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Effect of Fluoxetine on the Induction of Long-term Potentiation in Rat Frontal Cortex

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Serotonin (5-hydroxytroptamine, 5-HT) has been shown to affect the induction of long-term potentiation (LTP) in the cortex such as the hippocampus, the visual cortex and the prefrontal cortex. Fluoxetine, as a selective serotonin reuptake inhibitor, is used in the management of a wide variety of psychological diseases. To study the effect of fluoxetine on the induction of LTP, we recorded the field potential in layer II/III of the frontal cortex from 3-wk-old. LTP was induced in horizontal input by theta burst stimulation (TBS). TBS with two-folds intensity of the test stimulation induced LTP, which was blocked by application of D-AP5 (50 μM), an NMDA receptor antagonist. Whereas bath application of 5-HT (10 μM) inhibited the induction of LTP, treatment with the 5-HT depleting agent, para-chloroamphetamine (PCA, 10 μM), for 2hr did not affect the induction of LTP. Bath application of fluoxetine (1, 3, and 10 μM) suppressed the induction of LTP in concentration-dependent manner, however, fluoxetine did not inhibit the induction of LTP in 5-HT-depleted slices. These results indicate that fluoxetine may inhibit the induction of LTP by modulating serotonergic mechanism in the rat frontal cortex.

Key Words: Serotonin, Fluoxetine, Long-term potentiation, Frontal cortex

INTRODUCTION

Serotonin (5-hydroxytroptamine, 5-HT) is one of the major neuromodulators that regulate synaptic transmission in the central and peripheral nervous systems (Barnes & Sharp, 1999). Serotonergic fibers originating from the raphe region have widespread projections to the forebrain, and their activation is implicated in complex brain function, such as brain development (Vitalis & Parnavelas, 2003), and short-term (Roerig et al, 1997) and long-term information processing (Staubli & Otaky, 1994). It has been reported that 5-HT modulates the induction of long-term potentiation (LTP) in the cortex such as the hippocampus (Staubli & Otaky, 1994; Shakesby et al, 2002), the visual cortex (Kojic et al, 1997; Edagawa et al, 2001) and the prefrontal cortex (Ohashi et al, 2003). However, the effect of 5-HT on the induction of LTP in the frontal cortex has not been studied in detail except the prefrontal cortex.

5-HT is also involved in pathophysiological mechanisms, especially of the brain state-related diseases such as depression, sleep disorder, seizure, and schizophrenia (Statnick et al, 1996; Wong & Van Tol, 2003). Fluoxetine (Prozac), introduced as a selective serotonin reuptake inhibitor, is one of the most widely used agents in the management of depression (Wong et al, 1995). Despite of its relatively selective action on the serotonergic system, many studies have been conducted on its effect on variety of voltage- and ligand-dependent ion channels (Rae et al, 1995; Hahn et al, 1999; Choi et al, 2003), metabotropic receptors and calcium homeostasis (Jagadeesh & Subhash, 1998; Dwivedi et al, 2002; Wang et al, 2003). Since these mechanisms are the major determinants in the induction of LTP in the cerebral cortex, fluoxetine may affect the long-term synaptic plasticity. Recently, two contradictory reports have appeared: fluoxetine attenuats LTP induction in the hippocampus (Shakesby et al, 2002) and reverses the impairment of LTP induced by acute stress in the prefrontal cortex (Rocher et al, 2004). However, since these two studies were done *in vivo*, many complicating factors could be involved.

In the present study, therefore, to investigate more directly effect of fluoxetine on the induction of LTP, we studied the effect of fluoxetine on the induction of LTP in frontal cortex slices of rats. Furthermore, using 5-HT-depleted slices by the 5-HT-dpleting agent para-chloroamphetamine (PCA) (Lu & Gean, 1998), we examined whether the effect of fluoxetine on LTP induction involved the serotonergic mechanism.

METHODS

Chemicals

6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and D-

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; LTP, long-term potentiation; NMDA, N-methly-D-aspartate; PCA, para-chloroamphetamine; fEPSP, field excitatory postsynaptic potential.

aminopentanoic acid (D-AP5) were purchased from Tocris Cookson (Bristol, UK). All other chemicals, including 5-HT and PCA, were purchased from Sigma (MO, USA).

Slice preparation

Sprague-Dawley rats (3-week-old, Folas International, Seoul, Korea) were housed in standard conditions (23±1°C, 12/12 h light/dark cycle). After anesthesia with chloral hydrate (400 mg/kg, i.p.), the brain was quickly removed and placed in ice-cold (2~4°C) dissection medium, consisting of 125 NaCl, 4 KCl, 1 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 Glucose (in mM), and bubbled with 95% O_2 and 5% CO_2. A brain block including the frontal cortex was mounted on Teflon block with cyanoacrylate, and 400 μm-thick slices were cut on a vibrotome. The slices were allowed to recover for 40 min at 37°C in an incubation chamber containing dissection medium and stored at room temperature before the experiment. Then, the slices were transferred to a submerging type recording chamber and perfused continuously with an artificial cerebrospinal fluid (ACSF), containing 125 NaCl, 4 KCl, 1 $CaCl_2$, 2 $MgSO_4$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, and 10 Glucose (in mM), and bubbled with 95% O_2 and 5% CO_2, at a rate of 2 ml/min at 32~33°C.

Recording of field EPSP

Field excitatory postsynaptic potential (fEPSP) was recorded in layer II/III about 300 μm below the pia with a glass micropipette filled with ACSF, yielding impedances of 2.0~3.0 MΩ. A concentric bipolar stimulating electrode (100 μm in tip diameter) was positioned 250~500 μm lateral to the recording electrode. fEPSP was evoked with constant step current (0.1 msec) of amplitude inducing half amplitude of maximum fEPSP, which was given at 30 sec intervals. The signal was amplified, digitized at 10 kHz, stored in a Pentium PC, and analyzed with the LTP program (v2.3, www. ltp-program.com). After stable baseline response for 10 min, theta-burst stimulation (TBS) was applied to induce LTP. TBS consisted of 5 bursts at 5 Hz and each burst 10 stimuli at 100 Hz. fEPSP was recorded for more than 50 min after the induction. The effect was analyzed with the percent changes of peak amplitude of fEPSP at 30~40 min after the induction to the baseline fEPSP amplitude.

Statistical analysis

Data were pooled together for each experimental group and expressed as percent change from the baseline amplitude in mean±SE. Statistical comparison between two groups was determined with Student's *t*-test. P less than 0.05 was considered significant.

RESULTS

Induction of NMDA-dependent LTP in layer II/III of the frontal cortex

As shown in Fig. 1A, fEPSP was recorded in layer II/III of the frontal cortex and was evoked by the stimulation of neighboring layer II/III. According to Paxinos and Watson (1997), the frontal cortical area studied is a part of the motor cortex. The fEPSP evoked by the stimulation intensity to induce half maximal fEPSP amplitude showed the peak at 4.3±0.1 msec (n=18) after the stimulation (Fig. 1A). This fEPSP was completely blocked by CNQX (10 μM), an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist (n=2), indicating AMPA-mediated synaptic activation (Fig. 1B). To induce LTP, we applied TBS after the baseline recording for 10 min. Whereas TBS with test stimulus intensity did not change the amplitude of fEPSP (99.5±1.8% of the baseline amplitude at 30~40 min after TBS, n=10), TBS with two-folds intensity of the test stimulus induced LTP (115.6±2.0%, n=18, $P<0.05$) (Fig. 2). Bath application of D-AP5 (50 μM), an N-methyl-D-aspartate (NMDA) receptor antagonist, blocked the induction of LTP (93.0±3.2%, n=7, $P<0.05$)

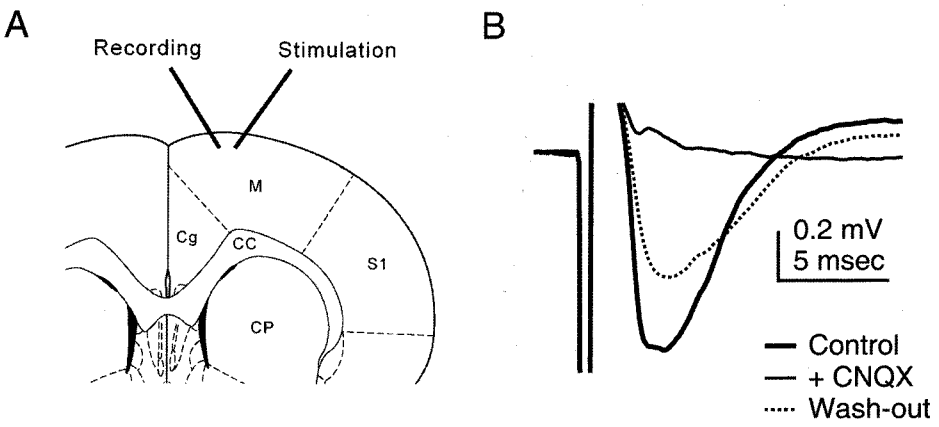

Fig. 1. Recording of fEPSP in layer II/III of the frontal cortex. A. Schematic drawing of the recording conditions. The coronal cut shown is at 1,6 mm from the bregma. M: motor cortex, S1: primary somatosensory cortex, Cg: cingulate cortex, CC: corpus callosum, and CP: caudate putamen. B. Effect of CNQX on the fEPSP. CNQX (10 μM) was applied into the bath after 10-min stable baseline recording (Control). Control response was evoked with stimulation intensity generating half maximal fEPSP amplitude. Wash-out of CNQX restored the fEPSP.

(Fig. 2). Thus, LTP induced by TBS of two-folds stimulus intensity in layer II/III of the rat frontal cortex is dependent on the activation of NMDA receptors. Therefore, in the following experiments, we used TBS with two-folds intensity of the test stimulation to induce LTP.

Effect of 5-HT and fluoxetine on the induction of LTP

We next tested whether 5-HT modulates the induction of NMDA-dependent LTP. Although application of 5-HT (10 μM) into the bath did not change the baseline EPSP amplitude (96.4±4.2%, n=6), the treatment blocked the LTP induced by TBS (103.3±3.2%, n=8) (Fig. 3A). Thus, as reported in the rat visual cortex (Edagawa et al, 2001), the induction of NMDA-dependent LTP in rats is inhibited by serotonergic activation. On the other hand, bath application of fluoxetine (10 μM) also blocked the induction of LTP (102.4±2.7%, n=11) (Fig. 3B), whereas it did not affect the baseline amplitude of fEPSP (101.4±5.0%, n=11). Application of 3 μM fluoxetine also inhibited the induction of LTP with less extent (108.2±5.7%, n=7, $P>0.173$), however, 1 μM fluoxetine did not (117.5±4.1%, n=8, $P<0.05$). These values yield a half inhibitory concentration (IC50) of 2.2 μM. Therefore, fluoxetine inhibited the LTP induced by TBS in layer II/III of the rat frontal cortex in concentration-dependent manner.

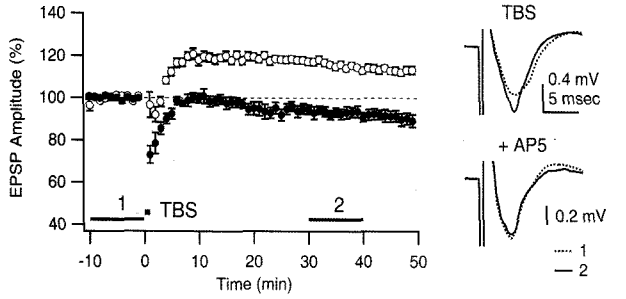

Fig. 2. Induction of LTP in layer II/III fEPSP evoked by TBS of neighboring layer II/III. LTP was induced by TBS with two-folds stimulation intensity of the test stimulation (n=18, open circle). Bath application of D-AP5 (50 μM) blocked the induction of LTP (n=7, closed circle). Numbers indicate the time period for the analysis of the effect of TBS. Right panels show the representative traces before (1) and after (2) the conditioning stimulus. Data represent mean±SE.

Effect of fluoxetine in 5-HT-depleted slices

IC50 value of 2.2 μM is far less than that on voltage-dependent ion channels reported, thus, suggesting that fluoxetine is not likely involved directly in the ion channels. To further investigate whether fluoxetine inhibition on the LTP induction involved 5-HT, we tested the effect of fluoxetine in 5-HT-depleted slices. To this end, we attempted to deplete 5-HT from the slice by incubation with PCA, a 5-HT-depleting agent, for 2 h before the experiment (Lu & Gean, 1998), and we confirmed the depletion of 5-HT with immunohistochemistry using anti-5-HT antibody (data not shown). However, PCA pretreatment did not change the induction of LTP (117.0±5.2%, n=8, $P<0.05$) (Fig. 4) as well as the shape and the peak time of fEPSP (4.3±0.2 msec, n=8). LTP was well induced with the application of fluoxetine (10 μM) in 5-HT-depleted slices (115.6±5.0%,

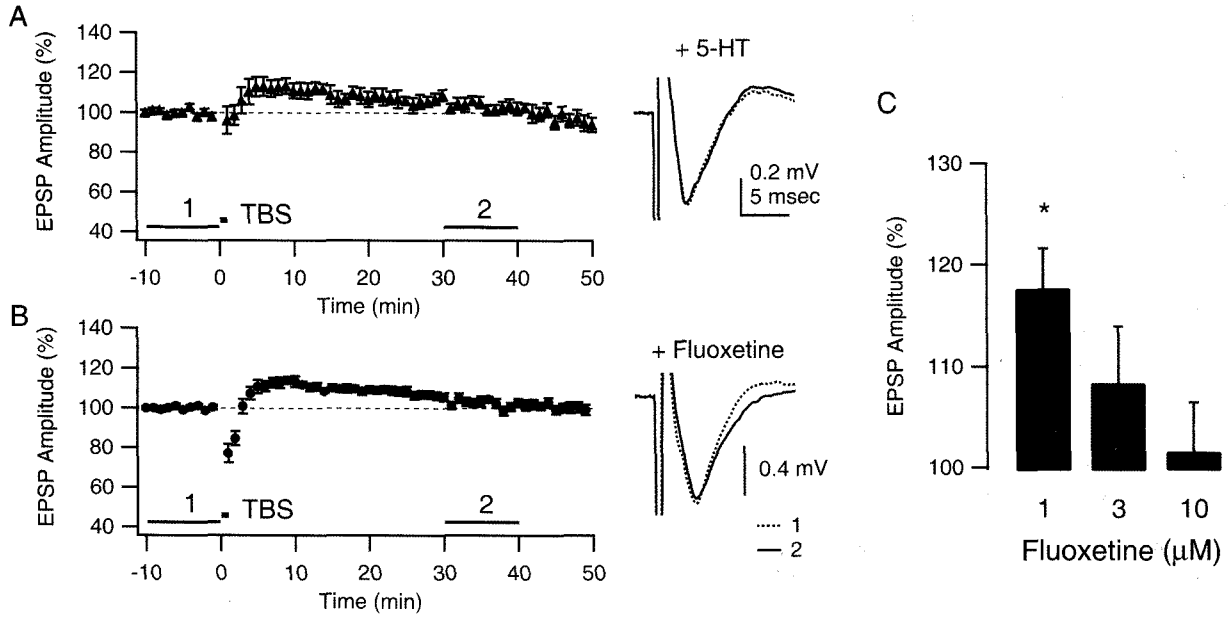

Fig. 3. Effect of 5-HT and PCA on the induction of LTP. A. Average response of 5-HT application. Application of 5-HT (10 μM) blocked the induction of LTP by TBS (n=8, closed triangle). B. Average response of fluoxetine application. Fluoxetine (10 μM, closed circle) blocked the induction of LTP (n=11). Numbers indicate the time period for the analysis of the effect of TBS. Right panels show the representative traces before (1) and after (2) the conditioning stimulus. C. Concentration response of fluoxetine on the induction of LTP. Fluoxetine was applied at concentration of 1 (n=8), 3 (n=7), and 10 μM. Data represent mean±SE. *: vs. baseline fEPSP amplitude, $P<0.05$.

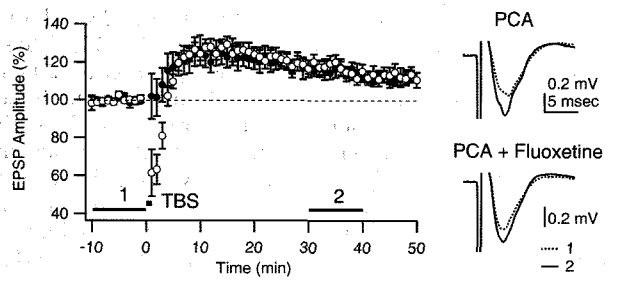

Fig. 4. Effect of fluoxetine on the induction of LTP in 5-HT-dpleted slices. Slices were incubated with PCA-containing storing solution for 2 h before the experiment. Incubation with PCA (10 μM) did not affect the induction of LTP by TBS (n=8. open circle). Application of fluoxetine (10 μM) did not change the induction of LTP in PCA-treated slices (n=6, closed circle), which was not different from that in the intact slices. Numbers indicate the time period for the analysis of the effect of TBS. Right panels show the representative traces before (1) and after (2) the conditioning stimulus. Data represent mean $\pm$ SE.

n=6, $P<0.05$), which was not different from that obtained in intact slices shown in Fig. 1 ($P>0.854$). These results indicate that fluoxetine inhibits the induction of LTP in the rat frontal cortex via the activation of 5-HT mechanism.

DISCUSSION

One of the major findings in our experiments is that 5-HT inhibits the induction of NMDA-dependent LTP by TBS in the rat motor cortex. It has been reported that 5-HT1A and 2A receptors in the motor cortex are altered in isolation-reared rats (Preece et al, 2004). There is accumulating evidence that altered functions of 5-HT and 5-HT receptors are involved in schizophrenia and epilepsy (Statnick et al, 1996; Preece et al, 2004). Supragranular neurons in the rat sensorimotor cortex are regulated by 5-HT (Foehring et al, 2002). To the best of our knowledge, this is the first report that 5-HT modulates the induction of LTP in the rat motor cortex. Altered modulation of the LTP induction by abnormal 5-HT function may contribute to the pathophysiology of many psychological disorders.

It has been reported that 5-HT activation facilitates the induction of LTP in the visual cortex of cat (Kojic et al, 1997; Kojic et al, 2000). In contrast, 5-HT suppresses the induction of LTP in the hippocampus (Staubli & Otaky, 1994), in the visual cortex (Edagawa et al, 2001), and in the prefrontal cortex (Ohashi et al, 2003) of rats. Generally, neuromodulators such as 5-HT, acetylcholine, and norepinephrine have been regarded as enabling factors to perform important function of gating experience-induced plasticity under certain behavioral states (Singer, 1995). Activation of cholinergic and noradrenergic receptors lowers the threshold of activity required for the induction of LTP and long-term depression (LTD) (Brocher et al, 1992; Kirkwood et al, 1999). However, the effect of 5-HT on LTP have been more difficult to nail down, partly due to the multiple subtypes of 5-HT receptor (Hoyer & Martin, 1997; Barnes & Sharp, 1999) and the heterogeneous distribution pattern of serotonergic fibers and receptors in the cortex (Dyck & Cynader, 1993; Kojic et al, 2000). Moreover, the amount and distribution pattern of 5-HT receptor expression change during postnatal development of the cortex (Li et al, 2004). Along with these changes, the effect of 5-HT on membrane potential in the prefrontal pyramidal neuron during the postnatal development shifts from depolarizing to hyperpolarizing during the third postnatal week, reflecting changes in receptor subtypes from 5-HT7 to 5-HT1A (Beique et al, 2004). Thus, the effect of 5-HT on the induction of LTP appears to depend on the regions, ages, and species of tested animals. More detailed studies on the involvement of 5-HT receptors on the inhibition of LTP induction in the frontal cortex are required.

5-HT inhibits NMDA receptors expressed in *Xenopus* oocytes (Masuko et al, 2004). However, 10 μM 5-HT in this study did not appear to directly inhibit the NMDA receptors, because the IC50 reported is 36 μM. It has been reported that 5-HT2 receptors are present on GABA-containing inhibitory interneurons in the cerebral cortex (Sheldon & Aghajanian, 1990) and 5-HT fibers preferentially innervate distinct subclasses of peptidergic inhibitory interneurons in the rat visual cortex (Paspalas & Papadopoulos, 2001). One of the major determinants in the induction of LTP is the inhibitory influences (Hensch et al, 1998; Huang et al, 1999). Thus, it may be possible that 5-HT inhibits the induction of LTP by modulating inhibitory interneurons in the frontal cortex.

In this study, we have found that fluoxetine inhibited the induction of LTP but did not inhibit the induction of LTP in 5-HT-depleted slices, implying that fluoxetine suppresses the induction of NMDA-dependent LTP by serotonergic activation. Moreover, IC50 was 2.2 μM, which is not much higher than the reported therapeutic concentration in plasma (about 1 μM) (Altamura et al, 1994), in comparison with reported IC50 of 13~36 μM values on the voltage-dependent ion channels (Rae et al, 1995; Hahn et al, 1999) and NMDA receptors (Masuko et al, 2004). Thus, in this study, the fact that fluoxetine even at 10 μM concentration did not inhibit the induction of LTP in 5-HT-depleted slices excludes the possibility of direct action of fluoxetine on voltage-dependent ion channels and NMDA receptors in inhibition of the LTP induction. However, K*i* value of fluoxetine to inhibit the 5-HT uptake has been reported to be about 20 nM (Bolden-Watson & Richelson, 1993; Wong et al, 1995). In our study, 1 μM concentration of fluoxetine did not inhibit the induction of LTP in intact slices. The discrepancy in concentrations between the known concentration for 5-HT reuptake inhibition and the IC50 value in this study might have been due to different experimental conditions between slice preparation and cultured single cells.

Synaptic plasticity is altered not only by stress paradigms, but also by pharmacological manipulations such as antidepressants and/or anxiolytics (Stewart & Reid, 2000; Rocher et al, 2004). Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (Shakesby et al, 2002) and fluvoxamine (Kojima et al, 2003) and the 5-HT1A receptor agonist tandospirone (Mori et al, 2001) inhibit LTP in the hippocampal CA1 field. We demonstrated in the present study that acute treatment of fluoxetine inhibits the induction of LTP by TBS in the frontal cortex. Fluoxetine as an antidepressant has a 2 to 3 week lag time before clinical improvement is apparent (Nierenberg et al, 2000), and this latency may be due to drug-induced changes in 5-HT receptors and their signaling pathways. Thus, further study in animal treated chronically with fluoxetine is required to find out the chronic effect of fluoxetine treatment.

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