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Anti-Anxiety Effects of Prelimbic 5-HT$_4$ Receptors in the Rat Model of Parkinson's Disease: Evidence from Behavioral and Electrophysiological Study

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Abstract: *Objective:* Clinical and laboratory studies have demonstrated that prelimbic (PrL) and serotonin-4 (5-HT$_4$) receptors may have the key role in regulating anxiety. However, the pathophysiology of anxiety in Parkinson's disease (PD) remains obscure. In this research, the effects of PrL 5-HT$_4$ receptors on anti-anxiety behaviors in hemiparkinsonian rats were investigated. *Methods:* PD model rats were used as the research subjects, starting with behavioral changes, from the point of view of electrophysiology, the regulatory effect of PrL 5-HT$_4$ receptors on PD-related anxiety and the possible mechanism were explored. *Results:* Anxiety-like behaviors were induced via MFB lesion in rats. Intra-PrL injection of 5-HT$_4$ receptors agonist RS67333 induced anti-anxiety effects in both sham and PD group. In the sham group, PrL administration of 5-HT$_4$ receptors antagonist SB204070 produce anti-anxiety effects, but in the PD group, the expression of anxiety-like behavior was increased. Compared to the sham group, the effective dose of the behavioral effects of the two drugs in the PD group was obviously higher. Electrophysiological data suggested that PrL administration of RS67333 (SB204070) increased (decreased) the firing activities of γ-aminobutyric acid (GABA) neurons in both groups. Compared with rats in sham group, lesioned rats had a shorter duration of the excitation (inhibition) effects on firing activities of GABA neurons. *Conclusion:* PrL 5-HT$_4$ receptors regulate anxiety behaviors in PD rats, and its mechanism may be related to the down-regulation of expression or function of PrL 5-HT$_4$ receptors in PD.

Keywords: 5-HT$_4$ receptors; Anxiety; Parkinson's disease; Electrophysiology

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1. Introduction

In rodents, the medial prefrontal cortex (mPFC) is divided into dorsal and ventral areas based on its physiological characteristics and afferent and efferent projections. The ventral mPFC linked with diverse emotional procedure includes the infralimbic (IL) and prelimbic cortices (PrL). Previous data has provided sufficient evidences that regulatory functions of PrL are involved in anxiety behaviors [1].

The 5-HT$_4$ receptors, the latest 5-HT receptors identified, belonging to the G-protein-coupled receptor super family, are different from all other 5-HT receptors subtypes attributing to its unclear pharmacological characteristics [2]. The 5-HT$_4$ receptor is highly expressed in the limbic and limbic-related brain areas. The location of 5-HT$_4$ receptors in the corticolimbic area, the relatively potent affinity, and antagonistic activity of several antianxietics towards these receptors suggest that the receptors may have a significant function in anxiety [3]. It should be noted that since 5-HT$_4$ receptors have emerged as an interesting molecular target

for drug development as there is still an unresolved paradox that both agonists and antagonists should have complicated effects on anxiety. There has been no satisfactory elucidation of the putative mechanisms responsible for the anti-anxiety effects of 5-HT_4 receptors.

Parkinson's disease (PD) is a neuropsychiatric disease characterized by prominent symptoms including tremor, rigidity, and bradykinesia. However, non-motor symptoms such as depression, anxiety and cognitive deficits are also commonly observed. According to recent data, more disability was caused by non-motor symptoms than by motor complications [4].

The most common mental health problem related with PD is anxiety [5]. Typical motor symptoms are caused by degeneration of dopaminergic (DA) neurons in the substantia nigra. It is also important to note that degeneration can also be seen in non-DA neurotransmitter systems, especially impairment of 5-HT neurotransmitter systems, which is considered as an important factor in anxiety. The medial forebrain bundle (MFB) has been demonstrated to induce anxiety-like behaviors in rats when unilaterally lesioned [6]. Furthermore, another study also suggested that stimulation of 5-HT_4 receptors may have anti-anxiety properties [7]. Considering the findings of the aforementioned researches, we reasonably hypothesize that PrL 5-HT_4 receptors may contribute to the regulation of anxiety behaviors, especially in PD-related anxiety.

Based on the hypothesis, this study aims to determine: (1) whether unilateral 6-OHDA lesion of MFB in rats could induce anxiety behaviors; (2) effects of PrL administration via 5-HT_4 receptor agonist and antagonist on anxiety behaviors in sham and PD group; (3) the firing activity of PrL GABA neurons and their response to 5-HT_4 receptor activation or blockade.

2. Methods and materials

2.1. Treatments and drugs

In this study, male Sprague-Dawley rats of 270–320 g were obtained from Xi'an Jiaotong University's experimental animal center (Xi'an, China). All experimental operations were carried out in strict accordance with the recommendations of the "Guide for the Care and Use of Laboratory Animals" by the National Institutes of Health, and approved by the Animal Care Committee of the university. SD rats were divided into two groups: sham group and PD group. 6-hydroxydopamine (6-OHDA) hydrochloride and apomorphine hydrochloride were purchased from Sigma-Aldrich. RS67333 (5-HT_4 receptor agonist) and SB204070 (5-HT_4 receptor antagonist) were purchased from Tocris Boscience.

2.2. Preparation and verification of PD models

The rat model of PD was established by unilateral 6-OHDA (12 μg/4 μl) lesions of the MFB. The location of MFB on the right side (AP: −4.4 mm, ML: 1.2 mm, DV: 7.8 mm) was determined according to the stereotaxic map of rat brain [8]. The rats in the sham group were only injected with the same amount of saline containing 0.02% ascorbic acid. The rat PD model was detected by APO-induced rotation behavior experiment. One week after MFB damage, APO (0.05mg/kg) was subcutaneously injected into the neck of rats. After injection, the rotation behavior of rats was observed in 1-15min. If the rats rotated to the opposite side of the injured side, and the number of internal rotation of 5min was more than 20 times, the model was considered to be successful.

2.3. Guide cannula implantation and intra-PrL injections

The location of PrL on the right side (AP: +3.3 mm, ML: 0.7 mm, DV: 2.0 mm) was determined according to the stereotaxic map of rat brain. The stainless-steel bushing will be positioned at the 1 mm above the PrL [8]. The cannula was fixed with dental cement and was implanted to wait for rats to recover for 1 week after operation. Before each behavioral record, drug was injected into PrL through a guide cannula, and the injection volume was 0.5 μl. The specific operation procedure of drug administration was as follows: The

steel needle was removed before the behavioral record, and the drug was slowly injected with a 1 μl syringe into the PrL through the cannula for 60 seconds. After the injection, the needle was left until the drug was completely diffused. Rats in both groups were injected with saline, 5-HT_4 receptor agonist RS67333 (0.25,0.5,1 μg / 0.5 μ l) and antagonist RS67333 (0.02,0.04 and 0.08 μg / 0.5 μl).

2.4. Anxiety-like behavior tests

We conducted behavioral tests in a quiet environment within 4 weeks after injection of saline or 6-OHDA containing ascorbic acid, from 9 a.m. to 12:00 pm. Digital cameras were used to record the entire process.

Open field test [8]: Rats were placed in the central grid, and the residence time of rats in the central area of open field (40 × 40 cm) in 5 min was recorded to reflect anxiety-like behavior. The calculation formula was as follows: percentage of residence time in central area = residence time in central area (s) / 300 (s) × 100%. The decrease of this value indicated the occurrence of anxiety-like behavior.

2.5. In vivo electrophysiological recordings

An electrophysiological study was conducted four weeks after 6-OHDA lesions on PrL GABA neurons [9]. Rats under 4% chloral hydrate anesthesia were mounted in a stereotaxic frame. Glass microelectrodes prefilled with 1% Pontamine Sky Blue were stereotaxically positioned in the right prion ligament. Neuronal firings were amplified, bandpass-filtered and displayed with the Labchart 6.1.3 analysis system. Local injection of RS67333 (0.2μg/0.1μl) and RS67333 (0.02μg/0.1μl) into PrL was performed and the firing activities were observed for 30 min.

2.6. Statistics

Sigma Stat software was used for statistical analysis, and all the data were expressed by mean ± SEM. In terms of statistical significance, the difference was significant ($P < 0.05$). The behavioral experimental data were compared by independent sample *t*-test between the two groups. After drug injection, the behavioral changes were analyzed by one-way ANOVA. Dunnett's test was utilized for multiple comparisons. The independent Student's t-test was conducted to analyze basal firing rates for electrophysiological data between two groups of rats.

3. Results

3.1. Open field test (OFT)

There were significant differences between the PD and sham groups in terms of how much time they spent in the central area, suggesting that PD can induce anxiety behavior in rats. (Fig. 1).

In the sham group, intra-PrL of 5-HT_4 receptor agonist RS67333 and antagonist SB204070 increased the percentage of time spent in the central area, suggesting that anti-anxiety behavior was induced in rats. In PD group, injection of RS67333 into PrL resulted in a higher percentage of time spent, suggesting that PD rats had anti-anxiety effects, while injection of SB204070 into PrL decreased the percentage of time spent in central area, suggesting that expression of anxiety behaviors was increased in rats of PD group. Among the effective doses of the behavioral effects of the two drugs, doses in the PD group were obviously increased than those in the sham group (**Figure 1**).

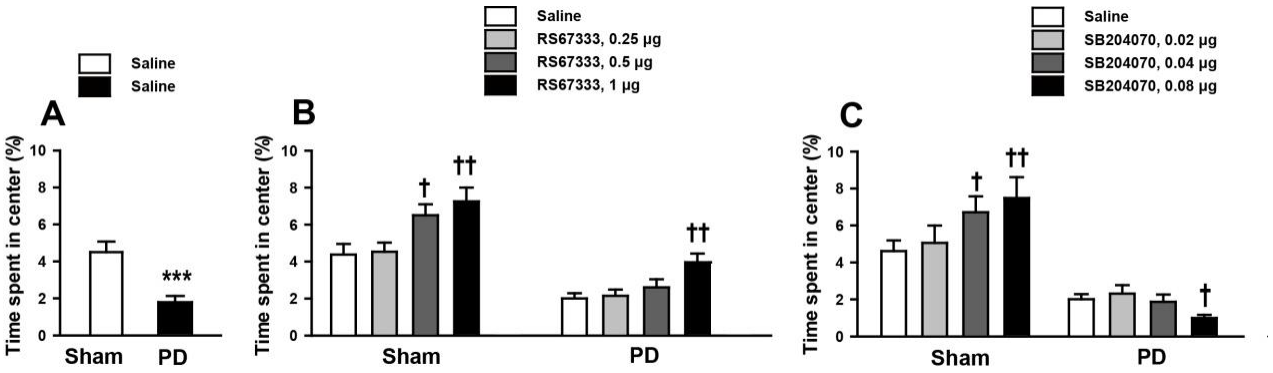

Figure 1. Effects of unilateral MFB lesions with 6-OHDA and intra-PrL administration of 5-HT_4 receptor agonist or antagonist on anxiety-like behaviors in rats. (*** P<0.001, compared with the sham group, independent sample t-test, The data were expressed as mean ±SEM. Compared with the same group of rats injected normal saline into PrL, † P<0.05, ††P<0.01, n= 6 / each group.)

3.2. Firing activity of GABA neurons

Injection of agonist RS67333 into PrL enhanced the firing rate of GABA neurons in the sham group significantly (**Figure 2A**), resulting in excitatory effects. And this excitatory effect appeared within 5min after administration, and sustained for 25 min (**Figure 2A**). In the PD group, the same dose of RS67333 enhanced the firing rate of GABA neurons (**Figure 2B**). The excitatory effect appeared within the 5min after administration, but only sustained for 15min which was significantly shorter than that of the sham group (**Figure 2B**). Injection of antagonist SB204070 into PrL showed an inhibitory effect. It enhanced the firing rate of GABA neurons in both groups significantly (**Figure 2C & D**). In the sham group, the inhibitory effect appeared within 5min and persisted for 20 min (**Figure 2C**). But in the PD group, the inhibitory effect also appeared within 5 min, but the duration was only for 10 min, which was significantly shorter than that in the sham group (**Fig. 2D**). The electrophysiological data showed that the inhibitory or excitatory duration of GABA interneurons expressing on PrL 5-HT_4 receptor of PD group was significantly shortened.

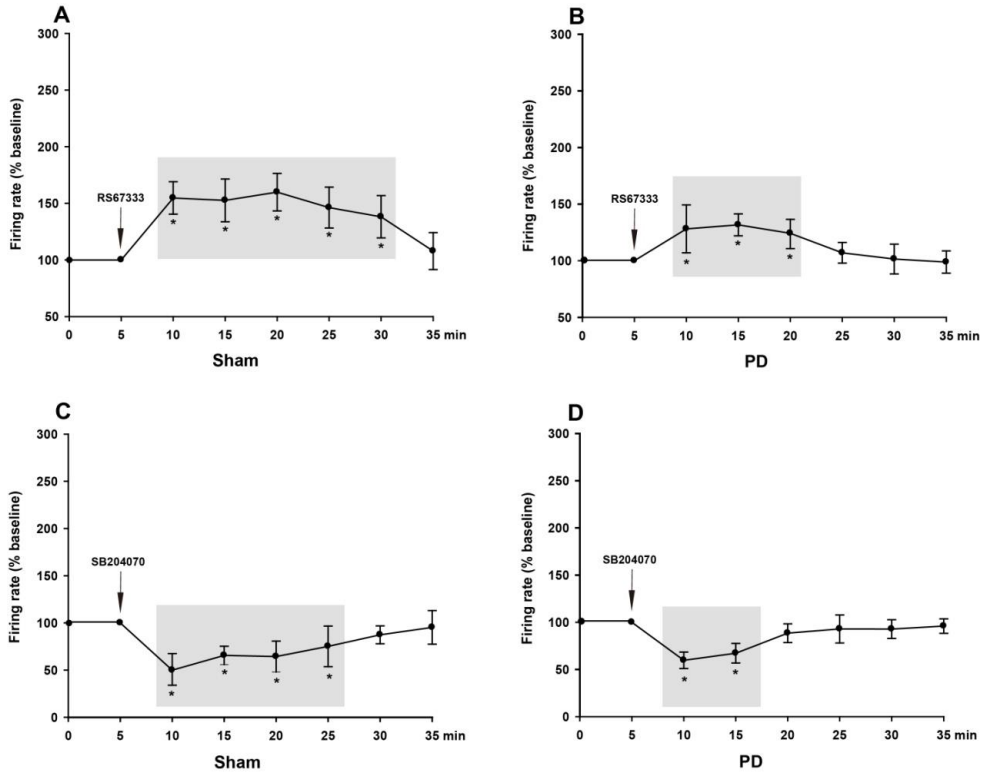

Figure 2. Effects of 5-HT_4 receptor agonist RS67333 and antagonist SB204070 on the firing activities of GABA neurons. Compared with the basic firing rate, * P <0.05, the data is expressed as mean ± SEM.

4. Discussion

Previous studies have confirmed that PrL and central 5-HT system contribute to the crucial role in anxiety-like behavior [10]. Furthermore, a large number of 5-HT nerve fibers in the raphe nucleus project to the PrL and innervate the neural activity in this brain area. There is a large amount of 5-HT_4 receptors expressed in PrL, and 5-HT_4 receptor has become a key target for the study of anxiety disorders. Recent data have suggested that subcutaneous injection with highly selective 5-HT_4 receptor antagonists SB204070 and SB207266 can improve the behavior of rats in elevated cross maze, suggesting that 5-HT_4 receptors play an important role in regulating anxiety behaviors. Recent results have demonstrated that local administration of mPFC to rats with highly selective 5-HT_4 receptor agonist RS67333 also produces anti-anxiety behavior. Recent studies have used 5-HT_4 receptor knockout mice to further verify the regulation of anxiety behavior by this receptor. These results suggested that 5-HT_4 receptor is very likely to be an important target for regulating anxiety behavior [11-13].

Recent studies have found that unilateral lesion of MFB via 6-OHDA can induce anxiety-like behavior in rats, and this behavior is closely associated with the PrL neurons' electrical activity [14]. It was found that in the state of PD, the firing rate of GABA neurons in PrL decreased, which led to the disinhibition of Glu neurons, which caused the overexcitation of the Glu neurons and the increase of the discharge frequency [15]. It is suggested that the occurrence of anxiety-like behavior in PD rats is closely related to the excessive firings of Glu neurons. 5-HT_4 receptor belongs to excitatory receptor, and its expression is abundant in PrL. From the cellular level, it can be found that a certain number of 5-HT_4 receptors can be expressed on Glu neurons and GABA neurons in PrL, especially on GABA neurons [16]. Therefore, the activation of 5-HT_4 receptor can increase the excitability of GABA neurons in PrL to some extent, thus regulate the electrical activity of Glu neurons through the local loop, reduce the excitability of Glu neurons in PD state, and thus reduce the expression of anxiety-like behavior. Blocking the receptor may have the opposite effect.

This study confirmed that intra-PrL administration of 5-HT_4 receptor agonist RS67333 could induce anti-anxiety-like behavior in rats in sham group and PD group. intra-PrL administration with 5-HT_4 receptor antagonist SB204070 to PrL produced anti-anxiety-like effects in sham-operated rats, but increased the expression of anxiety-like behavior in PD group. At the same time, it was also found that among the effective doses of the behavioral effects of the two drugs, significantly higher doses were given to the PD group than to the sham group. These results suggest that PrL 5-HT_4 receptors contribute an important role of the regulation in anxiety-like behavior in PD. However, the specific mechanism of the action of 5-HT_4 receptor agonists and antagonists in PD is not clear. From another point of view, the expression and / or function of 5-HT_4 receptor in PrL were down-regulated after MFB lesions. These changes can weaken the response of the neurons expressing the receptor to the stimulation of the receptor, and the change of the electrical activity of the neurons is more obvious after the administration of agonists or antagonists to the receptor. This result also supports the different behavioral effects produced by the administration of agonists or antagonists in the PrL under the condition of PD.

On the other hand, PrL has a direct fiber connection with the subcortical structures involved in anxiety (raphe nucleus and amygdala) and regulates the neural activity of these structures [17,18]. Studies have confirmed that the overactivity of 5-HT neurons derived from the raphe nucleus is closely related to the generation of anxiety-like behavior [19]. Previous studies also confirmed that the electrical activity of 5-HT neurons in the raphe nucleus increased in the state of PD [20]. Therefore, we theorize that after administration of 5-HT_4 receptor agonist, PrL stimulates GABA interneurons expressing 5-HT_4 receptor in PrL, and regulates the firing of Glu neurons through local loop, which affects the output of PrL to excitatory Glu of raphe nucleus, weakens the electrical activity of 5-HT neurons, and finally produces anti-anxiety effect in PD rats.

Therefore, a more reasonable explanation for the results of this experiment is that after MFB lesions, the expression or / and function of 5-HT_4 receptor in PrL was down-regulated, and the neurons expressing 5-HT_4 receptor were less responsive to receptor stimuli. After administration of receptor agonists or antagonists, the excitatory Glu output in PrL was changed, and the neural activity in limbic and limbic-related brain regions is also affected. Finally, the expression of anxiety-like or anti-anxiety-like behavior was regulated.

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Disclosure statement

The authors declare no conflict of interest.

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