Effects of Adrenergic Agonists on an Experimental Urinary Incontinence Model in Anesthetized Rabbits

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ABSTRACT—We have developed an experimental urinary incontinence model in anesthetized female rabbits, in order to study the effects of alpha-adrenergic receptor agonists on it in vivo. Micturition was induced artificially by electrical stimulation of the abdomen of rabbits receiving a continuous infusion of glucose-free Tyrode's solution into the urinary bladder. Alpha-1 adrenergic agonists, phenylephrine (1 mg/kg, i.v.) and the newly synthesized agent ST-1059 (1 mg/kg, i.v.) and its prodrug midodrine (10 mg/kg), which was intraduodenally administered, elevated the bladder pressure and arrested micturition induced by electrical stimulation. Prazosin (0.1 mg/kg, i.v.) inhibited these effects of phenylephrine. The effect of an alpha-2 agonist, clonidine (1 mg/kg, i.v.), on micturition induced by electrical stimulation was not clearly defined. This study demonstrates that alpha-1 adrenergic agonists can arrest artificially-induced micturition via urethral contraction. This method may be useful for evaluating the effect of a drug on urethral leakage in vivo.

The base of the urinary bladder and the proximal urethra have an important role in the storage and voiding mechanism of micturition. The distribution of receptors in the urinary bladder and urethra, and the responses of these organs to drugs, have been widely investigated in rabbits (1-14). These experiments were carried out in isolated muscle strips of these organs. In the present experiment, we tried to develop a model of urinary incontinence in experimental animals to study the effects of drugs on it in vivo. Since urine leakage during physical exercise is a frequent complaint in women and sympathomimetic drugs are used for the treatment of urinary incontinence, female rabbits were used in this experiment and we studied the effects of alpha-adrenergic agonists and an antagonist, including the new alpha-1-selective adrenergic agent ST-1059 and its prodrug midodrine, on the urinary incontinence induced by our method. As midodrine is absorbed from the intestine and then enzymatically changes to the active form ST-1059 in the liver (15), we studied the effect of midodrine, which was intraduodenally administered.

MATERIALS AND METHODS

The basic procedures for bladder infusion and recording of bladder motility were adapted from our experimental system designed for rats, as described in a previous paper (16). Female rabbits (weighing 1.5 to 2.5 kg) were anesthetized with urethane (1 g/kg, i.p.) and alpha chloralose (25 mg/kg, i.p.). An abdominal incision to the left of the midline was made, through which the bladder was exposed. A needle (19G), which was attached to a silicone tube (outside diameter, 2 mm; inside
diameter, 1 mm; 30–40 cm long), was inserted into the bladder through the left ureter. The ureter was ligated to the needle, the bladder was replaced in the abdominal cavity and the incision was sutured. The urine from the left kidney flowed into the abdominal cavity from the incision in the ureter through which the needle was inserted. The right ureter was left intact. When cystometrography was performed, glucose-free Tyrode's solution was infused into the bladder at a constant rate (approximately 8.2 ml/10 min) through the silicone tube, and the intravesical pressure signals were transformed by a pressure transducer (Nihon Kohden, LPU-0.1) connected to the silicone tube via a T-tube, delivered by an amplifier (Nihon Kohden, RP-5) and recorded by a DC recorder (Watanabe Sokki, SR 6204).

In order to press the bladder and induce micturition from the urethra artificially, the abdominal muscle was contracted by electrical stimulation. Two disk electrodes (1-cm diameter) were placed 5–6 cm apart on the abdominal skin virtually above the bladder to the right of the midline, and electrical stimulation consisting of 5-Hz pulses, 10 msec duration and 50 V intensity, was performed with an electrical stimulator (Nihon Kohden, S-5039). Micturition was detected by a drinkometer (Muromachi Kikai, model LA-1). The solution was collected by a funnel and dripped onto a pair of silver wires, which were connected to the drinkometer. The signals generated in the drinkometer when the electrodes short-circuited were recorded by a DC recorder together with the cystometrogram.

Electrical stimulation of the abdomen was performed about one hour after commencement of the bladder infusion. The selected minimum duration of electrical stimulation which induced micturition (5, 10 or 15 sec) in each rabbit was selected. Before injection of drugs, the electrical stimulation was repeated for the minimum time approximately every 5 or 10 min, and it was confirmed that micturition occurred reproducibly. In most of the rabbits that received electrical stimulation, spontaneous micturition did not occur. The rabbits which did not conform to the above protocol were not used. In the rabbits in which electrical stimulation performed about every 5 min caused micturition reproducibly, electrical stimulation was performed at an interval of about 5 or 10 min after drug injection. In the rabbits in which a 10-min interval was necessary to induce reproducible micturition, electrical stimulation was performed approximately every 10 min alter drug injection. To evaluate the effects of drugs on micturition induced by electrical stimulation, the time from drug injection to the first micturition induced by electrical stimulation or the spontaneous bladder contraction was measured. Intravenous (i.v.) injections were administered via an ear vein, and intraduodenal (i.d.) injections were administered via a cannula inserted into the duodenum at the time of surgery.

The drugs used in this experiment were: l-phenylephrine HCl (Tokyo Kasse), midodrine HCl (alpha-(2,5-dimethoxyphenyl)-beta-glycin-amido-ethanol HCl) and ST-1059 (alpha-(2,5-dimethoxyphenyl)-beta-aminoethanol) (gifts from Taisho Pharm. Co., Ltd.), clonidine HCl (Sigma) and prazosin (a gift from Pfizer Co., Ltd.).

Statistical analyses were carried out by Student's t-test; the values measured were expressed as means ± S.E., and differences at P values of less than 0.05 were considered to be significant.

RESULTS

Relation between bladder pressure and micturition

Fifty-one rabbits were used in this series of experiments. When Tyrode's solution was continuously infused into the bladder, continuous leakage from the urethra without elevation of bladder pressure occurred in six rabbits, and the rabbits were not included in this experiment. In 38 rabbits, the bladder produced spontaneous contractions which were accompanied with micturition (Fig. 1), and the peak
pressure during micturition ranged from 3.7 to 66.0 cmH2O. In these rabbits, electrical stimulation also caused both elevation of bladder pressure and micturition simultaneously. In an additional 4 rabbits, the bladders failed to develop spontaneous contraction, but electrical stimulation caused the elevation of bladder pressure followed by micturition. In 42 rabbits, electrical stimulation on the abdomen caused elevation of bladder pressure and micturition. The bladder pressure elevation induced by electrical stimulation ranged from 3.7 to 68.7 cmH2O, and micturition began during or just after the electrical stimulation (Figs. 1 and 2). In the other three rabbits, the bladders had contracted intensely and were small in size during surgery; therefore, bladder infusion alone generated pressure, and micturition was not well-associated with the increase in bladder pressure induced by spontaneous bladder contraction or electrical stimulation (Fig. 3).

Effects of drugs on the micturition induced by abdominal electrical stimulation

In 42 rabbits in which electrical stimulation on the abdomen induced micturition, effects of various drugs were examined (Figs. 1 and 2). In three rabbits, the effects of relative low and high doses of phenylephrine (0.1 mg/kg and 1 mg/kg, i.v.) on micturition induced by electrical stimulation were studied. In another five rabbits, the effects of a high dose of phenylephrine (1 mg/kg, i.v.) were investigated, since the first electrical stimulation performed after injection of phenylephrine (0.1 mg/kg, i.v.) induced micturition in all three rabbits. Phenylephrine (1 mg/kg, i.v.) elevated the bladder pressure in 16 of 23 rabbits, and the mean value of the peak response was 15.6 ± 3.5 cmH2O (n = 23). As shown in Fig. 1, urethral leakage did not occur during the
After injection of phenylephrine (1 mg/kg, i.v.), electrical stimulation elevated the bladder pressure, but micturition induced by electrical stimulation was arrested for 29.0 ± 4.7 min (n = 8). The antagonism of the action of phenylephrine by prazosin was investigated in 15 rabbits. The effect of phenylephrine (1 mg/kg, i.v.) on the arrested period of micturition was inhibited by prazosin at 0.1 mg/kg, i.v., but not at 0.01 mg/kg, i.v. (Fig. 1 and Table 1). In ten out of 15 rabbits in which the antagonistic effect of prazosin was studied, phenylephrine (1 mg/kg, i.v.) clearly elevated the bladder pressure. The values of the elevation of bladder pressure induced by phenylephrine before and after injection of prazosin (0.1 mg/kg, i.v.) were 32.9 ± 6.3 and 3.2 ± 2.0 cmH₂O (n = 7), respec-
Fig. 3. Cystometrogram traces from an anesthetized female rabbit in which bladder infusion alone generated high pressure, and the effects of prazosin on the cystometrogram. Tyrode's solution was instilled into the bladder from the left ureter at a constant rate. Trace (a) represents excretion of solution from the urethra and was obtained from signals which were generated by the drinkometer when leaking solution from the urethra short-circuited the input of the instrument. Trace (b) is the cystometrogram, and the vertical bar represents the bladder pressure (cmH₂O). At the points indicated by (v), the abdomen was stimulated electrically. The lower pair of traces are those recorded 36 min after the ends of the upper pair. A circle indicates injection of prazosin. At the end of the lower trace, reflexly induced-bladder contraction followed by spontaneous micturition is shown.

Table 1. Effect of prazosin on arrested period of micturition after i.v.-injection of drugs in anesthetized female rabbits

| Phenylephrine (1 mg/kg) | Prazosin (0.01 mg/kg) | Phenylephrine after injection of prazosin |
|------------------------|----------------------|----------------------------------------|
| (n = 5)                |                      |                                        |
| 35.7 ± 5.9 min         | 4.7 ± 0.4 min        | 35.5 ± 11.5 min                        |

| Phenylephrine (1 mg/kg) | Prazosin (0.1 mg/kg) | Phenylephrine after injection of prazosin |
|------------------------|----------------------|----------------------------------------|
| (n = 10)               |                      |                                        |
| 28.8 ± 4.8 min         | 18.2 ± 5.2 min       | 11.5 ± 2.7** min                       |

Drugs were injected in the order of phenylephrine, prazosin and phenylephrine. The time from injection of the drug to the first leakage induced by electrical stimulation or spontaneous bladder contraction was measured. The next drug was injected after leakage had resumed. Each value represents the mean ± S.E. The times for which phenylephrine arrested leakage before and after injection of prazosin were compared. **P < 0.01.
tively; and prazosin (0.1 mg/kg, i.v.) significantly inhibited the elevation of bladder pressure. In another three rabbits in which the effect of prazosin (0.01 mg/kg, i.v.) was studied, prazosin (0.01 mg/kg, i.v.) had no effect on the pressure elevation. Prazosin (0.1 mg/kg, i.v.) also arrested micturition induced by electrical stimulation in some rabbits (Table 1). Though there was no significant difference between the values of the bladder pressure induced by electrical stimulation before and after injection of prazosin (0.1 mg/kg, i.v.) in ten rabbits, in the two rabbits in which electrical stimulation did not induce micturition for 40–50 min, prazosin clearly inhibited the elevation of the bladder pressure.

After injection of ST-1059 (0.1 mg/kg, i.v.) the first electrical stimulation induced micturition, but a higher dose of ST-1059 (1 mg/kg, i.v.), which was injected after the effect of the lower dose of ST-1059 (0.1 mg/kg, i.v.) was investigated, arrested micturition for 23.5 ± 3.8 min. ST-1059 (1 mg/kg, i.v.) caused the elevation of bladder pressure, and the mean value was 17.3 ± 6.8 cmH₂O (n = 5) (Fig. 2A).

The effect of i.d. injection of midodrine (10 mg/kg) was studied in four rabbits. Though micturition was induced by electrical stimulation about 10–20 min after administration of midodrine, subsequent electrical stimulation did not induce micturition, and the mean time for which micturition was not observed was 57.4 ± 12.9 min. After administration of midodrine (10 mg/kg, i.d.), the bladder pressure rose gradually, and the mean pressure about 40 min after midodrine administration was 9.7 ± 2.7 cmH₂O (n = 4) (Fig. 2B).

To study the effect of clonidine on micturition induced by electrical stimulation, three rabbits were first injected with a low dose of the drug (0.1 mg/kg, i.v.) and then a high dose (1 mg/kg, i.v.); and another seven rabbits were only injected with one dose of clonidine (1 mg/kg, i.v.). After i.v.-injection of 0.1 mg/kg clonidine (0.1 mg/kg, i.v.), the first electrical stimulation induced micturition in all three rabbits. After injection of clonidine at 1 mg/kg, i.v., micturition was induced by the first electrical stimulation in five of ten rabbits, and in two of these five rabbits, micturition occurred immediately after injection. In the other five rabbits, micturition was induced by electrical stimulation about 20 to 40 min after injection of clonidine. The mean time for which clonidine (1 mg/kg, i.v.) arrested micturition was 17.8 ± 4.7 min (n = 10). Clonidine (1 mg/kg, i.v.) slightly elevated the bladder pressure, and the mean value was 1.9 ± 0.6 cmH₂O (n = 10) (Fig. 2C).

In the three rabbits in which bladder infusion alone generated high pressures, prazosin (0.1 mg/kg, i.v.) reduced the pressure to baseline levels, and electrical stimulation and spontaneous bladder contraction induced micturition (Fig. 3).

DISCUSSION

Using anesthetized female rabbits, we tried to develop a model of urinary incontinence. Urethral leakage occurs when bladder pressure exceeds the maximum urethral pressure. To elevate the bladder pressure, we performed electrical stimulation of the abdominal muscle. Since electrical stimulation not only contracted the abdominal muscle directly but also might have inflicted a noxious stimulus on the rabbit, bladder pressure rose and micturition occurred during or immediately after electrical stimulation. Using this method, we studied the effects of drugs on micturition induced by electrical stimulation in vivo. As in our method both the bladder and urethra are intact for the recording of the cystometrogram, the effects of drugs on both organs can be simultaneously investigated. Alpha-1 adrenergic agonists, phenylephrine, ST-1059 and midodrine not only arrested micturition induced by electrical stimulation but also elevated bladder pressure. Latency in the action of midodrine on micturition may be due to the time taken for the metabolic activation of midodrine (15). As prazosin inhibited both the effect of phenylephrine on micturition induced by electrical stimulation and elevation of blad-
der pressure, the arrest of leakage and the bladder pressure elevation induced by these alpha-1 adrenergic agonists are due, respectively, to contraction of the proximal urethra and bladder base where alpha-adrenergic receptors predominate (1, 4, 6). Although the alpha-1 adrenergic agents elevated the bladder pressure, leakage was not observed. Therefore, the increase in urethral pressure generated by these drugs may be greater than that in bladder pressure. Though the high density of alpha-2 receptors and high contractile response induced by alpha-2 agonists were reported in isolated urethra of female rabbits (4, 8, 12, 13), clonidine (1 mg/kg, i.v.) did not arrest leakage in any of the rabbits. There are other studies showing that the elevation of urethral tone is regulated predominantly by activation of the alpha-1 adrenergic receptor in the urethra (9, 10, 14) and that in the in vivo experiments, clonidine reduces urethral closure pressure by reducing the spinal sympathetic outflow to the organ (17, 18). As i.v.-injected clonidine may exert these contrary actions on the urethral closure pressure, the effect of clonidine on the micturition induced by electrical stimulation may not be consistent.

It was found that prazosin reduced the tonus of the bladder, as well as that of the urethra (Fig. 3). Also in rabbits, the sympathetic nervous system contributes to the generation of bladder pressure, as reported in dogs (19). Prazosin (0.1 mg/kg, i.v.) arrested micturition induced by electrical stimulation in some rabbits. Prazosin may reduce the bladder tonus, and electrical stimulation can not elevate the bladder pressure to the urethral pressure.

In conclusion, when the effects of alpha-1 and alpha-2 agonists on artificially induced micturition were studied, alpha-1 agonists arrested the micturition, but the alpha-2 agonist clonidine, which is reported to contract isolated urethral muscle, could not constantly arrest it in this in vivo preparation. This system may be useful for evaluating the effects of drugs on urinary incontinence.

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