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Dopaminergic mechanisms underlying bladder hyperactivity in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway

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1 This study was undertaken to elucidate dopaminergic mechanisms underlying bladder hyperactivity in a rat model of Parkinson's disease (PD) induced by a unilateral 6-OHDA injection into the substantia nigra pars compacta.

2 In 6-OHDA-lesioned rats, voided volume per micturition (0.41 ± 0.04 ml, mean $\pm$ s.e.m.) measured during 24 h in a metabolic cage was significantly smaller than in sham-operated rats (0.67 ± 0.07 ml).

3 Cystrometrograms (CMG) in conscious animals revealed significantly smaller bladder capacity (BC) (0.46 ± 0.03 ml) in 6-OHDA-lesioned rats than in sham rats (0.72 ± 0.06 ml).

4 SKF38393 (D1/D5 receptor agonist, i.v.) significantly increased BC in 6-OHDA rats without apparent effects in sham rats. SKF38393 applied intracerebroventricularly (i.c.v.) under urethane anesthesia also increased BC in 6-OHDA-lesioned rats and by a smaller increment in sham rats.

5 In contrast, quinpirole (D2/D3/D4 receptor agonist, i.v.) significantly reduced BC in sham and 6-OHDA-lesioned rats. Intrathecal injection of quinpirole similarly reduced BC in sham and 6-OHDA-lesioned rats.

6 PD128907 (D_3-receptor agonist) did not have significant effects on BC in 6-OHDA-lesioned rats.

7 These results indicate that a rat model of PD exhibited bladder hyperactivity as observed in patients with PD, and that stimulation of D1/D5 dopamine receptors at a supraspinal site can suppress bladder hyperactivity in PD, whereas stimulation of D2/D4, but not D3, dopamine receptors had the opposite effect to reduce bladder capacity. Thus, D1/D5 dopamine receptor agonists might be effective in treating neurogenic bladder hyperactivity in PD.
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Keywords: 6-hydroxydopamine; Parkinson's disease; bladder hyperactivity; dopamine receptors; SKF38393; quinpirole

Abbreviations: BC, bladder capacity; CMG, cystometrogram; i.c.v., intracerebroventricular; i.t., intrathecal; i.v., intravenous; 6-OHDA, 6-hydroxydopamine

Introduction

Since the function of the lower urinary tract, to store and periodically release urine, is controlled by complex mechanisms in spinal and supraspinal neural pathways, diseases or injuries of the central nervous system can produce voiding dysfunctions such as urinary frequency, incontinence or inefficient voiding (Yoshimura & Chancellor, 2002). Parkinson's disease (PD), characterized by dopamine depletion in the striatum (Hornykiewicz & Kish, 1986), is one disorder that causes serious disruption of lower urinary tract function. Patients with PD often exhibit irritable bladder symptoms such as urinary urgency, frequency and incontinence, which are induced by bladder hyperactivity (Pavlakis *et al*., 1983; Berger *et al*., 1987; Aranda & Cramer, 1993; Araki & Kuno, 2000; Araki *et al*., 2000). The incidence of lower urinary tract dysfunction is estimated to be as high as 50–70% in patients with PD (Pavlakis *et al*., 1983; Berger *et al*., 1987; Bonnet *et al*., 1997; Araki *et al*., 2000). However, little is known of the mechanisms inducing bladder hyperactivity in this disease.

It has been well documented that motor dysfunction such as bradykinesia or akinesia observed in PD results from the loss of striatal dopamine and in turn a reduction in D2 receptor-mediated inhibition of striatopallidal neurons, which leads to increases in the overall activity in the striatopallidal indirect pathways. However, it seems that autonomic dysfunction of the urinary bladder (e.g., bladder hyperactivity) is induced by mechanisms different from those inducing the motor dysfunction. Previous studies using rats, cats and monkeys have indicated that: (1) an activation of D1-like and D2-like dopamine receptors has different effects on bladder activity; (2) the dopaminergic pathway from substantia nigra exerts a tonic inhibition on bladder function through D1-like dopamine receptors; and (3) a deficiency in D1-like dopamine

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receptor activation rather than D2-like receptors in PD plays an important role in inducing bladder hyperactivity associated with parkinsonism (Kontani *et al*., 1990; Yoshimura *et al*., 1992; 1993; 1998; Yokoyama *et al*., 1999; Seki *et al*., 2001). Furthermore, in contrast to D1-receptor-mediated inhibition of micturition reflex in parkinsonian animals, an activation of D2-like receptors facilitated the micturition reflex (Kontani *et al*., 1990; Yoshimura *et al*., 1992; 1993; 1998; Yokoyama *et al*., 1999; Seki *et al*., 2001). In awake rats, systemic application of a D2-like receptor agonist (quinpirole) induced bladder hyperactivity which was blocked by a D2-like receptor antagonist (remoxipride) (Seki *et al*., 2001). Similarly, systemic application of quinpirole induced bladder hyperactivity in monkeys (Yoshimura *et al*., 1993; 1998), and treatment with bromocriptine, a D2 receptor agonist, exacerbated urinary frequency in humans with PD (Kuno *et al*., 1997). However, since D2 receptor-mediated facilitation of the micturition reflex was similarly found in normal and MPTP-induced parkinsonian monkeys (Yoshimura *et al*., 1993; 1998), and microinjection of dopamine to the pontine micturition center reduced bladder capacity and facilitated the micturition reflex in normal cats (de Groat *et al*., 1993), it is possible that D2-like receptor-mediated effects on bladder function might be mediated by dopaminergic mechanisms in systems other than the nigrostriatal dopaminergic pathways. A recent clinical study also suggested the possibility of an involvement of spinal dopamine receptors since sublingual application of apomorphine, a nonselective dopamine receptor agonist, induced bladder hyperactivity in patients with spinal cord injury in which reflex bladder contractions are triggered by spinal cord pathways (Steers *et al*., 2000).

Thus, the present study was performed to further clarify the pathophysiology of bladder dysfunction associated with PD by examining the location and subtypes of receptors involved in the dopaminergic control of micturition in rats with a 6-OHDA-induced unilateral lesion of the nigrostriatal pathway.

Methods

Animal preparation

Experiments were performed on adult male Sprague–Dawley rats (250–300 g). Care and handling of animals were in accordance with institutional guidelines and approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Unilateral lesions of the nigrostriatal pathway were induced by 6-OHDA injection (4 μg in 2 μl of 0.1% ascorbic acid in 0.9% saline solution) into the substantia nigra pars compacta on the left side (L: 2.2, H: −7.7, AP: −5.3 from bregma and skull) using a stereotaxic microinjector under ketamine/xylazine anesthesia (75 and 15 mg kg^{-1}, respectively, i.m.). Sham-operated animals were injected with vehicle solution in a similar manner. Animals were maintained for 2 weeks after treatment.

Measurement of micturition pattern

At 2 weeks after 6-OHDA injection, animals were kept in metabolic cages and micturition parameters including total urine output per 24 h, number of micturition per 24 h and voided volume per micturition were measured. Urine was collected on an electronic scale connected to a microcomputer. Data were stored using data acquisition software (Windaq, Dataq).

Cystometric evaluation of bladder function

Following the metabolic cage studies, a polyethylene tube (PE 50) was inserted into the bladder from the bladder dome with midline abdominal incision under halothane anesthesia. At 1–2 h after insertion of the bladder catheter, cystometrograms were obtained either in awake rats that received drugs intravenously (i.v.) or under urethane anesthesia (1.2 mg kg^{-1}, s.c.) in rats injected with drugs intracerebroventricularly (i.c.v.) or intrathecally (i.t.). Awake animals were placed in a restraining cage during cystometric evaluation. Bladder activity was monitored through an intravesical catheter connected to a pressure transducer. Physiological saline was continuously infused at a rate of 0.07 ml min^{-1}. Intravesical fluid voided from the urethral meatus was collected and measured to determine the voided volume. The data obtained in rats with 6-OHDA lesions were compared with those in sham-operated rats.

Drug administration

i.v. Injections were made through cannulas (PE-50) inserted into the right femoral vein. For i.t. injections, a catheter was inserted through a slit in the atlanto-occipital membrane to the level of L6-S1 spinal cord. The volume of fluid within the catheter was kept constant at 6 μl. Single doses of drugs were then administered in a volume of 2 μl followed by 7 μl flush with artificial cerebrospinal fluid. i.c.v. (intracerebroventricular) Injections were made into the lateral ventricle (L: 1.0, H: −5.1, AP: 0.3 from bregma and skull). Using a stereotaxic microinjector, a 30G needle attached with 10 μl Hamilton syringe was inserted into the lateral ventricle, and single doses of drugs were administered in a volume of 2 μl during 3 min. Before drug administration, either i.c.v or i.t., control injections of artificial CSF (2 and 10 μl, respectively) were tested to evaluate possible injection artifacts. The injection sites in the spinal cord and the lateral ventricle were confirmed by injection of dye (methylene blue) in every animal at the end of the experiments. Doses of each drug used in this study were determined according to the results of pilot experiments as well as previous experiments evaluating bladder function and behavioral changes in normal rats and parkinsonian monkeys (Yoshimura *et al*., 1992; 1993; 1998; Seki *et al*., 2001).

Histological analysis of 6-OHDA-induced lesion

After cessation of the experiments, the lesions induced by 6-OHDA injection were confirmed by the formaldehyde-induced fluorescence technique (Yoshimura *et al*., 1990). Under deep anesthesia with pentobarbital (100 mg kg^{-1}), the animal was perfused with phosphate buffer solution containing 4% formaldehyde, 0.5% glutaraldehyde, 0.2% picric acid and 2% glyoxylic acid to fix the nervous tissue and convert catecholamines to fluorescent derivatives. Then, the brain was removed, and cut into serial sections. Catecholamine fluorescence in the area of the substantia nigra was examined using a fluorescent microscope with ultraviolet filters (excitation wavelength: 365 nm) to confirm 6-OHDA-induced lesions.

Statistics

All data values are expressed as mean ± s.e. Statistical significance was determined with Mann–Whitney *U*-test. *P*-values less than 0.05 were considered to be significant.

Results

Histologic evaluation after 6-OHDA injection

A marked reduction in the number of neurons with catecholamine fluorescence was observed in the region of substantia nigra pars compacta on the lesioned side (left) injected with 6-OHDA, but not on the intact side (right), in all animals examined ($n=24$) (Figure 1). No difference in the number of neurons with catecholamine fluorescence was observed on the right and left sides of substantia nigra pars compacta of sham-operated rats (data not shown).

Micturition parameters in metabolic cages

In 6-OHDA-lesioned rats, voided volume per micturition averaged 0.41 ± 0.04 ml (mean ± s.e.m., $n=8$) in 24 h recordings using a metabolic cage (Figure 2a). This value was significantly smaller ($P<0.01$) than that in sham-operated rats (0.67 ± 0.07 ml, $n=7$). There was no significant difference in total urine volume per 24 h between sham and 6-OHDA-lesioned animals (11.2 ± 0.42 and 12.1 ± 0.37 ml day^{-1}, respectively).

Cystometric parameters in conscious animals

In sham-operated rats ($n=7$), a contraction of the urinary bladder with a maximum intravesical pressure of 41.0 ± 3.5 cmH_2O was induced at volume and pressure thresholds of 0.63 ± 0.07 ml and 6.3 ± 0.5 cmH_2O, respectively (Figure 2b and c). In contrast, in 6-OHDA lesioned rats ($n=8$), a bladder contraction was elicited with a significantly ($P<0.05$) smaller volume threshold (0.46 ± 0.05 ml) than that in sham-operated rats (Figure 2b and c). Pressure threshold and maximum voiding pressure in 6-OHDA-lesioned animals did not differ from those in sham-operated animals. In addition, no residual urine after voiding was found in either group of animals. These data together with those in the metabolic cage study indicate that 6-OHDA-induced lesion in the substantia nigra induces bladder hyperactivity as evidenced by a smaller bladder capacity for inducing the micturition reflex.

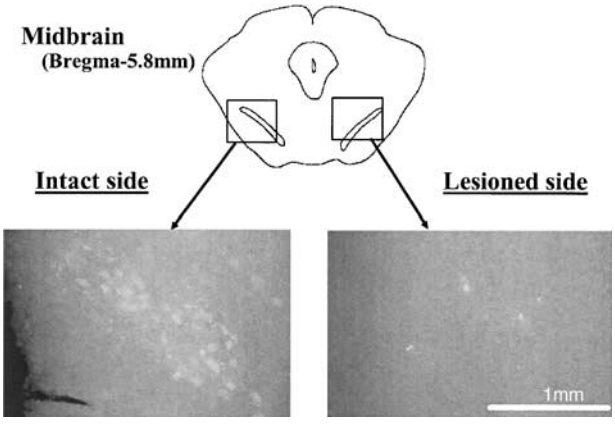

Figure 1 Photomicrographs of the substantia nigra showing cells with positive catecholamine fluorescence induced by the formaldehyde-induced fluorescence technique after 6-OHDA injection to the left side of substantia nigra (lesioned side). When compared with the intact side, there was a marked reduction of catecholamine-positive cells in the lesioned side where 6-OHDA was injected.

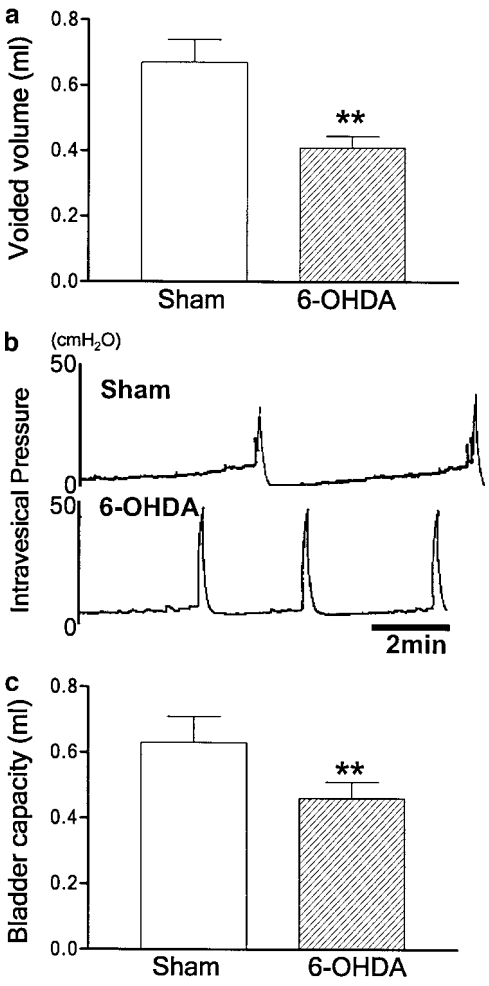

Figure 2 (a) Voided volume per micturition in a metabolic cage. Ordinate, voided volume (ml) per micturition. Note that 6-OHDA-treated rats ($n=8$) urinate with significantly smaller bladder capacity (0.41 ml) as compared with sham-operated rats (0.67 ml, $n=6$). $^{**}P<0.01$ vs sham-operated rats. (b) Representative traces of cystometrogram in sham (top) and 6-OHDA-treated (bottom) rats. Recordings were obtained in awake animals. Ordinate, bladder pressure in cmH_2O. (c) Averaged bladder capacity inducing voiding. Ordinate, bladder volume (ml). Note that the bladder volume threshold in 6-OHDA-treated rats (0.46 ml, $n=8$) was significantly smaller than in sham-operated rats (0.63 ml, $n=7$). $^{**}P<0.01$ vs sham-operated rats.

Effects of SKF 38393 (D1/D5 dopamine receptor agonist)

Systemic administration (conscious cystometry) In sham-operated awake rats, i.v. administration of SKF38393 at doses of 0.5 and 1.0 mg kg^{-1} elicited no apparent effects on the intercontraction interval (ICI) in cystometrograms ($n=5$) (Figure 3, Table 1). However, in 6-OHDA-lesioned rats,

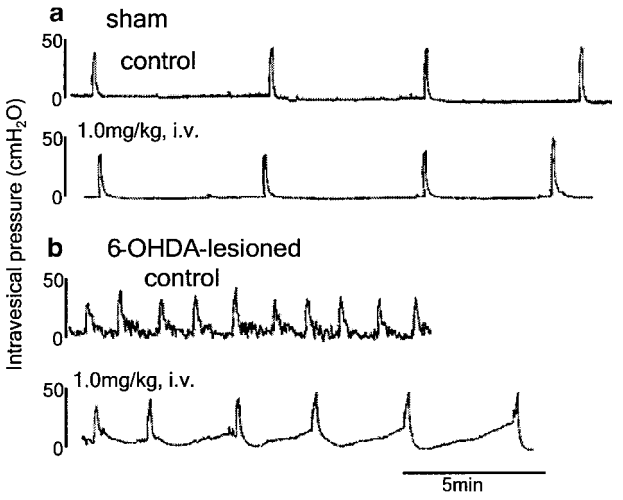

Figure 3 Effects of i.v. injection of SKF 38393 (1.0 mg kg^{-1}) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Upper panels show cystometrograms during control periods, and lower panels show the recordings 5 min after the drug application. Note that SKF 38393 increased intercontraction intervals in 6-OHDA-lesioned rats, but not in sham-operated rats.

SKF38393 produced an inhibitory effect on the micturition reflex in a dose-dependent manner (Figure 3). SKF38393 at doses of 0.5 and 1.0 mg kg^{-1}, i.v. significantly increased the ICI to 7.9±0.5 ($P<0.05$, $n=5$) and 9.3±0.7 min ($P<0.01$, $n=5$), respectively, from the control value (6.2±0.4 min) 5 min after the drug application. The ICI returned to control during the 30–40 min recording period. The mean relative increment in the ICI following SKF38393 application was 50.9±5.0% at the dose of 1.0 mg kg^{-1}. Pressure thresholds also significantly increased from 4.5±0.2 to 9.5±0.5 cmH_2O ($P<0.05$) after 1.0 mg kg^{-1} SKF 38393 (Figure 3). No significant changes were observed in voiding pressures following SKF 38393 application.

i.c.v. Administration Under urethane anesthesia, i.c.v. application of SKF38393 increased the ICI in both the groups of animals (Figure 4). The effects were observed almost immediately after injection, and were usually eliminated after 15–20 min in both the groups. SKF 38393 at a dose of 5 μg, but not 2 μg, slightly, but significantly increased ($P<0.05$) (21.1±6.2%) the ICI to 7.6±0.6 min from 6.3±1.1 min in sham-operated animals ($n=5$) (Figure 5, Table 1). In 6-OHDA-treated animals, the inhibitory effects induced by SKF38393 were greater than in sham-operated animals. SKF 38393 at doses of 2 and 5 μg (i.c.v.) significantly increased the

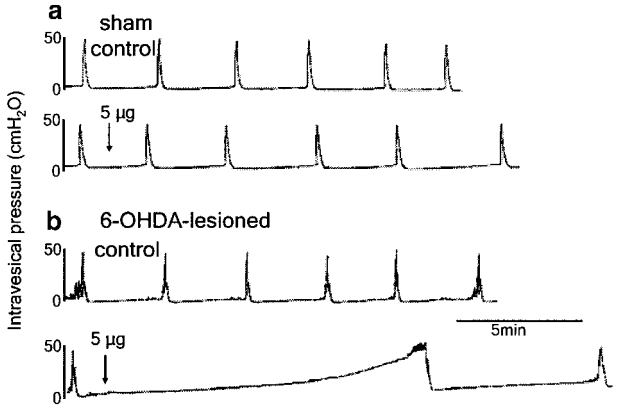

Figure 4 Effects of i.c.v. application of SKF 38393 (5.0 μg) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that SKF 38393 increased intercontraction intervals in both the groups of animals, and the effect was much greater in 6-OHDA-lesioned rats.

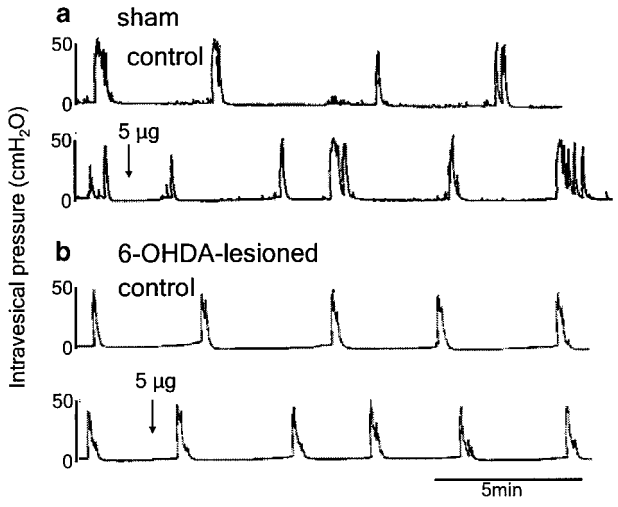

Figure 5 Effects of i.t. injection of SKF 38393 (5.0 μg) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that SKF 38393 did not induce significant changes in intercontraction intervals in either sham or 6-OHDA-lesioned rats.

Table 1 Effects of SKF 38393 on intercontraction intervals during cystometry

	i.v.	*i.c.v.*	*i.t.*
Sham	($N=5$)	($N=5$)	($N=4$)
Control	8.6±0.7	6.3±1.1	6.6±1.1
SKF 38393	8.5±0.9 (0.5 mg kg^{-1})	6.6±0.8 (2 μg)	6.8±1.0 (5 μg)
	8.9±0.6 (1.0 mg kg^{-1})	7.6±0.6* (5 μg)	
6-OHDA-treated	($N=5$)	($N=5$)	($N=4$)
Control	6.2±0.4	4.4±0.5	4.7±0.7
SKF 38393	7.9±0.5* (0.5 mg kg^{-1})	6.9±1.0* (2 μg)	4.9±0.6 (5 μg)
	9.3±0.7** (1.0 mg kg^{-1})	8.4±1.1** (5 μg)	

All values are expressed in minutes. *$P<0.05$, **$P<0.01$ compared with control values in each group of either sham-operated (sham) or 6-OHDA-treated rats.

ICI from 4.4±0.5 to 6.9±1.0 ($P<0.05$) and 8.4±1.1 min ($P<0.01$) with the mean relative ICI increments of 34.1±5.0 and 90.1±8.1%, respectively (Figure 4, Table 1).

i.t. Administration When SKF 38393 was administered i.t. at a dose of 5 μg, there was no significant change in the ICI in sham and 6-OHDA-lesioned groups (Figure 5, Table 1).

Effects of quinpirole (D2/D3/D4 dopamine receptor agonist)

Systemic administration (conscious cystometry) In sham-operated rats, i.v. administration of quinpirole at doses of 0.2 and 0.4 mg kg^{-1} significantly reduced the ICI in a dose-dependent manner to 5.3±0.6 ($P<0.05$, $n=5$) and 2.9±0.4 min ($P<0.01$, $n=5$), respectively, from control value (8.7±0.7 min) 5 min after the drug application (Figure 6, Table 2). The mean relative reduction of ICI by i.v. quinpirole (0.4 mg kg^{-1}) was 66.4±4.3%. The effects were observed 1–2 min after quinpirole application and partially recovered after 60–90 min. Similar results were obtained following i.v. quinpirole administration in 6-OHDA-lesioned rats (Figure 6, Table 2). Quinpirole at doses of 0.2 and 0.4 mg kg^{-1} significantly reduced the ICI to 4.1±0.5 ($P<0.05$, $n=5$) and 2.3±0.3 min ($P<0.01$, $n=5$), respectively, from the control value (6.3±0.6 min) 5 min after the drug application. The mean relative reduction of the ICI following quinpirole (0.4 mg kg^{-1}) injection in 6-OHDA-lesioned rats (62.9±5.0 %) was not different from that in sham-operated rats. No significant changes were found in micturition pressure thresholds or bladder contraction pressures following quinpirole application.

i.c.v. Administration Under urethane anesthesia, i.c.v. application of quinpirole reduced the ICI in both the groups of animals as found in i.v. administration (Figure 7, Table 2). Quinpirole at a dose of 2 μg, but not 1 μg, slightly, but significantly reduced the ICI from 6.9±1.0 to 6.1±0.8 min in sham-operated animals ($P<0.05$, $n=5$). Similarly, quinpirole (i.c.v.) at a dose of 2 μg significantly decreased the ICI from 4.0±0.6 to 3.4±0.4 min in 6-OHDA-lesioned rats ($P<0.05$, $n=5$) (Table 2). However, the stimulating effects on the micturition reflex were smaller than those induced by i.v. injection of quinpirole. The mean relative reduction of the ICI by i.c.v. quinpirole (2 μg) was not different between sham-operated (12.4±2.5%) and 6-OHDA-lesioned animals

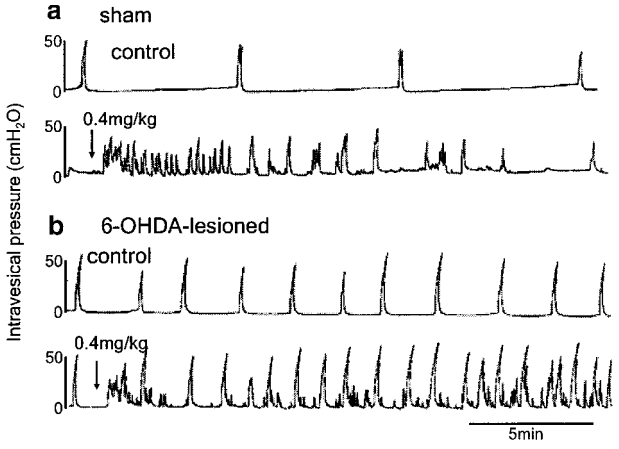

Figure 6 Effects of i.v. application of quinpirole (0.4 mg kg^{-1}) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that quinpirole reduced intercontraction intervals in both sham and 6-OHDA-lesioned rats.

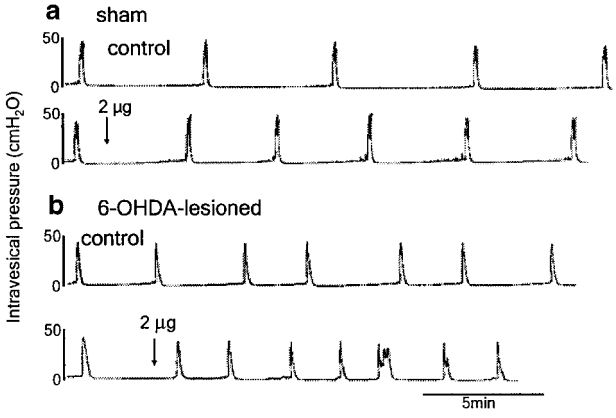

Figure 7 Effects of i.c.v. application of quinpirole (2.0 μg) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that quinpirole reduced intercontraction intervals in both sham and 6-OHDA-lesioned rats, but to a smaller degree than the effects seen after i.v. injection of quinpirole (Figure 6).

Table 2 Effects of quinpirole on intercontraction intervals during cystometry

	i.v.	*i.c.v.*	*i.t.*
Sham	($N=5$)	($N=5$)	($N=5$)
Control	8.7±0.7	6.9±1.0	6.5±1.1
Quinpirole	5.3±0.6* (0.2 mg kg^{-1})	6.7±0.9 (1 μg)	4.8±0.9* (1 μg)
	2.9±0.4** (0.4 mg kg^{-1})	6.1±0.8* (2 μg)	2.8±0.4 ** (2 μg)
6-OHDA-treated	($N=5$)	($N=5$)	($N=5$)
Control	6.3±0.6	4.0±0.6	4.2±0.8
Quinpirole	4.1±0.5* (0.2 mg kg^{-1})	3.9±0.7 (1 μg)	3.2±0.7* (1 μg)
	2.3±0.3** (0.4 mg kg^{-1})	3.4±0.4 * (2 μg)	1.7±0.2** (2 μg)

All values are expressed in minutes. *$P<0.05$, **$P<0.01$ compared with control values in each group of either sham-operated (sham) or 6-OHDA-treated rats.

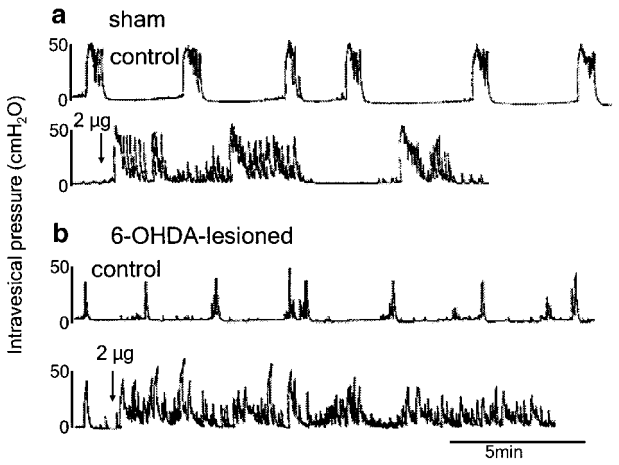

Figure 8 Effects of i.t. application of quinpirole (2.0 μg) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that quinpirole reduced intercontraction intervals in both sham and 6-OHDA-lesioned rats in a similar manner as observed after i.v. injection of quinpirole (Figure 6).

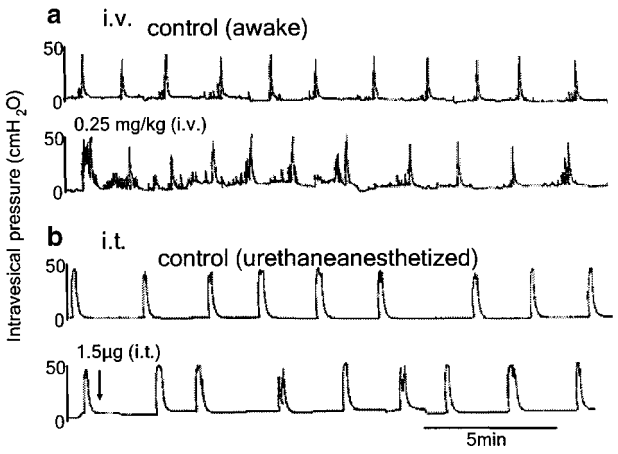

Figure 9 Effects of i.v. and i.t. application of PD128907 (0.25 mg kg^{-1} and 1.5 μg, respectively) on bladder activity in sham (a) and 6-OHDA-lesioned rats (b). Arrows indicate the time of drug administration. Note that PD128907 did not induce significant changes of intercontraction intervals in either sham or 6-OHDA-lesioned rats.

(16.0 $\pm$ 3.0%), but significantly smaller than those (64–66%) in animals receiving i.v. injection of quinpirole (0.4 mg kg^{-1}).

i.t. Administration In contrast to relatively small reduction in the ICI induced by i.c.v. injection of quinpirole, i.t. application of the drug (2 μg) decreased the ICI to a greater extent in sham-operated and 6-OHDA-lesioned animals (Figure 8). The ICI was decreased from 6.5 $\pm$ 1.1 to 2.8 $\pm$ 0.4 min and from 4.2 $\pm$ 0.8 to 1.7 $\pm$ 0.2 min in sham and 6-OHDA rats, respectively, almost immediately after i.t. injection of quinpirole (2 μg) (Figure 8, Table 2). The relative reduction in the ICI following 2 μg quinpirole (i.t.) in sham (56.8 $\pm$ 4.1%) and 6-OHDA rats (60.8 $\pm$ 5.0%) was comparable with those (64–66%) seen after i.v. injection of quinpirole (0.4 mg kg^{-1}).

Effects of PD 128907 (D3 dopamine receptor agonist)

When PD 128907 was administered i.v. (0.1 and 0.25 mg kg^{-1}) and i.c.v or i.t. (0.5 and 1.5 μg) in awake and urethane-anesthetized 6-OHDA-lesioned rats, respectively, no significant changes were observed in the ICI, pressure threshold and voiding pressure (Figure 9), suggesting that facilitatory effects of quinpirole on the micturition reflex were predominantly mediated by D2 or D4 dopamine receptors.

Discussion

The results of the present study indicate that degeneration of dopaminergic neurons in the nigrostriatal pathway causes bladder hyperactivity as demonstrated by a reduction in bladder capacity for inducing reflex bladder contractions, and that dopamine receptor agonists acting on D1/D5 receptors at a supraspinal site have therapeutic effects on bladder hyperactivity using a rodent model of 6-OHDA-induced parkinsonism. In contrast, dopamine receptor agonists acting on D2/D4, but not D3 receptors, had facilitatory effects on the micturition reflex, predominantly at a lumbosacral spinal site.

Idiopathic parkinsonism is known to be a disorder primarily caused by degeneration of dopaminergic neurons originating in the substantia nigra (Hornykiewicz & Kish, 1986). It has been documented that patients with Parkinson's disease often exhibit irritable urinary symptoms, such as urgency, frequency or urinary incontinence, and that bladder hyperactivity is the most common observation in urodynamic studies of these patients (Pavlakis *et al*., 1983; Berger *et al*., 1987; Aranda & Cramer, 1993). In the present experiments, awake rats with 6-OHDA-induced lesions in the nigrostriatal dopaminergic pathway exhibited bladder hyperactivity as evidenced by a reduction in bladder capacity during cystometrograms or in a metabolic cage. Thus, it seems that a rat model of Parkinson's disease produced by 6-OHDA-induced lesions in the nigrostriatal pathways exhibits bladder hyperactivity as observed in patients with PD as well as MPTP-lesioned parkinsonian monkey (Albanese *et al*., 1988; Yoshimura *et al*., 1993; 1998).

In this study, bladder hyperactivity in 6-OHDA-lesioned rats was suppressed by SKF 38393 that activates D1-like dopamine receptors (i.e., D1/ D5 subtypes). This is in line with previous studies using normal cats (Yoshimura *et al*., 1992) and MPTP-lesioned parkinsonian monkeys (Yoshimura *et al*., 1993; 1998) that central dopaminergic pathways have inhibitory effects on the micturition reflex through D1-like dopamine receptors. In the cat, the micturition reflex was inhibited by stimulation of the substantia nigra pars compacta, and this inhibition was antagonized by the D1/D5 dopamine receptor antagonist SCH 23390 injected into the lateral ventricle (Yoshimura *et al*., 1992). It has been also demonstrated in monkeys with parkinsonism produced by MPTP-induced damage of nigrostiatal dopaminergic pathways that SKF 38393 suppressed bladder hyperactivity (Yoshimura *et al*., 1993) and that D1- and D2-like receptor stimulation by pergolide or BAM-1110 produced D1-like receptor-mediated inhibition of bladder hyperactivity (Yoshimura *et al*., 1998). In addition, recent studies demonstrated that intravenous application of SCH 23390 facilitated the micturition reflex by

reducing bladder capacity, whereas SKF38393 did not change bladder volume thresholds for the micturition reflex in conscious rats (Yokoyama *et al*., 1999; Seki *et al*., 2001). These results are in accordance with our data in sham-operated rats that intravenous SKF 38393 had no effects on the micturition reflex, although it seems that direct stimulation of supraspinal D1/D5 receptors by i.c.v. SKF 38393 at a high dose can still suppress the micturition reflex. Overall, it is assumed that the dopaminergic pathway from the substantia nigra exerts a tonic inhibition on bladder function, which is nearly fully active under a normal condition. Thus, in this study, stimulation of D1/D5 dopamine receptors such as SKF 38393 had small effects in sham-operated rats. However, it is likely that this tonic inhibition becomes less active in PD, and therefore D1/D5 dopamine receptor agonists were more effective to suppress bladder activity in 6-OHDA-treated rats.

The present study also demonstrated that D1/D5 receptor-mediated inhibition of bladder hyperactivity in 6-OHDA-treated rats was observed when SKF 38393 was injected i.v. and i.c.v., but not when injected i.t. These results indicated that D1/D5 receptors located at a supraspinal site should be activated in order to suppress bladder hyperactivity in parkinsonism. It has been documented that the majority of dopaminergic neurons originating in the substantia nigra pars compacta project to neostriatal neurons in the basal ganglia, the predominant neurotransmitter of which is the inhibitory amino acid, γ-aminobutyric acid (GABA) (Di Chiara *et al*., 1994), and that an activation of D1/D5 receptors in these GABAergic neurons enhances cell excitability *via* stimulation of adenylyl cyclase activity (Nestler, 1994; Umemiya & Raymond, 1997). It has been shown that the supraspinal micturition reflex pathway is under tonic GABAergic inhibitory control (de Groat *et al*., 1993; de Groat & Yoshimura, 2001). Therefore, it could be speculated that the inhibitory effect on the micturition reflex induced by central D1/D5 receptors might be mediated by activation of the GABAergic system in the basal ganglia.

In contrast to the inhibitory effect through dopamine D1/D5 receptors, an activation of dopamine D2-like receptors (i.e., D2, D3 and D4 subtypes) by i.v. administered quinpirole exerted excitatory effects on the micturition reflex in both sham and 6-OHDA-treated rats. It was also noted in the previous experiments using cats and monkeys that quinpirole reduced the volume threshold for inducing bladder contractions (Yoshimura *et al*., 1992; 1993; 1998). In addition, a recent study by Seki *et al*. (2001) showed that i.v. application of quinpirole facilitated the micturition reflex by reducing bladder capacity in conscious rats, as found in this study. Thus, it seems likely that dopamine D2/D3/D4 receptors mediate the facilitatory effects on the micturition reflex. The present study further revealed that quinpirole-induced enhancement of bladder activity was more effective following i.t. injection at the lumbosacral spinal cord level, as compared to i.c.v. administration. Moreover, PD 128907 had no significant effects on bladder activity in sham and 6-OHDA-treated rats. It has been reported that PD 128907 binds with high affinity to D3 receptors, demonstrates at least 18–200-fold selectivity over other dopamine receptor subtypes such as D2 and D4 dopamine receptors (Witkin *et al*., 1998), and can selectively activate the D3 receptor when applied systemically at low doses between 0.01 and 0.3 mg kg^{-1} in previous studies examining extracellular dopamine levels in the ventral striatum (Zapata *et al*., 2001) and locomotor behavior (Pritchard *et al*., 2003). Thus, it is assumed that in the present study, PD 129807 at the dose up to 0.25 mg kg^{-1} selectively activated D3 dopamine receptors without apparent effects on bladder activity. Overall, these results indicated that activation of D2/D4, but not D3, receptor subtypes in the spinal cord can enhance bladder activity. The presence of D2-like dopamine receptors in the spinal cord has been reported previously by histochemical and functional studies (Kondo *et al*., 1985; 1986). In addition, a recent clinical study also suggested the possibility of an involvement of spinal dopamine receptors since sublingual application of apomorphine, a nonselective dopamine receptor agonist, induced bladder hyperactivity in patients with spinal cord injury in which reflex bladder contractions are triggered by pathways in the spinal cord (Steers *et al*., 2000).

Although L-dopa is still the mainstay in the treatment of symptoms in Parkinson's disease, its long-term use is associated with various adverse events, such as dyskinesia, motor fluctuations and psychiatric symptoms (Calne, 1993). Therefore, various attempts have been made to reduce the incidence and severity of motor fluctuations associated with long-term L-dopa therapy. Thus, centrally acting dopamine D2-like receptor agonists, such as bromocriptine, have been used and proven to be effective in the treatment of behavioral symptoms in patients with PD (Rinne, 1985), since a deficiency in stimulation of dopamine D2-like receptors is reportedly the major cause for the behavioral symptoms in PD (Baik *et al*., 1995). However, as demonstrated in this study, the dopamine D2-like receptor agonist might exacerbate urinary symptoms such as urinary urgency or frequency, since the D2-like receptor agonist quinpirole reduced the bladder volume threshold in 6-OHDA-treated parkinsonian rats. We have previously reported that patients with PD, who received the treatment with bromocriptine, exhibited urinary frequency and urgency, and that their urinary symptoms were relieved after substitution of bromocriptine with pergolide, a nonspecific dopamine receptor agonist (Kuno *et al*., 1997). Thus, it is assumed that additional D1/D5 receptor activation might be effective in treating irritative urinary symptoms in patients with PD.

Conclusion

These results indicate that a rat model of PD exhibited bladder hyperactivity as observed in patients with PD, and that stimulation of D1/D5 dopamine receptors at a supraspinal site can suppress bladder hyperactivity in PD. However, stimulation of D2/D4, but not D3, dopamine receptors at a spinal site had the opposite effect to induce bladder hyperactivity. Thus, D1/D5 dopamine receptor agonists might be effective in treating neurogenic bladder hyperactivity in PD.

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