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Augmentation of rat urinary bladder relaxation mediated by β_1-adrenoceptors in experimental diabetes

Yuko Kubota, Tsutomu Nakahara*, Akiko Mitani, Takeshi Maruko, Kenji Sakamoto, Kunio Ishii

Department of Molecular Pharmacology, Kitasato University School of Pharmaceutical Sciences, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

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Abstract

We examined how diabetes affects the β-adrenoceptor subtypes mediating relaxation of rat urinary bladder smooth muscle contracted with carbachol. The relaxant responses to isoproterenol were larger in muscles from rats 8 to 10 weeks after induction of diabetes with streptozotocin (80 mg/kg, i.p.) as compared to the control muscles. In contrast, forskolin-induced relaxations did not differ significantly in the control and diabetes groups. Propranolol (1 μM) abolished the diabetes-induced augmentation of relaxant responses to isoproterenol. The relaxant responses to T-0509 ((−)-(*R*)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)-amino]ethanol hydrochloride), a β_1-adrenoceptor agonist, were small but significantly augmented by diabetes. On the other hand, diabetes did not change the relaxations produced by clenbuterol, a β_2-adrenoceptor agonist, and BRL37344 ((±)-(*R**,*R**)-(4-[2-([2-(3-chlorophenyl)-2-hydroxyethyl]amino)propyl]phenoxy)acetic acid), a β_3-adrenoceptor agonist. These results suggest that diabetes selectively augments the β_1-adrenoceptor-mediated relaxation of the rat urinary bladder smooth muscle.

Keywords: β-Adrenoceptor; Diabetes; Urinary bladder, rat; Relaxation; Streptozotocin

1. Introduction

The function of the urinary bladder is often changed in patient with diabetes mellitus (Buck et al., 1976; Bradley, 1980; Ellenberg, 1980). The major clinical feature of this disorder is a gradual loss of bladder sensation and motor function resulting in a large bladder with a chronic residual urine volume (Buck et al., 1976; Ellenberg, 1980). The motor activity of the smooth muscle, especially that of the bladder dome, is mainly regulated by the sympathetic and parasympathetic nervous systems. Sympathetic nerves mediate relaxation of the urinary bladder smooth muscle through the activation of β-adrenoceptors, and this contributes to urine storage function. By contrast, parasympathetic nerves mediate contraction of the urinary bladder smooth muscle through the activation of muscarinic receptors. Thus, parasympathetic nervous system plays an important role in the voiding phase of urine.

Some investigators have demonstrated that experimental diabetes causes a significant alteration in the biochemical and functional characteristics of muscarinic and β-adrenoceptors in urinary bladder smooth muscles (Kolta et al., 1985; Kudlacz et al., 1989; Latifpour et al., 1989, 1991; Kanda et al., 1997). For example, the contractile responses to muscarinic receptor agonists were enhanced in the urinary bladder of rats with streptozotocin-induced diabetes mellitus (Kolta et al., 1985; Latifpour et al., 1989, 1991; Kanda et al., 1997). In these studies, the enhanced contractile responses were accompanied by significant increases in the densities of muscarinic receptors in urinary bladder (Latifpour et al., 1989, 1991; Kanda et al., 1997). On the other hand, other investigators have shown muscarinic receptor agonist-induced contractions to be reduced or unaltered by diabetes (Lincoln et al., 1984; Longhurst and Belis, 1986; Luheshi and Zar, 1991). In regard to β-adrenoceptors, the relaxant responses to β-adrenoceptor agonists were enhanced and the number of receptors was increased in the diabetic rat bladder (Kudlacz et al., 1989; Latifpour et al., 1991).

Muscarinic receptors and β-adrenoceptors have been subdivided into M_{1-5} and β_{1-3} subtypes, respectively. Up-regulation of muscarinic M_2 and M_3 receptors in the bladder of streptozotocin-induced diabetic rat has been demonstrated (Tong et al., 1999; Tong and Cheng, 2002). On the other hand, although it has been shown that three

* Corresponding author. Tel./fax: +81-3-3444-6205.
E-mail address: nakaharat@pharm.kitasato-u.ac.jp (T. Nakahara).

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subtypes of β-adrenoceptors are present in the urinary bladder smooth muscle (Seguchi et al., 1998; Fujimura et al., 1999; Takeda et al., 1999; Igawa et al., 1999), there seems to be no information on how diabetes affects β-adrenoceptor subtypes. The purpose of this study, therefore, was to compare the relaxant effects of isoproterenol and selective β-adrenoceptor agonists in rat urinary smooth muscles from streptozotocin-induced diabetic rats with those from control rats.

2. Material and methods

2.1. Animal model of diabetes

Male Wistar adult rats weighting 210–280 g were maintained on standard rat chow and tap water ad libitum with 12:12-h dark cycles in a quite environment. Diabetes was induced by a single intraperitoneal injection of streptozotocin (80 mg/kg) dissolved in sodium citrate buffer (pH 4.5). Age-matched control rats were treated with an injection of an equal volume of vehicle. Induction of diabetes was ascertained by determination of serum glucose concentrations 1 week after streptozotocin and confirmed by a serum glucose concentration >16.7 mM at death.

2.2. Preparation of rat urinary bladder strips

At 8–10 weeks after treatment with streptozotocin or vehicle (sodium citrate buffer), rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The urinary bladder was removed and placed in ice-cold Krebs-bicarbonate buffer (composition in millimolar: NaCl, 119; KCl, 4.8; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25 and glucose, 10.0) (pH = 7.4). The mucosa was removed by careful dissection under a dissecting microscope. Four longitudinal strips (approximately $1 \times 2 \times 10$ mm) were isolated from the bladder body. The strips were used for measurement of mechanical responses.

2.3. Measurement of mechanical activity

One end of each strip was attached to an isometric force displacement transducer (model TB-611T, Nihon Kohden, Tokyo, Japan) by a cotton thread, and the other end was tied to a stainless steel holder. Tension recorded was digitized at a sampling rate of 2 Hz with the use of a 12-bit analog-to-digital converter (model AD12-8(PM), Contec, Osaka, Japan) interfaced with a dedicated laboratory computer system (PC9821 Nr150, NEC, Tokyo, Japan). Strips were mounted in 20 ml of jacketed organ baths filled with Krebs-bicarbonate buffer gassed with 95% O_2–5% CO_2 at 37 °C. The preparations were placed under initial load (1 g) and the resting tension was adjusted every 15 min. The tissues were allowed to equilibrate for 60 min and the bath solution was exchanged every 15 min with fresh Krebs-bicarbonate buffer.

After this period, all preparations were contracted with acetylcholine (100 μM). The tissues were washed with fresh Krebs-bicarbonate buffer and allowed to return to their resting tone. When resting tone was established, the muscle preparations were contracted with carbachol (0.6 μM), which caused approximately 50% of the maximal contraction. Then, the concentration–response curves to β-adrenoceptor agonists and forskolin were established. In some experiments, isoproterenol-induced relaxations of rat urinary bladder smooth muscles contracted with carbachol (0.6 μM) were examined in the presence or absence of propranolol (1 μM). We also examined the effect of clenbuterol on the preparations contracted with 30 mM KCl. In experiments with high K^+ solution, Na^+ in the bathing medium was replaced by an equimolar concentration of K^+. Phentolamine (1 μM) was present throughout the experiments to block α-adrenoceptors.

2.4. Data analysis and statistics

Isolated bladder strips contract spontaneously with irregular frequency and varied amplitude under the experimental conditions adopted in the present study; therefore, the average tension during a steady-state period (1.5–3 min) for each concentration was used to assess contractility. Relaxant responses to β-adrenoceptor agonists and forskolin were expressed as percentages of carbachol-induced tension obtained just before the cumulative addition of drugs.

Data were expressed as the mean ± S.E.M. with N = number of rats and n = number of preparations. The maximal responses (E_{max}) and the half-maximum effective concentration values (EC_{50}) were calculated using the GraphPad Prism program (GraphPad Software, San Diego, CA, USA). The pD_2 values were calculated as the negative log of EC_{50}. Data were analyzed using unpaired Student's t-test (if required with Welch's correction). A P value smaller than 0.05 was considered significant.

2.5. Drugs

The following drugs were used: acetylcholine chloride, BRL37344 ((±)-(*R**,*R**)-(4-[2-([2-(3-chlorophenyl)-2-hydroxyethyl]amino)propyl]phenoxy)acetic acid), carbamylcholine chloride (carbachol), clenbuterol hydrochloride, (−)-isoproterenol (+)-bitartrate, forskolin, phentolamine hydrochloride, DL-propranolol hydrochloride, terbutaline hemisulfate (Sigma, St. Louis, MO, USA). T-0509 ((−)-(*R*)-1-(3,4-dihydroxyphenyl)-2-[(3,4-dimethoxyphenethyl)-amino]ethanol hydrochloride) was kindly provided by Tanabe Seiyaku (Osaka, Japan).

3. Results

Some of the characteristics of the rats used in this study are shown in Table 1. All control rats showed an increase in

Table 1
Serum glucose levels, body weights and bladder weights of control and streptozotocin-treated rats

	Control ($N=16$)	Streptozotocin ($N=16$)
Serum glucose (mM)	11.3 ± 0.6	32.0 ± 1.1[a]
Initial body weight (g)	259 ± 3	262 ± 3
Final body weight (g)	448 ± 10[b]	291 ± 7[a]
Bladder weight (mg)	125 ± 5	254 ± 14[a]

Values are means ± S.E.M.
[a] Significantly different from control group, $P<0.01$.
[b] Significantly different from initial body weight, $P<0.01$.

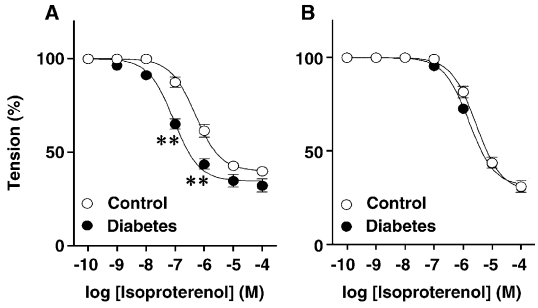

Fig. 2. Concentration–response curves for isoproterenol-induced relaxation of urinary bladder smooth muscles, in the absence (A) or presence (B) of propranolol (1 μM), from control and diabetic rats. The rat urinary bladder smooth muscle preparations were precontracted with carbachol (0.6 μM). Relaxant responses to isoproterenol were expressed as percentages of carbachol-induced tension obtained just before the cumulative addition of the drug. Each point with a vertical bar represents the mean ± S.E.M. from six to eight separate preparations. **$P<0.01$ vs. corresponding control values.

body weight, whereas the diabetic rats failed to gain weight over 8–10 week period following streptozotocin injection. The serum glucose level in the diabetic rats was higher than that in normal rats. Bladder weight in absolute terms was increased in the diabetic rats by approximately 2.0 times that of the controls.

Fig. 1 shows that isoproterenol concentration dependently relaxed the carbachol (0.6 μM)-induced contraction of the urinary bladder smooth muscle from control and diabetic rats. The tensions developed with carbachol (0.6 μM) were not significantly different between control (1.1 ± 0.1 g, $n=8$) and diabetic rats (1.0 ± 0.1 g, $n=6$). As summarized in Fig. 2A, diabetes caused leftward shifts of concentration–response curves for the relaxant responses to isoproterenol. The mean pD_2 values for isoproterenol in control and diabetic rats were 6.35 ± 0.11 and 7.04 ± 0.06, respectively ($P<0.01$, $n=6–8$). In contrast, the maximum relaxations induced by isoproterenol were not significantly different between control and diabetic rats.

As shown in Fig. 2B, propranolol (1 μM), which blocks β_1- and β_2-adrenoceptors (Kubota et al., 2002), significantly ($P<0.01$) shifted the concentration–response curves for isoproterenol-induced relaxations to the right, and this drug abolished the diabetes-induced augmentation of relaxant responses to isoproterenol (pD_2 values; control

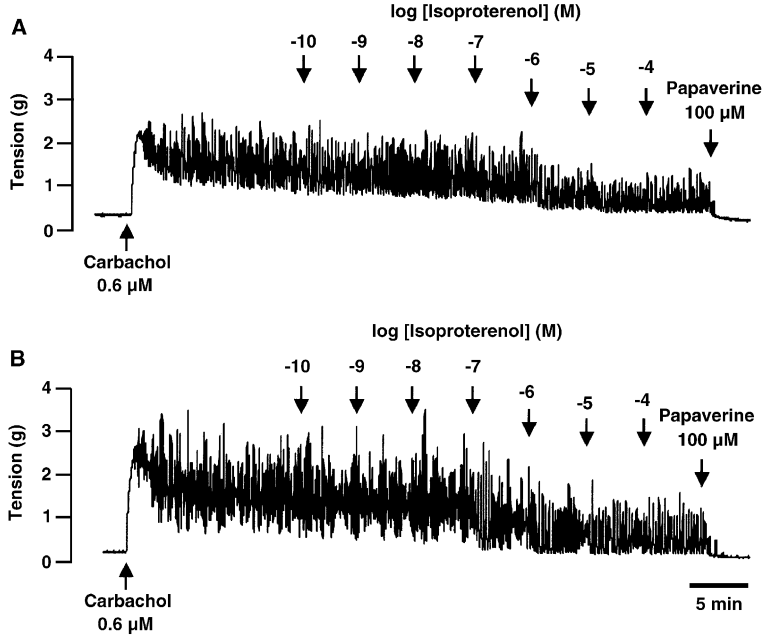

Fig. 1. Typical recordings of effect of isoproterenol on tension induced by carbachol (0.6 μM) in the urinary bladder smooth muscle from control (A) and diabetic rats (B). Isoproterenol was added cumulatively during steady-state contraction induced by carbachol in the presence of phentolamine (1 μM). Each arrow shows point of application of isoproterenol, whose concentrations are given above arrows.

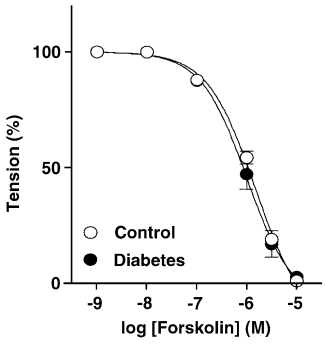

Fig. 3. Concentration–response curves for forskolin-induced relaxation of urinary bladder smooth muscles from control and diabetic rats. The rat urinary bladder smooth muscle preparations were precontracted with carbachol (0.6 μM). Relaxant responses to forskolin were expressed as percentages of carbachol-induced tension obtained just before the cumulative addition of the drug. Each point with a vertical bar represents the mean ± S.E.M. from eight separate preparations.

5.56 ± 0.06 vs. diabetes 5.79 ± 0.04, $n = 6$). Diabetes had no effect on the relaxant responses to forskolin in the rat urinary bladder smooth muscles (Fig. 3).

Next, we examined the effect of diabetes on the relaxations of urinary bladder smooth muscles evoked by subtype selective β-adrenoceptor agonists. The results are shown in Fig. 4. The β_1-adrenoceptor agonist, T-0509 (Sato et al., 1996), and the β_3-adrenoceptor agonist, BRL37344 (Granneman et al., 1991), caused a concentration-dependent relaxation in the urinary smooth muscle contracted with carbachol (0.6 μM). By comparison, the β_2-adrenoceptor agonist, clenbuterol (Hudman et al., 2000), only produced a small (~ 10%, $n = 4$) relaxant response at the highest concentration (100 μM) tested. Terbutaline, another β_2-adrenoceptor agonist (Longhurst and Levendusky, 1999), also had only small relaxant effects (E_{max}; 18.5 ± 0.8%, $n = 4$). Diabetes slightly but significantly augmented the T-0509-induced relaxation, whereas it did not affect the relaxant responses to clenbuterol and BRL37344.

In the preparations contracted with 30 mM KCl, clenbuterol produced an apparent relaxation (E_{max}; 61.2 ± 4.0%, pD_2; 6.99 ± 0.20, $n = 4$), although tensions developed with 30 mM K^+ (1.0 ± 0.1 g, $n = 4$) were not significantly different from those with 0.6 μM carbachol.

4. Discussion

Stimulation of sympathetic nerves causes relaxation of the urinary bladder smooth muscle through the activation of β-adrenoceptors. β-adrenoceptors couple to the stimulatory G protein (G_s) to activate adenylyl cyclase, and stimulation of these receptors could relax the urinary bladder through the activation of adenylyl cyclase and a consequent increase in cyclic AMP. The present study demonstrated that the isoproterenol-induced relaxations of the urinary smooth muscles were greater in diabetic rats than in control rats. On the other hand, forskolin, which directly stimulates adenylyl cyclase, relaxed control and diabetic bladders to the same extent. It is likely, therefore, that diabetes affected the events proximal to adenylyl cyclase activation in the rat bladder smooth muscle.

Recent studies demonstrated that the rat urinary bladder contains β_1-, β_2- and β_3-adrenoceptors, all of which mediate relaxation (Seguchi et al., 1998; Longhurst and Levendusky, 1999). Accordingly, isoproterenol-induced relaxation of the rat urinary bladder smooth muscle should be mediated by all of these β-adrenoceptor subtypes. However, in the present study, unlike T-0509 (a β_1-adrenoceptor agonist) (Sato et al., 1996) and BRL37344 (a β_3-adrenoceptor agonist)(Granneman et al., 1991), β_2-adrenoceptor agonists, such as clenbuterol and terbutaline, had only slight relaxant effects on the rat urinary bladder smooth muscles contracted with 0.6 μM carbachol. Longhurst and Levendusky (1999) showed that isoproterenol was more effective in attenuating 40 mM K^+-contracted rat urinary bladder smooth muscles than carbachol-induced ones. We also found that clenbuterol apparently relaxed the rat urinary bladder smooth muscles contracted with 30 mM K^+. Thus, stimulation of muscarinic receptors could suppress the relaxant responses to β-adrenoceptor agonists in the urinary bladder smooth muscle.

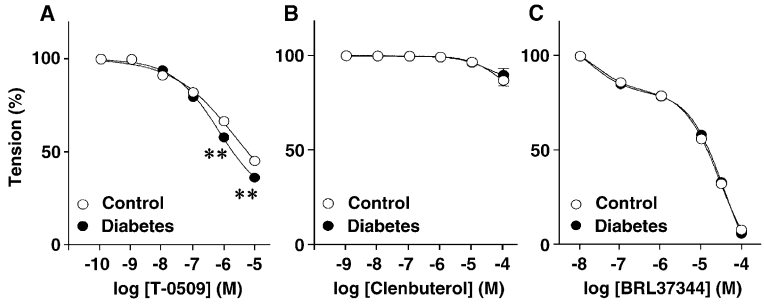

Fig. 4. Effects of selective β_1-, β_2- and β_3-adrenoceptor agonists on urinary bladder smooth muscles from control and diabetic rats. Graph shows relaxation with the β_1-adrenoceptor agonist, T-0509 (A), the β_2-adrenoceptor agonist, clenbuterol (B) and the β_3-adrenoceptor agonist, BRL37344 (C). Relaxant responses to β-adrenoceptor agonists were expressed as percentages of carbachol (0.6 μM)-induced tension obtained just before the cumulative addition of drugs. Each point with a vertical bar represents the mean ± S.E.M. from four to six separate preparations. $^{**}P < 0.01$ vs. corresponding control values.

Furthermore, these results suggest that β_2-adrenoceptors play a minor role in the isoproterenol-induced relaxation of rat urinary bladder smooth muscle contracted with carbachol (0.6 μM).

The present study demonstrated that propranolol (1 μM) abolished the enhanced response of diabetic muscles to isoproterenol. In addition, streptozotocin-induced diabetes slightly but significantly augmented the relaxant responses to T-0509 without changing the concentration–response curves for BRL37344 and clenbuterol. Thus, the observed increased isoproterenol-induced relaxation seems to be mainly due to the selective enhancement of β_1-adrenoceptors-mediated response.

The mechanism of enhancement of β_1-adrenoceptors-mediated relaxation in the diabetic rat urinary bladder smooth muscle remains to be elucidated. However, peripheral and/or autonomic neuropathy might be partly involved (Ellenberg and Weber, 1966; Faerman et al., 1973; Buck et al., 1976). In fact, denervation of sympathetic nerves by treatment with 6-hydroxydopamine of the rat enhanced the isoproterenol-induced relaxation of urinary bladder preparations (Ekström, 1979), though whether this procedure selectively augments β_1-adrenoceptor-mediated relaxation is unknown. Diabetes causes polyuria and bladder overdistension; therefore, these changes also may contribute to enhancement of β_1-adrenoceptor mediated relaxations.

A number of reports described the enhanced muscarinic receptor-mediated contraction in diabetic rat urinary bladder (Latifpour et al., 1989, 1991; Kanda et al., 1997). The enhanced contractile responses of the diabetic muscle to muscarinic receptor agonists are most evident at higher concentrations of the agonist. This seems to be the reason why the control and diabetic bladder smooth muscles exhibited similar absolute levels of force when stimulated with 0.6 μM carbachol (which caused approximately 50% of the maximal contraction).

In conclusion, this study demonstrated that the isoproterenol-induced relaxations of the urinary smooth muscles were enhanced in diabetic rats. The augmentation of isoproterenol-induced relaxation seems to be mainly due to the selective enhancement of β_1-adrenoceptors-mediated response.

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