Electrophysiological Effects of Neurotensin on Globus Pallidus Neurons of 6-Hydroxydopamine-Lesioned Rats

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Key Words

Globus pallidus • Neurotensin • 6-Hydroxydopamine • Parkinson's disease • Single unit recording

Abstract

The globus pallidus is a nucleus in the indirect pathway of the basal ganglia circuits. Neurotensin has been reported to play an important role in the central nervous system. Functional study revealed that systemic administration of neurotensin produced antiparkinsonian effects. The aim of the present study was to investigate the effects of neurotensin on the firing rate of globus pallidus neurons in 6-hydroxydopamine-lesioned parkinsonian rats. Micropressure ejection of neurotensin increased the spontaneous firing rate of globus pallidus neurons on both lesioned and unlesioned sides. Furthermore, the neurotensin-induced increase in firing rate on the unlesioned side (95.9%) was stronger than that on the lesioned side (37.3%). The neurotensin receptor antagonist, SR48692, prevented neurotensin-induced increase in firing rate. Based on the excitatory effects of neurotensin in globus pallidus of parkinsonian rats, we hypothesize that the pallidal neurotensinergic system may be involved in its possible therapy in Parkinson's disease.

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Introduction

Parkinson's disease is a neurodegenerative movement disorder characterized by the progressive loss of dopaminergic neurons in substantia nigra pars compacta [1]. The globus pallidus represents a key structure in the basal ganglia. There is evidence supporting the involvement of the globus pallidus in both normal motor function and movement disorders including Parkinson's disease. For example, previous electrophysiological studies have shown that the firing rate of the globus pallidus neurons decreased in parkinsonian patients and non-human primates (macaque) [2, 3]. Consistently, the average neuronal discharge rate decreased in globus pallidus of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys. In addition to the change of firing rate, MPTP treatment tended to induce pallidal neurons to discharge in oscillatory bursts [4]. The conventional anatomical models [5] have been applied to explain the abnormal hypoactivity of globus pallidus neurons, through the indirect pathway, leading to akinesia and hypokinetic symptoms of Parkinson's disease [6, 7]. Furthermore, the oscillatory bursts firing induced by the absence of normal dopaminergic innervation is related to the symptoms of parkinsonian tremor [8–11].

Neurotensin, first isolated by Carraway and Leeman [12], is an endogenous tridecapeptide that behaves as a neurotransmitter or neuromodulator in the central ner-

vous system [13]. Three neurotensin receptors have been identified, neurotensin type-1, type-2 and type-3 receptor (NTS3/sortilin) [14–16]. Morphological studies have revealed that the globus pallidus expresses both neurotensin type-1 and type-2 receptors [17, 18]. Recently, Martorana et al. [19] reported that neurotensin type-1 receptor is localized in both parvalbumin-positive and -negative globus pallidus neurons. The globus pallidus receives neurotensinergic innervation arising from the striatum [20, 21]. Previous studies have indicated that the expression of neurotensin and neurotensin receptor in globus pallidus changed in Parkinson's disease. In 6-hydroxydopamine (6-OHDA)-treated rats and parkinsonian patients, an increase in neurotensin-immunoreactivity has been found in globus pallidus [22, 23]. However, the expression of neurotensin receptors in globus pallidus decreased markedly in parkinsonian patients [24, 25]. Recently, a functional study revealed that systemic administration of a neurotensin analog, which can cross the blood-brain barrier, produced antiparkinsonian-like effects in 6-OHDA-treated rats [26]. Our previous studies have described the electrophysiological effects of neurotensin on globus pallidus neurons of normal rat [27, 28]. In the present study, single-unit recordings were used to observe the effects of neurotensin on neuronal activity of globus pallidus neurons in 6-OHDA-lesioned parkinsonian rats.

Materials and Methods

Animals

Experiments were performed according to the university guidelines on animal ethics. All efforts were made to minimize animal suffering and to reduce the number of animals used. Adult male Wistar rats (200–250 g) were individually housed with free access to food and water in a light- (12:12 h light/dark cycle) and temperature- ($22 \pm 1^\circ\text{C}$) controlled room.

6-OHDA Lesion

Rats were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and gently placed in a stereotaxic frame (Narishige SN-3, Tokyo, Japan). The skull was then exposed and a burr hole was drilled. A cranial burr hole (1 mm) was drilled into the skull over the injection site, and a microsyringe was lowered into the medial forebrain bundle: 3.2 mm posterior, 1.5 mm lateral to the bregma, 8.4 mm ventral to the skull surface [29]. A total dose of 14.5 μg 6-OHDA hydrochloride (H4381; Sigma, St. Louis, Mo., USA) in 4 μl sterile saline containing 0.01% ascorbic acid was then injected into the right medial forebrain bundle at a rate of 1.0 $\mu\text{l}/\text{min}$. The microsyringe was allowed to rest for 10 min to prevent backflow of the toxin. Rats were pretreated 30 min before the 6-OHDA infusion with 25 mg/kg desipramine to protect noradrenergic projections.

Rotational Behavior Tests

Behavioral testing was performed 14 days after the injection of 6-OHDA treatments, the rats were injected subcutaneously with 0.2 mg/kg apomorphine hydrochloride (A4393; Sigma) dissolved in 0.1% ascorbate saline solution. The numbers of contralateral turns were counted from the videotape. The lesion was considered successful in those animals that made at least 210 net contralateral rotations in 30 min.

Extracellular Single Unit Recordings

On the day of electrophysiological recordings, rats were deeply anesthetized with urethane (1 g/kg, i.p.) and placed in a stereotaxic apparatus (Narishige SN-3). An incision was made in the scalp, the skull exposed, and a burr hole drilled in the skull. Rectal temperature was maintained at $36\text{--}38^\circ\text{C}$ by a heated pad.

Three-barrel microelectrodes were fastened at each end with metal tubing and prepared using a Stoelting pipette puller (Stoelting Co., Wood Dale, Ill., USA). The tips of microelectrodes were broken to 3–10 μm under a microscope. The resistance of microelectrodes ranged from 10–20 M Ω . The recording electrode was filled with 0.5 M sodium acetate containing 2% pontamine sky blue dye. The other two micropressure ejection barrels connected to a 4-channel pressure ejector (PM2000B; Micro Data Instrument, South Plainfield, N.J., USA). The two micropressure ejection barrels respectively contained neurotensin and vehicle (normal saline), SR48692 and vehicle (DMSO), SR48692 and SR48692 with neurotensin or neurotensin and neurotensin with SR48692. The electrode was then placed into the globus pallidus with coordinates of 0.8–1.2 mm posterior, 2.5–3.5 mm lateral from the bregma, 5.5–7.2 mm ventral from the dura [29]. Neurons were identified as pallidal on the basis of their location and electrophysiological features. Drugs were ejected onto the surface of firing cells with short pulse gas pressure (1,500 ms, 5.0–15.0 psi).

The recorded electrical signals were amplified by a microelectrode 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 into a bioelectricity signal analyzer and computer. Spike times were preprocessed online and further analyzed offline using the program of Histogram Version 1.00 (Shanghai Medical University, Shanghai, China) for spike data analysis. The firing rates were recorded in 1-s bins. 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 onto 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. A change of at least 20% of basal firing rate during drug application was considered significant [30]. Drug application was performed only once for each recording, and a period of 30 min was allowed to pass before another recording in the same track.

Histology and Immunohistochemistry

To verify the position of single unit recording, pontamine sky blue was ejected from the recording electrode tip by iontophoresis (10 μA , 20 min). The rats were then sacrificed by chloral hydrate (600 mg/kg, i.p.) and perfused with 4% paraformaldehyde solu-

Fig. 1. Immunostaining of tyrosine hydroxylase in rat substantia nigra pars compacta in 6-OHDA-lesioned parkinsonian rats. **a** Low-magnification photomicrograph showing the immunostaining of tyrosine hydroxylase in substantia nigra pars compacta. **b, c** High-magnification photomicrograph of tyrosine hydroxylase in substantia nigra pars compacta of lesioned (**b**) and unlesioned (**c**) sides. Scale bars: (**a**) 0.5 mm, (**b, c**) 50 μ m.

tion transcidentally. The brains were dissected out and incubated in paraformaldehyde overnight. After that, the brains were frozen and sectioned to identify recording and microinjection sites under light microscope.

To determine the extent of nigral dopaminergic degeneration, rats receiving unilateral injection of 6-OHDA were examined for immunohistochemical staining of tyrosine hydroxylase. After perfusion, the brain containing the part of substantia nigra was removed from the cranium and fixed overnight. Tissues were fixed in 4% paraformaldehyde for 24 h and were dehydrated in a graded series of ethanol concentrations before paraffin embedding. Coronal sections (5 μ m) were made using a microtome and mounted on slides coated with 3-aminopropyltriethoxysilane and dried at 37°C for 24 h. The sections were deparaffinized and rehydrated. Endogenous peroxidase was quenched with 0.3% H_2O_2 (30 min). After washing in distilled water (3×5 min), the sections were incubated with 5% normal goat serum for 1 h. This was followed by application of monoclonal anti-tyrosine hydroxylase antibody (1:5,000; Sigma) incubated overnight at 4°C. After washing in PBS (3×5 min), the sections were incubated with the biotinylated secondary antibody (1:100) for 60 min, followed by washes with PBS (3×5 min). The immunoreactive sites were revealed by incubation in 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB; Sigma) in 0.1 M Tris buffer. The sections were then washed with tap water, dehydrated, cleared with xylene and coverslipped using mountant. Quantification of cells under the microscope was done. The loss of tyrosine hydroxylase-positive neurons on the lesioned side was more than 90% in the present study (fig. 1).

Drugs and Statistics

Neurotensin was obtained from Sigma. SR48692{2-[(1-(7-chloro-4-quinolinyl)-5-2(2,6-dimethoxyphenyl)pyrazol-3-

yl)carbonylamino]-tricyclo(3.3.1.1.3.7)-decan-2-carboxylic acid} was kindly provided by Dr. Danielle Gully (Sanofi Recherche, Toulouse, France). 6-OHDA hydrochloride, apomorphine hydrochloride and monoclonal anti-tyrosine hydroxylase antibody were obtained from Sigma.

The data are expressed as means $\pm$ SDs. Paired t test was used to compare the difference of firing rate before and after 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.

Results

All the neurons recorded in the present study showed a biphasic positive/negative waveform, which are characterized as type II globus pallidus neurons [31, 32]. In normal rats, the basal spontaneous firing rate of globus pallidus neurons ranged from 3 to 40 Hz (13.6 ± 7.2 Hz, $n = 21$). Micropressure ejection of 0.1 mM neurotensin significantly increased the spontaneous firing rate of pallidal neurons in normal rats. In 11 out of 21 pallidal neurons, 0.1 mM neurotensin increased the firing rate from 14.5 ± 4.7 to 21.3 ± 7.2 Hz ($n = 11$, $p < 0.001$, fig. 2a). The average increase was $48.3 \pm 20.7\%$, which was significantly different compared to that of vehicle (normal saline) injection (basal: 14.8 ± 4.4 Hz; vehicle: 15.1 ± 4.2 Hz; increase: $2.7 \pm 4.4\%$, $n = 11$, $p < 0.01$ compared to neurotensin).

Fig. 2. Effects of intrapallidal micropressure ejection of neurotensin on the spontaneous firing rate of globus pallidus neurons. Typical frequency histograms showing that neurotensin (0.1 mM) increased the firing rate of pallidal neurons in normal rats (**a**), lesioned side (**b**) and unlesioned side (**c**) of 6-OHDA parkinsonian rats. NS = Normal saline.

Effects of Neurotensin on Spontaneous Firing of Globus Pallidus in 6-OHDA-Lesioned Rats

To clarify the effects of neurotensin on globus pallidus neurons of 6-OHDA-lesioned parkinsonian rats, we monitored the spontaneous activity of 59 pallidal neurons sampled from 15 parkinsonian rats. On the lesioned side, pallidal neurons discharged with a mean firing rate of 14.4 ± 7.0 Hz ($n = 30$), which was not significantly different from that of normal rats (13.6 ± 7.2 Hz, $n = 21$, $p > 0.05$). In 13 out of 30 pallidal neurons, 0.1 mM neurotensin increased the frequency of spontaneous firing from 11.1 ± 6.7 to 15.1 ± 8.7 Hz ($p < 0.001$, fig. 2b). The average increase was $37.3 \pm 12.6\%$, which was significantly different from that of vehicle (normal saline) injection

Fig. 3. Comparison of neurotensin-induced increase in firing rate between 6-OHDA-lesioned rats and normal rats. * $p < 0.05$, ** $p < 0.01$, ns = not significant.

(basal: 11.1 ± 6.6 Hz; vehicle: 11.5 ± 7.0 Hz; increase: $3.9 \pm 8.1\%$, $n = 9$, $p < 0.01$ compared to neurotensin). There was no significant difference in neurotensin-induced excitation of pallidal neurons between the lesioned side ($37.3 \pm 12.6\%$, $n = 13$) of parkinsonian rats and that of normal rats ($48.3 \pm 20.7\%$, $n = 11$, $p > 0.05$). In another 17 neurons, local administration of neurotensin had no significant effect on the firing rate of pallidal neurons.

On the unlesioned side, pallidal neurons discharged with a mean firing rate of 13.5 ± 13.2 Hz ($n = 29$), which was not different from that of normal rats (13.6 ± 7.2 Hz, $n = 21$, $p > 0.05$). In 17 out of 29 pallidal neurons, 0.1 mM neurotensin increased the spontaneous firing from 11.3 ± 9.4 to 20.2 ± 14.7 Hz ($p < 0.001$, fig. 2c). The average increase was $95.9 \pm 65.9\%$, which was significantly different compared to vehicle (normal saline) injection (basal: 10.6 ± 8.5 Hz; vehicle: 10.9 ± 8.2 Hz, increase: $6.5 \pm 9.2\%$, $n = 10$, $p < 0.001$ compared to neurotensin). The increase in firing rate on the unlesioned side ($95.9 \pm 65.9\%$, $n = 17$) was much stronger than that on the lesioned side ($37.3 \pm 12.6\%$, $n = 13$, $p < 0.01$), as well as that in normal rats ($48.3 \pm 20.7\%$, $n = 11$, $p < 0.05$, fig. 3). There was no correlation found between the rotational behavior in each rat, or the size of the lesion, and the effect of neurotensin on the unlesioned side of the brain.

Consistent with the previous morphological study that neurotensin receptor was expressed in about 56% of

Fig. 4. Maps of recorded pallidal neurons with or without response to neurotensin. **a** Maps of recorded neurons in normal rats with (black square, $n = 11$) or without (white circle, $n = 10$) response to neurotensin. **b** Maps of recorded neurons in 6-OHDA-lesioned rats with (black square, $n = 30$) or without response to neurotensin (white circle, $n = 29$).

globus pallidus neurons [19], the present data showed that neurotensin only increased the firing rate of a proportion of pallidal neurons. Figure 4 showed the maps of recorded neurons with or without response to neurotensin in globus pallidus.

Effects of SR48692 on Neurotensin-Induced Excitation

The neurotensin receptor antagonist SR48692, which has a higher affinity for neurotensin type-1 receptor compared to neurotensin type-2 receptor, was used in the present study. On the lesioned side, in 19 pallidal neurons, 1 mM SR48692 alone had no effect on firing rate (basal: 13.2 ± 9.6 Hz; SR48692: 13.7 ± 9.2 Hz; increase: $6.1 \pm 12.2\%$, $n = 19$, $p > 0.05$). In 12 neurons that then received SR48692 with neurotensin, the firing rate did not change significantly (basal: 13.1 ± 10.5 Hz; neurotensin+SR48692: 14.3 ± 12.0 Hz, $p > 0.05$, fig. 5a). The average change of firing rate was $6.6 \pm 9.7\%$, which was significantly decreased compared to that of neurotensin alone ($p < 0.001$). In a separate set of rats, administration of vehicle (DMSO) did not alter the spontaneous firing rate of pallidal neurons (basal: 16.6 ± 8.9 Hz; vehicle: 17.9 ± 10.3 Hz; increase: $6.2 \pm 6.7\%$, $n = 8$, $p > 0.05$). On the unlesioned side, however, the effects of SR48692 were different from those on the lesioned side. In 5 out of 19 pallidal neurons, SR48692 decreased the spontaneous firing rate from 13.6 ± 8.9 to 7.2 ± 7.6 Hz. The average decrease was $48.3 \pm 26.8\%$. In 10 neurons, co-ejection of SR48692 with neurotensin did not alter the firing rate of pallidal neurons (basal: 13.7 ± 8.6 Hz; neurotensin+SR48692: 14.5 ± 9.3 Hz, $p > 0.05$, fig. 6a). The average change was $5.1 \pm 8.5\%$, which was significantly decreased compared to that of neurotensin alone ($p < 0.001$). In the control group, administration of vehicle (DMSO) did not alter the spontaneous firing rate of pallidal neurons (basal: 16.8 ± 11.9 Hz; vehicle: 17.6 ± 12.7 Hz; increase: $4.6 \pm 5.1\%$, $n = 6$, $p > 0.05$).

Considering that only a proportion of pallidal neurons responded to neurotensin, we further performed another group of experiments to apply neurotensin first and then SR48692 with neurotensin. On the lesioned side, in 7 pallidal neurons with response to micropressure ejection of 0.1 mM neurotensin (basal: 11.8 ± 3.3 Hz; neurotensin: 15.6 ± 3.9 Hz; increase: $33.6 \pm 9.1\%$), co-ejection of neurotensin with SR48692 did not change the firing rate significantly (basal: 11.3 ± 3.0 Hz; neurotensin+SR48692: 12.0 ± 3.3 Hz, $p > 0.05$). The average change was $6.3 \pm 7.3\%$, which was significantly decreased compared to that of neurotensin alone ($33.6 \pm 9.1\%$, $p < 0.001$, fig. 5b). On the unlesioned side, in 7 pallidal neurons with response to

neurotensin (basal: 9.6 ± 5.7 Hz; neurotensin: 17.3 ± 11.2 Hz; increase: $86.3 \pm 34.2\%$), co-ejection of neurotensin with SR48692 did not change the firing rate significantly (basal: 10.9 ± 7.2 Hz; neurotensin+SR48692: 10.8 ± 6.4 Hz, $p > 0.05$). The average change was $1.7 \pm 5.1\%$, which was significantly decreased compared to that of neurotensin alone ($86.3 \pm 34.2\%$, $p < 0.001$, fig. 6b). In control experiments, neurotensin was applied twice to another group of pallidal neurons. On the lesioned side, in 10 neurons with response to neurotensin, neurotensin increased the firing rate by $40.0 \pm 11.8\%$ (basal: 12.4 ± 6.7 Hz; neurotensin: 17.0 ± 8.6 Hz, $p < 0.001$) on the first time, and $36.1 \pm 11.5\%$ (basal: 13.1 ± 6.9 Hz; neurotensin: 17.7 ± 9.0 Hz, $p < 0.001$) on the second time ($n = 10$, $p = 0.16$ compared to that on the first time, fig. 5c). On the unlesioned side, in 8 neurons with response to neurotensin, neurotensin increased the firing rate by $98.0 \pm 63.3\%$ (basal: 12.2 ± 8.7 Hz; neurotensin: 22.0 ± 14.4 Hz, $p < 0.001$) on the first time, and $70.0 \pm 42.7\%$ (basal: 12.4 ± 8.0 Hz; neurotensin: 20.3 ± 13.1 Hz, $p < 0.001$) on the second time ($n = 8$, $p = 0.098$ compared to that on the first time, fig. 6c).

Discussion

The present findings revealed that the spontaneous firing rate of globus pallidus neurons in 6-OHDA-lesioned rats did not change significantly compared to that in normal rats, which is in line with the previous studies that there was no significant difference in pallidal firing between nigral 6-OHDA-induced parkinsonian rats and normal rats [33]. However, Chang et al. [34] have shown that unilateral 6-OHDA lesion decreased the spontaneous firing of globus pallidus neurons. Consistently, a decreased discharge rate of globus pallidus neurons was reported in parkinsonian monkey [4]. The anesthesia may play a potential role in this discrepancy on firing rate under parkinsonian state. The decreased firing of globus pallidus neurons was performed in freely-moving rats or monkey [4, 34], while unchanged firing of globus pallidus neurons was collected in anesthetized animals [33] including the present recording.

Previous morphological evidence indicated a decreased neurotensin binding in the globus pallidus of parkinsonian patients [25]. However, the present electrophysiological recordings showed that neurotensin-induced excitation on the lesioned side was slightly, but not significantly, decreased compared to that in normal rats, suggesting no significantly functional loss of neurotensin receptor

Fig. 5. Effects of SR48692 on neurotensin-induced increase in firing rate of pallidal neurons of 6-OHDA-lesioned side. **a** In this cell, SR48692 alone had no effect on the spontaneous firing rate. Co-ejection of SR48692 with neurotensin did not alter the firing rate. **b** In this cell which was activated by neurotensin, co-ejection of SR48692 with neurotensin prevented neurotensin-induced excitation. **c** In this cell, neurotensin was applied twice to the globus pallidus neuron with the same application interval as for the experiments **a** and **b**.

in the globus pallidus of 6-OHDA parkinsonian rats. Furthermore, the present study also showed that neurotensin exerted stronger excitatory effects in globus pallidus of the 6-OHDA unlesioned side, which may suggest the compensation in the globus pallidus of unlesioned side. The finding was supported by some morphological evidence. For example, unilateral striatal lesions which destroyed a certain proportion of dopaminergic terminals in the lesioned striatum led to slight, but significant, increase of neurotensin-binding site densities in the contralateral striatum [35]. Furthermore, in bilateral 6-OHDA-lesioned rats, the num-

Fig. 6. Effects of SR48692 on neurotensin-induced excitation of pallidal neurons of 6-OHDA unlesioned side. **a** In this cell, SR48692 alone had no effect on the spontaneous firing rate. Co-ejection of SR48692 with neurotensin did not alter the firing rate. **b** In this cell which was activated by neurotensin, co-ejection of SR48692 with neurotensin did not change the firing rate. **c** In this cell, neurotensin was applied twice to the globus pallidus neuron with the same application interval as for the experiments **a** and **b**.

ber of neurotensin-binding sites was increased in the lateral part of the prefrontal cortex [36]. However, Masuo et al. [37] reported that there was no significant change of neurotensin receptors in the contralateral striatum and substantia nigra of 6-OHDA-lesioned rats, although the expression on contralateral striatum and substantia nigra was much higher than that on the lesioned side.

Another finding of the present experiments was that SR48692 inhibited the spontaneous firing in some of the pallidal neurons on the unlesioned side, which indicated that endogenous neurotensinergic system may mod-

ulate the cellular activity of pallidal neurons. Consistently, functional studies revealed that intrapallidal microinjection of SR48692 modulated motor symptoms in tardive dyskinesia model [38]. Interestingly, SR48692 only decreased the firing rate of pallidal neurons on the unlesioned side rather than lesioned side. Our previous results have shown that SR48692 also decreased the basal firing of globus pallidus neurons in normal rats. One of the possibilities for the lack of the effect of SR48692 on the lesioned side may be that the tonic activity of neurotensinergic system on the lesioned side was decreased compared to that on the unlesioned side. Destruction of dopaminergic terminals may change the activity of the neurotensinergic system in globus pallidus. Previous studies have revealed that the neurotensin-immunoreactive territory of the globus pallidus on the 6-OHDA-lesioned side was larger than that on the unlesioned side. This accumulation may be caused by the decrease in release of neurotensin from striatopallidal terminals [21]. If this is true, the decreased tonic activity of endogenous neurotensinergic system in globus pallidus of 6-OHDA parkinsonian rats may be involved in the pathophysiology of Parkinson's disease. According to the functional scheme of basal ganglia circuit [7], hypoactivity of globus pallidus causes disinhibition of the subthalamic nucleus, leading to overactivity of the entopeduncular nucleus and the substantia nigra pars reticulata. The resulting inhibition of thalamocortical activity leads to bradykinesia and rigidity in Parkinson's disease.

Previous functional study revealed that systemic administration of a neurotensin analog produced antiparkinsonian effects in 6-OHDA-treated rats [26]. The present findings that neurotensin directly excited globus pallidus neurons under parkinsonian state may provide a rationale for further investigations into its potential in the treatment of motor disorders originating from the basal ganglia. However, it is well known that neurotensin has a close relationship with the dopaminergic system. Mor-

phological studies have revealed that neurotensin receptors, particularly neurotensin type-1 receptor, were highly prevalent in midbrain neurons. Approximately 80% of dopamine cells in the ventral tegmental area and substantia nigra pars compacta expressed neurotensin receptors [17, 39, 40]. In addition, there was a wide distribution of neurotensin-like immunoreactivity and neurotensin-binding sites on the surface of midbrain dopamine neurons [39, 41]. Microinjection of neurotensin into the substantia nigra pars compacta caused an increase in dopamine and dopamine metabolites in the globus pallidus and striatum [42]. Furthermore, intracerebroventricular administration of neurotensin elevated concentrations of dopamine in the striatum and amygdala [43]. The extracellular dopamine concentration in the nucleus accumbens of 6-OHDA-treated rats was significantly increased following neurotensin microinjection into the ventral tegmental area [44]. Therefore, the therapeutic effect of systemic administration of neurotensin would depend on the interaction of its effects on the whole basal ganglia nuclei.

In conclusion, our present findings demonstrated that neurotensin excited globus pallidus neurons in 6-OHDA-lesioned rats. The lack of tonic activity of endogenous neurotensinergic system in the globus pallidus of 6-OHDA-lesioned side may be involved in the pathophysiology of Parkinson's disease, while the excitatory effects of neurotensinergic system in globus pallidus of 6-OHDA-lesioned rats may provide a rationale for further investigations into its possible therapy in Parkinson's disease.

Acknowledgements

The authors wish to thank Dr. Danielle Gully (Sanofi Recherche, France) for kindly providing SR48692. This work was supported by grants from the National Natural Science Foundation of China to L. Chen (30470545 and 30670664) and B. Bai (30770676).

References

- 1 Ehringer H, Hornykiewicz O: Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Klin Wochenschr* 1960;38:1236-1239.
- 2 El-Deredy W, Branstetter NM, Samuel M, Schrag A, Rothwell JC, Thomas DG, Quinn NP: Firing patterns of pallidal cells in parkinsonian patients correlate with their pallidotomy clinical scores. *Neuroreport* 2000;11:3413-3418.
- 3 Starr PA, Rau GM, Davis V, Marks WJ, Ostrem JL, Simmons D, Lindsey N, Turner RS: Spontaneous pallidal neuronal activity in human dystonia: comparison with Parkinson's disease and normal macaque. *J Neurophysiol* 2005;93:3165-3176.
- 4 Soares J, Kliem MA, Betarbet R, Greenamyre JT, Yamamoto B, Wichmann T: Role of external pallidal segment in primate parkinsonism: comparison of the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism and lesions of the external pallidal segment. *J Neurosci* 2004;24:6417-6426.
- 5 Albin RL, Young AB, Penney JB: The functional anatomy of basal ganglia disorders. *Trends Neurosci* 1989;12:366-375.

- 6 Wichmann T, DeLong MR: Functional and pathophysiological models of the basal ganglia. *Curr Opin Neurobiol* 1996;6:751–758.
- 7 Wichmann T, DeLong MR: Functional neuroanatomy of the basal ganglia in Parkinson's disease. *Adv Neurol* 2003;91:9–18.
- 8 Bergman H, Feingold A, Nini A, Raz A, Slovin H, Abeles M, Vaadia E: Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. *Trends Neurosci* 1998;21:32–38.
- 9 Magnin M, Morel A, Jeanmonod D: Single-unit analysis of the pallidum, thalamus and subthalamic nucleus in parkinsonian patients. *Neuroscience* 2000;96:549–564.
- 10 Raz A, Vaadia E, Bergman H: Firing patterns and correlations of spontaneous discharge of pallidal neurons in the normal and the tremulous 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine vervet model of parkinsonism. *J Neurosci* 2000;20:8559–8571.
- 11 Wichmann T, Soares J: Neuronal firing before and after burst discharges in the monkey basal ganglia is predictably patterned in the normal state and altered in parkinsonism. *J Neurophysiol* 2006;95:2120–2133.
- 12 Carraway R, Leeman SE: The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalamus. *J Biol Chem* 1973;248:6854–6861.
- 13 Tyler-McMahon BM, Boules M, Richelson E: Neurotensin: peptide for the next millennium. *Regul Pept* 2000;93:125–136.
- 14 Tanaka K, Masu M, Nakanishi S: Structure and functional expression of the cloned rat neurotensin receptor. *Neuron* 1990;4:847–854.
- 15 Chalon P, Vita N, Kaghad M, Guillemot M, Bonnin J: Molecular cloning of a levocabastine-sensitive neurotensin binding site. *FEBS Lett* 1996;386:91–94.
- 16 Mazella J, Zsurgner N, Navarro V, Chabry J, Kaghad M, Caput D: The 100-kDa neurotensin receptor is gp95/sortilin, a non-G-protein-coupled receptor. *J Biol Chem* 1998;273:26273–26276.
- 17 Fassio A, Evans G, Grisshammer R, Bolam JP, Mimmack M, Emson PC: Distribution of the neurotensin receptor NTS1 in the rat CNS studied using an amino-terminal directed antibody. *Neuropharmacology* 2000;39:1430–1442.
- 18 Sarret P, Perron A, Stroh T, Beaudet A: Immunohistochemical distribution of NTS2 neurotensin receptors in the rat central nervous system. *J Comp Neurol* 2003;461:520–538.
- 19 Martorana A, Martella G, D'Angelo V, Fusco FR, Spadoni F, Bernardi G, Stefani A: Neurotensin effects on N-type calcium currents among rat pallidal neurons: an electrophysiological and immunohistochemical study. *Synapse* 2006;60:371–383.
- 20 Zahm DS, Heimer L: Ventral striatopallidal parts of the basal ganglia in the rat. I. Neurochemical compartmentation as reflected by the distributions of neurotensin and substance P immunoreactivity. *J Comp Neurol* 1988;272:516–535.
- 21 Brog JS, Zahm DS: Morphology and Fos immunoreactivity reveal two subpopulations of striatal neurotensin neurons following acute 6-hydroxydopamine lesions and reserpine administration. *Neuroscience* 1995;65:71–86.
- 22 Fernandez A, Jenner P, Marsden CD, de Ceballos ML: Characterization of neurotensin-like immunoreactivity in human basal ganglia: increased neurotensin levels in substantia nigra in Parkinson's disease. *Peptides* 1995;16:339–346.
- 23 Martorana A, Fusco FR, D'Angelo V, Sancesario G, Bernardi G: Enkephalin, neurotensin, and substance P immunoreactive neurons of the rat GP following 6-hydroxydopamine lesion of the substantia nigra. *Exp Neurol* 2003;183:311–319.
- 24 Chinaglia G, Probst A, Palacios JM: Neurotensin receptors in Parkinson's disease and progressive supranuclear palsy: an autoradiographic study in basal ganglia. *Neuroscience* 1990;39:351–360.
- 25 Fernandez A, de Ceballos ML, Jenner P, Marsden CD: Neurotensin, substance P, delta and mu opioid receptors are decreased in basal ganglia of Parkinson's disease patients. *Neuroscience* 1994;61:73–79.
- 26 Boules M, Warrington L, Fauq A, McCormick D, Richelson E: Antiparkinson-like effects of a novel neurotensin analog in unilaterally 6-hydroxydopamine-lesioned rats. *Eur J Pharmacol* 2001;428:227–233.
- 27 Chen L, Yung KK, Yung WH: Neurotensin depolarizes globus pallidus neurons in rats via neurotensin type-1 receptor. *Neuroscience* 2004;125:853–859.
- 28 Xue Y, Chen L, Cui QL, Xie JX, Yung WH: Electrophysiological and behavioral effects of neurotensin in rat globus pallidus: an in vivo study. *Exp Neurol* 2007;205:108–115.
- 29 Paxinos G, Watson C: *The Rat Brain in Stereotaxic Coordinates*. New York, Academic Press, 1986.
- 30 Querejeta E, Oviedo-Chavez A, Araujo-Alvarez JM, Quinones-Cardenas AR, Delgado A: In vivo effects of local activation and blockade of 5-HT_{1B} receptors on globus pallidus neuronal spiking. *Brain Res* 2005;1043:186–194.
- 31 Kelland MD, Soltis RP, Anderson LA, Bergstrom DA, Walters JR: In vivo characterization of two cell types in the rat globus pallidus which have opposite responses to dopamine receptor stimulation: comparison of electrophysiological properties and responses to apomorphine, dizocilpine, and ketamine anesthesia. *Synapse* 1995;20:338–350.
- 32 Ruskin DN, Rawji SS, Walters JR: Effects of full D₁ dopamine receptor agonists on firing rates in the globus pallidus and substantia nigra pars compacta in vivo: tests for D₁ receptor selectivity and comparisons to the partial agonist SKF 38393. *J Pharmacol Exp Ther* 1998;286:272–281.
- 33 Breit S, Bouali-Benazzouz R, Popa RC, Gasser T, Benabid AL, Benazzouz A: Effects of 6-hydroxydopamine-induced severe or partial lesion of the nigrostriatal pathway on the neuronal activity of pallido-subthalamic network in the rat. *Exp Neurol* 2007;205:36–47.
- 34 Chang JY, Shi LH, Luo F, Woodward DJ: Neural responses in multiple basal ganglia regions following unilateral dopamine depletion in behaving rats performing a treadmill locomotion task. *Exp Brain Res* 2006;172:193–207.
- 35 Masuo Y, Montagne MN, Pélaprat D, Scherman D, Rostène W: Regulation of neurotensin-containing neurons in the rat striatum. Effects of unilateral striatal lesions with quinolinic acid and ibotenic acid on neurotensin content and its binding site density. *Brain Res* 1990;520:6–13.
- 36 Herve D, Tassin JP, Studler JM, Dana C, Kitabgi P, Vincent JP, Glowinski J, Rostène W: Dopaminergic control of ¹²⁵I-labeled neurotensin binding site density in corticolimbic structures of the rat brain. *Proc Natl Acad Sci USA* 1986;83:6203–6207.
- 37 Masuo Y, Pélaprat D, Montagne MN, Scherman D, Rostène W: Regulation of neurotensin-containing neurons in the rat striatum and substantia nigra. Effects of unilateral nigral lesion with 6-hydroxydopamine on neurotensin content and its binding site density. *Brain Res* 1990;510:203–210.
- 38 McCormick SE, Stoessl AJ: Central administration of the neurotensin receptor antagonist SR48692 attenuates vacuous chewing movements in a rodent model of tardive dyskinesia. *Neuroscience* 2003;119:547–555.
- 39 Dana C, Vial M, Leonard K, Beauregard A, Kitabgi P, Vincent JP: Electron microscopic localization of neurotensin binding sites in the midbrain tegmentum of the rat. I. Ventral tegmental area and the interfascicular nucleus. *J Neurosci* 1989;9:2247–2257.
- 40 Tanji H, Araki T, Fujihara K, Nagasawa H, Itoyama Y: Alteration of neurotensin receptors in MPTP-treated mice. *Peptides* 1999;20:803–807.
- 41 Boudin H, Pélaprat D, Rostène W, Pickel VM, Beaudet A: Correlative ultrastructural distribution of neurotensin receptor proteins and binding sites in the rat substantia nigra. *J Neurosci* 1998;18:8473–8484.
- 42 Napier TC, Gay DA, Hulebak KL, Breese GR: Behavioral and biochemical assessment of time-related changes in globus pallidus and striatal dopamine induced by intranigally administered neurotensin. *Peptides* 1985;6:1057–1068.
- 43 Drumheller AD, Gagné MA, St-Pierre S, Jolicoeur FB: Effects of neurotensin on regional brain concentrations of dopamine, serotonin and their main metabolites. *Neuropeptides* 1990;15:169–178.
- 44 Ikegami M, Ichitani Y, Takahashi T, Iwasaki T: Compensatory increase in extracellular dopamine in the nucleus accumbens of adult rats with neonatal 6-hydroxydopamine treatment. *Nihon Shinkei Seishin Yakurigaku Zasshi* 2006;26:111–117.