Neuroscience 135 (2005) 299–303

STRIATAL DOPAMINE LEVEL INCREASES IN THE URINARY STORAGE PHASE IN CATS: AN *IN VIVO* MICRODIALYSIS STUDY

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Abstract—Parkinson's disease is a common neurodegenerative disease that shows not only movement disorder, but also profound urinary dysfunction. Bladder hyperactivity is the major urodynamic abnormality. Therefore, the basal ganglia have been thought to modulate the micturition reflex. In six male adult cats under ketamine anesthesia, in which spontaneous isovolumetric micturition reflexes had been generated, we measured levels of striatal dopamine, in micturition and storage phases, using *in vivo* microdialysis. The striatal dopamine level significantly increased in the storage phase as compared with that in the micturition phase. It is suggested that striatal dopamine may inhibit the micturition reflex via the dopamine D1 receptor-GABAergic *direct* striatal output pathway, and that disruption of this pathway may be what leads to bladder hyperactivity in patients with Parkinson's disease.

Key words: nigrostriatal dopaminergic neuron, microdialysis, basal ganglia, micturition reflex, Parkinson's disease.

Parkinson's disease (PD) is associated with degeneration of nigrostriatal dopaminergic neurons (NSDN) that originate in the substantia nigra pars compacta (SNc) (Bernheimer, 1973; Hornykiewicz and Kish, 1986). Degeneration of NSDN is accompanied by decreases in corresponding biochemical markers, including dopamine (Bernheimer, 1973; Hornykiewicz and Kish, 1986) and dopamine metabolites, as detected by noninvasive imaging with positron emission tomography (PET) (Morrish et al., 1996) and single-photon emission computed tomography (SPECT) (Cline et al., 1992). Patients with PD often show signs and symptoms of autonomic involvement (Martignoni et al., 1995). The most common are gastrointestinal and urinary systems, and the latter includes urinary frequency and urgency incontinence, both of which reflect bladder hyperactivity by urodynamic study (Murnaghan, 1961; Aranda and Cramer, 1993; Christmas et al., 1998). SPECT scanning of dopamine transporters with [^{123}I]-2β-carbomethoxy-3β-(4-iodophenyl) tropane (β-CIT) suggested more marked loss of NSDN in PD patients with urinary dysfunction than in those without it (Sakakibara et al., 2001a). In experimental settings, bladder hyperactivity can be reproduced in marmosets (Albanese et al., 1988) and in monkeys (Yoshimura et al., 1998) with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -induced Parkinsonism. Despite these facts, the role of dopamine on the micturitional reflex in humans is controversial. Levodopa (L-DOPA) is a precursor of dopamine, which is rapidly converted into dopamine by dopa-decarboxylase. Treatment with levodopa increased the concentration of dopamine within the brain, and restored the impaired urinary storage function in de novo PD patients (Sakakibara et al., 2001b), whereas in advanced PD patients, who had a wearing-off phenomenon, a single dose of levodopa worsened bladder hyperactivity (Uchiyama et al., 2003). However, a paucity of direct evidence of the role of NSDN in the micturition function leads to difficulties in interpreting the clinical and experimental literature. Thus, we measured striatal dopamine level in the urinary storage/micturition cycle in cats using *in vivo* microdialysis.

EXPERIMENTAL PROCEDURES

Animal subjects

Experiments were performed on six adult male cats under anesthesia with ketamine (initial injection of 20 mg/kg i.m., maintaining injections of 5 mg/kg when necessary with regard to blood pressure). The trachea was intubated and catheters were placed in the femoral artery to monitor blood pressure and in the femoral vein to administer drugs. A 20 gauge double-lumen urinary catheter was inserted into the bladder transurethrally to measure bladder pressure and regulate bladder volume. The animals were positioned in a stereotaxic frame and their positions adjusted to expose the surface of the left hemisphere. Artificial ventilation was applied and end-tidal CO_2 was maintained at 3–5%. At the end of the experiment, the animals were given an overdose of pentobarbital sodium. All efforts were made to minimize the numbers of animal used and their suffering. All experiments conformed to the international guidelines on the ethical use of animals (Phillips, 1996). Periodic isovolumetric bladder contraction was generated by infusion of saline (20–50 ml) into the bladder, which resulted in continuous cycles of bladder contraction against a closed outlet and relaxations. The micturition phase was defined as the period between onset and offset of the increase in bladder pressure. The storage phase was defined as the period between micturition phases.

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Abbreviations: GPe, external globus pallidus; GPi, internal globus pallidus; HPLC, high-performance liquid chromatography; NSDN, nigrostriatal dopaminergic neuron; PD, Parkinson's disease; PET, positron emission tomography; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; SPECT, single-photon emission computed tomography; STN, subthalamic nucleus.

0306-4522/05$30.00+0.00 © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2005.06.007

In vivo microdialysis and high-performance liquid chromatography (HPLC) system

A concentric I-type dialysis probe (0.22 mm diameter; 1.0 mm exposed membrane; A-I-50-1; Eicom Inc., Kyoto, Japan) was inserted stereotaxically into the caudate head (A=15 mm, L=5 mm, H=5 mm) using Horsley-Clarke coordinates (Okamoto et al., 2003; Snider and Niemer, 1961). The perfusion rate was kept at 2 μl/min, using modified Ringer's solution composed of Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM, and Cl^- 155.6 mM. Dialysate was collected during the micturition and storage phases, respectively, and stored at −80 °C. The HPLC system used for determination of dopamine was equipped with an electrochemical detector system (ECD-300, Eicom). The mobile phase was 0.1 M citric acid–0.1 M sodium acetate (pH 3.9) containing 140 mg/l sodium 1-octane sulfonate, 5 mg/l EDTA-2Na, and 15% methanol. A flow rate was 0.23 ml/min. Samples were injected manually into an analytical column (EICOMPAK SC-5ODS, 2.1 mm×150 mm; Eicom). Dopamine was electrochemically detected by a graphite electrode (WE-3G, Eicom) at 700 mV relative to an Ag/AgCl reference electrode.

Statistical analysis

Results are expressed as means±standard deviation. Student's *t*-test (two-tailed) was used for micturition and storage phase comparison. *P* values <0.05 were considered to be statistically significant.

RESULTS

The typical relationship between the urinary storage/micturition cycle and changes in striatal dopamine levels is depicted in Fig. 1. The dopamine level was first measured in the dialysate collected during the entire period of each storage or micturition phase, and then averaged in five to six cycles of storage or micturition phases in each animal. Thereafter, the mean value of the striatal dopamine levels of storage/micturition phases was combined and averaged in six animals. Mean duration of the storage phases was 13.0±4.8 min and mean duration of the micturition phases was 9.0+6.2 min. Mean number of the storage/micturition cycles in each animal was 5.3.

The striatal dopamine level periodically changed in relation to the urinary storage/micturition cycles, significantly increasing in the storage phase (13.7±9.6 pg/ml) compared with that in the micturition phase (8.7±6.6 pg/ml) ($P<0.05$). Intra-animal variability of the dopamine level was 4.1/2.4 pg/ml in storage/micturition phase, respectively. Inter-animal variability was 9.6/6.6 pg/ml in storage/micturition phase, respectively. There were no significant differences of striatal dopamine levels in relation to the bladder capacity of animals.

DISCUSSION

The basal ganglia have a critical role on extrapyramidal motor control. Recent studies also suggest that basal ganglia modulate the micturition reflex in both experimental animals (Yoshimura et al., 1992, 2003; Seki et al., 2001) and humans (Murnaghan, 1961; Aranda and Cramer, 1993; Christmas et al., 1998), since lesions in the basal ganglia including NSDN were shown to cause severe urinary dysfunction (Murnaghan, 1961) and PET scanning

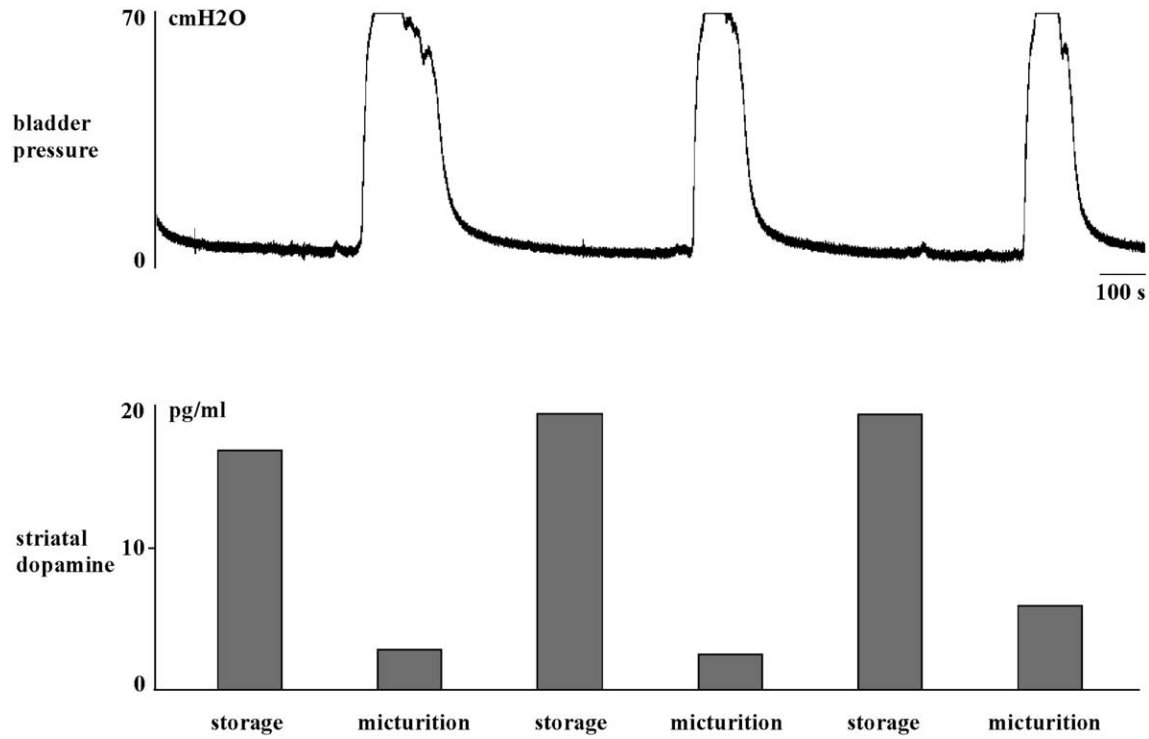

Fig. 1. Typical recording of bladder cycles and changes in striatal dopamine level. The upper trace shows the urinary storage/micturition cycle, and the lower trace shows temporal changes in the striatal dopamine level. The striatal dopamine level periodically changed in relation to urinary cycles, increasing in the storage phase and decreasing in the micturition phase.