Fluoxetine potentiates GABAergic IPSCs in rat hippocampal neurons

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Abstract

The GABA system is highly involved in the pathophysiology of mood disorders such as depression. Altered GABAergic function is evident in depressed patients and animal models of depression. Currently, the most widely used antidepressants are selective 5-HT reuptake inhibitors, such as fluoxetine. However, the effects of fluoxetine on GABAergic synaptic neurotransmission remain poorly investigated. Whole-cell patch-clamp recordings from cultured rat hippocampal neurons were therefore conducted to investigate the effects of fluoxetine on GABAergic neurotransmission. The spontaneous inhibitory postsynaptic current (sIPSC) was completely blocked by 10 μM bicuculline and reversibly potentiated by 30 μM fluoxetine. The fluoxetine potentiation on either amplitude or frequency of sIPSCs was dose-dependent, with the EC50 values of 0.96 and 14.26 μM, respectively. This potentiation was also TTX-insensitive, suggesting independence of presynaptic action potentials. The ritanserin (5 μM), a selective 5-HT2 receptor antagonist, did not alter the fluoxetine potentiation on miniature inhibitory postsynaptic currents. Taken together, our data suggest that fluoxetine can potentiate GABAergic neurotransmission without depending on presynaptic firing of action potentials and its elevating of 5-HT receptor activities. This potentiation by fluoxetine may normalize the hippocampal GABA deficit during depression and in part exert its antidepressant activity.

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The GABA in the central nervous system is a powerful modulator of emotional processes. Tremendous evidence has supported and formulated a GABAergic hypothesis that dysfunction of GABAergic neurotransmission is implicated in the pathogenesis of mood disorders such as depression [5,13,20,30]. The involvement of GABA in the pathogenesis of depression is also consistent with several clinical and preclinical findings. In depressed patients and animal models, a GABA deficit was found in cerebrospinal fluid [11], plasma [3,21] as well as several brain areas including cerebral cortex [9,26] and hippocampus [8]. Treating with antidepressants could reverse the GABA deficit [27,29]. Also, it is well-known that GABA agonists have antidepressant effects [14].

Currently, the most widely prescribed antidepressant drugs are selective 5-HT uptake inhibitors (SSRIs), such as fluoxetine. Molecular and cellular mechanisms that underlie the therapeutic action of these drugs still remain unclear. Although fluoxetine was found to normalize the GABA deficit during depression [7,27], the GABA concentration measured in these studies did not reflect the synaptic GABA concentration. In this study, we used whole-cell patch-clamp recording to investigate the effects of fluoxetine on GABAergic synaptic neurotransmission and the underlying mechanisms in cultured rat hippocampal neurons.

The use and care of animals in the present study followed the guidelines and protocols approved by the Institutional Animal Care and Use Committee of the University of Science and Technology of China. All efforts were made to minimize the number of animals used. Hippocampal neurons were isolated from Wistar rats (postnatal day 0). The neonatal rat was transferred to the chilled, Hank's buffered salt solution and decapitated. The whole brain was removed and placed in the iced Hank's solution. The hippocampus was collected carefully under a dissection microscope to avoid the contamination by other brain tissues.

The hippocampus was stored in culture medium for 24 h, the culture medium was replaced with neuron basal medium.

Abbreviations: ANOVA, analysis of variance; APV, 2-amino-5-phosphonovaleric acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DMEM, Dulbecco's modified Eagle's medium; mIPSC, miniature inhibitory postsynaptic current; S.E.M., standard error of the mean; sIPSC, spontaneous inhibitory postsynaptic current; SSRI, selective 5-HT reuptake inhibitor; TTX, tetrodotoxin.

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Fig. 1. Fluoxetine reversibly potentiated GABAergic sIPSC. (A) In the presence of 50 μM APV and 10 μM CNQX, recordings show sIPSCs before (control) and during the application of 30 μM fluoxetine. (B) Cumulative fraction plots for sIPSCs amplitude (left; K–S test, P<0.001, fluoxetine vs. control) and inter-event intervals (right, P<0.001) in the same experiment. (C) Relative amplitude (left) and frequency (right) of sIPSC before (control) and during the application of 30 μM fluoxetine (Student’s t-test, n=6, **P<0.01).
Fig. 2. Fluoxetine poteniations of sIPSCs were TTX-insensitive. (A) In the presence of 50 μM APV, 10 μM CNQX and 0.3 μM TTX, recordings show mIPSCs before (control) and during the application of 30 μM fluoxetine. (B) Cumulative fraction plots for mIPSCs amplitude (up; K–S test, \(P < 0.001\), fluoxetine 30 μM vs. control) and inter-event intervals (below, \(P < 0.001\)) in the same experiment. (C) Fluoxetine potentiation of sIPSC amplitude (left) and frequency (right) in the absence (control) and presence of TTX (Student’s \(t\)-test, \(n = 6\), **\(P < 0.01\), compared with the value before the application of fluoxetine; \(P > 0.05\), control vs. TTX during the application of fluoxetine).

GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in the presence of nonselective glutamate receptor antagonist, CNQX (10 μM) plus APV (50 μM). Alternatively, GABAergic postsynaptic currents were confirmed by a complete inhibition by bicuculline (10 μM), the GABA-A receptor antagonist (Fig. 1A).

The MiniAnalysis Program (Synaptosoft, Leonia, NJ) was used for the analysis of synaptic events. Each synaptic current selected by the software was visually inspected to eliminate potential detection errors. Quantitative comparisons of the extracted amplitude and inter-event interval distribution were conducted with the K–S test. The continuous theoretical curves for concentration–response relationships of fluoxetine on the amplitude and frequency of miniature inhibitory postsynaptic currents (mIPSCs) were drawn according to a modified Michaelis–Menten equation by the method of least-squares (the Newton–Raphson method) after normalizing the amplitude of the response:

\[
A = \frac{A_{max}C^h}{C^h + EC_{50}^h}
\]

where \(A\) is the normalized value of the amplitude or frequency, \(A_{max}\) the maximal response, \(C\) the drug concentration, \(EC_{50}\) the concentration which induced the half-maximal response and \(h\) the apparent Hill coefficient.

Data were statistically compared using Student’s \(t\)-test for comparison between two groups and one-way ANOVA for comparison between multiple groups. For all experiments, average values are expressed as mean ± S.E.M. Levels of statistical significance were assumed as \(P < 0.05\). \(P\) and \(n\) represent the value of significance and the number of neurons, respectively.

At a holding potential of −60 mV in all test neurons under our experimental conditions, fluoxetine (30 μM) significantly potentiated both the amplitude (142.9 ± 12.0%, \(P < 0.01\), \(n = 6\)) and frequency (556.2 ± 117.4%, \(P < 0.01\), \(n = 6\)) of sIPSCs (Fig. 1). The potentiated amplitude and frequency of sIPSCs could be recovered by 20 s washout with standard external solution (Fig. 1A). These results suggest that fluoxetine can reversibly potentiate the amplitude and frequency of sIPSCs in cultured rat hippocampal neurons.

The recorded populations of sIPSCs represent a mixture of action potential-dependent IPSCs and tetrodotoxin-independent (TTX-independent) mIPSCs. Therefore, we further examined the fluoxetine modulation of sIPSC in the presence of 0.3 μM TTX to block the action potential-dependent IPSCs (Fig. 2). We found that fluoxetine at 30 μM could also significantly potentiate both the amplitude and frequency of mIPSC (\(P < 0.01\), \(n = 6\)). Furthermore,
the amounts of fluoxetine potentiation on either amplitude (145.8 ± 7.5%) or frequency (457.5 ± 62.4%) of mIPSCs were not significant different from those seen in the absence of TTX (Fig. 2C, P > 0.05, n = 6).

We also examined the mIPSC in the presence of 0.3 μM TTX before and during the application of fluoxetine at different concentrations. As illustrated in Fig. 3, fluoxetine potentiated both the amplitude and frequency of mIPSCs in a dose-dependent manner, with the EC50 values of 10.96 and 14.26 μM, respectively.

Previous studies showed that activation of 5-HT2 receptors could potentiate GABAergic mIPSCs in the hippocampus [28]. Therefore, as a SSRI, fluoxetine may potentiate GABAergic mIPSCs through its modulation on serotonergic system, especially the activation of 5-HT2 receptors. To test this possibility, we examined the fluoxetine potentiation of mIPSCs in the presence of 5 μM ritanserin, a selective antagonist of 5-HT2 receptor. As illustrated in Fig. 4, 30 μM fluoxetine significantly potentiated both the amplitude and frequency of mIPSCs (P < 0.01, n = 6). The amounts of fluoxetine potentiation on either amplitude (161.4 ± 5.5%) or frequency (470.1 ± 23.7%) were not significant different from those seen in the absence of ritanserin (P > 0.05, n = 6), suggesting that the fluoxetine potentiation does not depend on the activation of 5-HT2 receptors.

As we know, the GABA is a major inhibitory neurotransmitter playing a ubiquitous and central part in the function of the central nervous system. A GABA deficit was found in the cerebrospinal fluid [11] and plasma [3,21] sample of the depressed subjects. Accumulating evidence indicates that most of treatment approaches for depression, including electroconvulsive therapy, pharmacotherapy with SSRIs or cognitive behavioral therapy, can increase GABA levels in the brain although their pattern may be different [24,25,27]. However, the GABA concentration measured in the cerebrospinal fluid or plasma did not reflect the synaptic GABA concentration. Unfortunately, the effects of fluoxetine on GABAergic synaptic transmission remain poorly investigated. In the hippocampus, the results of this study demonstrate that fluoxetine could significantly potentiate both the amplitude and frequency of GABAergic IPSCs in a dose-dependent manner, with the EC50 values of 10.96 and 14.26 μM, respectively. Furthermore, the potentiation was TTX-insensitive, suggesting independence of presynaptic action potentials.

It has been typically reported that plasma fluoxetine concentration in patients is about 1–2 μM [1,17,18]. In the brains of chronically treated patients, accumulation of fluoxetine can even increase the concentration nearly 20-fold compared with plasma levels [10]. In a magnetic resonance spectroscopy study the steady-state brain concentration of fluoxetine was found to be 13 ± 2 μM [4]. These data and our results suggest that fluoxetine is able to potentiate GABAergic neurotransmission during antidepressant treatment. Since accumulating evidence indicates that normalizing of GABA deficit might be beneficial in treatment of depression [24,25,27], it is reasonable to assume that this action of fluoxetine might contribute to its therapeutic effect.

Furthermore, the potentiation of fluoxetine on the amplitude of sIPSCs indicates a postsynaptic mechanism, which is not surprising given that fluoxetine could directly potentiate the activity of GABA type A receptors [6,7,22,31]. In contrast, the potentiation of fluoxetine on the frequency of sIPSCs indicates a presynaptic mechanism. Fluoxetine potentiated the GABAergic IPSCs in the presence of TTX, suggesting independence of action potential activity by acting at the presynaptic terminal.

Cross-talking between GABAergic and serotonergic systems has been observed in the hippocampus [2,19,23]. The activation of 5-HT3/5-HT2 or 5-HT1A receptors can increase [15,23,28] or decrease [12] the GABAergic neurotransmission. Thus, the potentiation of GABAergic IPSCs by fluoxetine may rely on its elevating of serotonergic system activity. Previous studies have shown that the potentiation of GABAergic neurotransmission by the activation of 5-HT3 receptors in hippocampus was abolished in the presence of TTX [23]. In this study, the fluoxetine potentiation of GABAergic IPSCs was TTX-insensitive, suggesting independence of 5-HT3 receptor activation. Furthermore, ritanserin, a selective antagonist
Fig. 4. The effect of ritanserin on the fluoxetine potentiation of mIPSC. (A) In the presence of 50 μM APV, 10 μM CNQX, 0.3 μM TTX, and 5 μM ritanserin, recordings show mIPSCs before (control) and during the application of 30 μM fluoxetine. (B) Cumulative fraction plots for mIPSCs amplitude (left; K–S test, \( P < 0.001 \), fluoxetine vs. control) and inter-event intervals (right, \( P < 0.001 \)) in the same experiment. (C) Fluoxetine potentiation of mIPSC amplitude (left) and frequency (right) in the absence (control) and presence of 5 μM ritanserin (Student’s t-test, \( n = 6 \), \( * * P < 0.01 \), compared with the value before the application of fluoxetine; \( P > 0.05 \), control vs. ritanserin during the application of fluoxetine).

of 5-HT2 receptor, also failed to alter the fluoxetine potentiation of GABAergic IPSCs. Taken together, our results suggest that fluoxetine may potentiate GABAergic IPSCs in a 5-HT receptor-independent manner.

In summary, fluoxetine can significantly potentiate both the amplitude and frequency of GABAergic IPSCs in a dose-dependent manner. Furthermore, the fluoxetine potentiation does not depend on the action potential activity and its elevating of 5-HT receptor activity, implicating a non-serotonergic mechanism of fluoxetine action.

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