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NEUROTENSIN SELECTIVELY FACILITATES GLUTAMATERGIC TRANSMISSION IN GLOBUS PALLIDUS

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Abstract—The tridecapeptide neurotensin has been demonstrated to modulate neurotransmission in a number of brain regions. There is evidence that neurotensin receptors exist in globus pallidus presynaptically and postsynaptically. Whole-cell patch-clamp recordings were used to investigate the modulatory effects of neurotensin on glutamate and GABA transmission in this basal ganglia nucleus in rats. Neurotensin at 1 μM significantly increased the frequency of glutamate receptor–mediated miniature excitatory postsynaptic currents. In contrast, neurotensin had no effect on $GABA_A$ receptor–mediated miniature inhibitory postsynaptic currents. The presynaptic facilitation of neurotensin on glutamatergic transmission could be mimicked by the C-terminal fragment, neurotensin (8–13), but not by the N-terminal fragment, neurotensin (1–8). The selective neurotensin type-1 receptor antagonist, 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}, blocked this facilitatory effect of neurotensin, and which itself had no effect on miniature excitatory postsynaptic currents. The specific phospholipase C inhibitor, U73122 {1-[6-[[17β-3-methoyyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione}, significantly inhibit neurotensin-induced facilitation on glutamate release. Taken together with the reported postsynaptic depolarization of neurotensin in globus pallidus, it is suggested that neurotensin excites the globus pallidus neurons by multiple mechanisms which may provide a rationale for further investigations into its involvement in motor disorders originating from the basal ganglia. © 2006 IBRO. Published by Elsevier Ltd. All rights reserved.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AP5, ±-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquino xaline-2,3-dione; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N,N,N′,N′*-tetraacetic acid; EPSC, excitatory postsynaptic current; HEPES, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid; IPSC, inhibitory postsynaptic current; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; SR142948A, 2-[(5-(2,6 dimethoxyphenyl)-1-(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropyl-phenyl)-1H-pyrazole-3-carbonyl)-amino]adamantine-2-carboxyoic acid; 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; TTX, tetrodotoxin; U73122, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione; 6-OHDA, 6-hydroxydopamine.

Key words: neurotensin, globus pallidus, postsynaptic currents.

Being a critical structure in the indirect pathway of basal ganglia, the globus pallidus plays an important role in the modulation of movement via its GABAergic innervation to all other nuclei of the basal ganglia. There is much evidence supporting the involvement of the globus pallidus in both normal motor function and movement disorders. Previous studies have demonstrated that the degeneration of nigrostriatal dopaminergic innervation leads to hypoactivity and decreased GABAergic output of the globus pallidus, which contribute to akinesia and hypokinetic symptoms of Parkinson's disease. Recently, the oscillatory activity within globus pallidus, which is likely produced by its complex interactions with subthalamic nucleus and cortex, has been proposed to be involved in the tremor seen in both animal parkinsonian models and parkinsonian patients (Bergman et al., 1998; Magill et al., 2001; Plenz and Kital, 1999). Although electrophysiological and morphological studies have demonstrated that GABA and glutamate are the principal neurotransmitters released in globus pallidus, other neuroactive substances have also been shown to exist in globus pallidus, which may play at least a modulatory role.

Neurotensin, a putative peptidergic neurotransmitter, was first isolated and characterized by Carraway and Leeman (1973). Previous studies have shown that neurotensin produces various effects in CNS and is suggested to play a role in the pathophysiology of several CNS disorders, including schizophrenia (Garver et al., 1991; Kinkead and Nemeroff, 2004) and Parkinson's disease (Bissette et al., 1985; Fernandez et al., 1994). It has been reported that neurotensin and neurotensin receptor levels are altered in the basal ganglia of both parkinsonian patients and animal models (Chinaglia et al., 1990; Yamada et al., 1995; Tanji et al., 1999). Recently, Boules et al. (2001) reported that systemic administration of a neurotensin analog, which can cross the blood–brain barrier, produced antiparkinson-like effects in 6-hydroxydopamine (6-OHDA) -lesioned rats.

Anatomical studies indicated that the globus pallidus receives neurotensinergic innervation arising from striatum (Zahm and Heimer, 1988) and extremely dense networks of neurotensin-containing fibers were found in dog globus pallidus (Atoji et al., 1995). In line with this, both neurotensin type-1 and type-2 receptors are present in the globus pallidus (Fassio et al., 2000; Sarret et al., 2003). These data suggest that the actions of neurotensin in globus pallidus may contribute to basal ganglia functions and

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dysfunctions. Indeed, in 6-OHDA-lesioned rats, neurotensin immunoreactivity in globus pallidus increased significantly (Martorana et al., 2003). Behavioral studies showed that intra-pallidal administration of neurotensin receptor antagonist attenuated motor symptoms in a model of tardive dyskinesia (McCormick and Stoessl, 2003). Our recent report that neurotensin depolarizes pallidal neurons by acting directly on postsynaptic neurotensin type-1 receptor (Chen et al., 2004) also supports this notion.

In addition to its well-known effects on dopamine transmission (Kasckow and Nemeroff, 1991; Wang et al., 2004), neurotensin has also been shown to modulate the release of glutamate and GABA in some brain areas. Previous *in vivo* and *in vitro* studies have demonstrated that neurotensin stimulates the release of glutamate in cortex (Sanz et al., 1993; Ferraro et al., 2000), striatum (Ferraro et al., 1995, 1998) and substantia nigra (Ferraro et al., 2001). In substantia nigra pars reticulata, neurotensin exerts different influences on glutamate and GABA levels *in vivo*, increasing glutamate release but decreasing GABA release (Ferraro et al., 2001). In globus pallidus, immunohistochemical studies have shown that neurotensin receptors are expressed at presynaptic terminals, especially neurotensin type-2 receptor (Sarret et al., 2003). To understand the functions of these presynaptic neurotensin receptors in globus pallidus, we studied the effects of neurotensin on glutamate and GABA release by directly examining the miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs), respectively.

EXPERIMENTAL PROCEDURES

In vitro slice preparation

The experimental procedures were approved by the Animal Ethics Committee of the Chinese University of Hong Kong and were performed in accordance to local and international guidelines on the ethical use of animals. Efforts were made to minimize the number of animals used and their suffering. Sprague–Dawley rats aged 13–14 days were used for the preparation of acute brain slices. The animals were killed by decapitation. The brains were immediately removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 125, KCl 4.0, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, glucose 11 and $NaHCO_3$ 26, which was continuously bubbled with 95% O_2 and 5% CO_2. Thin hemi-coronal slices (250 μm) containing the globus pallidus were sectioned using a vibrating microtome (Campden Instruments, Loughborough, UK). After equilibration in ACSF for at least 30 min, the slices were transferred to a small volume chamber mounted on an upright microscope (Zeiss Axioskop, Carl Zeiss, Oberkochen, Germany), and superfused with ACSF at a rate of 1.5–2.0 ml/min maintained at a temperature of 34±1 °C. Neuronal soma and proximal dendrites of neurons were directly visualized by a combination of differential interference contrast optics and contrast-enhanced video microscope.

Whole-cell patch-clamp recordings

Whole-cell patch-clamp recordings from total 52 globus pallidus neurons were obtained using a patch-clamp amplifier (LM/PCA, List Medical, Germany). Whole-cell pipettes (P-97, Sutter Instrument, Novato, CA, USA) typically had a resistance of 3–5 MΩ. To record glutamate receptor-mediated excitatory postsynaptic currents (EPSCs), the pipettes were filled with an internal solution of the following composition (in mM): K-gluconate 130, KCl 10, HEPES 10, EGTA 1, $MgCl_2$ 2, Na_2ATP 2, Tris GTP 0.4 and the pH was adjusted to 7.25–7.30 with 1 M KOH. Since the EPSCs were measured at −70 mV and in the presence of magnesium, the excitatory miniature events recorded in the present study are mainly non–*N*-methyl-D-aspartate (NMDA) receptor-mediated mEPSCs. To study the $GABA_A$ receptor-mediated inhibitory postsynaptic currents (IPSCs), the internal solution containing (in mM): KCl 140, HEPES 10, EGTA 1, $MgCl_2$ 2 Na_2ATP 2 and Tris GTP 0.4 was used. Since measurements were made at a holding potential of −70 mV the inclusion of 140 mM KCl in the recording pipette enhanced their detection by increasing the electrochemical driving force on Cl ions. Monitoring through a television connected to the camera, a pipette was placed on the soma of a pallidal neuron and conventional whole-cell recording was made. Normally no series resistance compensation was applied but the cell was rejected if the series resistance increased significantly (>20%) during recording. The current signals were filtered at 3 kHz and were taped using a DAT recorder (Sony, Tokyo, Japan) modified for recording AC and DC signals at a sampling rate of 32 kHz. On- or off-line digitization (10 kHz) was made via the Digidata-pClamp system (Axon Instruments, Foster City, CA, USA).

Analysis of synaptic currents

Computer files containing information of synaptic currents were analyzed by Minianalysis program (Synaptosoft, version 6), which automatically generates various parameters including the time of occurrence, peak amplitude and kinetics. Statistical comparison of two cumulative probabilities was based on the Kolmogorov-Smirnov test.

Drugs and statistics

Neurotensin (1–13) was obtained from Sigma (St. Louis, MO, USA). 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} and SR142948A {2-[(5-(2,6 dimethoxyphenyl)-1-(4-(N-(3-dimethylaminopropyl)-N-methylcarbamoyl)-2-isopropyl-phenyl)-1H-pyrazole-3-carbonyl)-amino]adamantine-2-carboxyoic acid} were kindly provided by Dr. Daniellie Gully (Sanofi Recherche, France). AP5 (±-2-amino-5-phosphonopentanoic acid), CNQX (6-cyano-7-nitroquino xaline-2,3-dione), bicuculline and tetrodotoxin (TTX) were obtained from Sigma/RBI (Natick, MA, USA). U73122 {1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1H-pyrrole-2,5-dione} was obtained from Calbiochem (La Jolla, CA, USA).

The data are expressed as means±S.E.M. Paired *t*-test was used to compare the difference between control group and treatment group. The level of significance was set at a *P* value of 0.05.

RESULTS

Effects of neurotensin on mEPSCs

To determine whether neurotensin modulates glutamatergic neurotransmission presynaptically, the effects of neurotensin on glutamate receptor-mediated mEPSCs were studied. Whole-cell patch-clamp recordings were made from globus pallidus neurons, which were clamped at −70 mV. The mEPSCs were then isolated by including 10 μM bicuculline in the superfusion solution to block $GABA_A$ receptor-mediated synaptic currents, and 0.5 μM TTX to eliminate action potential-dependent transmitter release. The raw traces shown in Fig. 1A are an example of the effect of neurotensin. Superfusion of neurotensin (1 μM)