Effects of Milnacipran on the Inhibitory Postsynaptic Potential in Neurons of the Rat Locus Coeruleus

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Summary: Effects of milnacipran (MIL), a serotonin and noradrenaline reuptake inhibitor (SNRI), on synaptic transmission were examined in the rat locus coeruleus (LC). Bath-application of MIL produced a hyperpolarization associated with a decrease in input resistance of LC neurons. The MIL-induced hyperpolarization reversed polarity near the equilibrium potential of K⁺. The MIL-induced hyperpolarization was blocked by yohimbine (1 µM). Clonidine, but not serotonin (5-hydroxytryptamine; 5-HT), produced a hyperpolarizing potential in LC neurons. The MIL-induced hyperpolarization reversed polarity at -114±3 mV (n=4). MIL (0.1-10 µM) depressed the amplitude of the excitatory postsynaptic potential (EPSP), while it enhanced the amplitude and duration of the inhibitory postsynaptic potential (IPSP). These results suggest that MIL hyperpolarizes LC neurons and enhances the IPSP by increasing endogenous noradrenaline (NA) concentration at synapses in LC neurons.

Key words SNRI, locus coeruleus, milnacipran, IPSP, hyperpolarization

INTRODUCTION

Noradrenergic neurons in the locus coeruleus (LC) have been known to play an important role in determining attention, the level of vigilance and the response to stress-induced by activity in mammals [1-4]. Failure of central noradrenaline (NA) reuptake is considered to be involved in an intensive processing of irrational belief, anxiety and depression [5-8]. Serotonin (5-hydroxytryptamine; 5-HT) has also been recognized as an important neurotransmitter related to anxiety, depression and panic disorder, and this has led to the recent development of selective serotonin reuptake inhibitors (SSRIs) for the treatment of these emotional disorders [9,10]. The LC receives a dense serotonergic fiber input coming from pericoerulear 5-HT neurons [4,11] and sends noradrenergic efferents to the dorsal raphe nucleus [12,13]. Interactions between serotonergic and noradrenergic systems have recently been implicated in emotional disorders, based on the clinical results obtained with the use of serotonin and noradrenaline reuptake inhibitors (SNRIs) for the therapy of depression [14-16]. Milnacipran (MIL), an SNRI [17-19], has a robust antidepressant activity which is possibly superior to that of SSRIs [20]. A previous study has shown that acute administration of MIL reduced the rate of spontaneous firing in rat LC neurons [21]. However, little is known whether SNRI modulates the function of noradrenergic neurons in the LC. The purpose of the present study was to investigate the effects of MIL on the membrane potential and synaptic transmission in the rat LC.

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Abbreviations: ACSF, artificial cerebrospinal fluid; EPSP, excitatory postsynaptic potential; 5-HT, 5-hydroxytryptamine; IPSP, inhibitory postsynaptic potential; LC, locus coeruleus; MIL, milnacipran; NA, noradrenaline; SNRI, serotonin and noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.
MATERIALS AND METHODS

Brain slices containing the LC were obtained from rats in a manner described previously [21]. Male Wistar rats, 150-200 g, were killed by a heavy blow to the chest, their brains were rapidly removed and immersed for 8-10 s in a cooled artificial cerebrospinal fluid (ACSF, 4-6°C) that was pre-bubbled with 95% O₂-5% CO₂. Horizontal brain slices (250-300 μm in thickness) were cut with a Vibroslice (Campden Instruments) in cooled ACSF and left to recover for one hour in oxygenated ACSF at room temperature (22-24°C). A hemisected slice was then transferred to a recording chamber and submerged in the ACSF at 32-33°C. The composition of the ACSF was as follows (in mM): 126 NaCl, 2.5 KCl, 2.4 CaCl₂, 1.2 MgCl₂, 21 NaHCO₃, 1.2 NaH₂PO₄, and 11 D-glucose (pH 7.4 and 295-305 mOsm). Intracellular recording methods were used with glass microelectrodes filled with 2 M KCl (tip resistance 26-40 MΩ). Voltage and current were recorded with an Axoclamp-2A amplifier and were monitored continuously with a memory oscilloscope (Nihon-Kohden, RTA-1100). NA, clonidine, 5-HT and yohimbine were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). MIL ((±)-cis-2-aminomethyl-N,N-diethyl-1-phenylcyclopropeneboxamide hydrochloride) was from Asahi Kasei Pharma (Tokyo, Japan). Drugs were directly dissolved in the ACSF. Each experimental value was presented as the mean ± S.E. (standard error of the mean) and was analyzed by unpaired Student’s t-test.

RESULTS

MIL-induced hyperpolarization in LC neurons

Figure 1A shows the effect of MIL on the membrane potential of an LC neuron, in which the membrane potential was initially hyperpolarized to −60
mV to block the firing of spontaneous action potentials. Application of MIL (10 μM) to LC neurons for 1-5 min produced a hyperpolarizing response with peak amplitude of 4.0±1.4 mV (n=9) at -60 mV (Fig. 1Aa). The neuron membrane potential recovered within 15-20 min after withdrawal of MIL from the superfusing solution. Figure 1Ab shows the effect of yohimbine, an α2-adrenoceptor antagonist, on the MIL-induced hyperpolarization in an LC neuron. In the presence of yohimbine (1 μM) application of MIL (10 μM) produced no hyperpolarization. Since MIL has been reported to block the reuptake of both NA and 5-HT [15,21], an increase in the concentration of either transmitter may be to the cause of the hyperpolarization induced by MIL in LC neurons. Figure 1Ba shows the effects of clonidine, an α2-adrenoceptor agonist, on the membrane potential in an LC neuron. Bath-application of clonidine (100 nM) produced a hyperpolarizing potential with peak amplitude of 13±1 mV (n=8) in LC neurons. By contrast, addition of 5-HT (30 μM) to the ACSF did not produce any change in the membrane potential in LC neurons (n=4) (Fig. 1Bb). These results suggest that NA mainly contributes to the MIL-induced hyperpolarization in LC neurons.

The input resistance of LC neurons was examined by measuring the electrotonic potentials produced by injection of hyperpolarizing current pulses with duration of 500 ms. MIL (1 μM) depressed the amplitude of the electrotonic potentials at membrane potential between -70 and -140 mV, indicating a decreased input resistance of LC neurons (Fig. 2A). Figure 2B shows the voltage-current relationships (V-I curves) obtained before and 5 min after application of MIL (1 μM). The V-I curve obtained in the presence of MIL had a less steep slope than the control curve at -114±3 mV (n=4). These results suggest that the MIL-induced hyperpolarization is

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**Fig. 2.** Relationship between the amplitude of the MIL-induced hyperpolarization and the membrane potential. (A) Electrotonic potentials (center and right traces) were produced by injection of hyperpolarizing current pulse with duration of 500 ms (left traces). Center and right traces were obtained before and 5 min after application of MIL (1 μM, respectively). (B) Voltage-current relationship (V-I curves) in an LC neuron. Open and closed circles were obtained before and 5 min after application of MIL (1 μM). All data were obtained from the traces shown in A.
produced by activation of a K⁺ conductance in LC neurons.

Effects of MIL on synaptic transmission in LC neurons

Recurrent collaterals of LC neurons project to the somatodendritic membrane of the same neurons or of other LC neurons. These collaterals release NA which produces an inhibitory postsynaptic potential (IPSP) via postsynaptic a₂-adrenoceptors [23,24]. A single focal stimulation of the caudal edge of the LC evoked a fast EPSP followed by a slow IPSP with duration of 50-60 ms and 1-2.5 s, respectively (Fig. 3). Figure 3A shows an example of the effects of MIL (3 µM) on the EPSP and IPSP in an LC neuron.

Bath-application of MIL for 10 min markedly increased the amplitude of the IPSP. In contrast, the EPSP was depressed by MIL (3 µM). The EPSP and IPSP recovered, when LC neurons were superfused with the ACSF containing no MIL (recovery solution) for approximately 30 min. Pooled data showed that MIL (3 µM) enhanced the IPSP to 280±10% (n=4) of control and depressed the EPSP to 35±6% (n=4) of control (Fig. 3B). The time course of the IPSP was markedly increased by MIL (100 nM-10 µM). The half-decay time was increased to 240±45% (n=4) of control by MIL (3 µM, Fig. 3C).

The possible contribution of 5-HT to the effect of MIL on the EPSP and IPSP was examined in LC neurons (Fig. 4). The EPSP was clearly depressed

![Figure 3](https://example.com/figure3.png)

**Fig. 3.** Effects of MIL on synaptic transmission in LC neurons. (A) Effects of MIL (3 µM) on the amplitude of IPSP (a1-c1) and EPSP (a2-c2) in LC neurons. Records are example of the EPSP followed by the IPSP obtained before (a) and 10 min after application (b) of MIL (1 µM). Records c1 and c2 were obtained 30 min after withdrawal of MIL. Records (a2-c2) are expanded records of a1-c1, respectively. (B) Effects of MIL (3 µM) on the amplitude of the IPSP. Open and closed columns were obtained before and 5 min after application of MIL (3 µM). (C) Effects of MIL (3 µM) on the half-decay time of the IPSP. Open and hatched columns were obtained before and 5 min after application of MIL (3 µM). Inset shows a sample record of the IPSP. In (B) and (C), the amplitude of the IPSP obtained before application of MIL is indicated as 1. Vertical lines on hatched column indicate S.E. of mean. Asterisk indicates statistical significance obtained by unpaired Student's t-test (*: P<0.01). The number of experiments is shown in parentheses.
within 2-4 min after introduction of 5-HT (10 μM) into the ACSF (Fig. 4A). Pooled data showed that 5-HT (10 μM) depressed the amplitude of the EPSP to 42±3% (n=4) of control (Fig. 4B). The EPSP recovered when LC neurons were superfused with the ACSF for approximately 30 min. 5-HT also produced depression of the IPSP in LC neurons (Fig. 4A).

DISCUSSION

The present study clearly showed that MIL, an antidepressant SNRI, produced a hyperpolarization with a decrease in input resistance in LC neurons. Yohimbine (1 μM), a selective α2-adrenoceptor antagonist, depressed the MIL-induced hyperpolarization. Clonidine, an α2-adrenoceptor agonist, produced a large hyperpolarization in LC neurons. It has been reported that NA produced a hyperpolarizing response in LC neurons by activating inward rectifier K+ channels via α2-adrenoceptors [24-28]. The MIL-induced hyperpolarization reversed polarity at a membrane potential close to the equilibrium potential of K+. Previous studies have shown that MIL selectively inhibits 3H-noradrenaline uptake into synaptosomes from rat cortex without any affinities for various transmitter receptors [15] and increases the extracellular level of NA [29]. MIL enhanced the outward current induced by exogenously applied NA in LC neurons (unpublished observation). These results suggest that NA mediates the MIL-induced hyperpolarization via activation of α2-adrenoceptors in LC neurons. The LC receives dense 5-HT projections coming from pericoerulear 5-HT neurons [4,11], which exert an inhibitory role on the LC [30,31]. Since MIL has been known to block both NA and 5-HT uptake systems [14,15], 5-HT possibly mediates the MIL-induced hyperpolarization in LC neurons. Previous studies have shown, however, that application of 5-HT did not affect the basal neuronal discharge [4] and the membrane potential in LC neurons [32]. In the present study, the application of 5-HT did not produce any hyperpolarizing response in LC neurons. It is, therefore, suggested that NA mainly contributes to the MIL-induced hyperpolarization in rat LC neurons.
The LC is a compact noradrenergic nucleus which sends extensive projections throughout the central nervous system [33,34]. Recurrent collaterals of the LC projections feed back onto the LC neuron themselves, and release NA which mediates the IPSP via postsynaptic α2-adrenoceptors [23,24]. The present study clearly showed that MIL enhanced the amplitude and the duration of the IPSP in LC neurons. The block of NA-reuptake increases the concentration of NA at synaptic cleft resulting in the enhancement of the IPSP in LC neurons. Endogenous 5-HT does not appear to contribute to the MIL-induced facilitation of the IPSP, because exogenous 5-HT rather depressed the IPSP. In contrast to the IPSP, MIL depressed the EPSP in LC neurons. It has been shown that MIL depresses open channels of N-methyl-D-aspartate receptors [35]. However, a previous report has shown that clonidine does not depress the EPSP in LC neurons [36]. Since MIL is known as an inhibitor of the 5-HT reuptake system, 5-HT may mediate the MIL-induced depression of the EPSP. The present study showed that exogenous 5-HT depressed the amplitude of the EPSP. We, therefore, suggest that MIL directly depresses the efficacy of excitatory neurotransmission via 5-HT in the LC.

Recent development of antidepressant is accomplished primarily through drugs that enhance 5-HT and NA neurotransmission by blocking monoamine transporters. MIL, as an SNRI, has robust antidepressant activity and possibly superior efficacy compared to SSRI [20]. In adrenergic neurons, acute or short-term (2 days) administration of SSRI did not change the neuronal activity of the LC [37-39], while short-term (2 days) administration of SNRI, such as MIL, decreased neuronal firing in rat LC neurons [21]. The present study showed that, at a relatively low concentration, MIL produced a hyperpolarization and depressed the firing activity of neurons. Furthermore, MIL enhanced the IPSP and depressed the EPSP in LC neurons. These results suggest that MIL has strong inhibitory effects on synaptic transmission in the rat LC.

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