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IN VIVO EFFECT OF 5-HT$_7$ RECEPTOR AGONIST ON PYRAMIDAL NEURONS IN MEDIAL FRONTAL CORTEX OF NORMAL AND 6-HYDROXYDOPAMINE-LESIONED RATS: AN ELECTROPHYSIOLOGICAL STUDY

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Abstract—The 5-hydroxytryptamine (5-HT)-7 receptor began to be cloned and pharmacologically characterized close to 20 years ago. It couples positively via G-proteins to adenylyl cyclase and activation of this receptor increases neuronal excitability, and several studies have shown that degeneration of the nigrostriatal pathway leads to an impairment of 5-HT system. Here we reported that systemic and local administration of 5-HT$_7$ receptor agonist AS 19 produced excitation, inhibition and no change in the firing rate of pyramidal neurons in medial prefrontal cortex (mPFC) of normal and 6-hydroxydopamine-lesioned rats. In normal rats, the mean response of the pyramidal neurons to AS 19 by systemic and local administration in mPFC was excitatory. The inhibitory effect by systemic administration of AS 19 was reversed by GABA$_A$ receptor antagonist picrotoxinin. Systemic administration of picrotoxinin excited all the neurons examined in normal rats, and after treatment with picrotoxinin, the local administration of AS 19 further increased the firing rate of the neurons. In the lesioned rats, systemic administration of AS 19, at the same doses, also increased the mean firing rate of the pyramidal neurons. However, cumulative dose producing excitation in the lesioned rats was higher than that of normal rats. Systemic administration of AS 19 produced inhibitory effect in the lesioned rats, which was partially reversed by picrotoxinin. The local administration of AS 19, at the same dose, did not change the firing rate of the neurons in the lesioned rats. Systemic administration of picrotoxinin and the local administration of AS 19 did not affect the firing rate of the neurons in the lesioned rats. These results indicate that activity of mPFC pyramidal neurons is regulated through activation of 5-HT$_7$ receptor by direct or indirect action, and degeneration of the nigrostriatal pathway leads to decreased response of these neurons to AS 19, suggesting dysfunction and/or down-regulation of 5-HT$_7$ receptor on the pyramidal neurons and GABA interneurons in the lesioned rats.

Key words: 5-hydroxytryptamine-7 receptors, pyramidal neurons, medial prefrontal cortex, Parkinson's disease, extracellular recording.

The medial prefrontal cortex (mPFC) is crucial for the control of a large number of higher brain functions (Miller and Cohen, 2001) and either pyramidal neurons or γ-aminobutyric acid (GABA) interneurons or glial cells in the area are the cellular targets for psychiatric drugs (Jacobs and Azmitia, 1992; Montgomery, 1994; Meltzer, 1999). Neurons of the mPFC are classified as pyramidal neurons and non-pyramidal neurons according to their morphology. Pyramidal neurons accumulate glutamate and are the main excitatory cortical neurons, whereas most of non-pyramidal neurons use GABA as a neurotransmitter and are believed to be inhibitory interneurons. It is well documented that the mPFC receives 5-hydroxytryptamine (serotonin, 5-HT) innervations from the midbrain raphe nuclei (Steinbusch, 1981; O'Hearn and Molliver, 1984), and expresses several 5-HT receptor subtypes, including 5-HT$_{1A}$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_7$ (Gustafson et al., 1996; Morales et al., 1996; Morales and Bloom, 1997; Santana et al., 2004). Furthermore, several studies have shown that the 5-HT system is involved in psychiatric illnesses (Blier and de Montigny, 1999; Mann, 1999). Therefore, dysfunction of the mPFC may be linked to abnormalities in 5-HT system of the brain.

Parkinson's disease (PD) is characterized by degeneration and loss of midbrain substantia nigra pars compacta (SNc) that produce the neurotransmitter dopamine (DA), resulting in tremor at rest, inability to initiate or complete movements, muscle rigidity and postural instability. However, there is growing evidence that the PD is associated with a wide variety of non-motor features, including depression, anxiety and cognitive decline (Löhle et al., 2009), which affect the vast majority of patients during the course of the disease and may even precede the onset of motor symptoms (Shulman et al., 2001; Ziemssen and Reichmann, 2007). Several studies have shown the changes in expression of 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_3$ receptor subtypes in the raphe nuclei, hippocampal formation and prefrontal cortex of patients with PD and parkinsonian animals (Cicin-Sain and Jenner, 1993; Chen et al., 1998; Frechilla et al., 2001; Doder et al., 2003). Furthermore, electrophysiological studies from our laboratory have found that the lesion of the nigrostriatal pathway leads to a hyperactivity of pyramidal neurons in the mPFC and the abnormality of

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Abbreviations: DA, dopamine; ISI, interspike interval; ISIH, interspike interval histogram; mPFC, medial prefrontal cortex; PD, Parkinson's disease; SN$_C$, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, TH-immunoreactive; VTA, ventral tegmental area; 5-HT, 5-hydroxytryptamine; 6-OHDA, 6-hydroxydopamine.

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response of these pyramidal neurons to 5-HT_{1A} and 5-$HT_{2A/2C}$ receptor stimulation in the rat (Wang et al., 2009a,b). In addition, our study has also shown a decreased response of mPFC interneurons to 5-$HT_{2A/2C}$ and 5-HT_3 receptor stimulation in the 6-hydroxydopamine (6-OHDA)-lesioned rats (Gui et al., 2010; Zhang et al., 2010). These findings strongly suggest that the dysfunction of the mPFC and 5-HT neurotransmitter system appears to be an etiologic and pathophysiological factor for depression, anxiety and cognitive impairment in PD.

The 5-HT_7 receptor is the latest 5-HT receptor subtype to be identified (Bard et al., 1993; Ruat et al., 1993). It has been implicated in various functions including mood regulation, circadian rhythmicity and sleep disturbances, which are related to affective disorders (Hedlund and Sutcliffe, 2004; Thomas and Hagan, 2004). *In situ* hybridization, immunolabelling and radioligand binding studies have shown that the 5-HT_7 receptors are expressed in rat neocortex (Gustafson et al., 1996; Stowe and Barnes, 1998; Neumaier et al., 2001; Hedlund and Sutcliffe, 2004). *In vitro* electrophysiological studies have also shown that activation of 5-HT_7 receptors increases the excitability of the hippocampal, thalamic and globus pallidus neurons (Bacon and Beck, 2000; Bickmeyer et al., 2002; Goaillard and Vincent, 2002; Matsumoto et al., 2002; Tokarski et al., 2003; Chen et al., 2008). In addition, activation of 5-HT_7 receptors induces an inward current that can depolarize and excite layer V pyramidal neurons in the developing rat prefrontal cortex (Béïque et al., 2004). However, limited knowledge is available regarding the effect of 5-HT_7 receptor on pyramidal neurons in the mPFC. To address this issue, the present study was undertaken to examine the *in vivo* effect of the potent 5-HT_7 receptor agonist AS 19 on firing activity of the pyramidal neurons in normal rats, and changes in response of the pyramidal neurons to 5-HT_7 receptor stimulation in rats with 6-OHDA lesions of the SNc by electrophysiological techniques.

EXPERIMENTAL PROCEDURES

Animals and drugs

The experiments were performed on male Sprague–Dawley rats (270–320 g). The animals were kept in groups of four to a cage at a temperature of 21±2 °C, and had free access to food and water. Animal care followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised 1996, and the guideline of the Institutional Animal Care Committee of the University. The experiments were performed on two groups of rats: normal (n=69) and 6-OHDA-lesioned rats (n=64). All efforts were made to minimize the number of animals used and their suffering.

The following drugs were used: desipramine hydrochloride, 6-OHDA hydrochloride, apomorphine hydrochloride and picrotoxinin, all from Sigma-Aldrich (St. Louis, MO, USA). AS 19 [(2*S*)-(+)-5-(1,3,5-Trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin] and SB 269970 [(2*R*)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine] hydrochloride were obtained from Tocris (Ellisville, MO, USA). 6-OHDA and apomorphine were prepared in 0.9% saline containing 0.02% ascorbic acid; desipramine and SB 269970 were prepared in 0.9% saline; AS 19 and picrotoxinin were dissolved in 0.9% saline with 1% and 5% dimethyl sulfoxide, respectively. These drugs were prepared on the day of the experiment.

6-OHDA-induced lesions of substantia nigra DA neurons

Unilateral 6-OHDA lesioning was carried out as previously described (Breit et al., 2001; Wang et al., 2009a,b). The stereotaxic coordinates (in millimeters) were taken from the atlas of Paxinos and Watson (2005) using bregma and dura mater as references. Briefly, animals were anesthetized with 4% chloral hydrate (400 mg/kg, i.p.), placed in a stereotaxic frame (SN-2N, Narishige, Tokyo, Japan) and 6-OHDA (8 μg/4 μL) was injected over 5 min unilaterally into the SNc. Stereotaxic infusion followed the coordinates of the Paxinos and Watson (2005) atlas: AP: −5.0–5.3, L: 1.9–2.1, D: 7.1–7.3. The injection was made at a rate of 0.5 μl/min using a glass micropipette connected to a 5 μl microsyringe (air-tight Hamilton). All animals were treated with injection of desipramine (25 mg/kg, i.p.) 30 min before surgery, in order to protect noradrenergic terminals from 6-OHDA toxicity. Two weeks after the surgery, animals were given apomorphine (0.05 mg/kg, s.c.) and those exhibiting >20 contralateral turns per 5 min were chosen for further study (Breit et al., 2001; Wang et al., 2009a,b). In addition, normal rats as control did not undergo desipramine injection, the sham surgery and apomorphine behavioral test.

In vivo electrophysiological recordings and firing pattern analysis

During the third week after the injection of 6-OHDA, rats were anesthetized with 4% chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame (SN-2N, Narishige, Tokyo, Japan). Supplemental doses of anesthetic were administered as needed (80 mg/kg, i.v.). Body temperature was maintained at 37±0.5 °C by a heating pad, and heart rate was monitored throughout the experiment. Glass microelectrodes (7–15 MΩ) filled with 2% Pontamine Sky Blue in 0.5 M sodium acetate were directed stereotaxically to the mPFC (AP: 2.7–3.4, L: 0.5–1.0, D: 1.5–4.0; Paxinos and Watson, 2005). The neuronal firings were amplified, bandpass-filtered using a pre-amplifier (AVB-11A, Nihon Kohden, Tokyo, Japan), displayed on an oscilloscope (VC-11, Nihon Kohden, Tokyo, Japan) and stored in a computer equipped with the Spike 2 analysis system (Cambridge Electronic Design, England) for off-line analysis. The neurons which met the following criteria were included for off-line analysis in this study: (i) neurons with slow firing rates (0.1–5 spikes/s) exhibiting long duration (>1 ms) and with biphasic or triphasic extracellular waveforms; (ii) irregular single-spike firing pattern with burst events containing 2–4 spikes with decreasing amplitude and increasing duration in each burst (Hajós et al., 2003; Tseng et al., 2006); and (iii) the recorded neurons location was histologically confirmed from the mPFC.

In electrophysiological experiments, most of the neurons were recorded in the prelimbic prefrontal area (PrL) of the mPFC in normal (59 of 69, 86%) and the lesioned (57 of 69, 82%) rats, although a few neurons were also recorded in the cingulate cortex, area 1 (normal rats: 5 of 69, 7%; lesioned rats: 8 of 69, 12%) and infralimbic area of the mPFC (normal rats: 5 of 69, 7%; lesioned rats: 4 of 69, 6%; Paxinos and Watson, 2005). Additionally, the majority of the recorded neurons were localized in the layer V in normal (59 of 69, 86%) and the lesioned (55 of 69, 80%) rats, while a few neurons were found in the layers III or VI (layer III, normal rats: 3 of 69, 4%; lesioned rats: 3 of 69, 4%; layer VI, normal rats: 7 of 69, 10%; lesioned rats: 11 of 69, 16%). In experiments of the response of the neurons to 5-HT_7 receptor stimulation, all neurons observed in normal and the lesioned rats were localized in the PrL and these neurons were observed in the layer V (AP: 2.7–3.4, L: 0.5–0.7, D: 1.5–3.5; Swanson, 1998; Paxinos and Watson, 2005).