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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 EC_{50} values of 10.96 and 14.26 μM, respectively. This potentiation was also TTX-insensitive, suggesting independence of presynaptic action potentials. The ritanserin (5 μM), a selective 5-HT_2 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.

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. Then hippocampal neurons were isolated by a standard enzyme treatment protocol. Briefly, hippocampal tissues were dissociated with trypsin–EDTA solution (0.25% trypsin and 1 mM EDTA, Gibco, Grand Island, NY, USA) and then the neurons were plated ($(1–5) \times 10^6$ cell/ml) on poly-L-lysine (Sigma, St. Louis, MO, USA) coated cover glasses in DMEM (Gibco) with L-glutamine plus 10% fetal bovine serum (Gibco), 10% F-12 nutrient mixture (Gibco) and 100 units/ml penicillin/streptomycin (Gibco). After the attachment of neurons to the glasses within 24 h, the culture medium was replaced with neuron-basal medium

Abbreviations: ANOVA, analysis of variance; APV, 2-amino-5-phosphovaleric 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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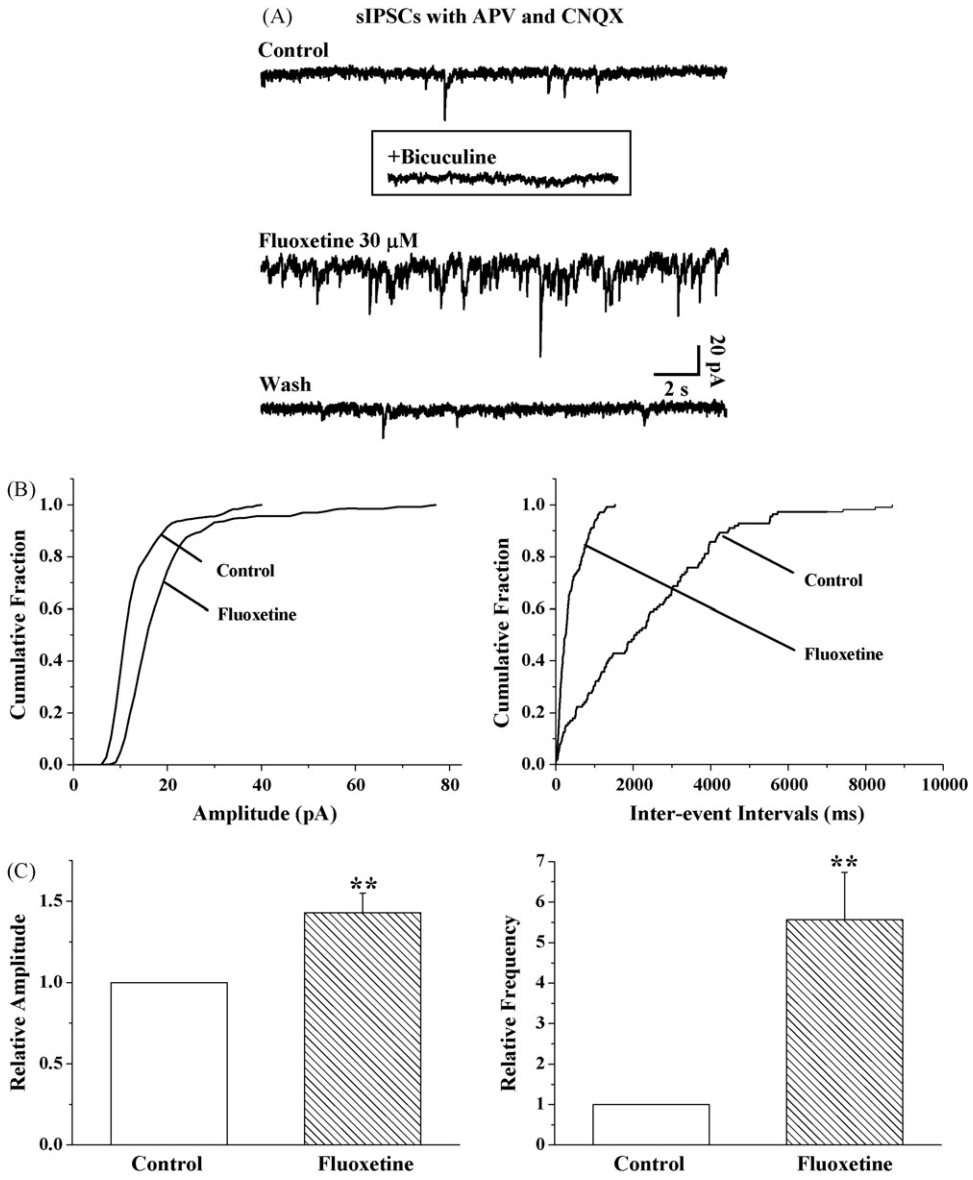

Fig. 1. Fluoxetine reversibly potentiated GABAergic sIPSC. (A) In the presence of 50 μM APV and 10 μM CNQX, recordings show sIPSCs before (control) and during the application of 30 μM fluoxetine. (B) Cumulative fraction plots for sIPSCs amplitude (left; K–S test, $P < 0.001$, fluoxetine vs. control) 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, $n = 6$, $^{**}P < 0.01$).

(1 ml) with 2% B_{27} (Gibco) every 3–4 days. To stabilize the cell population, the culture was treated with 5-fluoro-5′-deoxyuridine (20 μg/ml, Sigma) on the fourth day after plating to block cell division of non-neuronal cells. The cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere. Cells for electrophysiological recordings were used at 7–14 days after plating.

The standard external solution contained (in mM): 150 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 Hepes. The pH was adjusted to 7.4 with Tris base. The osmolarity of the solutions was adjusted with sucrose to 310–320 mOsm/l detected with a micro-osmometer (Model 3300, Norwood, MA, USA). The pipette solution for whole-cell patch-clamp recording contained (in mM): 150 KCl, 1 $MgCl_2$, 0.5 $CaCl_2$, 5 EGTA, 2 Mg-ATP, and 10 Hepes. The pH was also adjusted to 7.4 with Tris base. The drugs were first dissolved in ion-free water and then diluted to the final concentrations in the standard external solution just before use. All drugs for electrophysiological experiments were purchased from Sigma and applied using a rapid application technique termed as 'Y-tube' method throughout the experiments [16]. This application system allows a complete exchange of external solution surrounding a neuron within 20 ms.

The electrophysiological recordings were performed in conventional whole-cell patch-clamp recording configuration under voltage-clamp mode. Patch pipettes were pulled from glass capillaries with an outer diameter of 1.5 mm on a two-stage puller (PP-830, Narishige, Tokyo, Japan). The resistance between the recording electrode filled with pipette solution and the reference electrode was 3–5 MΩ. Membrane currents were measured using a patch-clamp amplifier (Axon 200B, Molecular Device, CA, USA), sampled using a Digidata 1320A interface connected to a personal computer and analyzed with Clampex and Clampfit software (Version 9.0.1, Axon Instruments). In all experiments, 70–90% series resistance was compensated. Unless otherwise specified, the membrane potential was held at −60 mV on hippocampal neurons throughout the experiment. All the experiments were carried out at room temperature (22–25 °C).