Neuropharmacology 137 (2018) 275–285

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Activation and blockade of basolateral amygdala 5-HT$_6$ receptor produce anxiolytic-like behaviors in an experimental model of Parkinson's disease

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ARTICLE INFO

Article history:
Received 27 November 2017
Received in revised form
16 April 2018
Accepted 11 May 2018

Keywords:
Basolateral amygdala
Serotonin$_6$ receptor
Anxiety
Glutamate neurons
γ-aminobutyric acid
Parkinson's disease

ABSTRACT

Although the basolateral amygdala (BLA) and serotonin$_6$ (5-HT$_6$) receptor are involved in modulation of anxiety, their roles in Parkinson' disease (PD)-related anxiety are still unknown. Thus we perform this study to examine the involvement of BLA 5-HT$_6$ receptor on anxiety in unilateral 6-hydroxydopamine-induced PD rats. The lesion of the medial forebrain bundle (MFB) induced anxiety-like behaviors, and decreased the basal firing rate of BLA glutamate neurons and dopamine (DA) levels in tissues of the medial prefrontal cortex (mPFC), amygdala and ventral part of hippocampus (vHip) in rats. Activation of BLA 5-HT$_6$ receptor by local infusion of WAY208466 induced anxiolytic-like effects and increased extracellular γ-aminobutyric acid (GABA) level in the BLA in the lesioned rats. Blockade of BLA 5-HT$_6$ receptor by SB258585 produced anxiolytic-like effects and increased extracellular GABA levels in the BLA in two groups of rats. Activation and blockade of BLA 5-HT$_6$ receptor resulted in increases in DA levels and decreases in noradrenaline levels in tissues of the mPFC, amygdala and vHip in two groups of rats, and induced opposite effects on the firing activity of glutamate neurons between sham-operated and the lesioned rats. The results suggest that decreased DA levels in the limbic brain regions and the enhanced sensitivity of the 5-HT$_6$ receptor on the BLA neurons might be etiological and pathophysiological factors for anxiety in PD. The anxiolytic-like effects may due to elevated extracellular GABA levels in the BLA and altered monoamine levels in the limbic regions, which were induced by WAY208466 and SB258585 through different mechanisms.

1. Introduction

Parkinson's disease (PD) is a neurological disorder characterized by motor disabilities, such as tremor, rigidity and bradykinesia. However, a series of non-motor symptoms including depression, anxiety, and cognitive decline can precede the classic motor defects, and have a significant impact on patients' quality of life. Anxiety is commonly concomitant with depression, but when the depression is treated, anxiety may remain (Marsh, 2000). Therefore, anxiety is known as an increased risk factor for PD patients (Prediger et al., 2012).

Several pieces of data indicate that degeneration of dopamine (DA) neurons of the substantia nigra pars compacta (SNc) underlies the motor symptoms, whereas degeneration of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) and noradrenaline (NA) neurons in the locus coeruleus (LC) associated with the non-motor symptoms (Delaville et al., 2012; Huot et al., 2011). Particularly, the 5-HT neurotransmitter system undergoes degeneration in PD, not only including loss of 5-HT neurons in the DRN, but also lower cortical 5-HT levels and alterations in various subtypes of 5-HT receptor (Huot et al., 2011).

The 5-HT$_6$ receptor is G-protein-coupled receptor, which is positively coupled to adenylyl cyclase and increases cAMP production upon activation (Monsma et al., 1993; Ruat et al., 1993). It is located almost exclusively within the central nervous system, with moderate to high levels in the striatum, nucleus accumbens, hippocampus, cortex, hypothalamus and amygdala (Monsma et al., 1993; Ruat et al., 1993; Ward et al., 1995). Previous studies illustrated that several antipsychotic agents and antidepressants had

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https://doi.org/10.1016/j.neuropharm.2018.05.016
0028-3908/

high affinity for the 5-HT_6 receptor and most of them were antagonists of 5-HT_6 receptor (Monsma et al., 1993; Roth et al., 1994). These findings indicate that the 5-HT_6 receptor might be involved in various neuropsychiatric disorders, such as anxiety, depression and schizophrenia (Hamon et al., 1999; Svenningsson et al., 2007). There are only a few studies that have explored the involvement of 5-HT_6 receptor in anxiety, and the reported findings are inconsistent. Intracerebroventricular injection of 5-HT_6 receptor antisense oligonucleotides suppressed the conditioned fear stress-induced 5-HT release in the rat prefrontal cortex, which may suggest an anxiolytic-like response (Yoshioka et al., 1998). Other studies indicated anxiogenic-like activity in elevated plus-maze (EPM) and social interaction tests in rats (Hamon et al., 1999; Otano et al., 1999). Furthermore, using selective ligands of the 5-HT_6 receptor, several studies have found that both 5-HT_6 receptor agonists and antagonists produce anxiolytic-like activities as measured by established behavioral tests in rodents (Carr et al., 2011; Nikiforuk et al., 2011; Wesolowska, 2007, 2008; Wesolowska and Nikiforuk, 2007). However, the mechanisms involved in the anxiolytic-like effects of 5-HT_6 receptor agonists and antagonists are still unclear.

The basolateral amygdala (BLA) has been identified to play a crucial role in the regulation of anxiety-like behavior (Hale et al., 2006). There are two major cell types in the BLA, which are classified as pyramidal-like glutamate projection neurons and non-pyramidal γ-aminobutyric acid (GABA) interneurons (Carlsen, 1988; McDonald, 1992). The interneurons display extensive axonal arborization with many terminals onto the soma of the projection neurons. Therefore, they can exert powerful regulation in the firing activity of the projection neurons (Carlsen, 1988). It is reported that the amygdala exhibits significant pathological changes in PD, including atrophy and Lewy body formation (Harding et al., 2002). Electrophysiological data from our previous study illustrated that GABA interneurons in the BLA showed a more burst-firing pattern after unilateral 6-OHDA lesioning of the SNc (Sun et al., 2013). It is suggested that the BLA might participate in the modulation of anxiety-related behaviors in PD. The BLA receives 5-HT innervation arising primarily from the DRN and the BLA neurons express a moderate level of 5-HT_6 receptor (Gerard et al., 1997; Ma et al., 1991; Ward et al., 1995). However, at present, there are no studies that have investigated the modulatory mechanism of the 5-HT_6 receptor in the BLA on PD-associated anxiety-like behaviors.

6-hydroxydopamine (6-OHDA) is a selective catecholaminergic neurotoxin mainly used to generate lesions in the nigrostriatal pathway in rats (Ungerstedt, 1968). The most common use of 6-OHDA is via unilateral injection into the rat medial forebrain bundle (MFB). DA depletion, nigral DA cell loss, and neurobehavioral deficits have been successfully achieved using this model. Therefore, this study was carried out on rats with complete unilateral 6-OHDA lesion of the MFB to investigate (i) effects of activation and blockade of BLA 5-HT_6 receptor on PD-associated anxiety-like behaviors, the firing activity of BLA putative glutamate neurons, extracellular GABA and glutamate levels in the BLA, and release of three monoamine levels in the limbic and limbic related brain regions, and (ii) changes in the expressions of 5-HT_6 receptor on glutamate and GABA neurons in the BLA after lesioning of the MFB.

2. Materials and methods

2.1. Animals and drugs

Adult male Sprague-Dawley rats (270–320 g) obtained from Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China) were used in this study. All experiments were performed in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011), and were approved by the Animal Care and Use Committee of Xi'an Jiaotong University. Rats were kept in a controlled environment (12 h light/dark cycle and 22 ± 2 °C temperature) with food and water *ad libitum*. All efforts were made to reduce the number of animals used and to minimize their suffering.

6-OHDA hydrochloride, apomorphine hydrochloride and desipramine hydrochloride were obtained from Sigma-Aldrich (Sigma-Aldrich, MO, USA). 3-[(-3-Fluorophenyl) sulfonyl]-*N*,*N*-dimethyl-1H-pyrrolo[2,3-*b*]pyridine-1-ethanamine dihydrochloride (WAY208466; high affinity and selective 5-HT_6 receptor agonist) and 4-Iodo-*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl] benzenesulfonamide hydrochloride (SB258585; potent and selective 5-HT_6 receptor antagonist) were purchased from Tocris (Bristol, UK). Apomorphine and 6-OHDA were dissolved in saline containing 0.02% ascorbic acid. Desipramine was dissolved in distilled water. WAY208466 and SB258585 were prepared in sterile saline. All drugs were prepared on the day of the experiment.

2.2. Unilateral 6-OHDA lesions in the MFB

Under chloral hydrate anesthesia (400 mg/kg, i.p.), rats were fixed in a stereotaxic frame (SN-2N, Narishige, Tokyo, Japan), and were received 25 mg/kg of desipramine (i.p.) to protect the NA neurons 30 min prior to the injection of 6-OHDA. Then, 6-OHDA (12 μg/4 μl) or vehicle (4 μl of saline containing 0.02% ascorbic acid) was injected into the right MFB (AP −2.8 mm, L 2.0 mm, D 8.1 mm relative to bregma; Paxinos and Watson, 1998). The solution was infused through a glass micropipette connected with a 10 μl microsyringe (air-tight Hamilton) at a rate of 0.5 μl/min. The apomorphine (0.05 mg/kg, s.c.) inducing rotation test was performed two weeks after the MFB lesions. Rats exhibiting more than 20 contralateral turns per 5 min were selected for the further investigation. All rats used in the present study turned consistently towards the side contralateral to the side of the lesion at > 38 turns per 5 min.

2.3. Guide cannula implantation and intra-BLA injections

Two weeks after the MFB lesions, rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and a 20 mm long stainless-steel guide cannula was implanted stereotaxically 2.0 mm above the right BLA (AP −2.5 mm, L 4.9 mm, D 7.0 mm relative to bregma; Paxinos and Watson, 1998). The cannula was stabilized to the skull with three stainless steel screws and dental cement, and then a steel stylet was inserted into the cannula to prevent obstruction. After surgery, animals were allowed a recovery period of two weeks before behavioral tests.

For drug injections, the needle that was used for injection was connected to a 1 μl syringe (7002H, Hamilton Co., Reno, NV, USA) through PE-10 tubing and was 2.0 mm longer than the guide cannula. Intra-BLA injections of vehicle (0.5 μl/rat)/vehicle (0.5 μl/rat), vehicle (0.5 μl/rat)/WAY208466 (1.5, 3 and 6 μg/rat), vehicle (0.5 μl/rat)/SB258585 (1, 2 and 4 μg/rat) or SB258585 (4 μg/rat)/WAY208466 (6 μg/rat) were performed on sham-operated and the 6-OHDA-lesioned rats. The solution volume (0.5 μl) was injected during the course of 60 s. The needle was left in position a further 60 s for drug diffusion before it was removed. The time between the two injections was 5 min, and the behavioral tests were performed 10 min after intra-BLA injection.

2.4. Behavioral tests

All behavioral tests were performed during the fifth week after