Role of the β₃-Adrenoceptor in Urine Storage in the Rat: Comparison between the Selective β₃-Adrenoceptor Agonist, CL316,243, and Various Smooth Muscle Relaxants

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ABSTRACT

The objective of this study was to compare the effects of a β₃-adrenoceptor (β₃-AR) agonist on bladder function and cardiovascular parameters in rats with those of several drugs that act on smooth muscle. CL316,243 (β₃-AR agonist), isoproterenol (non-selective β-AR agonist), procaterol (β₂-AR agonist), verapamil (Ca²⁺ antagonist), and papaverine (antispastic drug) each evoked a concentration-dependent relaxation of the detrusor in vitro. They also reduced bladder pressure in anesthetized rats, the β-AR agonists apparently being more potent than the other drugs. Atropine (muscarinic antagonist) neither relaxed detrusor strips nor reduced bladder pressure. In anesthetized rats, CL316,243 and atropine each had only a slight influence on blood pressure and heart rate, but isoproterenol, procaterol, verapamil, and papaverine significantly affected cardiovascular function at the same dose range as that required to reduce bladder pressure. In cystometry experiments, CL316,243 (10 μg/kg i.v.), verapamil (1 mg/kg i.v.), and papaverine (1 mg/kg i.v.) all significantly prolonged micturition interval and increased bladder capacity, but did not change the residual urine volume after a micturition contraction. Procaterol (100 μg/kg i.v.) prolonged the micturition interval and increased both bladder capacity and residual urine volume (all significantly). Atropine (100 μg/kg i.v.) reduced micturition pressure and increased residual urine volume (both significantly). Because the human detrusor, like the rat detrusor, relaxes on β₃-AR stimulation, we conclude that this β₃-AR agonist may have potential in pollakiuria (frequent urination) as a therapeutic agent without cardiovascular side effects.

Urinary bladder function is controlled by both the parasympathetic and sympathetic nervous systems, their activation mediating bladder contraction and relaxation, respectively (Andersson, 1993). It is considered that pathophysiologic conditions such as pollakiuria, urgency, and incontinence arise from disturbances of this dual control mechanism (Andersson, 1988). Consequently, drugs such as muscarinic antagonists (Boman and von Garrelts, 1973; Blaivas et al., 1980), Ca²⁺ antagonists (Palmer et al., 1981), and antispastic drugs (Stanton, 1973; Delaere et al., 1977) are considered useful for the treatment of patients with pollakiuria caused by a hyperactive bladder. However, because they have little or no selectivity for the detrusor, such drugs often produce adverse systemic effects.

In the bladder, the β-adrenoceptor (β-AR) subtypes mediating sympathetic relaxation of the detrusor differ substantially from species to species. For example, relaxation of the detrusor in cats (Nerglass et al., 1977) and guinea pigs (Li et al., 1992) is mediated mainly via β₁-AR, whereas in rabbits (Anderson and Marks, 1984; Levin et al., 1988; Yamazaki et al., 1998) it is said to be mediated entirely via β₂-AR. Moreover, the rat detrusor relaxes through not only β₂-AR, but also β₁-AR (Yamazaki et al., 1998) even though all three β-AR subtype mRNAs are expressed in the detrusor in this species (Seguchi et al., 1998). We recently confirmed that although all three β-AR subtype mRNAs are positively expressed in the human detrusor, the major β-AR subtype responsible for its relaxation is neither the β₁- nor the β₂-AR, but the β₃-AR (Igawa et al., 1997, 1998, 1999).

In this study, we used rats to investigate the usefulness of a selective β₃-AR agonist on aspects of bladder function closely related to urine storage, and we compared its effects with those of other drugs expected to be useful clinically for the treatment of such bladder dysfunctions as pollakiuria and urinary incontinence.

Materials and Methods

Animals

This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co. Ltd.

Male and female Sprague-Dawley rats (SLC, Hamamatsu, Japan), weighing from 200 to 380 g at the beginning of the experiments, were used in this study. All rats were group-housed in cages for at least 1 week before the experiment, and they were fed laboratory chow and water ad libitum. The temperature of the room was 23 ± 1°C, and a 12-h light/dark cycle (lights on at 8:20 AM) was used.

Isolated Preparations

Male rats, weighing from 250 to 380 g, were anesthetized with diethyl ether. They were then sacrificed, and the urinary bladder was isolated and placed in Krebs solution of the following composition: 118.1 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 25.0 mM NaHCO3, 1.2 mM KH2PO4, and 11.1 mM glucose (pH 7.4). The bladder was opened longitudinally, and a detrusor strip approximately 10 × 2 mm was taken and suspended in a 10-ml organ bath containing Krebs solution. This was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of 0.5 g was applied to the preparation at the beginning of the experiment, and the tissue was equilibrated for at least 60 min. The isometric tension generated by the tissue was measured using a force-displacement transducer (SB-1T; Nihon-Kohden, Tokyo, Japan) and recorded on a rectigraph (Rectigraph SK; NEC San-ei, Tokyo, Japan). Concentration-response curves were obtained by cumulative addition of the appropriate drug to the bathing fluid. Each preparation was exposed to only one drug. All experiments were conducted in the presence of 10−6 M phenolamine, an α-adrenoceptor antagonist.

In Vivo Experiments

Bladder Pressure in Anesthetized Rats. Male rats, weighing from 300 to 350 g, were anesthetized with urethane (1.5 g/kg s.c.). Through a midline abdominal incision, the pelvic viscera were exposed, and the ureter on each side was ligated and cut proximal to the ligature so as to allow urine to drain into cotton wads. After the urethra had been ligated, a polyethylene catheter (PE-50; Nihon-Becton Dickinson, Tokyo, Japan) was inserted into the bladder dome and connected through a three-way connector to a pressure transducer (SPB-108; NEC San-ei) and a syringe filled with warmed saline. The initial bladder pressure was adjusted to 6 cm H2O by instillation of warmed saline (37°C) in 0.05-ml increments. An arterial catheter was inserted into the left carotid artery (PE-90; Nihon Becton Dickinson) and connected to a pressure transducer (SPB-108) for the measurement of blood pressure. Heart rate was measured via a tachometer (I321; NEC San-ei) connected to the transducer amplifier (1829; NEC San-ei). Blood pressure, heart rate, and bladder pressure were recorded continuously on a rectigraph (Recti-Horiz-8K; NEC San-ei). Drug effects on bladder pressure, blood pressure, and heart rate were quantified by expressing each postadministration value as a percentage of the value before drug administration. Each animal was either exposed to only one dose of each drug, or exposed to more than one dose of a given drug with an interval of 60 min allowed between applications so that each parameter could recover to its initial value. No animal was exposed to more than one of the test drugs. A venous catheter was inserted into the left femoral vein (PE-50; Nihon Becton Dickinson) for drug injection.

Cystometry in Anesthetized Rats. Female rats, weighing from 200 to 230 g, were anesthetized with urethane (1.0 g/kg s.c.). Through a midline abdominal incision, the ureter on each side was ligated and cut proximal to the ligature. A polyethylene catheter (PE-50) was inserted into the urinary bladder and connected through a three-way connector to: 1) a pressure transducer (SPB-108) for measurement of bladder pressure, and 2) a syringe infusion pump (KD Scientific model 100; Muromachi Kikai, Tokyo, Japan) for continuous infusion of saline into the bladder. Micturition volumes were measured by means of a fluid collector connected to a force displacement transducer (Type 45196A; NEC San-ei). During cystometry, warmed saline was infused at a rate of 3.6 ml/h. Bladder pressure and micturition volumes were recorded continuously on a rectigraph (Recti-Horiz-8K). The following cystometric parameters were obtained: micturition interval, micturition pressure (maximum bladder pressure during micturition), micturition volume (volume of urine expelled), residual volume (residual volume at the previous micturition plus volume of saline infused up to the time of micturition minus micturition volume), and bladder capacity (micturition volume plus residual volume). Two reproducible micturition cycles were recorded before drug administration and used to provide a baseline value to be compared with the first two micturition cycles just after drug administration. Relative values for the various cystometric parameters were calculated as follows: (mean value from two micturition cycles just after drug administration/mean value from two micturition cycles just before drug administration). Each animal was exposed to only one dose of one drug. A venous catheter was inserted into the left femoral vein for drug injection.

Analysis of Data

The results are expressed as mean ± S.E. Statistical analysis was performed using a one-way ANOVA followed by Dunnett’s multiple-comparison method. A probability level less than .05 was accepted as significant. The JMP Statistics and Graphics Guide (version 3.1; SAS Institute Inc., Cary, NC) was used as the resource text for the statistical analysis.

Drugs

The following drugs were used: atropine sulfate monohydrate (Wako Pure Chemical, Osaka, Japan), (−)-isoproterenol (−)-bitartrate, procaterol hydrochloride, (±)-verapamil hydrochloride, papaverine hydrochloride, urethane (Sigma Chemical Co, St. Louis, MO), and phenolamine mesylate (Ciba-Geigy, Basel, Switzerland). CL316,243 [(R,R)-5-[2-[[(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate] was synthesized in our laboratories (Kissei, Hotaka, Japan). The drugs were dissolved in distilled water for the in vitro study, but in saline for the in vivo study. The solutions were prepared on the day of the experiment and kept in dark vessels to minimize light-induced degradation.

Results

Isolated Preparations

The β-AR agonists isoproterenol, procaterol, and CL316,243 all caused concentration-dependent relaxation of the detrusor (Fig. 1). The EC50 values were (9.64 ± 2.29) × 10−6 M for isoproterenol, (1.62 ± 0.76) × 10−5 M for procaterol, and (7.08 ± 1.56) × 10−5 M for CL316,243. Verapamil and papaverine relaxed the detrusor only at the high end of their concentration range, the EC50 values being (5.29 ± 1.25) × 10−6 and (2.00 ± 0.28) × 10−5 M, respectively. The intrinsic activities of these agents (relative to that of isoproterenol, 1.0) were calculated to be 1.02 for procaterol, 0.96 for CL316,243, 0.94 for verapamil, and 1.08 for papaverine. Atropine had only a weak relaxing effect on the detrusor. As the relative intrinsic activity of atropine was 0.11 even at concentrations of 10−5 M or more, we could not calculate an EC50 value for this drug.

In Vivo Studies

Effects on Bladder Pressure and Cardiovascular Variables in Anesthetized Rats. Figure 2 shows representative recordings of bladder pressure, blood pressure, and heart rate in rats given isoproterenol or CL316,243 by i.v. administration. The mean values for bladder pressure, blood pressure, and heart rate before drug administration were...
Cystometry in Anesthetized Rats. Figure 3 shows representative recordings of cystometrograms taken from anesthetized rats. The mean values for the various cystometric parameters before drug administration (n = 133 in all animals tested) were as follows: micturition interval, 6.34 ± 0.20 min; micturition pressure, 30.86 ± 0.54 cm H2O; micturition volume, 0.36 ± 0.01 ml; residual urine volume, 0.40 ± 0.02 ml; bladder capacity, 0.75 ± 0.02 ml. Table 4 shows relative values for these cystometric parameters after drug administration. Injection of vehicle (saline, 1 ml/kg i.v.) had no effect on the cystometric parameters. Procatelol (1 to 100 μg/kg i.v.) increased bladder capacity, prolonged micturition interval, and increased residual urine volume in a dose-dependent manner. Relative values for bladder capacity, micturition interval, and residual urine volume were 1.39 ± 0.09, 1.30 ± 0.08, and 1.70 ± 0.30, respectively, after the administration of procatelol at 100 μg/kg. CL316,243 (10 and 100 μg/kg i.v.) significantly increased bladder capacity, and prolonged micturition interval by 1.3-fold or more, effects comparable in size to those produced by procatelol. With CL316,243 at these doses, micturition pressure was reduced by about 10%, but residual urine volume was not increased significantly. Combined administration of procatelol (100 μg/kg) with CL316,243 (100 μg/kg) increased both bladder capacity and residual volume, and prolonged micturition interval (all significantly). Atropine (10 μg/kg to 1 mg/kg i.v.) reduced micturition pressure and increased residual volume to a dose-dependent manner; residual volume was 2 times the control value at 1 mg/kg. Verapamil (1 mg/kg i.v.) and papaverine (1 and 10 mg/kg i.v.) each increased bladder capacity and prolonged micturition interval (both significantly), but of these two drugs, only papaverine (10 mg/kg i.v.) produced a significant increase in residual volume.

Discussion

Although it is accepted that the sympathetic nervous system plays an important role in the control of micturition in a variety of species, including humans, its role in human bladder function has been the subject of much debate, partly because of the paucity of adrenergic innervation of the human detrusor. Recently, attention has focused on the β3-AR after the publication of evidence of its existence in the detrusor and of its importance in the relaxation of the human detrusor during adrenergic stimulation (Igawa et al., 1997, 1998, 1999). It has been reported that β3-AR also play an important role in the relaxation of the detrusor in rats in association with β3-AR (Yamazaki et al., 1998).

In this study, we attempted to gain some insight into the potential usefulness of a β3-AR agonist as a therapeutic agent for pollakiuria by comparing its effects in rats with those of several smooth muscle relaxants including a β2-AR agonist, a muscarinic antagonist, a Ca2+ antagonist, and an antispastic agent.

All of the β-AR agonists tested (isoproterenol, procatelol, and CL316,243) not only relaxed the detrusor in vitro, but also reduced bladder pressure in vivo in rats. The maximal reduction in bladder pressure induced in vivo did not differ among the three drugs. The rank order of potency in vivo was isoproterenol (nonselective β-AR agonist) > procatelol (β2-AR agonist, Yoshizaki et al., 1976) > CL316,243 (β3-AR agonist, Bloom et al., 1992). Combination of procatelol with

6.00 ± 0.26 cm H2O, 103 ± 2 mm Hg, and 414 ± 7 beats/min, respectively. Tables 1, 2, and 3 show the maximum response during the observation for 30 min after the drug administration. Injection of vehicle (saline, 1 ml/kg i.v.) had no effect on these parameters. Isoproterenol (10 μg/kg i.v.) significantly reduced both bladder pressure and blood pressure, and significantly increased heart rate. CL316,243 (100 μg/kg i.v.) also reduced bladder pressure significantly, but the changes in blood pressure and heart rate were minimal. Table 1 summarizes the effects of the test drugs on bladder pressure. The data for each parameter are expressed as a percentage of the preadministration value. All three β-AR agonists produced a dose-dependent reduction in bladder pressure. CL316,243 had the longest duration of action among these drugs, bladder pressure did not return to preadministration value at 120 min after CL316,243 (100 μg/kg) administration. At their highest dose, isoproterenol, procatelol, and CL316,243 significantly reduced bladder pressure to 77.7, 72.8, and 65.8%, respectively, of the resting pressure. Procatelol (100 μg/kg) plus CL316,243 (100 μg/kg) reduced bladder pressure to 63.6% of the resting pressure (data not shown). Isoproterenol lowered blood pressure to 69.2% of the resting value (10 μg/kg) and increased heart rate to 122.6% (at 1 μg/kg) (Tables 2 and 3). Procatelol produced a decrease in blood pressure to 72.3% (at 100 μg/kg i.v.) and a slight increase in heart rate to 106% (at 100 μg/kg). CL316,243 had only weak effects on blood pressure and heart rate; the values obtained were 87.9 and 100.1%, respectively, of the basal values (at 100 μg/kg). Blood pressure and heart rate 120 min after CL316,243 (100 μg/kg) administration were 86.9 ± 3.4 and 105.8 ± 3.3%, respectively, of their basal values. Atro-
CL316,243 at the highest dose of each drug (100 μg/kg) did not produce any additional reduction in bladder pressure beyond the level achieved with CL316,243 alone. In terms of their cardiovascular effects, isoproterenol increased heart rate, whereas both isoproterenol and procaterol lowered blood pressure significantly as a result of their respective stimulating effects on β₁- and β₂-ARs (Lands et al., 1967a,b). In the rat heart, β₃-AR mRNA is not detectable in the atria or in the right ventricular myocardium, and there is a low level of β₃-AR mRNA in the left ventricle, which contains major blood vessels (Evans et al., 1996). Furthermore, there has been no report of the existence of β₃-AR mRNA in aorta or veins. In this study, CL316,243 produced only slight effects on heart rate and blood pressure, presumably because of its excellent β₃-AR selectivity (Bloom et al., 1992; Dolan et al., 1994). In the cystometry test, CL316,243 increased bladder capacity, leading to an apparent prolongation of micturition interval. Micturition pressure was slightly but significantly reduced, and residual urine volume tended to increase only at higher doses of CL316,243. The major neuronal stimulus

### Table 1

Effects of isoproterenol, CL316,243, procaterol, atropine, verapamil, and papaverine on bladder pressure in urethane-anesthetized rats

Results are expressed as a percentage of the value before drug administration, and are given as the mean ± S.E. of data from seven to eight animals. The values show the maximum response during the observation for 30 min after the drug administration.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (i.v.)</th>
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<th>0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
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<td>μg/kg</td>
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<tr>
<td>Isoproterenol</td>
<td>98.1 ± 0.9</td>
<td>91.4 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.9 ± 2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CL316,243</td>
<td>98.8 ± 2.1</td>
<td>101.7 ± 2.7</td>
<td>89.7 ± 2.7</td>
<td>81.3 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.8 ± 5.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Procaterol</td>
<td>101.8 ± 2.4</td>
<td>96.7 ± 1.2</td>
<td>86.2 ± 1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.4 ± 1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.8 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Atropine</td>
<td>98.1 ± 1.2</td>
<td>95.8 ± 0.7</td>
<td>93.5 ± 0.2</td>
<td>96.5 ± 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Verapamil</td>
<td>99.1 ± 0.7</td>
<td>99.8 ± 0.6</td>
<td>97.1 ± 1.8</td>
<td>91.4 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Papaverine</td>
<td>97.4 ± 1.2</td>
<td>100.1 ± 1.0</td>
<td>99.3 ± 0.7</td>
<td>84.5 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> Indicates significant difference from saline control (0 μg/kg) (P < .05).
TABLE 2
Effects of isoproterenol, CL316,243, procaterol, atropine, verapamil, and papaverine on blood pressure in urethane-anesthetized rats
Results are expressed as a percentage of the value before drug administration, and are given as the mean ± S.E. of data from seven to eight animals. The values show the maximum response during the observation for 30 min after the drug administration.

<table>
<thead>
<tr>
<th>Drug</th>
<th>0</th>
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<tr>
<td>Isoproterenol</td>
<td>103.3 ± 0.9</td>
<td>86.4 ± 3.2</td>
<td>77.5 ± 3.3</td>
<td>69.2 ± 1.7</td>
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<tr>
<td>CL316,243</td>
<td>99.7 ± 1.1</td>
<td>98.7 ± 1.9</td>
<td>90.5 ± 2.7</td>
<td>87.9 ± 2.6</td>
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<td>Procerterol</td>
<td>104.0 ± 0.4</td>
<td>86.1 ± 1.1</td>
<td>83.4 ± 4.1</td>
<td>72.3 ± 4.6</td>
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<td>Atropine</td>
<td>103.1 ± 0.7</td>
<td>105.5 ± 2.1</td>
<td>104.4 ± 1.6</td>
<td>93.0 ± 1.3</td>
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<td>Verapamil</td>
<td>103.6 ± 1.0</td>
<td>101.4 ± 0.2</td>
<td>86.6 ± 1.5</td>
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<td>Papaverine</td>
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<td>99.4 ± 2.0</td>
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* Indicates significant difference from saline control (0 µg/kg) (P < .05).

TABLE 3
Effects of isoproterenol, CL316,243, procaterol, atropine, verapamil, and papaverine on heart rate in urethane-anesthetized rats
Results are expressed as a percentage of the value before drug administration, and are given as the mean ± S.E. of data from seven to eight animals. The values show the maximum response during the observation for 30 min after the drug administration.

<table>
<thead>
<tr>
<th>Drug</th>
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<tr>
<td>Isoproterenol</td>
<td>100.3 ± 0.2</td>
<td>107.6 ± 3.2</td>
<td>122.6 ± 1.8</td>
<td>121.4 ± 4.1</td>
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<tr>
<td>CL316,243</td>
<td>100.1 ± 0.2</td>
<td>101.8 ± 0.4</td>
<td>101.2 ± 0.4</td>
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<tr>
<td>Procerterol</td>
<td>100.9 ± 0.5</td>
<td>103.2 ± 1.3</td>
<td>103.8 ± 1.5</td>
<td>106.2 ± 1.9</td>
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<tr>
<td>Atropine</td>
<td>99.9 ± 0.1</td>
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<td>Verapamil</td>
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<td>95.1 ± 2.2</td>
<td>78.9 ± 4.5</td>
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<tr>
<td>Papaverine</td>
<td>100.0 ± 0.3</td>
<td>100.4 ± 0.2</td>
<td>101.4 ± 0.4</td>
<td>79.5 ± 2.7</td>
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* Indicates significant difference from saline control (0 µg/kg) (P < .05).

Fig. 3. Representative recordings from cystometric investigations in urethane-anesthetized rats. At the arrow, saline (A), CL316,243 (B), procaterol (C), atropine (D), verapamil (E), or papaverine (F) was administered i.v. Asterisk (+) indicates adjustment to baseline position.

for physiological bladder contraction is an acetylcholine-induced stimulation of parasympathetically innervated muscarinic receptors on the detrusor. Because isoproterenol (30 µM) does not affect the (+)-cis-dioxolane (muscarinic agonist)-induced concentration-dependent contraction of rat detrusor preparations (Hegde et al., 1997), a specific β3-AR agonist, such as CL316,243, might be expected to lack a significant influence over the bladder contraction induced by acetylcholine at the time of micturition. The results of this study demonstrate that in the rat, CL316,243, a β3-AR agonist, is the most selective at prolonging the micturition interval among the β-AR agonists we tested, and that it has the weakest cardiovascular side effects among these agents. Procaterol, a β2-AR agonist, prolonged micturition interval and significantly increased residual urine volume without reducing micturition pressure. Because procaterol has been found to increase the contractile force induced in the rabbit external urethral sphincter by electrical field stimulation (Morita et al., 1995), it seems likely that this agent produces an increase in residual urine volume as a consequence of its stimulating effect on the external sphincter. The changes in cystometric parameters observed after the combined administration of CL316,243 with procaterol could be explained as a result of a simple summation of their effects (Table 4).

Atropine, a nonselective muscarinic antagonist, relaxed the detrusor muscle hardly at all in vitro or in vivo. In the cystometry experiment, atropine reduced micturition pressure and shortened micturition interval in a dose-dependent manner. Although atropine delayed the first micturition occurring just after drug administration, it simultaneously lowered micturition pressure. As a consequence, residual urine volume was increased and the next micturition occurred sooner, without an increase in bladder capacity. Urinary bladder smooth muscle is rich in muscarinic receptors, both M2- and M3-subtypes being present in the rat (Hegde et al., 1997), and their stimulation leads to contraction of the bladder. Our study suggests that atropine reduces micturition pressure by antagonizing micturition stimuli arriving through
the pelvic nerve, leading to an augmented retention of urine, but that when the bladder pressure is below threshold pressure, the collecting phase for urine, this drug has no effect on bladder pressure.

It is well known that Ca²⁺ is essential for producing contractile activity in smooth muscle. Verapamil, a Ca²⁺ antagonist, also relaxes the detrusor in a concentration-dependent manner in vitro, and reduced bladder pressure in vivo, in rats. In the cystometry experiment, verapamil increased bladder capacity and prolonged micturition interval at a dose that reduced bladder pressure (1 mg/kg i.v.), but it did not affect micturition pressure at this dose. It has been reported that verapamil attenuated the intensity of both spontaneous and carbachol-induced contractions of the rat detrusor (Maggi et al., 1982). In addition, there is another report showing that verapamil (1 mg/kg i.v.) increased bladder capacity without reducing micturition pressure in anesthetized dogs (Kaneko et al., 1989). Our data are mostly in accord with these reports. However, the effect of verapamil was not selective for bladder function; indeed, it produced significant decreases in both blood pressure and heart rate. In actual fact, a significant decrease in blood pressure was induced by verapamil at doses lower than those producing an apparent reduction in bladder pressure in anesthetized rats.

In this experiment, papaverine relaxed the detrusor in vitro in a concentration-dependent manner and reduced bladder pressure in vivo, just as verapamil did. In the cystometry experiment, papaverine increased bladder capacity and prolonged the micturition interval in a dose-dependent manner. Neither micturition pressure nor micturition volume changed after an i.v. injection of papaverine at 10 mg/kg. However, residual urine volume was increased significantly and prolonged the micturition interval in a dose-dependent manner. Moreover, its cardiovascular side effects were so severe as to lead to the death of some of the rats.

Papaverine, an antispastic drug, is known to be a nonspecific phosphodiesterase inhibitor (Kukovetz and Pöch, 1970); it produces a nonselective relaxation of smooth muscle. In terms of its influence on bladder function in rats, papaverine proved to be a less selective drug. In fact, although papaverine increased residual urine volume, it does not have a contraction-repressing action as muscarinic antagonists do, and its systemic side effects were so severe as to lead to the death of some of the rats.

In conclusion, this study has clearly shown that the effects of β-AR agonists in reducing bladder pressure in rats are more pronounced than those of a muscarinic antagonist, a Ca²⁺ antagonist, and an antispastic drug. Although the β₂-AR agonist and the Ca²⁺ antagonist we tested were each effective in increasing bladder capacity and in prolonging the micturition interval without increasing residual urine volume, the β₂-AR agonist had much weaker cardiovascular side effects. In humans, as in the rat, β₂-AR mRNA has been detected in the bladder (Igawa et al., 1999), and only a low level of β₂-AR mRNA is present in the human heart (Krief et al., 1993; Berkowitz et al., 1995) and probably in blood vessels. Consequently, β₂-AR agonists would be expected to have fewer and weaker cardiovascular side effects than those produced by β-AR agonists acting on β₁- and/or β₂-AR. Our data strongly support this idea. Because the human detrusor relaxes mainly through β₂-AR stimulation, we suggest that the β₂-AR agonist may have a potential role in pollakiuria as a useful therapeutic drug without cardiovascular side effects.

References
Role of $\beta_3$-Adrenoceptor in Urine Storage


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