In Vivo Effects of Drugs that Act on the Autonomic Nervous System on the Rat Urinary Bladder Contraction Accompanying Micturition

Hitoshi KONTANI, Hiroshi KOBAYASHI, Yoichi KAWABATA
and Ryozo KOSHIURA
Department of Pharmacology, Hokuriku University, School of Pharmacy,
Ho-3 Kanagawa-machi, Kanazawa 920-11, Japan

Accepted September 27, 1986

Abstract—We prepared an experimental system to study the effects of drugs on urinary bladder contraction and micturition simultaneously in rats anesthetized with urethane (1 g/kg, s.c.) and α-chloralose (25 mg/kg, s.c.). When Tyrode's solution was infused at a constant rate (0.8–1 ml/10 min) through a needle inserted into the bladder from the left ureter, the bladder pressure gradually and then steeply rose, and micturition took place. These changes in bladder pressure and micturition were constantly repeated. In this model, drugs which partially inhibited the bladder contractile force, e.g., atropine (0.01–1 mg/kg, i.v.) and hexamethonium (C6, 5 mg/kg, i.v.) increased the frequency of bladder contraction instead of decreasing the amount of solution excreted from the penis by bladder contraction. The rate of afferent discharges during bladder filling was increased after injection of atropine or C6, and this increase was considered to be responsible for the induction of the increase in the frequency of bladder contraction. Drugs which inhibited the bladder contraction and interrupted micturition, e.g., C6 (20 mg/kg, i.v.) raised the bladder pressure until the solution leaked from the penis. As phentolamine (5 mg/kg, i.v.) or propranolol (1 mg/kg, i.v.) did not facilitate bladder motility but rather inhibited it, the inhibitory action of sympathetic nerves on bladder motility was considered to be weak in rats. This model was useful for studying the effect of drugs on bladder motility and micturition reflex.

There have been many studies carried out to investigate the effects of drugs on urinary bladder contraction or the micturition reflex in rats. Most of them have investigated the effects of drugs on the rhythmic contractions induced by saline loading of the urinary bladder (1–10). With the saline loading method, however, micturition cannot be observed, since the cannula is inserted through the urethra. Barrington (11) described the act of micturition as being a combination of six reflexes, five of them being triggered by urine flowing through the urethra. We therefore developed a model using rats in which urinary bladder contraction was induced by the infusion of solution from the ureter, so that micturition could be observed simultaneously. Using this model, we investigated the effects of drugs which had previously been examined by the saline loading method, and we compared both sets of results.

Materials and Methods

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 urinary bladder was exposed through a midline incision of the abdomen, and the abdominal muscle was transected. The urinary bladder was then prepared for the recording of intravesicular pressure as follows: a needle (1/4) attached to one end of a silicone tube (O.D., 1.0 mm, I.D., 0.5 mm;
30–40 cm in length) was inserted into the urinary bladder through the left ureter. The left ureter was ligated around the needle so that the urine from the left kidney flowed out from the incision. The right ureter was kept intact. The other end of the tube was connected to a syringe and a pressure transducer (Nihon Kohden, MPU-0.5) by means of a T tube. The whole system was filled with Tyrode’s solution lacking glucose. In order to infuse the Tyrode’s solution into the urinary bladder at a constant rate (0.8–1.0 ml/10 min), the plunger of the syringe was continuously pushed by that of another syringe into which water was infused with a peristaltic pump (Atto, SJ-1211). The pressure signals were delivered by an amplifier (Nihon Kohden, RP-5) and recorded by a D.C. recorder (Watanabe Sokki, SR 6204). Cotton-wool swabs soaked with Tyrode’s solution were laid on the bladder to keep it moist, and the swabs were warmed with a lamp.

In order to record the afferent activities from the urinary bladder receptors, the left pelvic nerve fibers were separated from the fibers innervating the rectum and resected. The peripheral ends of the fibers were placed on a pair of platinum electrodes and covered with paraffin oil. Afferent impulses were amplified with an amplifier (Nihon Kohden, AVB-2) and displayed on an oscilloscope (Nihon Kohden, VC-9). The same impulses were then transformed into unit square-wave pulses and fed into an integrator (time constant, 0.1 sec). The amplitudes of these afferent impulses were very small and the spikes could not be completely separated from background noise, so the trigger level of the integrator was adjusted so that it counted only the spikes with a voltage higher than an arbitrary threshold. The output voltage of the integrator was recorded by a D.C. recorder (Hitachi, 056) together with the bladder pressure measured by a pressure transducer (Nihon Kohden, LPU-0.1). The oscilloscope display was photographed on a moving film (Nihon Kohden, RLG-6101). When the left pelvic nerves were stimulated, rectangular pulses of 1 msec duration and 5 V strength, generated from a stimulator (Nihon Kohden, S-5039), were applied at 5 Hz for 30 sec.

All of the drugs except tetrodotoxin (TTX) were injected into the femoral vein. The solution containing TTX was dropped onto the cotton wool laid over the bladder by means of a syringe. The drugs for i.v. or topical administration were dissolved in saline or Tyrode’s solution, respectively. Each drug concentration was examined in at least 4 different rats.

Drugs used were: atropine sulfate (Merck), tetrodotoxin (Seikagaku Kogyo), hexamethonium chloride (Wako Pure Chem.), phentolamine methanesulfonate (Regitine, Takeda Pharm., Co.) and propranolol hydrochloride (Inderal, I.C.I. Pharm.). All drug concentrations were expressed as those of each respective salt.

Results

1) General: When Tyrode’s solution was infused into the bladder, the pressure in the bladder initially began to rise gradually but only slightly, and then it rose steeply to 30–50 cm H₂O. When the sharp bladder contraction occurred, with subsequent spasmodic contractions, micturition took place, and there was a stream-like emission of four to ten drops of solution from the penis. After each micturition, the pressures returned to almost the same level as that existing before bladder filling began. Bladder contraction was induced at almost a constant interval in each preparation, but the numbers of bladder contractions over a 10-min period ranged from two to six in different animals.

Figure 1A shows the afferent discharges recorded from the left pelvic nerves, and Fig. 1B shows the afferent discharges and pressure change in the bladder simultaneously. The high-frequency afferent discharges synchronized with bladder contraction could be recorded, and the rate of afferent discharges decreased before the bladder pressure reached its peak.

When the peripheral ends of the left pelvic nerves were electrically stimulated, the bladder pressure rose to almost the same level as that induced by the infusion of solution and then fell after micturition (Fig. 1C).

2) Effects of atropine on bladder con-
traction was decreased and it flowed along the penis. The bladder pressure after micturition returned to a slightly higher level, as compared with that before atropine injection. In two out of six rats that were injected with atropine (0.01 mg/kg, i.v.), repetitive contractions occurred. During the contractions, the solution was not excreted by every bladder contraction and only leaked from the penis. The contractions continued for 30 to 60 min. When the infusion of solution was interrupted for one or two minutes, the patterns of repetitive contraction were almost unchanged (Fig. 2B).

3) Effects of hexamethonium (C6) on bladder contraction: C6 (5 mg/kg, i.v.) reduced the peak pressure during bladder contraction and significantly increased the frequency of bladder contraction (Table 1 and Fig. 3). Although the solution was excreted from the penis by every bladder contraction, the amount of solution excreted by one contraction was decreased, and it flowed along the penis. The effects of C6 at this dose were very similar to those of atropine. C6 (10 mg/kg, i.v.) inhibited the bladder contraction in half of the rats; and at a higher dose (20 mg/kg, i.v.) C6 inhibited it in all of four rats. During interruption of micturition, the bladder pressure gradually rose until the solution leaked from the penis. Although small bladder contractions appeared, the solution was not excreted by the contractions, but merely leaked from the penis. Micturition was resumed 10 to 30 min after the injection (Fig. 3). When atropine was injected in incremental doses (0.01−1 mg/kg, i.v.), the bladder contractions continued but C6 (20 mg/kg, i.v.) inhibited the contractions and interrupted micturition for a while (Fig. 4).

4) Effects of phentolamine and propranolol on bladder contraction: Although after injection of phentolamine (5 mg/kg, i.v.) the peak pressure during bladder contraction was slightly but significantly reduced, the solution was excreted by every bladder contraction, and the numbers of bladder contractions over a 10-min period hardly changed before and after injection (Table 1). Propranolol (1 mg/kg, i.v.) inhibited the bladder contraction within a time range of from 3.5 to 20 min in four rats (Fig. 5).
Fig. 2. The effects of atropine on the response induced by infusion of Tyrode’s solution into the rat urinary bladder. A: Typical tracing showing the bladder contraction accompanying micturition after injection of atropine. B: Typical tracing showing repetitive bladder contraction after the injection of atropine (0.01 mg/kg, i.v.). Vertical bar: bladder pressure. Horizontal bar: time after atropine injection. For the period indicated by the dashed line in (B), infusion of Tyrode’s solution was interrupted.

5) Effects of TTX, atropine or C6 on afferent activity: The infusion of solution was begun to examine the effects of drugs on afferent discharges at least 60 min after the left pelvic nerve resection. Each effect of atropine or C6 on the rate of afferent discharges synchronized with bladder contraction was studied in eight rats. In three (atropine) or four (C6) out of eight rats, a gradual increase in the rate of afferent discharges according to the bladder filling could be recorded; in the preparations, the rate of afferent discharges gradually increased in parallel with a gradual rise in bladder pressure, and then high-frequency afferent discharges that synchronized with a steep rise in the bladder pressure appeared. When TTX (1 x 10^-5 M) was topically applied to
Table 1. The effects of drugs on the frequency of bladder contraction and the peak pressure at the urinary bladder contraction induced by constant infusion of solution into the urinary bladder in anesthetized rats

<table>
<thead>
<tr>
<th>(mg/kg, i.v.)</th>
<th>Numbers of bladder contractions during 10 min drug administration</th>
<th>% of peak pressure during bladder contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
</tr>
<tr>
<td>Atropine sulfate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>3.0±0.2</td>
<td>4.8±0.3**</td>
</tr>
<tr>
<td>0.1</td>
<td>3.2±0.4</td>
<td>5.8±0.3**</td>
</tr>
<tr>
<td>1</td>
<td>4.5±0.8</td>
<td>7.8±0.7*</td>
</tr>
<tr>
<td>Hexamethonium HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>4.8±1.0</td>
<td>11.4±1.4*</td>
</tr>
<tr>
<td>Phentolamine methanesulfonate</td>
<td>3.1±0.1</td>
<td>3.4±0.6</td>
</tr>
</tbody>
</table>

Each value is a mean±S.E. The peak pressure in the bladder was the mean pressure for 10 min before and after administration of drugs (n=4). *P<0.05, when compared with the value before drug administration. **P<0.01, when compared with the value before drug administration.

Fig. 3. The effects of hexamethonium on the response induced by infusion of Tyrode's solution into the rat urinary bladder. Vertical bar: bladder pressure. Horizontal bar: time after hexamethonium injection.

The bladder dome, the bladder contraction and afferent discharges disappeared in four out of five rats (Fig. 6A). Since the spikes could not be completely separated from background noise, the level of the integrator did not return to zero after the disappearance of afferent discharges. In rats with the left pelvic nerve resected, atropine (1 mg/kg, i.v.), as well as C6 (20 mg/kg, i.v.), inhibited the bladder contraction. After interruption of micturition, the rate of afferent discharges occurring during bladder filling showed a sustained increase. When the bladder slightly contracted, afferent discharges that synchronized with the bladder contraction appeared (Fig. 6, B and C).
Fig. 4. The effects of increasing doses of atropine and hexamethonium on the response induced by the infusion of Tyrode's solution into the rat urinary bladder. Vertical bar: bladder pressure. Horizontal bar: time after first injection of atropine.

Discussion

Tang and Ruch (12) reported that the cystograms in unanesthetized cats consisted of three segments: an initial rise, an initial limb and an ascending limb. In our present experiment using anesthetized rats, the gradual rise followed by a steep rise in the bladder pressure which was recorded may correspond to the initial limb followed by the ascending limb in the above cat cystograms, and no pressure change corresponding to the...
initial rise was found, since the solution remained in the bladder after micturition. In the saline loading method, changes in the bladder pressure due to the continuous production of urine have been described (9), but it was possible to neglect these in the present model. In other previous studies on rats, stimulation of the pelvic nerve has been performed using electrodes placed around the ureter a few millimeters from the bladder (10, 13). In the present model, as the bladder pressure change induced by stimulation of the resected left pelvic nerve was very similar to that induced by excitation of the intact right nerves, ligation of the left ureter did not injure the nerves (Fig. 1C). With regard to the afferent discharge from the bladder receptors recorded in this model, the discharge rate increased according to the bladder contraction, and then decreased before the bladder pressure had reached its peak (Fig. 1B). These changes in the rate of afferent discharges were consistent with those described by Iggo (14) in the bladder of anesthetized cats. Since TTX inhibited the bladder contraction in the present method, the bladder contractions in our model are considered to be of neurogenic origin, like those in the saline loading method (5, 7). From these results, it was considered that an almost normal micturition reflex could be induced by the infusion of solution through the ureter into the bladder. Therefore, the effects of drugs on bladder contraction were studied using this model. Atropine, at a low dose (0.01 mg/kg, i.v.), reduced the contractile force of the bladder, and this was not significantly decreased further by increasing the dose of atropine (Table 1 and Fig. 2A). The decrease in the amount of solution excreted as a result of the suppressed bladder contraction could be compensated for by the observed increase in the frequency of bladder contraction. It has been reported that atropine at similar doses completely suppresses the rhythmic contractions induced by the saline loading method (6, 7). In our study, atropine (0.01 mg/kg, i.v.) sometimes caused these repetitive contractions, during which no micturition occurred (Fig. 2B). Barrington (11) reported that the reflex produced by running water through the urethra relaxed the urethra and effected bladder contraction. Accordingly, when micturition takes place, it may be necessary for the urethra or the outlet of the bladder to be relaxed. Since the bladder contraction in this model and the rhythmic contractions produced in the saline loading method were inhibited by C6 at a dose of 20 mg/kg, i.v. (Fig. 3), this dose of C6 was necessary for blocking the transmissions of pelvic ganglia (6). As the solution was continuously infused into the bladder, the bladder pressure rose until the solution leaked from the penis when the bladder contraction was inhibited by the
Fig. 6. The effects of topical application of tetrodotoxin (A) and i.v. injection of atropine (B) or hexamethonium (C) on the afferent activity (top trace) from the left pelvic nerve and the response (bottom trace) induced by the infusion of Tyrode's solution into the rat urinary bladder in rats with the left pelvic nerve resected. Vertical bar: spike frequency of discharges and bladder pressure. Horizontal bar: time after drug injection.

drug. When drugs which partially suppress the contractile force of the bladder, for example C6 (5 mg/kg, i.v.) or atropine, were applied in the present method, the frequency
of bladder contraction was increased (Table 1). Bladder contraction is triggered by the afferent activities from the bladder receptors (14, 15). When the effects of these drugs on the afferent discharges were investigated, the rate of afferent discharges during bladder filling showed a sustained increase after the interruption of micturition by atropine or C6 (Fig. 6, B and C). The increase in the rate of afferent discharges would be related to the increase in the frequency of bladder contraction. The distension of the bladder wall due to solution retention would stimulate the bladder receptors.

With regard to the sympathetic nerve innervation of the rat bladder, an excitatory α-receptor (2) and an inhibitory β-receptor (2, 4) have been reported on the detrusor muscle, and it has also been reported that reserpine induces hyperreflexia (5). In cat and dog bladder (16, 17), sympathetic inhibition is mediated by an α-receptor in the pelvic ganglia and by a β-receptor on the detrusor muscle. In the present study, phentolamine (5 mg/kg, i.v.) and propranolol (1 mg/kg, i.v.) inhibited bladder contraction (Fig. 5). When these drugs were injected at a lower dose, no facilitation of bladder motility could be detected (our unpublished data). In rats, stimulation of hypogastric nerves had no effect on bladder motility (13). The inhibitory influence on bladder motility mediated by sympathetic nerves was weak, and so these sympatholytic drugs were considered not to facilitate bladder motility. In addition, the inhibitory effects of these drugs would not result from effects on the bladder.

As in the present method the urethra was left intact, and bladder contraction and micturition could be observed simultaneously, it would seem to be a useful approach for investigating the effects of drugs on both bladder motility and the micturition reflex.

References
11 Barrington, F.J.F.: The component reflexes of micturition in the cat. Parts I and II. Brain 54, 177-188 (1931)