Electrophysiological and behavioral effects of neurotensin in rat globus pallidus: An in vivo study

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Abstract

The globus pallidus plays a critical role in movement regulation. Morphological study has indicated that the globus pallidus receives neurotensinergic innervation from the striatum. The present study investigated the effects of activating neurotensin receptor in globus pallidus. In vivo single unit electrophysiological recordings showed that micro-pressure ejection of neurotensin into the globus pallidus increased spontaneous firing of pallidal neurons. The excitatory effect of neurotensin could be mimicked by the C-terminal fragment, neurotensin (8–13), but not by the N-terminal fragment, neurotensin (1–8). Local administration of both the non-selective neurotensin receptor antagonist, SR142948A, and the selective neurotensin type-1 receptor antagonist, SR48692, blocked the excitatory effect induced by neurotensin. In the behaving rats, we observed the postural effects of neurotensin in the globus pallidus. Unilateral microinjection of neurotensin into the globus pallidus induced a SR48692-sensitive contralateral dystonic posturing in the presence of systemic haloperidol administration, which could be accounted for by the electrophysiological effect of neurotensin in increasing the firing rate of pallidal neurons. Our in vivo electrophysiological and behavioral findings suggest that pallidal neurotensin receptor plays a role in the basal ganglia motor circuit by mediating an excitation of spontaneous activity in the globus pallidus.

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Introduction

Neurotensin is a tridecapeptide which was first isolated and characterized by Carraway and Leeman (1973). It is well known that neurotensin plays an important role in the central nervous system by acting either as a neurotransmitter or a neuromodulator. Previous studies have shown that neurotensin produces various effects on the brain, leading to the suggestion that it may play a role in the pathophysiology of several central nervous system disorders, including schizophrenia (Garver et al., 1991; Kinkead and Nemeroff, 2004) and Parkinson’s disease (Bissette et al., 1985; Fernandez et al., 1994). Three subtypes of neurotensin receptor, type-1, type-2 and type-3, have been described within the brain (Le et al., 1996; Nicot et al., 1994; Sarret et al., 2003a,b). Morphological studies have indicated a relatively high level of neurotensin and its receptor expression in the basal ganglia, a region involved in the regulation of motor behavior. For example, neurotensin receptors are densely expressed in the substantia nigra (Palacios and Kuhar, 1981) and significantly decreased in brains of parkinsonian subjects (Chinaglia et al., 1990; Fernandez et al., 1994) or 6-hydroxydopamine-treated rats (Cadet et al., 1991). In agreement with this, systemic administration of a neurotensin analog that can cross the blood–brain barrier produced anti-parkinson-like effects in 6-hydroxydopamine-lesioned rats (Boules et al., 2001).

The globus pallidus is another important structure in the basal ganglia. It plays a role in processing and distributing signals from the striatum (Chan et al., 2005). There is much evidence supporting a function of globus pallidus in normal movements and in the pathophysiology of basal ganglia disorders (Bergman et al., 1998; DeLong, 1971; Hebb and Robertson, 1999; Lozano et al., 1996). Anatomical studies indicated that the globus pallidus receives neurotensinergic
innervation arising from striatum (Zahm and Heimer, 1988). Dense networks of neurotensin-containing fibers (Atoji et al., 1995) and both neurotensin type-1 and type-2 receptors are present in the globus pallidus (Fassio et al., 2000; Hokfelt et al., 2000; Sarret et al., 2003b). In 6-hydroxydopamine-treated rats and Parkinson’s disease, the immunoreactivity of neurotensin in globus pallidus is increased which may reflect a compensatory mechanism (Fernandez et al., 1995; Martorana et al., 2003). These findings suggest that neurotensin in globus pallidus may play a role in motor behavior. The fact that intrapallidal administration of neurotensin antagonist attenuates motor symptoms in a model of tardive dyskinesia (McCormick and Stoesl, 2003) supports this hypothesis. Recently, a significant relationship between the firing variability of globus pallidus neurons and the severity of Parkinson’s disease has been reported (El-Deredy et al., 2000). Therefore, it is of interest to fully understand the role of neurotensin in globus pallidus. Our previous in vitro studies have shown that neurotensin depolarizes pallidal neurons and modulates synaptic transmission. However, the in vivo electrophysiological and behavioral effects of neurotensin in globus pallidus have not yet been described. In the present experiment, single unit recordings were performed in anesthetized rats to study the effects of neurotensin on the firing properties of pallidal neurons. We also microinjected neurotensin directly into the globus pallidus of awake rats to observe any changes in motor behavior. Our results indicate that activation of neurotensin receptors exerts excitatory effects on globus pallidus neurons in vivo and results in postural changes.

Methods

Animals

Male adult Wistar rats (250–300 g) were used. Experiments were performed according to the University guidelines on animal ethics. Care was taken to minimize pain or sufferings of the animals. The animals were housed in a temperature-regulated (22 ± 1 °C) room and maintained on a 12-h light/dark cycle. All rats had free access to food and water.

Electrophysiological recordings

On the day of surgery, the rats were anesthetized with urethane (1 g/kg, i.p.) and placed in a stereotaxic frame (Narishige SN-3, Tokyo, Japan). According to a standard stereotaxic atlas (Paxinos and Watson, 1986), a craniotomy was performed at coordinates of 0.8–1.2 mm posterior, 2.5–3.5 mm lateral from the bregma. Rectal temperature was maintained at 36–38 °C by a heated pad.

Three-barrel microelectrodes were fastened at each end with metal tubing and prepared using a Stoelting pipette puller (Illinois, USA). The electrodes were stereotaxically positioned within the globus pallidus for single-unit recordings and micropressure ejection, and the tips were broken back under a microscope to 3–10 μm and had a resistance of 10–20 MΩ. The recording barrel of the electrode was filled with 0.5 M sodium acetate and 2% pontamine sky blue. The other two micro-pressure ejection barrels connected to 4-channel pressure ejector (PM2000B, Micro Data Instrument, Inc., USA) respectively contained either: neurotensin (different concentrations) and vehicle, neurotensin (8–13) and vehicle, neurotensin (1–8) and vehicle, SR142948A and SR142948A with neurotensin, or SR48692 and SR48692 with neurotensin. 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 (1500 ms, 5.0–15.0 psi) (Chen et al., 2005).

The recorded electrical signals were amplified by a micro-electrode amplifier (MEZ-8201, Nihon Kohden) and passed through low and high pass filters between 0.3 and 3 kHz. Electrical signals were displayed on a memory oscilloscope (VC-11, Nihon Kohden) and monitored on an audiomonitor. The amplified electrical signals were sent through an A/D interface (Power 1404, CED, United Kingdom) into a laboratory computer, which was used to analyze the data online. Spike times were preprocessed online and further analyzed offline using the program of Histogram ver 1.00 (Shanghai University) for spike data analysis. A change at least 20% of basal firing rate during drug application was considered significant (Querejeta et al., 2005). 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.

At least 5 min of baseline firing was collected from each cell before drug ejection into the globus pallidus. The frequency of control was determined by the average frequency of 120-s baseline data before drug application. The maximal change of frequency within 50 s following drug application was calculated as drug effect.

Lesions of pallidal cholinergic neurons

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Immunotoxin 192 IgG-saporin solution (0.1 μg in 0.5 μl of 0.1 M PBS; Chemicon) was unilaterally or bilaterally microinjected into the globus pallidus, according to the following coordinates based on the atlas of Paxinos and Watson (1986): 1.0 mm posterior, 3.0 mm lateral from the bregma, 6.9 mm ventral from the skull surface. The rats were then allowed 10–14 days to recover from the surgery before electrophysiological recording. After recording, choline acetyltransferase (ChAT) immunohistochemistry was performed to verify the lesion.

Postural test

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. A guide cannula constructed from stainless steel (o.d., 0.4 mm; i.d., 0.3 mm) was implanted into the globus pallidus on either side. The cannulae were fixed to the skull with stainless steel screws and dental acrylic. Stainless steel stylets were used to keep the cannulae sealed.

Following at least 3 days of recovery, the rats were tested for postural behavior. The rats were placed in an observation cage to
which they had already become habituated. Postural behavior was recorded by a digital camera. Drugs (0.5 μl) were microinjected into the globus pallidus in the awake animal over a 2-min period. Injection cannulae were connected to a 1.0 μl microsyringe and a drug was injected. At the end of injection, the cannula was left in the globus pallidus for an additional 1 min before removal and then replaced by a stylet. Ten minutes after the intrapallidal injection, haloperidol (5 mg/kg), a potent D2 receptor antagonist, was administrated intraperitoneally. According to the angle between the nose-back line and the back-tail line, the postural asymmetry was quantitatively scored as follows: 0, no fixed postural alteration; 1, less than 30°; 2, 30–59°; 3, 60–89°; 4, 90° and greater (Miwa et al., 2000).

**Histological controls**

To identify the position of single unit recording, pontamine sky blue was ejected from the recording electrode tip by iontophoresis (10 μA, 20 min). All the rats used in electro-physiological and behavioral experiments were sacrificed and perfused with 4% paraformaldehyde solution transcardially. Brains were frozen, sectioned at 50 μm and all the recording and microinjection sites were verified under light microscope.

**Drugs and statistics**

Neurotensin (1–13) was obtained from Sigma. Neurotensin (1–8) and neurotensin (8–13) were obtained from Bachem. SR48692[2-{{1-(7-chloro-4-quinolinyl)-5-2(2,6-dimethoxy-phenyl)pyrazol-3-yl}carbonylamino}tricyclo(3.3.1.1.3.7)-decan-2-carboxylic acid] and SR142948A {2-[[5-(2,6 dimethoxyphenyl)-1-(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropyl-phenyl)-1H-pyrazole-3-carbonyl]-amino]adamantane-2-carboxyoic acid] were kindly provided by Dr. Daniellie Gully (Sanofi Recherche, France). 192 IgG-saporin was obtained from Chemicon. Haloperidol was obtained from Sigma.

The data are expressed as means±S.E.M. Paired t test was used to compare the difference of firing rate before and after

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**Fig. 1.** Intrapallidal micro-pressure ejection of neurotensin increases the spontaneous firing rate of globus pallidus neurons. (A) Typical frequency histograms showing that 0.1 mM neurotensin increased the firing rate by 42.3%. The firings of the neuron, displayed at a faster time base, at different stages of the experiment are shown in lower trace. (B) The increase in firing rate induced by neurotensin is in a concentration-dependent manner within the range from 0.001 mM to 1 mM.
treatment. Statistical comparisons between or among groups were determined with Student’s t test and one-way ANOVA. The scores of deviated posturing were analyzed by the non-parametric one-way Kruskal–Wallis test followed by the Mann–Whitney test. The level of significance was preset by using a P value of 0.05.

Results

Effects of neurotensin on spontaneous firing in globus pallidus

The neurons recorded in the present experiment are type II globus pallidus neurons (Kelland et al., 1995; Ruskin et al., 1998). To determine the effect of striato-pallidal neurotensinergic system, local application of neurotensin in the globus pallidus was performed. Micro-pressure ejection of neurotensin excited a significant portion of globus pallidus neurons. In 24 out of 49 (49.0%) globus pallidus neurons, 0.1 mM neurotensin increased the frequency of spontaneous firing rate from 10.3±1.4 Hz to 14.9±2.0 Hz. The average increase was 47.4±4.4% (Fig. 1A). This increase was statistically significant compared to vehicle injection control (P<0.001). The mean duration of the excitatory effect was about 176.9±23.1 s. In another 24 globus pallidus neurons (49.0%), micro-pressure ejection of neurotensin did not alter the firing rate of pallidal neurons significantly. Local administration of neurotensin decreased the firing rate in only one pallidal neuron (2.0%).

It has been reported that cholinergic neurons in globus pallidus are very densely occupied by neurotensin receptors. To observe the effects of neurotensin on GABAergic neurons, 192 IgG-saporin was microinjected into the globus pallidus to lesion cholinergic neurons. Under this condition, 0.1 mM neurotensin still increased the firing rate in 9 out of 21 globus pallidus neurons. The average increase was 36.7±7.3%, which was not

Fig. 2. Effects of neurotensin fragments on the spontaneous firing rate of globus pallidus neurons. (A) Typical frequency histograms showing that intrapallidal micro-pressure ejection of 3 mM neurotensin (8–13) increased the spontaneous firing rate of pallidal neurons (26.0%). The firings of the neuron, displayed at a faster time base, at different stages of the experiment are shown in lower trace. (B) Typical frequency histograms showing that 3 mM neurotensin (1–8) did not induce any effect on the spontaneous firing rate. The firings displayed at a faster time base are shown in lower trace.
statistically different compared to that without 192 IgG-saporin treatment \((P>0.05)\).

**Concentration-dependent response induced by neurotensin**

Further experiments were performed to study the effects of different concentrations of neurotensin on pallidal neurons. As shown in Fig. 1B, within the range of concentration from 0.001 mM to 1 mM, neurotensin produced a clear bell-shaped concentration-dependent effect in increasing the firing rate. Micro-pressure ejection of 0.001 mM neurotensin increased the firing rate by 28.4±3.2% (2 out of 17 neurons). At a concentration of 0.01 mM, the firing rate was increased by 38.1±8.7% (6 out of 14 neurons). Neurotensin at 0.03 mM increased the firing rate by 46.5±9.4% (7 out of 13 neurons). At a higher concentration of 1 mM, the increase in firing rate induced by neurotensin was 26.6±1.8% (6 out of 19 neurons) which was significantly less strong than that induced by the concentrations of 0.1 mM \((P<0.05)\).

**Effects of neurotensin fragments on spontaneous firing**

Neurotensin (8–13), the C-terminal hexapeptide fragment of neurotensin, could mimic the excitatory effect of neurotensin. At 3 mM, neurotensin (8–13) increased the firing rate from 9.7±1.6 Hz to 15.0±2.8 Hz in 21 out of 38 (55.3%) globus pallidus neurons (Fig. 2A). The average increase was 60.3±9.0% \((P<0.001)\). The excitatory effect induced by neurotensin (8–13)
lasted about 222.4 ± 37.8 s. In contrast, the N-terminal octapeptide neurotensin (1–8) at 3 mM was without any effect on the firing rate of 11 globus pallidus neurons tested (Fig. 2B).

**Neurotensin type-1 receptor is involved in neurotensin-induced excitation**

To determine the subtype of neurotensin receptor that mediated the excitatory response, the effects of two specific neurotensin receptor antagonists were studied. Micro-pressure ejection of SR142948A (1 mM), a non-selective neurotensin receptor antagonist, alone had no effect on the firing rate in 16 globus pallidus neurons. However, co-ejection of SR142948A and neurotensin could completely block neurotensin-induced excitatory effects in 15 out of 16 neurons. The average change and neurotensin could completely block neurotensin-induced excitatory effects in 15 out of 16 neurons. The average change was 4.0 ± 1.9% of basal firing (n = 15, Fig. 3A). Application of SR48692 (1 mM), a specific antagonist of neurotensin type-1 receptor, alone decreased spontaneous firing rate in 4 out of 27 pallidal neurons (Fig. 3B). The average inhibition was 36.8 ± 4.5% (P < 0.01, n = 4). Similarly, in 17 neurons with long, stable recording, co-ejection of SR48692 and neurotensin completely prevented the excitation induced by neurotensin (5.3 ± 1.2% of basal firing, Fig. 3C).

**Effects of neurotensin on postural behavior**

To provide evidence that neurotensin in globus pallidus modulates movement, the effects of neurotensin on behavior were studied in awake animals. Unilateral microinjection of neurotensin (0.1 mM, 0.5 μl) into globus pallidus did not produce a clear rotational behavior. However, neurotensin induced significant contralateral dystonic posturing under the conditions of haloperidol intraperitoneally administration (n = 7). The deviated posturing appeared almost immediately and persisted for at least 1 h. In the control group, vehicle injection did not cause any fixed deviated posturing in rats receiving haloperidol treatment (n = 5). Statistical analysis showed a significant difference between vehicle and neurotensin microinjection (P < 0.001). To confirm that neurotensin acted on the type-1 neurotensin receptor, the effect of SR48692 was studied. When microinjected into the globus pallidus unilaterally, SR48692 (1 mM) alone did not induce any dystonic posturing under conditions of haloperidol administration (n = 5). In another group of animals, in which SR48692 and neurotensin were co-injected, there was no significant contralateral posturing (n = 5, P < 0.001 compared with neurotensin alone, P > 0.05 compared with SR48692 alone). These data are summarized in Fig. 4.

**Discussion**

The principal finding of the present study is that local administration of neurotensin increased the spontaneous firing rate of globus pallidus neurons recorded in intact rats. This effect is consistent with its action in pallidal slices where activation of neurotensin receptors depolarizes the neurons (Chen et al., 2004). The excitatory effect of neurotensin demonstrated a bell-shaped concentration–response relationship comparable with the previous studies. For example, Ferraro et al. (2000) reported that neurotensin enhanced potassium-evoked glutamate release in a bell-shaped concentration response curve in rat cortical slices. Similar bell-shaped dose response induced by neurotensin was also found in its mitogenic effect in prostate cancer cell line (Seethalakshmi et al., 1997).

Previous morphological studies revealed contrary opinions about the distribution of neurotensin receptors in the globus pallidus. Some reports demonstrated that neurotensin receptors were distributed within globus pallidus in a pattern characteristic of cholinergic neurons (Morin et al., 1996; Alexander and Leeman, 1998). In human and pigeon brain, a very low quantity of neurotensin receptor binding level was revealed in globus pallidus (Brauth et al., 1986; Palacios et al., 1991). However, recent immunohistochemical study reported that neurotensin type-1 receptor is localized in most of both subpopulations of globus pallidus neurons (Martorana et al., 2006). By using electron microscopy, Fassio et al. (2000) reported that neurotensin type-1 receptors were mainly localized at dendrites that were typically postsynaptic to many terminals forming...
symmetrical synapse in globus pallidus. The present findings that neurotensin increases the firing rate of pallidal neurons in a SR48692-sensitive manner provided evidence for the existence of neurotensin type-1 receptor in globus pallidus. Consistent with our findings, Martorana et al. (2006) recently reported that neurotensin type-1 receptor affects high-voltage-activated calcium current in isolated globus pallidus neuron. Morphological study also revealed that neurotensin type-2 receptor is mainly localized in nerve terminals of globus pallidus neurons (Sarret et al., 2003b). The contribution of this receptor in globus pallidus is unclear at the moment.

Early anatomical studies revealed that the globus pallidus receives neurotensinergic innervation from the striatum. The present finding that neurotensin type-1 receptor antagonist inhibited the spontaneous firing in some of pallidal neurons indicated that endogenous neurotensin system may modulate the cellular activity of pallidal neurons. Consistently, McCormick and Stoessl (2003) reported that intrapallidal microinjection of SR48692 modulated motor symptoms in tardive dyskinesia model. However, SR142948A could not affect the spontaneous firing of globus pallidus neurons. It is known that SR142948A is non-selective neurotensin receptor antagonist, which could block both neurotensin type-1 and type-2 receptors. The neurotensin type-2 receptors located at striatopallidal terminals (Sarret et al., 2003b) may have a behavior as a presynaptic autoreceptor to inhibit the neurotensin release. In this way, blockade of presynaptic neurotensin type-2 receptor may increase the neurotensin release which in turn would compensate its possible inhibitory effect induced by blockade of postsynaptic neurotensin type-1 receptor.

There is much evidence supporting the involvement of the globus pallidus in both normal motor function and movement disorders. Since neurotensin increased the firing rate and thus excited globus pallidal neurons, one would expect that neurotensin may be involved in the motor regulation. It has been suggested that the mechanism underlying rotational behavior is based on the asymmetric alteration of basal ganglia output activity (Pycock, 1980). Unilateral excitation in the activity of globus pallidus would contribute to the generation of contralateral circling behavior, presumably resulting in disinhibition from the indirect pathway of the basal ganglia on the ipsilateral cortex. Therefore, the contralateral posturing induced by intrapallidal microinjection of neurotensin indicated that neurotensin exerts an excitatory effect on globus pallidus. It is possible that intrapallidal application of neurotensin compensates the hyoactivity of the globus pallidus induced by haloperidol, and induced a significant difference in the neuronal activity of globus pallidus between the intact side and the neurotensin injection side, thereby causing contralateral dystonic posturing.

Previous studies have indicated that neurotensin system may contribute to antipsychotics-induced motor side effects. Early in situ hybridization studies have shown that long-time antipsychotics treatment increases neurotensin mRNA expression in striatum which raises the possibility that increased striatopallidal neurotensin system may contribute to the motor side effects of the drugs (Augood et al., 1991; Merchant et al., 1989; Merchant and Dorsa, 1993; Holtom et al., 2000). Indeed, recent functional study revealed that intrapallidal administration of neurotensin receptor antagonist attenuated neuroleptic-induced tardive dyskinesias (McCormick and Stoessl, 2003). It is reported that intrapallidal administration of GABAA receptor antagonist induced dyskinesia (Matsumura et al., 1995), suggesting the involvement of globus pallidus in motor dyskinesia. Thus, our findings that neurotensin increases the excitability of globus pallidus neurons further provide a possible electrophysiological evidence for the involvement of striatopallidal neurotensin system in antipsychotics-induced motor side effects.

In addition to the contribution in antipsychotics-induced motor side effects, neurotensin may also be involved in the pathophysiology of Parkinson’s disease (Bissette et al., 1985; Fernandez et al., 1994), which is in line with the fact that neurotensin and its receptors are distributed in basal ganglia and have a close interaction with dopamine system (Kasckow and Nemeroff, 1991). Immunohistochemical studies also indicated that 6-hydroxydopamine lesions increased striatopallidal neurotensin immunoreactivity (Brog and Zahm, 1996; Zahm, 1992). The present findings that neurotensin excited globus pallidus neurons prompt us to hypothesize that the increased striatopallidal neurotensin immunoreactivity may reflect a compensatory mechanism. However, more information and experiments are needed before we could fully understand the participation of pallidal neurotensin system in any pathophysiological condition.

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