Therapeutic Effects of Dopamine D$_1$/D$_2$ Receptor Agonists on Detrusor Hyperreflexia in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Lesioned Parkinsonian Cynomolgus Monkeys$^1$

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ABSTRACT
The effects of dopamine receptor agonists on urinary bladder function were evaluated in normal and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian cynomolgus monkeys to investigate the therapeutic efficacy in the treatment of urinary symptoms in Parkinson’s disease. Under ketamine anesthesia, cystometrograms exhibited significant reduction in the volume threshold for the micturition reflex in MPTP-lesioned parkinsonian monkeys when compared with those of normal monkeys. The selective dopamine D$_2$ receptor agonist bromocriptine significantly reduced the bladder volume threshold for the micturition reflex by 25 to 30% in both normal and MPTP-lesioned animals. The nonspecific D$	ext{1}$/D$	ext{2}$ receptor agonist pergolide significantly reduced the bladder volume threshold by 22% in normal monkeys, but increased the volume threshold by 50% in MPTP-lesioned parkinsonian monkeys.

Another D$_1$/D$_2$ agonist (5$^R$,8$^R$,10$^R$)-6-methyl-8-(1,2,4-triazol-1-ylmethyl) ergoline maleate (BAM-1110) also increased the bladder volume threshold (by 80%) in parkinsonian monkeys without significant effects on the micturition reflex in normal monkeys. The reduction in the volume threshold by bromocriptine in both normal and MPTP-treated groups and by pergolide in normal monkeys was suppressed by pretreatment with the selective D$_2$ antagonist sulpiride, whereas the increment in the volume threshold by pergolide and BAM-1110 in parkinsonian monkeys was antagonized by pretreatment with the selective D$_1$ antagonist SCH 23390, but not by sulpiride. These findings suggest that concurrent activation of D$_1$/D$_2$ receptors, rather than selective stimulation of D$_2$ receptors, might be beneficial for treating urinary symptoms caused by detrusor hyperreflexia in Parkinson’s disease.

The primary neuropathologic feature of Parkinson’s disease is a degeneration of the dopaminergic neurons in the substantia nigra pars compacta, which results in a marked loss of striatal dopamine concentration (Hornykiewicz and Kish, 1986). It has been well documented that patients with Parkinson’s disease often exhibit lower urinary tract dysfunctions, the predominant symptoms of which are irritative, such as urinary urgency, frequency or incontinence. In those patients, the most common finding of urodynamic study is detrusor hyperreflexia, defined as an involuntary contraction of the urinary bladder during the storage phase (Pavlakis et al., 1983; Berger et al., 1987; Aranda and Cramer, 1993).

Central dopamine receptors are categorized into two subfamilies, the D$_1$-like and D$_2$-like dopamine receptors (hereafter simply called D$_1$ and D$_2$) based on their positive or negative coupling to adenylyl cyclase, although five subtypes of dopamine receptors have been identified in various dopaminergic systems of mammalian brains (Civelli et al., 1993; Seeman and Van Tol, 1994). A study with D$_2$-receptor-deficient mice indicated that the lack of dopamine D$_2$ receptor activation primarily contributes to the emergence of behavioral symptoms in Parkinson’s disease (Baik et al., 1995). However, previous studies with normal cats and MPTP-lesioned monkeys revealed that bladder dysfunction associated with Parkinson’s disease is likely to be induced by a loss of input to dopamine D$_1$ receptors rather than D$_2$ receptors. In the cat, the micturition reflex was inhibited by stimulation of...
the substantia nigra pars compacta, and this inhibition was antagonized by the D₂ selective antagonist SCH 23390 injected into the lateral ventricle (Yoshimura et al., 1992). The inhibition of the micturition reflex also was mimicked by an injection of the D₁ selective agonist SK&F 38393 (Yoshimura et al., 1992). It also has been demonstrated that SK&F 38393 suppressed detrusor hyperreflexia found in MPTP-lesioned parkinsonian monkeys (Yoshimura et al., 1993).

Although L-dopa has been accepted as the most effective drug for the treatment of symptoms of Parkinson's disease, long-term therapy with L-dopa leads to a loss of drug efficacy and the development of drug-induced adverse effects such as dyskinesia, fluctuations in mobility and development of psychotic symptoms (Calne, 1993). Thus, various dopamine D₂ agonists such as bromocriptine or lisuride have been used to reduce these L-dopa-induced side effects. However, the clinical trials demonstrated that monotherapy with the dopamine D₂ receptor agonist is effective but insufficient to produce an adequate therapeutic effect in patients with Parkinson's disease compared with L-dopa therapy, which suggests that stimulation of dopamine D₂ receptors is essential, but concurrent stimulation of dopamine D₁ receptors also plays a pivotal role in the treatment of Parkinson's disease (Rinne, 1985, 1989; Lieberman et al., 1983). Previous studies showed that pergolide, a dopamine receptor agonist acting on both dopamine D₁ and D₂ receptors (Koller and Herbst, 1988) often can give rise to improvement in cases in which bromocriptine does not control the patients' symptoms satisfactorily (Lieberman et al., 1983; Goetz, 1985; Factor et al., 1988; Jenner, 1995), although pergolide exhibited higher affinity for D₂ receptors than for D₁ receptors by as much as 50- to 100-fold (Okumura et al., 1988; Fuller and Clemens, 1991). It also is reported that another D₁ and D₂ dopamine receptor agonist, BAM-1110, which exhibits binding selectivity to D₁ receptors twice as high as pergolide (D₂/D₁ ratio: 50 vs. 100) in radioligand binding experiments (Okumura et al., 1988), ameliorated behavioral symptoms in MPTP-lesioned parkinsonian monkeys without side effects such as psychiatric and gastrointestinal symptoms (Kuno et al., 1992). However, therapeutic effects of these dopamine D₁/D₂ receptor agonists on bladder dysfunction in parkinsonism has not yet been evaluated. In addition, our previous study suggested that treatment with the D₂ receptor agonist might aggravate the urinary symptoms in parkinsonian patients, because the selective D₂ receptor agonist quinpirole reduced the bladder volume threshold for the micturition reflex in MPTP-lesioned parkinsonian monkeys that exhibited bladder hyperreflexia (Yoshimura et al., 1993). Therefore, the present study examined the effects of dopamine receptor agonists such as bromocriptine, pergolide and BAM-1110 on detrusor activity in MPTP-lesioned parkinsonian monkeys to elucidate the therapeutic efficacy of these drugs on the irritable urinary symptoms observed in Parkinson's disease.

**Methods**

**Animal preparation.** Ten adult male cynomolgus monkeys (*Macaca fascicularis*, Clea, Japan) weighing 3.8 to 5.8 kg were used. As described previously, five animals were treated with three consecutive injections of MPTP hydrochloride (0.3 mg/kg i.v.; Aldrich, Milwaukee, WI) at an interval of 3 or 4 days, followed by several injections of the same dose of MPTP at 7-day intervals, with the cumulative dose of MPTP ranging between 26 and 34 mg (Yoshimura et al., 1993; Akai et al., 1995a, b). These five monkeys were confirmed to develop persistent parkinsonian symptoms such as mild to severe degree of postural tremor and poverty of movements, which continued more than 24 months after the last MPTP injection. The remaining five monkeys, which received no injections of MPTP, were used as controls. All animals were fed with fresh fruits and had free access to commercial pellets and water.

**Assessment of bladder function.** Under ketamine anesthesia (initial dose, 10 mg/kg i.m.; supplemental doses, 5 mg/kg i.m. when required), cystometrograms were obtained with two catheters (3-Fr and 4-Fr Ureteric catheters, Rusch, Germany) inserted into the urinary bladder through the urethra. Intravesical pressure was recorded via one catheter (3 Fr size) using a pressure transducer (Nihon Kohden, TP-200T) connected to an amplifier (Nihon Kohden, AP-600G) and a pen recorder (Rikadenki Kohyo, R-031) with continuous infusion of physiological saline into the urinary bladder via the other catheter (4 Fr size) at a rate of 5 ml/min. To compare changes among cystometrograms, three parameters such as volume and pressure thresholds of the bladder for inducing reflex bladder contractions, and the maximum intravesical pressure during bladder contractions were measured on each cystometrogram. Bladder contractions were confirmed by fluid leakage around the catheter. Cystometrograms examining the effect of dopamine receptor agonists were carried out 15, 30, 60, 90 and 120 min after each drug injection.

**Drug administration.** Bromocriptine mesylate (Sigma, St. Louis, MO); pergolide mesylate (Research Biochemicals Inc. (RBI), Natick, MA) and BAM-1110 (Maruco, Aichi, Japan) were injected subcutaneously in physiological saline. SCH 23390 (RBI) and sulpiride (Dogmatyl Injection; Fujisawa Pharmaceutical Co., Osaka, Japan) were administered subcutaneously 15 and 60 min before the injection of test drugs, respectively. Doses of each drug used in this study were determined according to those reported previously to be effective in behavioral studies with MPTP-lesioned parkinsonian monkeys (Kuno et al., 1992; Akai et al., 1995b). All drugs were administered with an injection volume of 0.1 mg/ml, except for sulpiride which was administered in an appropriate volume at the concentration of 50 mg/kg. A washout period of at least 3 days, which had been confirmed as long enough to eliminate the effect of each test drug in the previous behavioral studies, elapsed between drug treatments (Kuno et al., 1992b; Akai et al., 1995b).

**Statistics.** All data values are expressed as mean ± S.E. Statistical significance was determined with Mann-Whitney U test, one-way repeated measures analysis of variance and Dunnett's tests for multiple comparisons where appropriate. P values less than 0.05 were considered to be significant.

**Results**

**Cystometric parameters of normal and MPTP-lesioned monkeys.** Before drug administration, three consecutive cystometrograms in each animal were obtained at 15-min intervals. The averages of parameters such as volume and pressure thresholds for reflex micturition, and maximum intravesical pressure during the bladder contractions in three consecutive cystometrograms of each animal were used as predrug control values. In normal monkeys, a contraction of the urinary bladder with a maximum intravesical pressure of 41.0 ± 3.5 cm H₂O (n = 5) was induced at volume and pressure thresholds of 61.8 ± 9.5 ml (range; 41–90 ml) and 6.3 ± 0.5 cm H₂O, respectively (fig. 1). In contrast, in MPTP-treated monkeys, a bladder contraction was elicited with a significantly (P < .05) smaller volume threshold (29.8 ± 2.5 ml; range, 25–38 ml; n = 5) than that in control monkeys,
indicating that parkinsonian animals exhibited hyperreflexic bladder function (fig. 1). Pressure threshold and maximum intravesical pressure in MPTP-lesioned animals did not differ from those in normal animals.

**Effects of bromocriptine.** Subcutaneous administration of bromocriptine at doses of 0.5 and 1.0 mg/kg in normal monkeys significantly reduced the volume threshold for bladder contractions to 47.7 ± 6.3 ml (n = 4) and 42.9 ± 7.7 ml (n = 4), respectively, from control values (60.9 ± 7.0 ml and 61.1 ± 9.6 ml, respectively) 60 min after drug application in a dose-dependent manner. These effects induced by bromocriptine were observed 30 min after drug administration and lasted more than 120 min (fig. 2). Similarly, in MPTP-lesioned monkeys the volume threshold for bladder contractions was reduced significantly to 20.9 ± 0.5 ml and 19.4 ± 2.2 ml (n = 4) from control (27.3 ± 1.5 ml and 27.7 ± 1.6 ml, respectively) 60 min after drug application in a dose-dependent manner. These effects induced by bromocriptine were observed 30 min after drug administration and lasted more than 120 min (fig. 2). Similarly, in MPTP-lesioned monkeys the volume threshold for bladder contractions was reduced significantly to 20.9 ± 0.5 ml and 19.4 ± 2.2 ml (n = 4) from control (27.3 ± 1.5 ml and 27.7 ± 1.6 ml, respectively) 60 min after drug application in a dose-dependent manner. The mean relative reductions of volume threshold by bromocriptine (1.0 mg/kg) were not different between normal (29.8 ± 5.5%) and MPTP-lesioned parkinsonian monkeys (30.2 ± 3.2%) (fig. 2). Subcutaneous administration of sulpiride (30 mg/kg) 60 min before the application of bromocriptine at a dose of 1.0 mg/kg suppressed the reduction in volume thresholds by bromocriptine in both normal (n = 3) and MPTP-lesioned animals (n = 3) (fig. 2).

**Effects of pergolide.** In normal monkeys, subcutaneous administration of pergolide at doses of 0.025 and 0.05 mg/kg significantly reduced the volume threshold for inducing bladder contractions to 52.1 ± 4.4 ml (n = 4) and 48.1 ± 4.0 ml (n = 4), respectively, from control values (60.9 ± 7.0 ml and 61.4 ± 7.3 ml, respectively) 30 min after the drug application (fig. 3). The mean relative reduction of volume threshold by pergolide (0.05 mg/kg) was 21.7 ± 5.3% (fig. 3). The effects were observed 15 min after pergolide application and partially recovered after 120 min. This reduction in volume threshold was antagonized by sulpiride (30 mg/kg) administered 60 min before pergolide (n = 3) (fig. 3). On the contrary, in MPTP-lesioned parkinsonian monkeys, subcutaneously administered pergolide produced an inhibitory effect on the micturition reflex in a dose-dependent manner (fig. 3). Pergolide at doses of 0.025 and 0.05 mg/kg significantly increased the volume threshold for inducing bladder contractions to 37.4 ± 2.9 ml (n = 4) and 45.3 ± 7.7 ml (n = 4), respectively, from control (28.9 ± 2.9 ml and 30.3 ± 2.0 ml, respectively) 30 min after the drug application, and the effect was eliminated partially during the recording period of 120 min (fig. 3). The mean relative increments in volume thresholds after pergolide application were 26.1 ± 7.8% (0.025 mg/kg) and 49.2 ± 15.4% (0.05 mg/kg). The
increment in volume threshold by pergolide in MPTP-lesioned parkinsonian monkeys was blocked when SCH 23390 (0.03 mg/kg) was administered subcutaneously 15 min before the 0.05 mg/kg dose of pergolide (fig. 3). However, the pre-treatment with sulpiride (30 mg/kg) exhibited no significant effects on pergolide-induced increment of volume threshold \((n = 3)\) (fig. 3).

Effects of BAM-1110. When BAM-1110, at doses of 0.2 and 0.4 mg/kg, was injected subcutaneously in normal monkeys, no significant changes in any parameters of cystometrograms were observed (fig. 4). However, in MPTP-lesioned parkinsonian monkeys, subcutaneously administered BAM-1110 resulted in inhibition of the micturition reflex in a dose-dependent manner, as seen, but to larger extent, with pergolide. BAM-1110 at doses of 0.2 and 0.4 mg/kg, respectively, suppressed the micturition reflex by increasing volume thresholds to 43.5 ± 2.5 ml \((n = 4)\) and 54.1 ± 7.4 ml \((n = 4)\) from control value \((30.0 ± 2.9 \text{ ml and } 30.2 ± 2.5 \text{ ml, respectively})\) 30 min after the drug application (fig. 4). The relative increments in volume threshold after BAM-1110 administration averaged 47.0 ± 6.5% \((0.2 \text{ mg/kg})\) and 79.3 ± 12.2% \((0.4 \text{ mg/kg})\). The increased bladder volume by BAM-1110 usually returned to the control value 90 to 120 min after drug application. As noted with pergolide, the increment in volume threshold by BAM-1110 \((0.4 \text{ mg/kg})\) in MPTP-lesioned parkinsonian monkeys was blocked by SCH 23390 \((0.4 \text{ mg/kg s.c.; } n = 3)\) but not by sulpiride \((30 \text{ mg/kg, } n = 3)\) (fig. 4).

Discussion

The results of the present study indicate that dopamine receptor agonists acting on both dopamine D1 and D2 receptors have therapeutic effects on detrusor hyperreflexia in a primate model of MPTP-induced parkinsonism. This effect was in contrast to the treatment with the selective D2 agonist in which the micturition reflex was facilitated.

Idiopathic parkinsonism is a disorder primarily caused by degeneration of dopaminergic neurons originating in the substantia nigra (Hornykiewicz and 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 in urodynamic studies detrusor hyperreflexia is the most common observation in these patients (Pavlakis et al., 1983; Berger et al., 1987; Aranda...
and Cramer, 1993). MPTP-lesioned parkinsonian monkeys in our study also exhibited bladder hyperreflexia detected by a reduction in cystometric bladder capacity. Bladder hyperreflexia also was reported in marmosets with MPTP-induced parkinsonism (Albanese et al., 1988). Thus the present experiments, together with results from previous studies, indicate that MPTP-lesioned parkinsonian monkey is a suitable model for studying bladder dysfunction associated with Parkinson’s disease.

The micturition reflex is mediated by a spino-bulbo-spinal pathway. In this pathway, the sensory signal arising from the urinary bladder is conveyed to the micturition center located in the rostral pons, and its output then reaches the urinary bladder through the sacral parasympathetic preganglionic neurons, thereby producing contractions of the urinary bladder (de Groat et al., 1993; Yoshimura and de Groat, 1997). Electrical stimulation of the basal ganglia including the substantia nigra pars compacta inhibits the micturition reflex in the cat (Lewin et al., 1967; Yoshimura et al., 1992). In addition, this inhibition of the micturition reflex by stimulation of the substantia nigra was blocked by an injection of the D1 selective antagonist SCH 23390 into the lateral ventricle, and also was mimicked by an intracerebroventricular application of the D1 selective agonist SK&F 38393 (Yoshimura et al., 1992). Thus it is plausible that dopaminergic neurons originating in the substantia nigra pars compacta inhibit the micturition reflex mediated by central dopamine D1 receptors, and that the bladder hyperactivity observed in patients with Parkinson’s disease can be explained by degeneration of dopaminergic neurons in the substantia nigra, which leads to a removal of inhibitory effects on the micturition reflex mediated by dopamine D1 receptors. This is in line with our previous finding that SK&F 38393 suppressed detrusor hyperreflexia found in MPTP-lesioned parkinsonian monkeys (Yoshimura et al., 1993).

The mechanism underlying the D1 receptor-mediated inhibition of the micturition reflex remains unclear. However, it has been documented that most 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 GABA (Di Chiara et al., 1994), and that an activation of D1 receptors in these GABAergic neurons potentiate cell excitability via stimulation of adenylyl cyclase activity (Nestler, 1994; Umemiyia and Raymond, 1997). It has been documented that supraspinal micturition reflex pathway is under tonic GABAergic inhibitory control (de Groat et al., 1993). Therefore, the inhibitory effect by central D1 receptors on the micturition reflex might be mediated by the potentiation of the GABAergic system in the basal ganglia.

In contrast to the inhibitory effect through dopamine D1 receptors, an activation of central dopamine D2 receptors by bromocriptine exerted excitatory effects on the micturition reflex in normal and MPTP-lesioned monkeys. It also was noted in the previous experiments with cats and monkeys that the D2 selective agonist quinpirole reduced the volume threshold for inducing bladder contractions (Yoshimura et al., 1992, 1993). Thus it seems likely that dopamine D1 and D2 receptors mediate the opposite effects on the micturition reflex. The present study also suggested that in the normal monkey stimulation of dopamine D1/D2 receptors elicits predominantly a D2-receptor-mediated excitatory effect on the micturition reflex, because the reduction of volume threshold by pergolide was antagonized by the selective D2 receptor antagonist sulpiride, although the other D1/D2 receptor agonist BAM-1110 did not show significant changes in the micturition reflex in normal monkeys. The differences between the effects of pergolide and BAM-1110 in the normal animal might be explained by the higher selectivity of BAM-1110 for D2 receptors, which mediate inhibitory effects on the micturition reflex, than pergolide (Okumura et al., 1988). The facilitatory effects of other nonselective D1/D2 receptor agonists on the micturition reflex also have been demonstrated in the previous experiments with L-dopa or apomorphine in the normal rat, in which L-dopa applied systemically or apomorphine applied either systemically, intrathecally or intracerebroventricularly induced bladder hyperactivity (Sillén et al., 1981; Kontani et al., 1990a, b). It, therefore, seems reasonable to assume that the D2-receptor-mediated facilitation of the micturition reflex is the predominant action in the normal condition.

The present study demonstrated a remarkable difference in the responses to D1/D2 receptor stimulation in the MPTP-lesioned parkinsonian monkey. In contrast to the predominance of the D2-receptor-mediated facilitatory effect in the normal animal, the D1/D2 receptor stimulation by pergolide or BAM-1110 in MPTP-lesioned parkinsonian monkeys produced inhibitory effects on the micturition reflex mediated by dopamine D1 receptors, because the inhibitory effects by pergolide or BAM-1110 in parkinsonian monkeys were antagonized by the administration of the selective D1 receptor antagonist SCH 23390, but not by that of sulpiride. In addition, BAM-1110, whose binding efficacy to D1 receptors is twice as potent as pergolide (Okumura et al., 1988), was more effective in increasing the bladder volume threshold of parkinsonian monkeys than pergolide (relative increase, 80% vs. 50%). Profound inhibitory effects mediated by dopamine D1 receptors in parkinsonism also have been demonstrated in previous experiments in which the selective D1 receptor agonist SK&F 38393 increased the bladder volume threshold in MPTP-lesioned monkeys, but not in normal monkeys (Yoshimura et al., 1993). Although the precise reason for the predominant inhibitory effect via D1 receptors on D1/D2 receptor stimulation in MPTP-lesioned monkeys is unknown, it might be explained by receptor supersensitivity after degeneration of dopaminergic neurons because binding studies have shown that the number of D1 receptors is increased significantly in the brain of parkinsonian patients (Rinne et al., 1985). In a recent study, an alteration in the responses to D1/D2 receptor stimulation also has been found directly by measuring neuronal activity in the subthalamic nucleus with the rat with 6-hydroxydopamine-induced lesions of the nigrostriatal pathway (Kresis et al., 1997).

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. Centrally acting dopamine D2 receptor agonists, such as bromocriptine, have been used and proven to be effective in the treatment of behavioral symptoms in patients with Parkinson’s disease (Rinne, 1985). This is in accordance with the previous findings that a deficiency in stimulation of
dopamine D2 receptors is the major cause for inducing behavioral symptoms in Parkinson’s disease (Baik et al., 1995). However, as demonstrated in this study, the selective dopamine D2 receptor agonist might exacerbate the urinary symptoms such as urinary urgency or frequency, because the D2 receptor agonist bromocriptine reduced the bladder volume threshold in parkinsonian monkeys with detrusor hyperreflexia. Indeed, our recent preliminary study revealed that patients with Parkinson’s disease who received bromocriptine treatment suffered from urinary frequency and urgency, especially during the night, and that their urinary symptoms were greatly relieved 4 weeks after substitution of bromocriptine with pergolide (Kuno et al., 1997). Recent experimental and clinical studies also have demonstrated that a combination of D1 and D2 receptor activation might have greater benefit in treatment of behavioral symptoms associated with Parkinson’s disease than activation of either receptor type alone (Lieberman et al., 1983; Goetz, 1985; Factor et al., 1988; Jenner, 1995; Akai, 1995a, b). In addition to this clinical suggestion that the D1/D2 receptor agonists such as pergolide or BAM-1110 also have therapeutic efficacy in the improvement of urinary symptoms in patients with Parkinson’s disease.

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References


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