Effects of substance P on neuronal firing of pallidal neurons in parkinsonian rats

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Abstract

Substance P is an important neurotransmitter or neuromodulator in central nervous system. Morphological studies have revealed the existence of substance P and its high affinity receptor, neurokinin-1 receptor, in globus pallidus. The expression of neurokinin-1 receptor in external globus pallidus has been reported to be decreased or unchanged in parkinsonian patients. To further investigate the effects of pallidal neurokinin-1 receptor in Parkinson’s disease, an in vivo extracellular recording in 6-hydroxydopamine parkinsonian rats was performed. Micro-pressure ejection of selective neurokinin-1 receptor agonist, [Sar9,Met(O2)11] substance P (0.1 mM), increased the spontaneous firing rate of pallidal neurons by 9.1% on the lesioned side, which was significantly weaker than that on the unlesioned side (20.7%), and that in normal rats (30.0%). The selective neurokinin-1 receptor antagonist, SR140333B, prevented the excitatory effects induced by [Sar9,Met(O2)11] substance P. Based on the action of substance P in globus pallidus of parkinsonian rats we hypothesize that the activity of neurokinin-1 receptors in globus pallidus may be decreased under parkinsonian state. This finding may provide a rationale for further investigations into the potential of pallidal substance P system in the treatment of Parkinson’s disease.

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1. Introduction

It is well known that Parkinson’s disease is closely related to the dysfunction of basal ganglia circuit. As a critical structure in the basal ganglia, the globus pallidus has been supposed to be involved in the manifestation of parkinsonian motor symptoms. In Parkinson’s disease and its animal models, depletion of dopamine in substantia nigra results in the abnormal hypoactivity of globus pallidus neurons, through the indirect pathway, leading to akinesia and hypokinetic symptoms of Parkinson’s disease (Albin et al., 1989; Chesselet and Delfs, 1996; Wichmann and DeLong, 1996). Furthermore, in the absence of normal dopaminergic innervation, increased synchronized oscillatory discharge presented in the globus pallidus may underlie resting tremor in parkinsonism (Nini et al., 1995; Bergman et al., 1998; Magnin et al., 2000; Raz et al., 2000). All these findings suggest that alteration of the firing characteristics of globus pallidus neurons constitutes the central origin of parkinsonian symptoms.

As a neuromodulator, neurotransmitter or neurotrophic-like factor (Diez-Guerra et al., 1988; Maggi et al., 1993; Otsuka and Yoshioka, 1993; Barker, 1996), substance P has been shown to be involved in the etiology of many central nervous system diseases, including Parkinson’s disease. For example, electrophysiological studies revealed that substance P excited nigral dopaminergic neurons (Walker et al., 1976; Nalivaiko et al., 1997). Substance P has also been revealed to promote dopamine release from striatal dopamine terminals (Humpel et al., 1991; Humpel and Saras, 1993), which may be involved in maintaining the integrity of neuronal populations (Barker, 1996). In addition, Barker (1986, 1991) reported that a loss of trophic peptidergic neurotransmitter, probably substance P, in the substantia nigra may lead to a secondary degeneration of dopaminergic neurons. Furthermore, studies on experimental parkinsonian syndrome suggested that intracaudate injection of substance P increased the motor activity and almost completely abolished the rigidity (Kryzhanovskii et al., 1989). Therefore, the involvement of substance P in Parkinson’s disease deserves more attention.
Earlier anatomical and morphological studies have revealed the existence of substance P system in globus pallidus (Mantyh et al., 1984; Chen et al., 2001; Mounir and Parent, 2002; Levesque et al., 2006). While in Parkinson’s disease, the expression of substance P and neuropeptide Y receptor displayed different alteration. Although some studies showed unchanged substance P in external globus pallidus of parkinsonian patients (Perez-Otano et al., 1992; de Ceballos et al., 1993), Mauborgne et al. (1983) reported the decreased expression of substance P in external globus pallidus of parkinsonian patients. However, a recent study showed that 6-hydroxydopamine (6-OHDA) lesion significantly enhanced substance P expression in globus pallidus (Martorana et al., 2003). On the other hand, the expression of neuropeptide Y receptor in external globus pallidus has been revealed to be decreased or unchanged in parkinsonian patients (Riou and Joyce, 1993; Fernandez et al., 1994). Our previous studies have indicated that substance P excited pallidal neurons via neuropeptide Y receptor in intact rats (Cuí et al., 2007). To further investigate the involvement of pallidial substance P system in Parkinson’s disease, in vivo extracellular recording was employed in the present study to observe the electrophysiological effects of substance P on pallidal neurons of 6-OHDA-lesioned rats.

2. Material and methods

2.1. Animals

Adult male Wistar rats (180–220 g) were used for this experiment. Animals were housed in a temperature controlled (22 ± 1 °C) room and maintained on a 12-h light/12-h dark cycle. Food and water were available at all times. Experiments performed in this study were carried out in strict accordance with the University guidelines on animal ethics. Care was taken to minimize pain or sufferings on the animals.

2.2. Unilateral lesions of the medial forebrain bundle

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame gently (Narishige SN-3, Tokyo, Japan). Then the scalp was aseptically prepared and reflected laterally. A stereotaxic frame gently (Narishige SN-3, Tokyo, Japan) was applied to the skull, and a microsyringe was lowered into the medial forebrain bundle. The coordinates of 1.2 mm posterior and 2.5–3.5 mm lateral from the bregma (Paxinos and Watson, 1998), a craniotomy was performed at coordinates of 0.8–1.2 mm posterior and 2.5–3.5 mm lateral from the bregma.

2.3. Rotational behavior tests

Two weeks after the 6-OHDA treatments, the rats were injected subcutaneously with 0.2 mg/kg apomorphine hydrochloride (A4393, Sigma) dissolved in 0.1% ascorbate saline solution. Animals accomplishing no less than 210 turns contralaterally in half an hour were included in this study.

2.4. Electrophysiological recordings

Rats were anesthetized with urethane (1 g/kg, i.p.; supplemented as needed) and positioned gently in the stereotaxic apparatus. A heating pad was used to maintain rectal temperature at 36–38 °C. According to the stereotaxic atlas (Paxinos and Watson, 1998), a craniotomy was performed at coordinates of 0.8–1.2 mm posterior and 2.5–3.5 mm lateral from the bregma.

Three-barrel microelectrodes were fastened at each end with metal tubing and prepared using a Stoeiing pipette puller (IL, USA). The tips were then broken back under a microscope to 3–10 μm and had a resistance of 10–20 MΩ. Afterwards, the electrodes were stereotaxically positioned into the globus pallidus. The recording barrel of the electrode was filled with 0.5 M sodium acetate containing 2% pontamine sky blue dye. The other two micro-pressure ejection barrels connected to four-channel pressure injector (PM2000B, Micro Data Instrument, Inc., USA) respectively contained either: [Sar9,Met(O2)11] substance P (SMSP) (0.1 mM) and vehicle, or SR140333B (0.5 mM) and SR140333B (0.5 mM) with SMSP (0.1 mM). The spontaneous firing neurons were identified as pallidal neurons according to the location and electrophysiological features. Drugs were ejected onto the surface of neurons with short pulse gas pressure (1500 ms, 5.0–15.0 psi).

The recorded electrical signals were amplified by a micro-electrode amplifier (MEZ-8201, Nihon Kohden, Tokyo, Japan) and displayed on a memory oscilloscope (VC-11, Nihon Kohden), while being fed to an audiomonitor. The amplified electrical signals were passed through low and high pass filters to a bioelectricity signal analyzer and computer. Spike times were preprocessed online and further analyzed offline using the program of Histogram ver 1.00 (Shanghai Medical University, Shanghai, China) for spike data analysis. Drug infusion was performed only once for each recording and a period of 30 min at least was allowed to pass before another recording in the same track.

At least 5 min stable basal firing was collected from each neuron before drug ejection into the globus pallidus. The frequency of basal firing was determined by the average frequency of 120 s baseline data before drug administration. The maximal change of frequency within 50 s following drug application was considered as drug effect.

2.5. Drugs and statistics

SMSP was obtained from Tocris (Avonmouth, UK). SR140333B was kindly provided by Sanofi-Aventis-Chilly-Mazarin. 6-OHDA hydrochloride and apomorphine hydrochloride were purchased from Sigma (St. Louis, MO, USA).

The data are expressed as mean ± S.E.M. Paired t test was used to compare the difference of firing rate before and after drug treatment. Statistical comparisons between or among groups were determined with Student’s t test and one-way ANOVA. The level of significance was preset by using a P value of 0.05.

3. Results

3.1. Effects of SMSP on firing rate of bilateral globus pallidus neurons in 6-OHDA-lesioned rats

The neurons recorded in the present experiment are type II globus pallidus neurons, with the characteristic of biphasic positive/negative waveforms (Kelland et al., 1995; Ruskin et al., 1998). Local administration of SMSP was performed in 6-OHDA-lesioned rats. As shown in Fig. 1, on the lesioned side, micro-pressure ejection of 0.1 mM SMSP increased the spontaneous firing rate of pallidal neurons from 19.2 ± 1.7 Hz to 21.0 ± 1.8 Hz (n = 36, P < 0.001). The average increase was 9.1 ± 2.0%, which was significantly different compared to vehicle injection (basal: 19.6 ± 4.1 Hz; vehicle: 20.1 ± 4.3 Hz; increase: 1.6 ± 1.5%, n = 10). More than 20% increase in firing rate was observed in 5 out of the 36 neurons receiving SMSP administration.

With respect to the unlesioned side, as shown in Fig. 2, SMSP (0.1 mM) increased the firing rate of pallidal neurons from 24.4 ± 1.9 Hz to 28.7 ± 2.2 Hz (n = 38, P < 0.001). The average increase was 20.7 ± 3.3%, which was significantly different from that of vehicle injection (basal: 25.0 ± 6.1 Hz; vehicle: 25.6 ± 6.2 Hz; increase: 1.7 ± 1.3%, n = 10). Sixteen
out of 38 neurons receiving SMSP administration displayed at least 20% increase in firing rate. As summarized in Fig. 3, SMSP-induced increase in firing rate on lesioned side (9.1 ± 2.0%) was significantly weaker than that on the unlesioned side (20.7 ± 3.3%, P < 0.01) and that obtained in intact rats (basal: 18.2 ± 2.2 Hz; SMSP: 22.2 ± 2.5 Hz; increase: 30.0 ± 5.6%, n = 26, P < 0.01). Besides, there is no significant difference between the SMSP-induced increase of firing rate on unlesioned side of 6-OHDA rats and that in intact rats (P > 0.05).

3.2. Neurokinin-1 receptor is involved in SMSP-induced excitation

To identify the subtype of neurokinin receptors that mediated the excitatory response, the effect of SR140333B, a selective neurokinin-1 receptor antagonist, was investigated in 6-OHDA parkinsonian rat. As shown in Fig. 4, on the lesioned side, application of SR140333B (0.5 mM) alone inhibited the firing rate of globus pallidus neurons (basal: 17.3 ± 5.0 Hz; SR140333B: 15.2 ± 5.1 Hz, n = 11, P < 0.05). The average decrease was 19.3 ± 5.8% (P < 0.01 compared to vehicle). Co-ejection of SR140333B and SMSP could block SMSP-induced excitatory effects. The average change was 3.9 ± 3.1% with regard to basal firing (n = 10, P < 0.05 compared to SMSP alone on lesioned side). As regard to the unlesioned side, application of SR140333B (0.5 mM) alone inhibited the firing rate from 18.8 ± 2.4 Hz to 17.4 ± 2.4 Hz in 11 neurons (P < 0.001). The average decrease was 8.6 ± 2.3% (P < 0.01 compared to vehicle; P > 0.05 compared to that on the lesioned side). Co-ejection of SR140333B and SMSP could block SMSP-induced excitation. The average change was
6.1 ± 4.8% with regard to basal firing (n = 10, P < 0.05 compared to SMSP alone on unlesioned side). Fig. 5 summarized the effects on unlesioned side.

4. Discussion

The present works showed that the increase in firing rate induced by substance P in globus pallidus is weaker in 6-OHDA treated rats, revealed a decreased expression of functional neurokinin-1 receptors in globus pallidus under parkinsonian state. Our finding is supported by some morphological evidences. For example, a significant reduction of substance P receptor was observed in external globus pallidus of patients with Parkinson’s disease (Fernandez et al., 1994). However, by using autoradiographic techniques, Rioux and Joyce (1993) reported no significant change of pallidal substance P receptor in parkinsonian patients. This difference may result from the distinct radiation labels as well as experimental conditions.

Fig. 2. Effects of SMSP on the spontaneous firing of globus pallidus neurons on unlesioned side. (A) Frequency histogram illustrating that 0.1 mM SMSP increased the firing rate of globus pallidus neuron by 24.4% on unlesioned side. Lower part showing the firings at different stages of the experiment. (B) Pooled data summarizing the effects of SMSP and vehicle on the firing rate of globus pallidus neurons on unlesioned side. ***P < 0.001; ns: not significant.

Fig. 3. Comparison of the increase in firing rate induced by intrapallidal microinjection of SMSP between 6-OHDA-lesioned rats and normal rats. **P < 0.01; ns: not significant.
Earlier studies by Mantyh and Hunt (1986) proved that striatum lesioning decreased 74% of substance P receptors in globus pallidus. Morphological evidences have revealed that 6-OHDA lesion of substantia nigra pars compacta or medial forebrain bundle significantly decreased the density of dendritic spines on the medium spiny striatum neurons (Bugiani et al., 1980; McNeill et al., 1988; Ingham et al., 1993; Solis et al., 2007). Therefore, decreased neurokinin-1 receptors in globus pallidus may be a consequence of denervation due to the loss of intrinsic striatal neurons or atrophy of striatal dendrites under parkinsonian state.

Another finding of the present experiments is that the selective neurokinin-1 receptor antagonist, SR140333B, blocked SMSP-induced excitation on both sides, confirming the involvement of neurokinin-1 receptors. In addition, in the same parkinsonian rat model, we observed that SR140333B significantly decreased the firing rate of pallidal neurons. Combined with previous studies that globus pallidus receives substance P-ergic projection from the striatum (Mounir and Parent, 2002; Levesque et al., 2003), we hypothesize that endogenous substance P system may play a role in modulating electrical activity of pallidal neurons in Parkinson’s disease.

The decreased morphological and functional expression of neurokinin-1 receptors in globus pallidus observed in previous and present studies is considered to be closely related to the etiopathogenesis of Parkinson’s disease. It has been confirmed that substance P exerts excitatory effects in the basal ganglia (Le Gal La Salle and Ben-Ari, 1977; Overton et al., 1992; Minabe et al., 1996; Nalivaiko et al., 1997), including the globus pallidus (Cui et al., 2007). Since the globus pallidus receives endogenous substance P innervation from the striatum, the loss of neurokinin-1 receptors would make this excitatory
effect weaker, which is in accordance with our findings. It is well known that the hypoactivity of pallidal neurons is in positive correlation with motor symptoms presented in Parkinson’s disease (Chesselet and Delfs, 1996; Wichmann and DeLong, 1996). Therefore, the decrease of neurokinin-1 receptors may be involved in the manifestation of parkinsonian symptoms.

In fact, substance P system has been revealed to be involved in Parkinson’s disease through multiple ways. Morphologically, abundant substance P and neurokinin-1 receptor exist in many nuclei of basal ganglia (Mantyh et al., 1984; Gallagher et al., 1992; Sutoo et al., 1999; Saffroy et al., 2003; Levesque et al., 2006). Under parkinsonian state, substance P and neurokinin-1 receptor displayed altered expression in these nuclei (Perez-Otano et al., 1992; de Ceballos et al., 1993; Rioux and Joyce, 1993; Fernandez et al., 1994; Martorana et al., 2003). Therefore, substance P may affect the function of several nuclei and then co-participate in pathophysiology of Parkinson’s disease. Functionally, by using in vitro electrophysiological recording, Aosaki and Kawaguchi (1996) reported that substance P could evoke depolarization on neostriatal cholinergic neurons and somatostatin/nitric oxide synthase-positive neurons in a dose-dependent manner. Besides, substance P could excite GABAergic and dopaminergic neurons in the guinea-pig substantia nigra (Nalivaiko et al., 1997). Combined with our previous findings that substance P evoked excitatory effects on GABAergic neurons in rat globus pallidus, we hypothesize that the role of substance P in Parkinson’s disease may be an integration of its effects on multiple neurons in basal ganglia.

Previous studies have indicated that intranigral injection of substance P or selective neurokinin-1 receptor agonist

Fig. 5. Effects of SR140333B on SMSP-induced increase in firing rate of globus pallidus neurons on unlesioned side. (A) SR140333B alone decrease the spontaneous firing rate by 8.8% in this cell. However, the presence of SR140333B depressed the increase in firing rate induced by SMSP to 4.1%. (B) Pooled data summarizing the effects of SMSP alone and SMSP with SR140333B on the firing rate of globus pallidus neurons on unlesioned side. *P < 0.05.
increased striatal dopamine metabolism (Humpel et al., 1991; Humpel and Saria, 1993). Glowinski et al. (1993) also reported that the selective neurokinin-1 receptor agonist presynaptically modulates dopamine release in striatum. In 6-OHDA-lesioned rats, intracerebroventricular administration of substance P increased the content of dopamine and its metabolites in the striatum (Krasnova et al., 2000). Since Parkinson’s disease is characterized by impaired dopamine transmission, substance P is therefore potentially useful in treating this basal ganglia motor disorder. However, in terms of its complicated effects in basal ganglia, more information and experiments are required to fully understand the functions of substance P and corresponding mechanisms.

In conclusion, our results indicated that the activity of neurokinin-1 receptors decreased in globus pallidus under parkinsonian state, which implies an involvement of substance P system in the etiology and treatment of Parkinson’s disease.

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