Neuroscience 311 (2015) 45–55

ACTIVATION AND BLOCKADE OF SEROTONIN$_7$ RECEPTORS IN THE PRELIMBIC CORTEX REGULATE DEPRESSIVE-LIKE BEHAVIORS IN A 6-HYDROXYDOPAMINE-INDUCED PARKINSON'S DISEASE RAT MODEL

Q. J. ZHANG,[a] C. X. DU,[a] H. H. TAN,[a] L. ZHANG,[b] L. B. LI,[a] J. ZHANG,[b] X. L. NIU[c] AND J. LIU[b]*

[a] *Department of Rehabilitation Medicine, The Second Hospital, Xi'an Jiaotong University, Xi'an 710004, China*

[b] *Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Xi'an 710061, China*

[c] *Department of Medicine, The Second Hospital, Xi'an Jiaotong University, Xi'an 710004, China*

Abstract—The role of serotonin$_7$ (5-HT$_7$) receptors in the regulation of depression is poorly understood, particularly in Parkinson's disease-associated depression. Here we examined whether 5-HT$_7$ receptors in the prelimbic (PrL) sub-region of the ventral medial prefrontal cortex (mPFC) involve in the regulation of depressive-like behaviors in sham-operated rats and rats with unilateral 6-hydroxydopamine lesions of the medial forebrain bundle. The lesion induced depressive-like responses as measured by the sucrose preference and forced swim tests when compared to sham-operated rats. Intra-PrL injection of 5-HT$_7$ receptor agonist AS19 (0.5, 1 and 2 μg/rat) increased sucrose consumption, and decreased immobility time in sham-operated and the lesioned rats, indicating the induction of antidepressant-like effects. Further, intra-PrL injection of 5-HT$_7$ receptor antagonist SB269970 (1.5, 3 and 6 μg/rat) decreased sucrose consumption, and increased immobility time, indicating the induction of depressive-like responses. However, the doses producing these effects in the lesioned rats were higher than those in sham-operated rats. Neurochemical results showed that intra-PrL injection of AS19 (2 μg/rat) increased dopamine, 5-hydroxytryptamine (5-HT) and noradrenaline (NA) levels in the mPFC, habenula and ventral hippocampus (vHip) in sham-operated and the lesioned rats; whereas SB269970 (6 μg/rat) decreased 5-HT levels in the habenula and vHip, and the levels of NA in the mPFC, habenula and vHip in the two groups of rats. The results suggest that 5-HT$_7$ receptors in the PrL play an important role in the regulation of these behaviors, which attribute to changes in monoamine levels in the limbic and limbic-related brain regions after activation and blockade of 5-HT$_7$ receptors.

Key words: 5-HT$_7$ receptors, prelimbic cortex, depression, Parkinson's disease, rat.

INTRODUCTION

The medial prefrontal cortex (mPFC) includes dorsal and ventral regions. The ventral mPFC can be further divided into prelimbic (PrL) and infralimbic cortices (Gabbott et al., 2003; Vertes, 2004). Further, an increasing body of evidence indicates that the ventral mPFC is involved in the regulation of depression in rats (Hamani and Nobrega, 2010; Hamani et al., 2010a,b; Scopinho et al., 2010; Hui et al., 2014; Lim et al., 2015). However, the role of the PrL sub-region of ventral mPFC in depressive-like behaviors is only now beginning to be understood.

It has long been established that central serotonin (5-hydroxytryptamine, 5-HT) neurotransmitter system plays a major role in various brain functions, as well as in the pathophysiology of neuropsychiatric disorders, such as depression. 5-HT mediates its effect by more than 14 molecularly identified receptor subtypes (Barnes and Sharp, 1999); however, the role of different 5-HT receptor subtypes in the pathophysiology and treatment of neuropsychiatric disorders has still to be clarified. The mPFC, including the PrL, is highly innervated by 5-HT fibers from the midbrain raphe nuclei (Steinbusch, 1981; O'Hearn and Molliver, 1984), and expresses several 5-HT receptor subtypes, such as 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_3$ and 5-HT$_7$ (Gustafson et al., 1996; Morales et al., 1996; Neumaier et al., 2001; Santana et al., 2004). The 5-HT$_7$ receptor belongs to a recently identified 5-HT receptor subtype. It is a seven-transmembrane domain G-protein-coupled receptor, which is positively linked to adenylyl cyclase (Ruat et al., 1993; Hedlund and Sutcliffe, 2004). Although 5-HT$_7$ receptors may involve in the regulation of the multiple brain functions, the precise role of the receptors is still not fully understood. Regarding the role of 5-HT$_7$ receptors in depression, several studies have found that 5-HT$_7$ receptor antagonists SB269970 and JNJ-18038683 show an antidepressant-like effect in the forced swim test (FST) and tail

*Corresponding author. Address: Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Xi'an Jiaotong University Health Science Center, Yan Ta Xi Lu 76, Xi'an 710061, China. Tel/fax: +86-29-82655160.
E-mail address: liujian@mail.xjtu.edu.cn (J. Liu).

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT$_7$, serotonin$_7$; 6-OHDA, 6-hydroxydopamine; DA, dopamine; DMSO, dimethyl sulfoxide; FST, forced swim test; MFB, medial forebrain bundle; mPFC, medial prefrontal cortex; NA, noradrenaline; PD, Parkinson's disease; PrL, prelimbic; SNc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-ir, TH immunoreactive; vHip, ventral hippocampus; VTA, ventral tegmental area.

http://dx.doi.org/10.1016/j.neuroscience.2015.10.016
0306-4522/

suspension test in rodents (Hedlund et al., 2005; Wesolowska et al., 2006a,b; Bonaventure et al., 2012). In addition, 5-HT_7 knockout mice also showed antidepressant-like activity in both these tests used (Guscott et al., 2005; Hedlund et al., 2005). These findings suggest the involvement of 5-HT_7 receptors in depression.

Although Parkinson's disease (PD) is well characterized by motor symptoms including akinesia, rigidity and tremor, it is often accompanied by non-motor symptoms, such as depression, anxiety and dementia, and depression is the most common encountered non-motor symptom of PD (Zesiewicz and Hauser, 2002; Lohle et al., 2009). The classical hallmark of PD pathology is the degeneration of nigrostriatal dopamine (DA) neurons, however, the degenerative process is far more extensive and also affects non-DA neurotransmitter systems, which are involved in the non-motor symptoms in PD. Further, a dysfunctional 5-HT neurotransmitter system is generally regarded as a risk factor for depression, and a wide body of evidence suggests that degeneration of the nigrostriatal pathway causes an impairment of 5-HT neurotransmitter system, with evidence including the loss of 5-HT neurons in the raphe nuclei, reduction of brain 5-HT level, and alterations in the firing activity of 5-HT neurons and various types of 5-HT receptors (Scholtissen et al., 2006; Huot et al., 2011; Huot and Fox, 2013). In addition, recent studies from our laboratory have found that unilateral lesions of the medial forebrain bundle (MFB) or substantia nigra pars compacta (SNc) in rats induce depressive-like behaviors (Hui et al., 2014; Han et al., 2015; Liu et al., 2015), and activation of 5-HT_{1A} receptors in the PrL produces antidepressant-like effects as measured by the sucrose preference test and FST (Hui et al., 2014). From all the above mentioned, it seems that 5-HT_7 receptors in the PrL may be involved in the regulation of depressive-like behaviors, particularly in PD-associated depression.

Therefore, in the present study we examined (i) the effects of 5-HT_7 receptor agonist or antagonist injected into the PrL on depressive-like behaviors by commonly used paradigms the sucrose preference test and FST in sham-operated rats and rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the MFB, and (ii) changes in monoamine levels in the limbic and limbic-related brain regions after intra-PrL injection of the agonist or antagonist in the two groups of rats.

EXPERIMENTAL PROCEDURES

Animals and drugs

Male Sprague–Dawley rats weighing 270–320 g were used (Experimental Animal Center of Xi'an Jiaotong University, Xi'an, China). Animals were kept in a controlled environment (12-h light–dark cycle and 22 ± 2 °C room temperature) with food and water provided *ad libitum*. Animal care followed the National Institute of Health Guide for the Care and Use of Laboratory Animals, and was approved by the Animal Care Committee of the University. All efforts were made to minimize the number of animals used and their suffering.

Desipramine hydrochloride, 6-OHDA hydrochloride and apomorphine hydrochloride were purchased from Sigma–Aldrich (Sigma–Aldrich, St. Louis, MO, USA). AS19 [(2S)-(+)-5-(1,3,5-Trimethylpyrazol-4-yl)-2-(dimethylamino)tetralin, potent 5-HT_7 receptor agonist] and SB269970 [(2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride, potent and selective 5-HT_7 receptor antagonist] were obtained from Tocris Bioscience (Bristol, UK). 6-OHDA and apomorphine were prepared in 0.9% saline containing 0.02% ascorbic acid; AS19 and SB269970 were dissolved in dimethyl sulfoxide (DMSO). These drugs were prepared on the day of the experiment.

6-OHDA lesioning of rats

The unilateral 6-OHDA lesioning was carried out as previously described (Hui et al., 2014). Briefly, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.), pretreated with desipramine (25 mg/kg, i.p.), placed in a stereotaxic instrument (SN-2N, Narishige, Tokyo, Japan) and injected with 6-OHDA (12 μg/4 μl) into the right MFB. The stereotaxic coordinates of the injection site were as follows: AP −2.8 mm, ML 2.0 mm, and DV 8.1 mm relative to bregma (Paxinos and Watson, 1998). Sham-operated rats were subjected to the same protocol except that 0.9% saline containing 0.02% ascorbic acid was injected instead of 6-OHDA. One week after the surgery, the efficacy of the 6-OHDA lesioning was examined by the test of contralateral rotational behavior induced by apomorphine (0.05 mg/kg, s.c.). The rotation rate indicating a good lesion was >20 turns/5 min (Wang et al., 2009a). All rats used in this study turned consistently toward the side contralateral to the side of the lesion of >30 turns/5 min.

Guide cannula implantation and intra-PrL injections

Two weeks after injection of 0.9% saline containing 0.02% ascorbic acid or 6-OHDA into the right MFB, the rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and fixed in a stereotaxic instrument (SN-2N, Narishige). A stainless steel guide cannula was implanted stereotaxically 1 mm above the center of the right PrL. Coordinates for cannula implantation into the PrL were AP + 3.3 mm, ML 0.7 mm, and DV 2.0 mm relative to the bregma (Paxinos and Watson, 1998). The cannula was attached to the bones with stainless steel screws and dental acrylic cement. A stylet was inserted into the cannula to prevent obstruction. One week after the surgery, the rats were subjected to behavioral testing.

The intra-PrL injection was performed with a needle that was introduced through the guide cannula until its tip was 1.0 mm below the cannula end. The injection needle was attached by a polyethylene tube to a 1-μl microsyringe. The injection solutions were administered in a total volume of 0.5 μl. In all experiments, the drugs were administered over a period of 60 s, and the intracerebral needle remained in place for another 60 s before it was removed. Sham-operated and the lesioned rats were injected in the PrL with vehicle (DMSO), AS19, SB269970/AS19 or SB269970. The rats were