of locus coeruleus neuronal activity was accomplished using a commercial multichannel neuronal acquisition processor (MNAP system, Plexon Inc., Dallas, TX, USA) that allows us to monitor groups (up to 4 neurons per wire) of neurons simultaneously. Identifying different neurons on a single wire was accomplished by real-time discrimination of individual waveforms using template analysis procedures provided by the MNAP system. To ensure that neurons recorded by different wires were distinct, we compared the shape of their waveforms, firing rates, and patterns (e.g. interspike interval histograms) before further analysis. Locus coeruleus neurons were identified using previously established criteria, i.e., low spontaneous firing rates, responses to noxious stimuli, and changes in firing rates in response to morphine (Korf et al., 1974; Bird and Kuhar, 1977; Aghajanian, 1978; Valentino and Wehby, 1988). At the end of recording, currents (20 μA, 10 s) were passed through the microwires to create lesions that were verified histologically.

2.3. Data analysis

Mean firing rates, auto-correlograms and cross-correlograms were analyzed using Neuroexplorer (Nex Technologies, Lexington, MA, USA) and Matlab (Mathworks, Natick, MA, USA) software. The degree of oscillation was quantified by an oscillatory index, which was computed as the ratio of the amplitude of the first satellite peak to the offset of the auto-correlogram (König, 1994). The strength of synchrony was quantified by a synchrony index, which was computed as the ratio of the amplitude of the central peak to the offset of the cross-correlogram (König, 1994). The effects of morphine and excitatory amino acid receptor antagonists on the mean firing rates, oscillatory indexes, and synchrony indexes were analyzed by paired *t*-test or one-way repeated measures ANOVA. Data are presented as mean ± S.E.M.

3. Results

A total of 175 locus coeruleus neurons were recorded from 11 rats before and after i.c.v. injection of morphine. The mean spontaneous firing rates of the locus coeruleus neurons decreased by 55%, from 2.4 ± 0.1 to 1.1 ± 0.1 spikes/s ($n = 175$, $P < 0.001$, paired *t*-test) 10 min after morphine injection. In agreement with our previous studies (Zhu and Zhou, 2001), a subpopulation of the locus coeruleus neurons (88/175, 50%) not only exhibited a decrease in their mean firing rates, but also

exhibited synchronous oscillatory activities. The discharge patterns of 2 representative locus coeruleus neurons recorded simultaneously are shown in Fig. 1A. Auto-correlation analysis showed that 22.6±1.6% of the locus coeruleus neuronal activity was deemed oscillatory 10 to 20 min after morphine injection, compared to 0.2±0.1% of the activity ($n=88$, $P<0.0001$) before morphine. The degree of synchrony between possible pairs of oscillatory locus coeruleus neurons in each rat was analyzed. Cross-correlation analysis revealed that all of the 88 locus coeruleus neurons that exhibited oscillatory discharges were synchronized with at least one other neuron. Ten to 20 min after morphine injection, 27.4±1.1% of the locus coeruleus

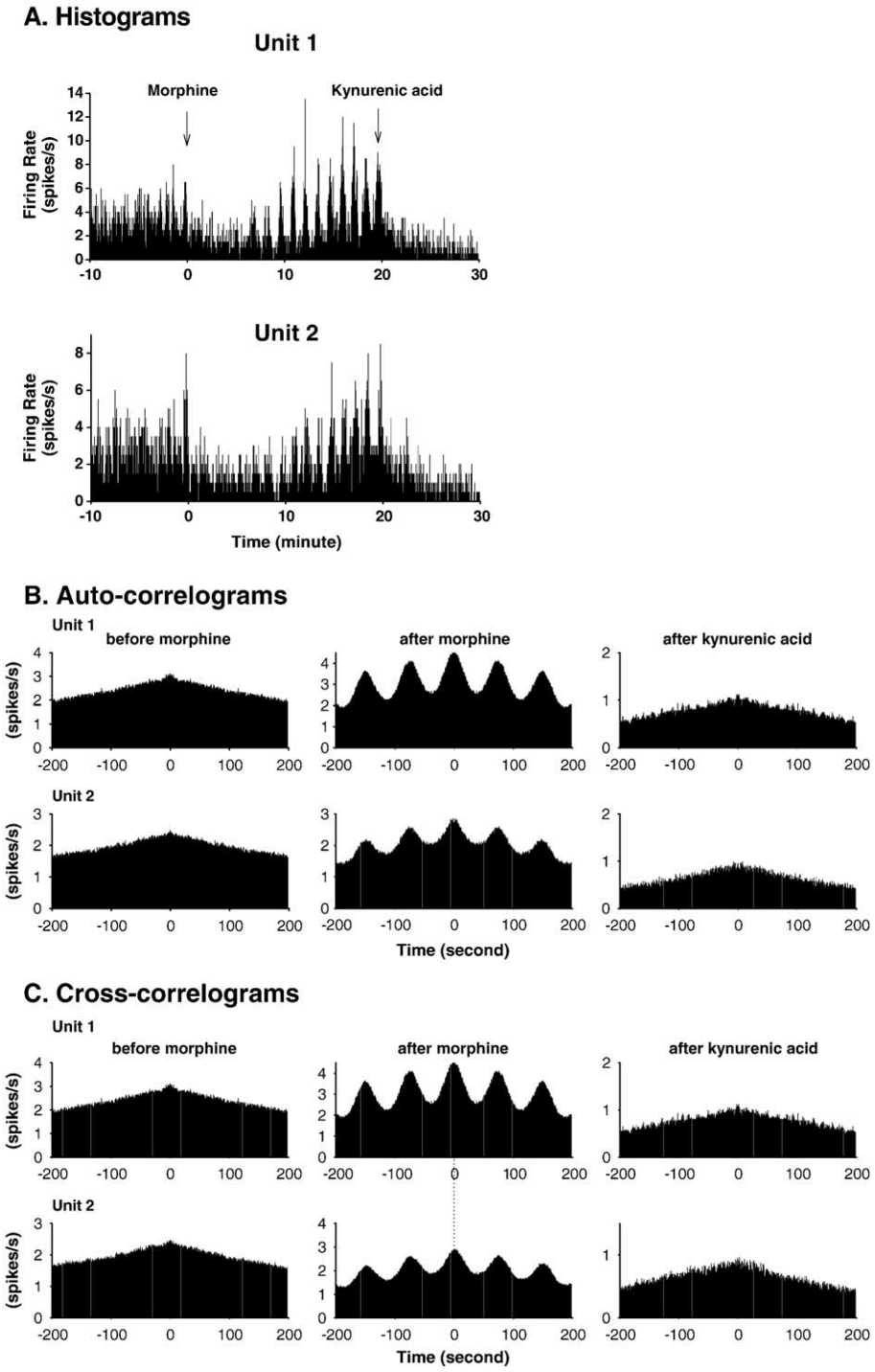

Fig. 1. Effects of the excitatory amino acid receptor antagonist kynurenic acid on the morphine-induced synchronous oscillatory discharges in the locus coeruleus. (A) Histograms of spontaneous firing rates of 2 simultaneously recorded locus coeruleus neurons (Unit 1 and Unit 2). Kynurenic acid (500 nmol, i.c.v.) was injected 20 min after morphine administration (0 time, 26 nmol, i.c.v.). (B) Auto-correlograms of the 2 neurons before versus 10 min after kynurenic acid. Note the large satellite peaks after morphine and lack of satellite peaks after kynurenic acid. (C) Cross-correlograms of the 2 locus coeruleus neurons before versus 10 min after kynurenic acid. Note the large central peaks after morphine and lack of central peaks after kynurenic acid.

neuronal activity was synchronous, compared to 5.4±0.3% (n=364 pairs, P<0.0001, paired t-test) before morphine injection.

To test the effects of excitatory amino acid receptor antagonists on the morphine-induced synchronous oscillatory discharges in the locus coeruleus, the non-selective excitatory amino acid receptor antagonist kynurenic acid, the NMDA receptor antagonist AP-5, or the non-NMDA receptor antagonist CNQX was injected 10 to 20 min after the onset of the morphine-induced synchronous oscillation. The morphine-induced synchronous oscillations in the locus coeruleus were significantly blocked by injection of each of the three excitatory amino acid receptor antagonists (Fig. 1, Tables 1 and 2). Fig. 1 shows the effects of kynurenic acid on the firing patterns of 2 simultaneously recorded locus coeruleus neurons. In addition to a decreased firing rate, about 10 min after morphine injection, the 2 locus coeruleus neurons exhibited repeated burst activities (Fig. 1A). The regularity of the burst activities was confirmed by the auto-correlograms, which show distinctive satellite peaks after morphine injection (Fig. 1B). The burst activity of the pair of locus coeruleus neurons was synchronous, which was confirmed by computing the cross-correlation of the discharge activity of the pair of locus coeruleus neurons. Their cross-correlograms show a significant central peak after morphine compared with lack of central peak before morphine (Fig. 1C). Kynurenic acid, which was injected 20 min after the morphine injection, decreased the firing rate of the locus coeruleus neurons and blocked the morphine-induced synchronous oscillatory discharges (Fig. 1A). The auto-correlograms show lack of satellite peaks and the cross-correlograms show lack of central peaks after kynurenic acid injection (Fig. 1B, C). Among the 46 locus coeruleus neurons recorded from 3 rats, a total of 24 neurons exhibited both decreases in mean firing rate and synchronous oscillatory activities after morphine. Injection of kynurenic acid blocked the morphine-induced locus coeruleus synchronous oscillation in all of the 24 locus coeruleus neurons. Their oscillation indexes were significantly decreased from 13.6±1.2% before kynurenic administration to 0.1±0.1% after kynurenic acid (n=24, P<0.001, one-way repeated measures ANOVA and Bonferroni t-test, Table 1). Their synchrony indexes were significantly decreased from 18.7%±1.1% to 3.2±1.0% after kynurenic acid administration (n=93 neuron pairs, P<0.001, one-way repeated measures ANOVA and Bonferroni t-test, Table 2).

Table 1
Effects of excitatory amino acid (EAA) antagonists on the morphine-induced synchronous oscillatory discharges: auto-correlation analysis

	Baseline	After morphine	After EAA antagonist or saline control
KYN (n=24)	0.4±0.4%	13.6±1.2%[a]	0.1±0.1%[b]
AP-5 (n=30)	0±0%	30.5±3.3%[a]	0±0%[b]
CNQX (n=17)	0±0%	19.6±2.5%[a]	0±0%[b]
Saline (n=17)	0.6±0.4%	24.4±4.0%[a]	22.3±4.0%

Data are expressed as mean oscillation index±S.E.M. (%). The oscillatory index was computed as the ratio of the amplitude of the first satellite peak to the offset of the auto-correlogram (Materials and methods). Numbers in the parentheses refer to the number of locus coeruleus neurons. [a]P<0.001, compared to baseline values. [b]P<0.001, compared to the values after morphine (one-way repeated measures ANOVA and Bonferroni t-test). KYN, kynurenic acid.

Table 2
Effects of excitatory amino acid (EAA) antagonists on the morphine-induced synchronous oscillatory discharges: cross-correlation analysis

	Baseline	After morphine	After EAA antagonist or saline control
KYN (n=93)	5.5±0.4%	18.7±1.1%[a]	3.2±1.0%[b]
AP-5 (n=117)	4.9±0.59%	32.3±1.6%[a]	2.8±0.9%[b]
CNQX (n=76)	4.5±0.4%	24.0±1.1%[a]	8.1±1.8%[b]
Saline (n=78)	6.9±0.7%	34.3±2.7%[a]	30.0±1.9%

Data are expressed as mean synchrony index±S.E.M. (%). The synchrony index was computed as the ratio of the amplitude of the central peak to the offset of the cross-correlogram (Materials and methods). Numbers in the parentheses refer to the number of locus coeruleus neuronal pairs. [a]P<0.001, compared to baseline values. [b]P<0.001, compared to the values after morphine (one-way repeated measures ANOVA and Bonferroni t-test). KYN, kynurenic acid.

Administration of the NMDA receptor antagonist AP-5 or the non-NMDA receptor antagonist CNQX produced similar effects on the morphine-induced synchronous oscillation in the locus coeruleus. Their firing rate histograms, auto-correlograms and cross-correlograms were similar to those of kynurenic acid (Fig. 1). A total of 47 locus coeruleus neurons recorded from 6 rats exhibited both decrease in mean firing rate and synchronous oscillatory activities after morphine. The morphine-induced synchrony and oscillatory activity were blocked by injections of AP-5 (30 neurons recorded from 4 rats) or CNQX (17 neurons recorded from 2 rats). The results are summarized in Tables 1 and 2.

In the control group (2 rats), after the onset of morphine-induced synchronous oscillation, the rats received saline instead of excitatory amino acid receptor antagonists injection. Saline vehicle injection did not significantly affect the morphine-induced synchronous oscillatory activity in the locus coeruleus (Tables 1 and 2), suggesting that the blockade of synchronous oscillations in the locus coeruleus by the excitatory amino acid antagonists was not attributable to disturbance by the injection procedure.

4. Discussion

The results of the present study indicate that excitatory amino acid transmission is involved in the mechanism of the morphine-induced synchronous oscillatory discharges in the locus coeruleus. Both oscillation and synchrony were blocked at the same time, suggesting that they share the same mechanism. Pharmacology studies of the excitatory amino acid receptor antagonist blockade of the synchronous oscillation in the locus coeruleus show that either AP-5 or CNQX can block the morphine-induced locus coeruleus synchronous oscillation, indicating both NMDA receptors and non-NMDA receptors in the locus coeruleus are involved in this process. Since only one concentration of each excitatory amino acid receptor antagonist was tested in the present study, it is unable to compare the involvement of different excitatory amino acid receptors in the morphine-induced synchronous oscillation in the locus coeruleus. A future concentration–response study may find out

whether NMDA receptors and non-NMDA receptors are equally involved in the process.

The mechanism for the involvement of excitatory amino acid transmission in the morphine-induced synchronous oscillation in the locus coeruleus is not known. One possibility is that central administration of morphine activates neurons in other brain areas that send excitatory amino acid inputs to the locus coeruleus. The nucleus paragigantocellularis in the rostral ventrolateral medulla is the major excitatory amino acid input to the locus coeruleus (Aston-Jones et al., 1986). Electrical stimulation of the nucleus paragigantocellularis produces robust, predominantly excitation of majority of the locus coeruleus neurons that can be blocked by excitatory amino acid receptor antagonists (Ennis and Aston-Jones, 1987, 1988; Ennis et al., 1992). Morphological studies have demonstrated the existence of μ-opioid receptors in the locus coeruleus-projecting paragigantocellularis neurons (Van Bockstaele et al., 1999). Electrophysiology studies suggest that some of the neurons in the nucleus paragigantocellularis can be activated by opioids (Satoh et al., 1979; Azami et al., 1981; Rosenfeld, 1994; Saiepour et al., 2001). It is possible that central administration of morphine periodically activates locus coeruleus-projecting neurons in the nucleus paragigantocellularis and results in an increase in glutamate release, which drives the synchronous oscillatory activities in the locus coeruleus. The effects of morphine on excitatory amino acid neurotransmitter release in the locus coeruleus have been studied by microdialysis (Zhang et al., 1994; Aghajanian et al., 1994). However, a single dose of morphine caused a slight but not significant increase in the level of glutamate in the locus coeruleus (Zhang et al., 1994). Since it was necessary to collect samples for microdialysis in 20-min periods, transient changes in glutamate release could be missed with microdialysis measurement.

A number of behavioral, electrophysiology and neurochemistry studies have demonstrated that excitatory amino acid transmission plays a role in the development of opioid tolerance and dependence. Naloxone-precipitated withdrawal from opioids induces an increase in glutamate release in the locus coeruleus (Zhang et al., 1994; Aghajanian et al., 1994). The withdrawal-induced hyperactivity of locus coeruleus neurons can be attenuated by administration of excitatory amino acid receptor antagonists (Rasmussen et al., 1990; Akaoka and Aston-Jones, 1991). Chronic treatment with morphine induces changes in gene expression of the NMDA receptors in the locus coeruleus (Zhu et al., 1999). Administration of excitatory amino acid antagonists (Trujillo and Akil, 1991) or NMDA receptor antisense oligo (Zhu and Ho, 1998) can inhibit the development of tolerance to and dependence on morphine. However, the mechanism underlying the inhibitory effects of excitatory amino acid antagonists on the development of opioid tolerance and dependence is unknown. Future behavioral studies should examine whether blockade of the locus coeruleus synchrony plays a role in the inhibitory effects of excitatory amino acid antagonists on the development of opioid tolerance and dependence.

The well-known inhibitory action of morphine on the locus coeruleus neuronal activities and the recent discovered morphine-induced locus coeruleus synchrony may have different functional influence on locus coeruleus target areas. The inhibitory action of morphine on locus coeruleus neurons results in a decrease in neurotransmitter norepinephrine release in locus coeruleus target areas, whereas the morphine-induced locus coeruleus synchrony could facilitate norepinephrine release. Our hypothesis is that the morphine-induced locus coeruleus synchrony is an important neuronal signal that has significant implications in the development of opioid addiction. The present data show that the excitatory amino acid antagonists selectively blocked the morphine-induced synchronous oscillation in the locus coeruleus, but did not affect the inhibitory action of morphine on the locus coeruleus neurons. In the future study, the excitatory amino acid antagonists could be used as a pharmacology tool to study of the influence of the morphine-induced locus coeruleus synchrony on locus coeruleus target areas.

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