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Attenuation of 5-HT_{1A} regulation in medial prefrontal cortex GABA system of early postnatal-stressed rats

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Abstract

Electrophysiological studies showed that 5-HT_{1A} receptor-mediated membrane hyperpolarization is impaired in the medial prefrontal cortex (mPFC) layer V pyramidal neuron of early postnatal stressed rats, exposed to foot shock stress (FS) at 2 weeks old of age (2w-FS rats). However, the role of 5-HT_{1A} receptor in the local GABA synaptic circuits is not known in mPFC of 2w-FS rats.

In the present study, we examined 5-HT_{1A} receptor-mediated responses in GABAergic synaptic transmission in mPFC pyramidal neuron of adult (10 week-old) rats. Stimulation of 5-HT_{1A} receptor with 8-OH-DPAT decreased frequency of GABAergic synaptic event in control rats without early postnatal FS (Non-FS), whereas 8-OH-DPAT did not change the frequency in 2w-FS group. 8-OH-DPAT did not change the amplitude of GABAergic

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synaptic events neither in Non-FS nor 2w-FS group.
It was indicated that aversive stress exposure during the early postnatal period decreases 5-HT_{1A} receptor-mediated inhibition of GABAergic mechanisms in adult rat mPFC. Early postnatal 2w-FS attenuated 5-HT_{1A} control of GABAergic regulation which may contribute to abnormal behavioral response in adult rats.

INTRODUCTION

Dysfunction of serotonergic (5-HTergic) neurotransmission has long been implicated in the pathogenesis of neuropsychiatric disorders. Maturation of serotonergic innervation of the cortex has been shown to occur during the second postnatal week (Lidov & Molliver 1982). As the first two-weeks after birth is a critical period for the maturation of cortical pyramidal neurons (Zhang 2004), early postnatal stress may modulate serotonin (5-HT)-mediated function of central nervous system. The rats exposed to electrical foot shock stress during 2nd week old of age (2w-FS rats) exhibited abnormal behavioral responses, i.e, low levels of fear expression to emotional stressors and loss of hippocampal 5-HT_{1A} receptor-mediated suppression of long-term synaptic potentiation in adult age (Matsumoto *et al.* 2005).

The prefrontal cortex (PFC) is a key component of the neural circuit mediating responses to stressful situation. The medial PFC (mPFC) in rodents corresponds to the prelimbic area of the PFC in humans (Pratt & Mizumori 2001; Floresco *et al.* 2006). Layer V pyramidal neurons are principal output neuron in mPFC and determines the overall cortical tone (Lambe *et al.* 2003; Neafsey *et al.* 1986; Dalsass *et al.* 1981). 5-HTergic system has been shown to modulate activities of PFC glutamatergic pyramidal neurons through different types of receptor (Beiq *et al.* 2004; Tanaka & North 1993). The 5-THergic regulation of pyramidal neurons has been shown to be modified by early-day stress in rats. Maternal separation in postnatal 2–14 days increased 5-HT_{1A}-mediated outward currents in postnatal third and forth weeks, which recovered at adulthood in mPFC layer II/III pyramidal neuron (Goodfellow *et al.* 2009). Early postnatal FS stress (2w-FS) produced loss of 5-HT_{1A}-mediated hyperpolarization in mPFC layer V pyramidal neuron in 2w-FS rats (Shiozawa *et al.* 2008; Kimura *et al.* 2011).

Concerning GABAergic interneurons in the adult rat mPFC, psychological stress has been shown to increase GABA release (Matsumoto *et al.* 2005;

Ballini *et al.* 2008). However, little information is available on 5-HTergic regulation in the local GABAergic mechanism in mPFCs of early postnatal stressed adult rats. In the present study, we tried to clarify whether aversive stimuli experienced during the second postnatal week (PND 14–18) affects the neural mechanisms in the layer V of mPFC of adult rats, with a focus on cortical 5-HT$_{1A}$ receptor-mediated GABAergic function.

MATERIALS AND METHODS

Animals

Wistar rats were originally supplied by Sankyo Labo Service (Tokyo, Japan) and bred in our laboratory. Rats were housed in a room with a 12 h light-dark cycle under constant temperature (21 ± 2 °C). All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Animal Research Committee of Health Science University of Hokkaido.

Male pups were divided into two groups: a 2 week footshock (2w-FS) group in which rats received foot shocks at post natal day (PND) 14–18 and a non-FS control (Non-FS) group. Pups in the 2w-FS group were acclimated to the FS box (Freeze Frame-41, Neuroscience Co. Ltd, Tokyo, Japan) for 5 min and subjected to five FSs every 30 s (shock intensity, 1 mA; shock duration, 2 s). After the last FS stimulation, each rat remained in the FS box for 5 min and returned to its home cage. FS stimulation occurred once each day for 5 days. We chose at least two pups in the same colony of 2w-FS group to serve as Non-FS control. Non-FS control rats were subjected to a treatment similar to the FS groups but did not receive FS. Thus, Non-FS control rats remained in the FS box for 12.5 min without FS stimulation for days. The 2w-FS and Non-FS pups were housed in cages with their mothers until weaning. After weaning on PND 28, the pups of both groups were housed individually (2–4 pups per cage) in standard plastic cage until used.

Membrane potential recording

Adult (ten-week-old) rats were anesthetized with ketamine (50 mg/kg i.p.) and perfused transcardially with 50 mL of ice-cold cutting solution (Endo & Isa 2001). Electrophysiological experiment was conducted as our previous report (Kang *et al.* 2007). Briefly, Forebrains were transferred to the ice-cold cutting solution within 1 min. According to the atlas of Paxinos and Watson (1986) and Swanson (2004), coronal slices of the cingulate cortex of the mPFC

(300 μm thickness) were prepared with a vibrating microslicer (5000 mz, Campden, U.S.A.) in a cutting solution. Preparations were allowed to recover for one to 4 h in the artificial cerebrospinal fluid (ACSF) at room temperature (20–25 °C). Each slice was transferred to a recording chamber on the stage of an upright microscope (BX-51, Olympus, Tokyo, Japan) and superfused with ACSF (30°C) at a rate of 1.2 mL/min. The composition of the cutting solution was (in mM); sucrose 228, glucose 30, KCl 3, $NaHCO_3$ 26, $MgCl_2$ 10, $CaCl_2$ 0.5, aerated 95% O_2 and 5% CO_2, pH 7.35. The composition of the ACSF was (in mM); NaCl 124, KCl 1.8, $NaHCO_3$ 26, KH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.5 and glucose 10, aerated with 95% O_2 and 5% CO_2, pH 7.35.

Pyramidal neurons of the mPFC were visually identified in layer V of the cingulated cortex using infrared video contrast microscopy with a 60× water-immersion objective. Recording pipettes with a resistance of 4 MΩ were made from glass capillaries (GD 1.5, Narishige, Tokyo, Japan) by a Flaming/Brown type puller (P-97, Sutter Instruments, Novato, CA, USA) and connected to the head stage of a patch clamp amplifier (Axopatch 200B, Axon Instruments, Foster City, CA, USA). The signal was digitized by an A/D converter (Digidata 1322A, Axon Instruments) and stored on a computer using Axograph 4.8 software (Axon Instruments). Data were obtained by a standard whole-cell recording in current-clamp mode. During recording, a hyperpolarizing current of 100 pA amplitude with 100 ms width was administered to estimate membrane input resistance. The composition of the pipette solution was (in mM); CsCl 140, HEPES 10, EGTA 1, ATP·2Na 3, GTP·2Na 0.2, leupeptin 0.1, pH 7.3.

To isolate spontaneous GABAergic potential (IPSP), glutamatergic AMPA receptor and NMDA receptor were inhibited by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) 10 μM and DL-2-amino-5-phosphovaleric acid (AP5) 10 μM, resepectively. Voltage-dependent Na channel was inhibited by tetrodotoxin (TTX) 1 μM to observe miniature IPSP (mIPSP). CNQX, AP5 and TTX were included in the perfusate during the experiment. The 5-HT_{1A} receptor agonist, 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) and 5-HT_{1A} receptor antagonist Way-100135 were added to the perfusate.

Drugs

8-OH-DPAT was obtained from Sigma (St. Louis, MO, USA). Way-100135 was obtained from Tocris (Ellisville, MO, USA). All other drugs were analytical grade and obtained from Wako (Osaka, Japan).

Statistical analysis

Data in the absence and presence of 8-OH-DPAT were collected during 3 min before and 3 min after start of application of 8-OH-DPAT, respectively. Synaptic event was analyzed with Mini-Analysis software (Synaptosoft Inc, Leonia, NJ, USA). Data are expressed as mean ± SEM with number of neurons in parenthesis. Effect of drugs are expressed in % relative value as compared to values before application of drug. Statistical evaluation was analyzed by Kruskal-Wallis test as a non-parametric ANOVA (GraphPad Prizm, San Diego, CA, USA) otherwise stated. Values of $p<0.05$ were considered statistically significant.

RESULTS

The resting membrane potential of layer V pyramidal neurons of mPFC in Non-FS and 2w-FS rats was –63.4 ± 0.5 mV (n=25) and –64.3 ± 0.5 mV (n=18), respectively. Resting membrane potential in the absence and presence of 8-OH-DPAT 10 μM was –63.7 ± 1.0 mV (n=7) and –63.5 ± 1.1 mV (n=7), respectively in Non-FS rats. Resting membrane potential in the absence and presence of 8-OH-DPAT 10 μM was –64.3 ± 2.1 mV (n=6) and –63.3 ± 1.0 mV (n=6), respectively in 2w-FS rats. Thus, there was no difference in resting membrane potential between Non-FS and 2w-FS rats, and 8-OH-DPAT 10 μM did not change the resting membrane potential of pyramidal neuron either in Non-FS and 2w-FS rats indicating successful potassium channel inhibition by cesium in pipette.

The membrane resistance of layer V pyramidal neurons of mPFC in Non-FS and 2w-FS rats was 132.6 ± 5.5 MΩ (n=25) and 130.6 ± 4.2 MΩ (n=18), respectively. In Non-FS rats, the membrane resistance in the absence and presence of 8-OH-DPAT 10 μM was 137.1 ± 13.0 MΩ (n=7) and 131.9 ± 12.5 MΩ (n=7), respectively. In 2w-FS rats, the membrane resistance in the absence and presence of 8-OH-DPAT 10 μM was 144.7 ± 8.8 MΩ (n=6) and 144.5 ± 10.2 MΩ (n=6), respectively. Thus, there was no difference in membrane resistance between Non-FS and 2w-FS rats, and 8-OH-DPAT 10 μM did not change the resting membrane resistance of pyramidal neuron either in Non-FS and 2w-FS rats again due to potassium channel block by cesium in pipette.

Spontaneous synaptic events (transient membrane depolarization) with amplitude of 1.69 ± 0.03 mV (n=25) and 1.80 ± 0.02 mV (n=18) were observed in Non-FS and 2w-FS rats, respectively. The 10-90% rise time of the event was 7.13 ± 0.40 ms (n=12) and 7.09 ± 0.34 ms (n=10), respectively. Their decay time

was 27.91 ± 1.50 ms (n=12) and 27.86 ± 1.15 ms (n=10), respectively. They were completely abolished by the selective $GABA_A$ receptor antagonist, bicuculline 10 μM, indicating that they were $GABA_A$ergic synaptic potentials.

In Non-FS rat, the amplitude of GABAergic synaptic event before and after 8-OH-DPAT 10 μM application was 1.69 ± 0.07 mV (n=7) and 1.72 ± 0.06 mV (n=7), respectively. In 2w-FS rat, the amplitude of GABAergic synaptic event before and after 8-OH-DPAT 10 μM application was 1.82 ± 0.06 mV (n=6) and 1.82 ± 0.06 mV (n=6), respectively. Thus, 8-OH-DPAT 10 μM did not change the amplitude of GABAergic synaptic event either in Non-FS and 2w-FS rats (Figure 1A).

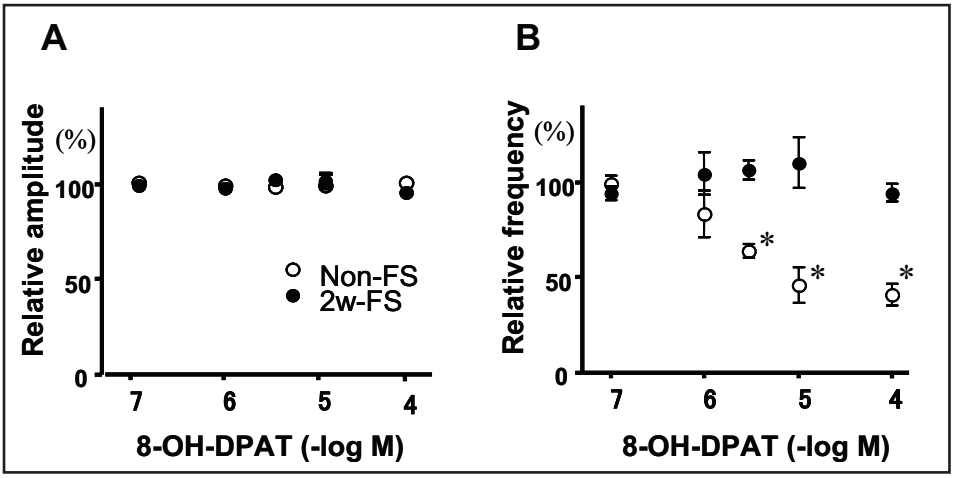

Fig. 1. Concentration-response curve of the effect of 8-OH-DPAT on the amplitude and frequency of GABAergic synaptic event in mPFC layer V pyramidal neuron of ten week-old rats recorded in the presence of TTX.
A: Effect of different concentration of 8-OH-DPAT on the relative amplitude of synaptic event was summarized. The amplitude before 8-OH-DPAT application for 3 min was considered as control amplitude (100%) and their amplitude after start of application of each concentration of 8-OH-DPAT for 3 min was represented as a relative value of control in each neuron. Data obtained from Non-FS (open circles) and 2w-FS(+) (filled circles) adult rats in number of 6 to 8 neurons, respectively. Each symbol represents average of data with vertical bars indicating SEM. Size of SEM bar was smaller than symbol size.
B: Effect of different concentration of 8-OH-DPAT on the relative frequency of synaptic event was summarized. The frequency before 8-OH-DPAT application for 3 min was considered as control frequency (100%) and their frequency after start of application of each concentration of 8-OH-DPAT for 3 min was represented as a relative value of control in each neuron. Data obtained from Non-FS (open circles) and 2w-FS (filled circles) adult rats in number of 6 to 8 neurons, respectively. Each symbol represents average of data with vertical bars indicating SEM. Asterisks show statistical significance with *p*-value smaller than 0.05 by nonparametric evaluation (Kruskal-Wallis test).

In Non-FS rat, the occurrance of GABAergic synaptic event before 8-OH-DPAT application was 0.71 ± 0.09 Hz (n=25). In Non-FS rats, 8-OH-DPAT 3 μM and 10 μM decreased the rate of occurrance of GABAergic synaptic event to 62.7 ± 3.6 % (n=7) and 49.0 ± 9.5 % (n=7) of pre-drug control level, respectively. From the concentration-response relation, the least effective concentrations of 8-OH-DPAT was above 0.1 μM and the response reached a maximum around at 10 μM. The value of IC_{50} for 8-OH-DPAT was 2.1 μM. Thus, in Non-FS rats, 8-OH-DPAT decreased the frequency of GABAergic synaptic event in a concentration-dependent manner (Figure 1B open circles).

In 2w-FS rats, the occurrance of GABAergic synaptic event before 8-OH-DPAT application was 0.65 ± 0.07 Hz (n=18). The rate of occurrance of GABAergic synaptic event in the presence of 8-OH-DPAT 3 μM and 10 μM was 106.3 ± 5.2 % (n=6) and 110.2 ± 5.2 % (n=6) of pre-drug control level respectively in 2w-FS rats. (Figure 1B filled circle). Thus, in 2w-FS rats, 8-OH-DPAT did not change the frequency of GABAergic synaptic event in a concentration between 0.1 and 100 μM (Figure 1B filled circles).

To examine the site of action of 8-OH-DPAT, we examined effect of 8-OH-DPAT on the distribution of GABAergic synaptic event amplitudes and inter-event interval (data not shown). The cumulative probability plots of GABAergic synaptic event amplitude were similar between the absence and presence of 8-OH-DPAT either in Non-FS or 2w-FS rats. The cumulative probability plots of GABAergic synaptic inter-event interval were shifted to the right by 8-OH-DPAT in Non-FS rats, indicating 8-OH-DPAT decreased frequency via presynaptic mechanism (Kolmogorov-Smirnov test). The cumulative probability plot of GABAergic synaptic inter-event interval was similar between the absence and presence of 8-OH-DPAT in 2w-FS rats.

To identify the involved 5-HT receptor subtype involved in the 8-OH-DPAT-induced responses, we observed effect of 8-OH-DPAT in the presence of a specific 5-HT$_{1A}$ receptor antagonist, WAY 100135 in Non-FS rats. WAY 100135 was reported to antagonize the effect of 8-OH-DPAT on neurons (Fletcher *et al.* 1993; Song *et al.* 2007). WAY 100135 30 μM did not affect amplitude or frequency of the GABAergic synaptic event. In the presence of WAY 100135 30 μM, 8-OH-DPAT 10 μM failed to change the frequency of GABAergic synaptic event (91.2 ± 5.6 % of before application, n=4), indicating 8-OH-DPAT acted mainly via 5-HT$_{1A}$ receptors.

DISCUSSION

5-HT is a major modulator of PFC function and 5-HT_{1A} receptor in PFC has been studied as a putative target for antipsychiatric drugs (Puig & Gulledge 2011). In *in vivo* experiments in anesthetized rats, systemic administration of 5-HT_{1A} agonists (8-OH-DPAT and BAYx3702) increased PFC pyramidal cell firing (Diaz-Mataix *et al.* 2006; Llado-Pelfort *et al.* 2011). However, in *in vitro* brain slice experiments, stimulation of 5-HT_{1A} receptor hyperpolarized the PFC pyramidal neuron membrane to decrease neuronal firing with membrane resistance decrease (Tanaka & North 1993; Beique *et al.* 2004; Kimura *et al.* 2011). 5-HT_{1A} receptor agonists, tandospirone and 8-OH-DPAT, have been shown to induce K^+ current and decrease the membrane resistance in rat dorsal raphe neurons, hippocampal pyramidal neurons and septal nucleus neurons (Okuhara & Beck 1994; Katayama *et al.* 1997; Jin & Akaike 1998; Yamada *et al.* 2001). As pyramidal membrane potential hyperpolarization by K^+ channel opening cannot increase cell firing, involvement of the decrease in GABAergic inhibitory tone has been proposed as a possible mechanism to understand the increase in pyramidal neuronal firing by administration of 5-HT_{1A} agonist in vivo (Diaz-Mataix *et al.* 2005; Gronier 2008; Llado-Pelfort *et al.* 2011).

Direct evidence of GABAergic tone decrease by 5-HT_{1A} agonist was unavailable in PFC of early life stress model. GABAergic inhibitory synaptic potential has been observed as a transient depolarizing (reversed) event because $GABA_A$-mediated Cl^- flux is outward at resting membrane potential as reported in hippocampal neurons (Alger & Nicoll 1980; Schmitz *et al.* 1995) and PFC pyramidal cells (Hirsch & Crepel 1990). In the present study, we observed spontaneous transient depolarizing potentials in the presence of TTX as well as CNQX and AP5. These spontaneous potential was inhibited by bicuculline, indicating it was mediated by $GABA_A$ receptor. Thus, the depolarizing $GABA_A$-mediated miniature potential observed in the present study is considered as miniature inhibitory postsynaptic potential (mIPSP).

In the present study, cesium, a K^+ channel blocker, was included in recording pipettes to inhibit pyramidal cell hyperpolarization by 8-OH-DPAT (Kishimoto *et al.* 2001) to minimize membrane hyperpolarization and resistance changes which might disturb the assessment of synaptic potential amplitude.

About 20% to 60% of GABAergic interneurons have been shown to express 5-HT_{1A} receptors in rat PFC (Amargos-Boschi *et al.* 2004; Santana *et al.* 2004). However, in the present study, 8-OH-DPAT decreased the frequency of mIPSP

in all recorded pyramidal neurons in relatively uniform fashion in Non-FS rats. It is of great contrast to 8-OH-DPAT-induced hyperpolarization observed in only 30% of pyramidal neurons in Non-FS rats (Kimura *et al.* 2011). The difference could be due to high convergence of interneuron synapses on a pyramidal neuron, because number of GABAergic interneuron is about 20 % of pyramidal neuron in PFC (Santana *et al.* 2004). It has been shown that most inhibitory neurons in layer V supply convergent inhibitory synapses to each pyramidal cell body (Hirsch & Crepel 1990; Szentagothai 1978; 1979).

Regulation of GABAergic transmission has been studied by recording spontaneous inhibitory postsynaptic current (IPSC) or miniature IPSC (mIPSC). Stimulation of 5-HT$_{1A}$ receptor decreased frequency of mIPSC in rat amygdala, periaqueductal grey, visual cortex and paraventricular nucleus (Koyama *et al.* 1999; Kishimoto *et al.* 2001; Xiang & Prince 2003; Lee *et al.* 2008), however, little information was available about rat mPFC interneurons (Yan 2002). Present study showed that stimulation of 5-HT$_{1A}$ receptor decreased frequency of mIPSP in rat mPFC and the decrease was attenuated in ealy life stress.

In the present study, postsynaptic mechanism may not be involved because amplitude of synaptic potential was not changed by 8-OH-DPAT. Previous studies on the postsynaptic 5-HTergic regulation of GABAergic transmission also showed that 8-OH-DPAT did not affect GABA$_A$ current in mPFC pyramidal neuron (Feng *et al.* 2001) or IPSC amplitude in deep cerebellar nucleus neuron (Saitow *et al.* 2009). Similarly in hippocampcal CA1 neuron, 8-OH-DPAT did not affect membrane response to exogenous GABA (Schmitz *et al.* 1995).

CONCLUSION

Present study indicates that 5-HT$_{1A}$ receptor is involved in the inhibitory regulation of GABAergic transmission in mPFC pyramidal neuron of adult rats. Early postnatal 2w-FS attenuated 5-HT$_{1A}$ control of GABAergic regulation which may contribute to abnormal behavioral responses in adulthood.

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