Effects of Apomorphine on Urinary Bladder Motility in Anesthetized Rats

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Abstract—We studied the effects of apomorphine (AM) on bladder motility in anesthetized rats in which Tyrode’s solution was continuously infused into the bladder at a constant rate, inducing an almost constant rate of bladder contraction accompanying micturition. AM at a dose of 1 mg/kg, i.v., caused a hyperactive bladder response, during which micturition disappeared. AM (12.5 μg for intracerebroventricular (i.c.v.) injection or 50 μg for intrathecal (i.t.) injection also caused a hyperactive response in about half of the rats. Supersensitization to AM appeared in reserpine-treated rats (2.5 mg/kg, i.p., 48 and 24 hr before the experiment). Haloperidol (1 mg/kg, i.v.) or SCH 23390 (5 mg/kg, i.v.) completely suppressed the hyperactive bladder response induced by AM (5 mg/kg, i.v.), and then the bladder contraction accompanying micturition reappeared after administration of these drugs. Pretreatment with sulpiride (100 mg/kg, i.p.) for 60 min, which hardly affected the bladder contraction induced by infusion of Tyrode’s solution, suppressed the hyperactive bladder response induced by AM. These results suggest that the hyperactive bladder response induced by i.v.-injected AM results from synchronous stimulation of the micturition reflex centers in the brain stem and sacral cord and that the hyperactive bladder response is elicited via both D1 and D2 receptors.

With regard to the dopaminergic neurons in rats that affect urinary bladder motility, Sillén et al. (1-3) have shown that dopamine receptors exist in the micturition reflex center of the mesencephalic-pontine region and that a hyperactive urinary bladder response is induced upon stimulation of these receptors with dopaminergic agonists. Although urinary bladder motility is also controlled by the micturition reflex center in the sacral cord, very little is known about the effects of dopamine agonists on this center. Recently, we studied the effects of diazepam, baclofen and morphine on the micturition reflex centers in both the brain stem and sacral cord and found that the inhibitory effects of these drugs on bladder motility were related to both of these micturition reflex centers (4-6). In the present experiments, the effects of apomorphine (AM), which stimulates dopaminergic receptors, on urinary bladder motility were examined in an attempt to provide additional evidence for dopaminergic innervation in the micturition reflex centers of the brain stem and sacral cord.

Materials and Methods

The methods used for recording bladder contraction were described in our previous papers (4-6). Male Wistar rats (weighing 250–350 g) were anesthetized with urethane (1.0 g/kg, s.c.) and α-chloralose (25 mg/kg, s.c.). The bladder was exposed through a midline incision in the abdomen, and a needle (1/4) attached to a silicone tube (O.D., 1.0 mm and I.D., 0.5 mm, 30–40 cm in length) was inserted into the bladder through the left ureter. After ligation of the left ureter around the needle, the bladder was put back into the abdominal cavity, and the incision was sutured. Bladder contraction was induced by infusion of Tyrode’s solution lacking glucose.
into the bladder through the silicone tube at a constant rate (0.8–1 ml/10 min), and the pressure signals were measured by a pressure transducer (Nihon Kohden, LPU-0.1) connected to the silicone tube via a T-tube.

Drugs were administered intravenously through the femoral vein or into the lateral ventricle by intracerebroventricular (i.c.v.) injection or subarachnoid space of the sacral cord by intrathecal (i.t.) injection according to the methods described in our previous papers (4, 5).

After i.v. injection of AM, the duration from the appearance to the disappearance of the pattern which clearly indicated a hyperactive bladder response was measured in the cystometrogram. After i.c.v. or i.t. injection of AM, the latency to the appearance of the hyperactive bladder response was also measured.

Reserpine (2.5 mg/kg, i.p.) was administered two times, 48 hr and 24 hr before the experiment.

Drugs used were apomorphine HCl and sulpiride (Sigma). haloperidol (Serenace®, Dainippon Pharmaceutical Co., Ltd.), reserpine (Apoprone®, Daiichi Pharmaceutical Co., Ltd.) and SCH 23390 (a gift from Essex Nippon Co., Ltd.). AM and SCH 23390 were dissolved in saline adjusted to about pH 5 with HCl. Sulpiride was suspended in 0.5% carboxymethylcellulose solution. All drug concentrations in this report are expressed as those of each respective salt.

Statistical analyses were carried out by Student's t-test at P<0.05 or P<0.01. Experimental values were expressed as the mean±S.E.

Results

1) Effects of i.v. injection of AM on bladder contraction induced by infusion of solution into the bladder: Figure 1 shows a typical cystometrogram after i.v. injection of AM (1 mg/kg). Injection of 1 mg/kg AM (i.v.) produced repetitive bladder contraction, and the collecting phase of the solution almost disappeared. The peak pressure level of the repetitive bladder contraction was higher than that of the contraction before AM injection, and solution leaked continuously from the penis during repetitive contraction. The pattern of pressure change during the repetitive bladder contraction was different in each preparation, and repetitive bladder contraction was interrupted periodically. Then the bladder contraction accompanying micturition reappeared when the hyperactive bladder response disappeared. The duration of the hyperactive bladder response was 40.5±3.3 min (n=6). AM at 0.1 mg/kg (i.v.) caused repetitive bladder

Fig. 1. Typical urinary bladder response induced by i.v.-injected apomorphine in an anesthetized rat. Tyrode's solution was instilled into the bladder from the left ureter at a constant rate, and an almost constant rate of bladder contraction accompanying micturition was induced before injection of apomorphine. Vertical bar: bladder pressure, Horizontal bar: time after apomorphine injection. The solid circle indicates injection of the drug.
contraction in only one out of five rats, and the duration was about 10 min. After AM injection (5 mg/kg, i.v.), repetitive bladder contraction continued for 40–60 min, and then contraction and relaxation of the bladder appeared alternately at intervals of about 30 sec. The peak pressure during the bladder contraction was higher than that before injection of AM. Though the time at which the hyperactive bladder response disappeared was not clear, it took 85.3±2.8 min (n=4) after injection of AM (5 mg/kg, i.v.) for the appearance of a cystometrogram pattern showing a clear collecting phase followed by a contraction capable of producing micturition.

Haloperidol (1 mg/kg, i.v.) or SCH 23390 (5 mg/kg, i.v.) raised the micturition threshold, i.e., the level at which the bladder pressure rose steeply, and prolonged the micturition interval; and the inhibitory effects of these drugs continued for 30–40 min, although they did not interrupt micturition (Fig. 2 and Table 1). As these dopamine antagonists inhibited the bladder motility, the hyperactive bladder response was induced by AM (5 mg/kg, i.v.) beforehand, and then the effects of these drugs on the response were studied. In all four rats, haloperidol (1 mg/kg, i.v.) or SCH 23390 (5 mg/kg, i.v.) suppressed the repetitive bladder contraction, and then the bladder contraction accompanying micturition reappeared (Fig. 3). On the other hand,

![Image](image_url)

**Fig. 2.** Effects of i.v.-injected haloperidol and SCH 23390 on the urinary bladder contraction induced by infusion of Tyrode's solution into the bladder in anesthetized rats. For details, see Fig. 1 legend.

| Table 1. Effects of haloperidol, SCH 23390 and sulpiride on urinary bladder contraction |
|-----------------------------------------------|
|                                | Haloperidol (1 mg/kg, i.v.) (n=4) | SCH 23390 (5 mg/kg, i.v.) (n=4) | Sulpiride (100 mg/kg, i.p.) (n=9) |
|                                | before injection | after injection 15 min | after injection 60 min | before injection | after injection 15 min | after injection 60 min | before injection | after injection 15 min | after injection 60 min |
| MI mean                        | 5.3 | 10.1** | 5.8 | 8.0 | 24.0** | 11.4 | 7.3 | 7.0 |
| S.E.                           | 1.3 | 0.9 | 1.1 | 2.7 | 2.2 | 3.8 | 3.0 | 1.5 |
| MT mean                        | 4.9 | 15.5* | 6.7 | 5.5 | 20.1* | 4.6 | 4.6 | 4.2 |
| S.E.                           | 1.2 | 3.2 | 1.1 | 1.4 | 2.7 | 1.6 | 1.7 | 1.5 |
| PP mean                        | 31.1 | 33.6 | 28.9 | 31.2 | 30.1 | 26.9 | 29.3 | 25.6* |
| S.E.                           | 0.9 | 1.3 | 2.6 | 1.8 | 1.7 | 1.0 | 2.4 | 2.3 |

MI: micturition interval (min), MT: micturition threshold pressure (cmH\(_2\)O), PP: peak pressure during bladder contraction (cmH\(_2\)O). Bladder contraction was induced by continuous infusion of Tyrode's solution into the bladder at a constant rate. *P<0.05, **P<0.01, statistical difference from the value before drug injection.
AM (1 mg/kg, i.v.) caused repetitive bladder contraction when sulpiride (10 mg/kg, i.p.) was injected two times about 15 min before and after injection of AM in all four rats. Though at 60 min after injection of sulpiride (100 mg/kg, i.p.) the peak pressure during bladder contraction was slightly reduced (Table 1), AM (1 mg/kg, i.v.) hardly induced repetitive bladder contraction when it was added 60 min after injection of sulpiride in all four rats (Fig. 4). AM (5 mg/kg, i.p.) caused repetitive bladder contraction when it was added 60 min after injection of sulpiride (100 mg/kg, i.p.), and the duration was 31.4±4.2 min (n=5).

2) Effects of i.c.v. or i.t. injection of AM and haloperidol: Although, as shown in Fig. 5, i.c.v. or i.t. injection of AM (12.5 µg or 50 µg, respectively) also caused repetitive bladder contractions like that observed after i.v. injection of AM; such contractions were only induced in 5 out of 9 rats after i.c.v. injection and in 3 out of 5 rats after i.t. injection (Fig. 5). The latency of the hyperactive response after i.c.v. injection of AM (12.5 µg) and the duration of repetitious bladder contraction in the 5 rats for which a clear hyperactive response could be observed were 8.2±1.7 and 11.8±1.5 min, respectively. In the case of i.t. injection of AM (50 µg), the latency and the duration in the 3 rats for which a clear hyperactive response could be observed were 10.0±2.8 and 21.3±3.6 min, respectively. Haloperidol (10 µg, i.c.v. or 20 µg, i.t.) also exerted inhibitory effects on bladder contraction like those observed after i.v. injection, and it did not
interrupt micturition in all four rats. As i.c.v. or i.t. injection of AM did not always induce repetitive bladder contraction, the antagonistic effect of i.c.v. or i.t. injection of haloperidol on the hyperactive bladder response induced by i.v. injection of AM (5 mg/kg) was examined. Repetitive bladder contraction induced by AM (5 mg/kg, i.v.) was suppressed by i.c.v.-injected (10 μg) or i.t.-injected (20 μg) haloperidol, after the appearance of the hyperactive bladder response induced by AM (Fig. 6) in all four rats. SCH 23390 (25 μg, i.c.v. or 100 μg, i.t.) also suppressed the hyperactive bladder response induced by AM (5 mg/kg, i.v.) in all four rats.

3. Response to AM in reserpine-treated rats: There was no significant difference in the micturition interval and the peak pressure during bladder contraction between control rats and reserpine-treated rats, and the micturition threshold in reserpinized rats was high in comparison with that in control rats.

### Table 2. Cystometrogram in control rats and reserpine-treated rats

|                          | Control (n=6) | Reserpine-treated (n=8) |
|--------------------------|--------------|-------------------------|
| Micturition interval (min)| 6.9±1.6      | 8.4±2.1                 |
| Micturition threshold pressure (cmH2O) | 3.7±0.3 | 6.8±1.4*                |
| Peak pressure during bladder contraction (cmH2O) | 32.8±2.4 | 32.2±1.5                |

Bladder contraction was induced by continuous infusion of Tyrode's solution into the bladder at a constant rate. When the cystometrogram became constant or the interval of bladder contraction was almost constant, AM was injected. The three parameters were measured in the bladder contraction before the injection. Reserpine (2.5 mg/kg, i.p.) was administered two times, 48 and 24 hr before the experiment. Each value indicates the mean±S.E. * Statistical difference in variance from the control group (P<0.01).
In all four rats pretreated with reserpine, AM injected i.v. (0.1 mg/kg) (Fig. 7), i.c.v. (12.5 µg) or i.t. (50 µg) caused repetitive bladder contractions. The mean latency and S.E. after i.c.v. injection of AM and the mean duration of repetitive bladder contraction and S.E. for four rats were 0.9±0.2 and 46.5±7.4 min, respectively, whereas the corresponding values after i.t. injection of AM were 5.5±1.1 and 36.3±5.5 min, respectively.

**Discussion**

In this experiment, AM changed the pattern of bladder contraction from one with accompanying micturition to a repetitive one. The repetitive bladder contraction in our model was thought to correspond to the hyperactive bladder response described by Sillén et al. (1–3). As i.t., as well as i.c.v., injection of AM could induce a hyperactive bladder response like that after i.v. injection (Fig. 5), it appears that the micturition center in the sacral cord is also involved in the generation of this response and that the severe hyperactive bladder response after i.v. injection is due to synchronous stimulation of both micturition centers in the brain stem and sacral cord. In addition, it was observed that the peak pressure during repetitive bladder contraction was higher than the peak pressure before injection of AM. This suggests an in-
crease in the muscle tone of the urethra, which in turn would contribute to the generation of repetitive bladder contraction because the stretch receptors in the bladder wall are distended when the bladder contracts without expelling the solution, and high-frequency afferent discharges generated in the receptors would continuously stimulate the micturition centers (7).

When haloperidol and SCH 23390 abolished the repetitive bladder contraction induced by AM, the bladder contraction accompanying micturition reappeared (Fig. 3). This finding suggests that there is a dopaminergic nervous system which elevates the excitability of neurons in the micturition reflex centers and suggests that this dopaminergic nervous system together with the afferent pathway from the bladder separately stimulate the micturition reflex centers. Both D₁ and D₂ receptor subtypes are related to the generation of the hyperactive bladder response because SCH 23390 and sulpiride, and haloperidol, specific dopamine D₁ and D₂ receptor antagonists, respectively (8, 9), could suppress the hyperactive bladder response. With regard to the effect of sulpiride, a high dose (100 mg/kg, i.p.) was necessary to suppress

Fig. 6. Antagonistic effects of i.c.v. (A) and i.t. (B)-injected haloperidol on the hyperactive urinary bladder response induced by apomorphine in anesthetized rats. For details, see Fig. 1 legend.
the response. The different effects of various doses of sulpiride: 20 mg/kg, i.p. and 40 and 80 mg/kg, i.p. on passive avoidance response in mice have been explained by blockade of the dopamine autoreceptor and the postsynaptic receptor, respectively (10). If this explanation could be applied to our results in rats, the hyperactive bladder response induced by AM would result from stimulation of the postsynaptic dopamine receptor, but not stimulation of the autoreceptor.

In reserpine-treated rats, the cystometrogram pattern was not significantly different from that in control rats (Table 2). On the other hand, the effects of AM on bladder motility were sensitized by reserpine treatment. This suggests that the dopaminergic system does not tonically influence the micturition reflex centers. Detrusor hyperreflexia was reported in reserpine-treated rats when bladder contraction was induced by a method different from ours (11). As in our rat preparation (12) and in that of others (13), the inhibitory influence of hypogastric nerves on bladder motility was weak in rats, depletion of catecholamine would not necessarily induce the hyperreflexia.

In some patients with Parkinson's disease, levodopa has been reported to aggravate the detrusor hyperreflex (14). Study of the hyperactive bladder response induced by a dopamine agonist in rats would help to elucidate the mechanism of the hyperreflex in such patients. An investigation of the interaction of dopamine receptor subtypes is now in progress.

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