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Involvement of cyclic AMP-dependent and -independent mechanisms in the relaxation of rat detrusor muscle via β-adrenoceptors☆

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Abstract

We investigated the cAMP-dependent and -independent mechanisms of relaxation via β-adrenoceptor in rat detrusor muscle with and without pre-contraction. A microdialysis technique was used to measure detrusor tension and cAMP level on the same detrusor tissue. In non-contracted tissue, isoproterenol, clenbuterol (β_2-adrenoceptor agonist) and FR165101, ((8S)-8-{[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl] amino}-6,7,8,9-tetrahydro-5H-benzocyclohepten-2-yl)oxy]acetic acid hydrochloride (β_3-adrenoceptor agonist) relaxed detrusor muscle and cAMP levels also increased in a concentration dependent manner. SQ22536 (adenylyl cyclase inhibitor) markedly suppressed relaxation, suggesting that β-adrenoceptor-mediated relaxation may be attributed mainly to cAMP-dependent mechanism. In high K^+ pre-contracted tissue, although relaxation advanced in a concentration dependent manner, cAMP production reached a plateau at concentrations of more than 10^{-7} M. SQ22536 had only a small inhibitory effect. However, large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channel inhibitors, charybdotoxin and iberiotoxin markedly suppressed relaxation. These results suggest that in addition to cAMP-dependent pathway, BK_{Ca} channels are involved in the β-adrenoceptor agonists-induced relaxation in pre-contracted detrusor muscle.

Keywords: Bladder smooth muscle; β-adrenoceptor; cAMP; Ca^{2+}-activated K^+ channel

1. Introduction

The detrusor muscle is relaxed by the activation of β-adrenoceptors distributed on bladder smooth muscle in various species including humans (Yamazaki et al., 1998; Yamaguchi, 2002; Nomiya and Yamaguchi, 2003). This relaxation of detrusor muscle via β-adrenoceptors is thought to contribute to the urine storage during bladder filling.

The mechanism by which β-adrenoceptor agonists induce relaxation of smooth muscle is not fully understood, but an intracellular pathway for smooth muscle relaxation is believed to be activated by adenosine 3′:5′-cyclic monophosphate (cAMP). Activation of β-adrenoceptors couples via Gs proteins to adenylyl cyclase, leading to an increase in intracellular cAMP levels and a subsequent activation of cAMP-dependent protein kinase A (PKA) (Gilman, 1987; Murray, 1990). Then, PKA phosphorylates myosin light chain kinase, which suppresses a calcium-calmodulin-dependent interaction of myosin with actin. The increase in cAMP production also results in attenuation of cytoplasmic calcium ion concentration ($[Ca^{2+}]_I$) by removal of Ca^{2+} from cytoplasm. Besides the above cAMP-dependent mechanisms, it has been suggested that in vascular (Scornik et al., 1993), gastrointestinal (Horinouchi and Koike, 2002) and airway smooth muscle (Kume et al., 1993), β-adrenergic transduction pathways exist that are independent of cAMP formation, involving direct interaction of Gs proteins with potassium (K^+) channels. However, with regard to bladder smooth muscle, the cAMP-independent mechanism remains unclear.

Most in vitro relaxation studies are performed using pre-contracted muscle strips because the basal tone of smooth

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muscle is very low. Therefore, the relaxing effect of β-adrenoceptor agonists has been studied in the presence of various pharmacological constrictors (carbachol, prostaglandin, high K^+, etc.) that produce pre-contraction. However, the cellular signaling processes in response to β-adrenoceptor agonists may differ according to whether the detrusor muscle is contracted or not.

Thus, in the present study, we investigated the cAMP-dependent and -independent mechanisms of β-adrenoceptor agonist-induced relaxation in the two different states of detrusor muscle, i.e., with or without pre-contraction.

2. Materials and methods

2.1. Animals and tissue preparation

The experimental protocol complies with the guidelines for animal experiments approved by the Fukushima Medical University. Male Sprague–Dawley rats (8 weeks, 200–250 g wt.) were anaesthetized with diethyl ether. The animals were killed by rapid exsanguination and the urinary bladder was isolated. After removal of the fat and adventitia, four equally sized longitudinal strips of approximately 2×8 mm were cut from the bladder body.

2.2. cAMP measurements

This study used a microdialysis technique to measure detrusor muscle tension and cAMP level on the same detrusor tissue. Extracellular cAMP in the detrusor tissue was sampled by the technique of microdialysis and measured later by enzyme immunoassay (EIA).

2.2.1. Microdialysis measurement of cAMP

The dialysis probe (O-P-100-10, Eicom Co., Kyoto, Japan) used in this study had a 0.22×10 mm dialysis membrane with a molecular cutoff of 50 kDa. The probe was inserted through the detrusor muscle strip and the inlet cannula of the probe was connected to a microinfusion syringe pump (EP-60, Eicom Co.). Krebs solution was continuously perfused at the rate of 3.4 μl/min. The detrusor strip, having the dialysis probe, was suspended in the organ bath. At various concentrations of β-adrenoceptor agonist, dialysate was collected in a microtube every 30 min. Dialysate collected 30 min before adding isoprotrenol was used for control. Each dialysate sample was stored at −80 °C and the cAMP content in dialysate was determined using cAMP EIA kit.

Since the microdialysis method measures extracellular cAMP, we briefly studied whether this technique can detect the changes in intracellular cAMP level in both non-contracted and pre-contracted detrusor tissue. Thus, the cAMP content in detrusor tissue, which represents intracellular cAMP level, was determined at various concentrations of isoproterenol (10^{-9} to 10^{-4} M). The isoproterenol–cAMP response curve was created and compared to that obtained from the microdialysis measurement.

2.2.2. cAMP content in detrusor tissue

One of the divided detrusor muscle strips was used for the stimulation of cAMP production by isoproterenol, and the other one was used for control. The detrusor strips were incubated in Krebs solution at 37 °C for 30 min. After incubation with isoproterenol and 10^{-5} M 3-isobutyl-1-methylxanthine (IBMX: phosphodiesterase inhibitor) in Krebs solution and also in 40 mM K solution at 37 °C for 30 min, the detrusor strips were rapidly frozen in liquid nitrogen. Trichloroacetic acid (6%, 1.0 ml) was added to the frozen detrusor strips and the tissues were then homogenized with a Teflon homogenizer. The homogenate was centrifuged for 10 min (9000 *g*) at 4 °C. The supernatant was washed with water saturated diethyl ether 4 times and assayed for cAMP content using cAMP EIA system kit (Amersham, Buckinghamshire, UK). The pellet was dissolved in 1 M NaOH and assayed for protein content using Dc Protein Assay Kit (Bio-Rad, Hercules, CA).

2.3. Functional studies

Detrusor strips were suspended in a 25 ml organ bath containing Krebs solution maintained at 37 °C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. Muscle tension was measured isometrically using a force displacement transducer (TB-621, Nihon Kohden, Tokyo). An initial tension of 9.8 mN was applied to the tissues, and the tissues were allowed to equilibrate for at least 30 min before any experimental procedure was begun.

Functional studies were performed on both pre-contracted and non-contracted detrusor muscle. When the detrusor strip was pre-contracted, 40 mM KCl was used. This high K^+ solution was made by replacing equimolar concentrations of Na^+ in Krebs solution with K^+ to retain isotonicity. In studies on non-contracted detrusor muscle, the strip was gradually stretched until a stable tension of approximately 10 mN was obtained.

2.3.1. Relaxing effects of β-adrenoceptor agonists

Concentration–response curves for β-adrenoceptor agonists were obtained by the cumulative addition of each substance to the bathing fluid. The relaxing effect of each agonist is expressed as a percentage of the maximal relaxation induced by 10^{-4} M papaverine. The pD_2 values (negative logarithm of EC_{50} value) were calculated for each agonist from its concentration–relaxation curve. β-adrenoceptor agonists used for this experiment were isoproterenol (β-adrenoceptor non-selective agonist), dobutamine (β_1-adrenoceptor selective agonist), clenbuterol (β_2-adrenoceptor selective agonist) and FR165101, ((8S)-8-{[(2R)-2-(3-chlorophenyl)-2-hydroxyethyl]amino}-6,7,8,9-tetrahydro-5H-benzocyclohepten-2-yl)oxy]acetic acid hydrochloride (β_3-adrenoceptor selective agonist). FR165101 is a carboxylic acid form of a β_3-adrenoceptor agonist, FK175 (Yamamoto et al., 1997; Fujimura et al., 1999), with a higher selectivity for β_3-adrenoceptor. The selectivity of FR165101 for activation of β_3-adrenoceptor versus that of β_1-adrenoceptor and β_2-adrenoceptor was calculated at >200 and >630-fold, respectively.

2.3.2. Measurements of muscle tension and cAMP production on the same detrusor tissue

The microdialysis technique was used to evaluate cAMP production and detrusor muscle relaxation on the same detrusor tissue in response to β-adrenoceptor agonists. Our preliminary study showed that insertion of the microdialysis probe into the muscle strip did not influence the muscle contractility (data not

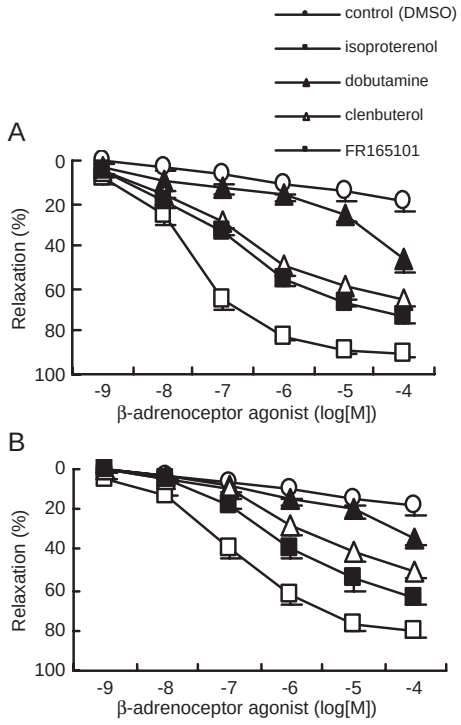

Fig. 1. Relaxing effects of isoproterenol, dobutamine, clenbuterol and FR165101 in non-contracted (A) and high K^+ pre-contracted (B) detrusor muscle. Data are expressed as percent relative to maximal relaxation induced by papaverine (10^{-4} M). Isoproterenol, clenbuterol and FR165101 caused statistically significant effects compared to control ($P<0.05$). Values are means±S.E.M. of 8 experiments.

shown). As described, the probe was inserted through the muscle strip and suspended in the organ bath. Each strip was connected to the force displacement transducer to measure muscle tension. Sampling was started 30 min before adding β-adrenoceptor agonist and dialysate was collected in a microtube every 30 min, which was the same interval as that between the successive administration of different concentrations of β-adrenoceptor agonist. The dialysate of each sample was stored at −80 °C and used for cAMP assay later. β-adrenoceptor agonists used in this experiment were isoproterenol, clenbuterol and FR165101.

2.3.3. Effects of adenylyl cyclase inhibitor and K^+ channel inhibitors

An adenylyl cyclase inhibitor, SQ22536 (3×10^{-4} M) was used. We examined whether SQ22536 suppresses the increase in detrusor tissue cAMP content produced by 10^{-5} M isoproterenol. After incubation with SQ22536 (3×10^{-4} M), 10^{-5} M isoproterenol was added. After 30 min, the detrusor strips were rapidly frozen in liquid nitrogen and stored at −80 °C. As described above, cAMP in tissues was extracted by trichloroacetic acid and assayed by using cAMP EIA system kit. Charybdotoxin (10^{-7} M) and iberiotoxin (10^{-7} M) were used as the large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channel inhibitors. Glibenclamide (10^{-5} M) was also used as ATP-activated K^+ (K_{ATP}) channel inhibitor. These inhibitors were added to the bath 30 min before the addition of β-adrenoceptor agonists. Concentration–response curves for β-adrenoceptor agonists were thus obtained in the presence of the inhibitors. When the relaxing response of each agonist was estimated, the relaxing effect is usually expressed as a percentage of the maximal relaxation induced by papaverine (phosphodiesterase inhibitor). However, in this experiment, we could not use papaverine to cause a maximal relaxation because the increase in cAMP content was already inhibited by pretreatment of SQ22536 (adenylyl cyclase inhibitor). Thus, the relaxing effect of each β-adrenoceptor agonist was expressed as a percent of the decrease from the initial tension.

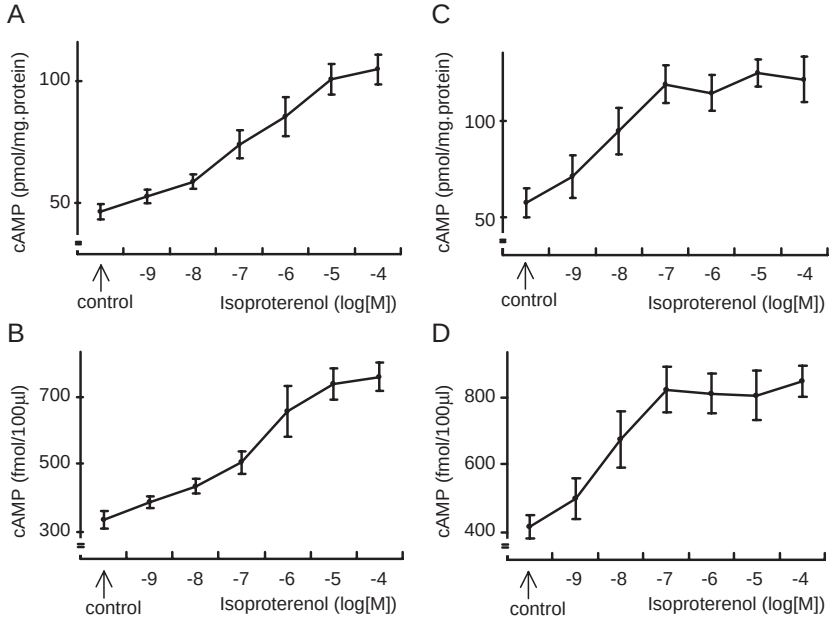

Fig. 2. Concentration-dependent effects of isoproterenol on tissue cAMP content and dialysate cAMP in non-contracted tissue (A,B) and pre-contracted tissue (C,D). In non-contracted tissue, the basal level of cAMP was 52.1±3.4 pmol/mg protein in tissue content, 354±24 fmol/100 μl in dialysate. In pre-contracted tissue, the basal level of cAMP was 63±8.3 pmol/mg protein in tissue content, 430±26 fmol/100 μl in dialysate. Values are means±S.E.M. of 5–8 experiments.

2.4. Drugs and solutions

The following drugs were used: (±)-isoproterenol hydrochloride, 3-isobutyl-1-methylxanthine (IBMX), papaverine hydrochloride, (±)-dobutamine hydrochloride, clenbuterol hydrochloride, charybdotoxin, iberiotoxin, glibenclamide (Sigma-Aldrich, St. Louis, MO, USA); 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ22536) (Research Biochemicals International, Natick, MA, USA); (FR165101) (Fujisawa, Osaka, Japan). FR165101 was dissolved in 100% dimethyl sulfoxide and other drugs were dissolved in distilled water. Reported concentrations are the calculated final concentrations in the bath solution. The composition of the Krebs solution used was 118.1 mM sodium chloride, 4.7 mM potassium chloride, 2.5 mM calcium chloride, 1.2 mM magnesium sulfate, 1.2 mM dipotassium phosphate, 25 mM sodium bicarbonate and 11.1 mM glucose, pH 7.4.

2.5. Data analysis

The results are expressed as means ± S.E.M. of the number (n) of experiments. Agonist potency is expressed as the pD_2 value. The statistical significance of the drug effects was analyzed by comparing the control and treated preparations using repeated measures two-way analysis of variance (ANOVA). When the mean values of the same group before and after stimulation were compared, Student's t test for paired observations was used. The correlation between cAMP level and relaxation of the detrusor muscle was performed using Pearson's correlation coefficient. P values of 0.05 or less were regarded as statistically significant.

3. Results

3.1. Relaxing effects of β-adrenoceptor agonists

Fig. 1 shows the concentration–response curve for β-adrenoceptor agonists-induced relaxation in rat detrusor muscle. In non-contracted detrusor strips, isoproterenol, clenbuterol (β_2-adrenoceptor selective agonist) and FR165101 (β_3-adrenoceptor selective agonist) produced a concentration dependent relaxation and caused statistically significant effects compared to control group (Fig. 1-A). The pD_2 value of each β-adrenoceptor agonist was 7.54 ± 0.09 in isoproterenol, 6.80 ± 0.13 in clenbuterol and 6.86 ± 0.11 in FR165101, respectively. On pre-contracted detrusor strips, isoproterenol, clenbuterol and FR165101 also relaxed detrusor muscle in a concentration dependent manner (Fig. 1-B). The pD_2 value of each β-adrenoceptor agonist was 6.94 ± 0.13 in isoproterenol, 6.20 ± 0.15 in clenbuterol and 6.35 ± 0.15 in FR165101, respectively. The rank order of β-adrenoceptor agonist potency for relaxing detrusor muscle was isoproterenol > FR165101 > clenbuterol. The relaxing effect of dobutamine (β_1-adrenoceptor selective agonist) was observed only when applied at a high dose (10^{-4} M) on both non-contracted and pre-contracted strips. Since this effect did not attain a maximum at a concentration of 10^{-4} M, pD_2 value was not determined.

3.2. Dialysate cAMP concentration and tissue cAMP content

Fig. 2 shows the changes in dialysate cAMP concentration and tissue cAMP content induced by isoproterenol in non-contracted tissue (A,B) and pre-contracted tissue (C,D). In non-contracted tissue, isoproterenol (10^{-9} to 10^{-4} M) produced a significant increase in cAMP level in the tissue as well as in dialysate. The pD_2 values were 6.90 ± 0.22 in tissue content, 6.65 ± 0.25 in dialysate (Fig. 2-A,B). In pre-contracted tissue, a similar response profile was obtained. The pD_2 values were 8.10 ± 0.19 in tissue content, 8.20 ± 0.27 in dialysate (Fig. 2-C,D). Thus, both dialysate cAMP concentration and tissue cAMP content showed approximately the same changes in response to isoproterenol. This relationship has made it possible to utilize changes in dialysate concentration of cAMP as an index of changes in its intracellular levels.

3.3. Effects of β-adrenoceptor agonists on tension and cAMP level

We measured tension and cAMP levels on the same detrusor tissue when isoproterenol, clenbuterol and FR165101 were each administered. In non-contracted isolated detrusor muscle, while β-adrenoceptor agonists (isoproterenol, clenbuterol and FR165101) relaxed detrusor muscle in a concentration dependent manner, cAMP levels also increased in a concentration dependent manner.

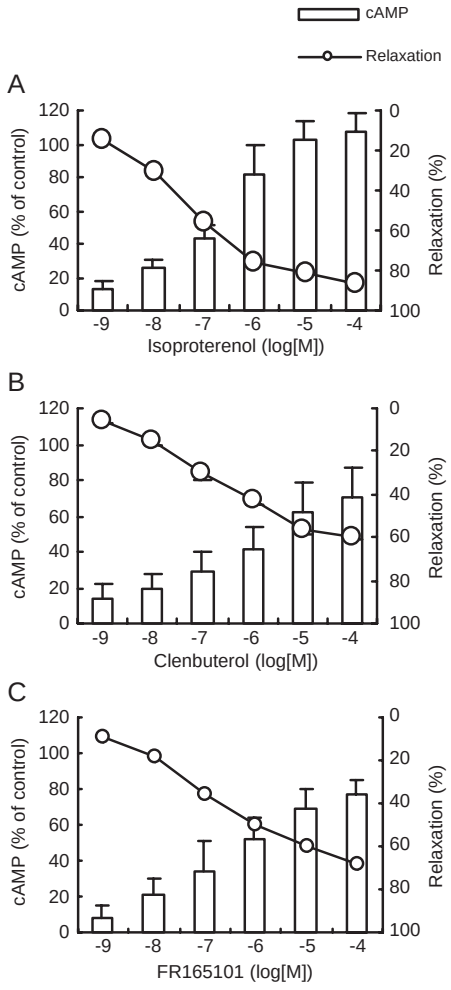

Fig. 3. Effects of isoproterenol (A), clenbuterol (B) and FR165101 (C) on tension and cAMP levels in non-contracted detrusor muscle. Relaxation responses are expressed as percent relative to maximal relaxation induced by papaverine (10^{-4} M). cAMP levels are expressed as percent increase of control. The basal level of cAMP was 320 ± 18 fmol/100 μl in dialysate. Values are means ± S.E.M. of 8 experiments.

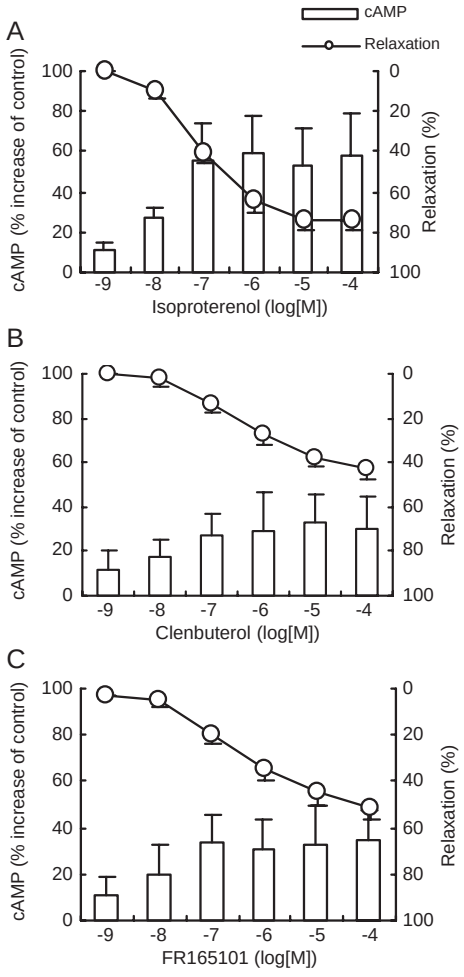

Fig. 4. Effects of isoproterenol (A), clenbuterol (B) and FR165101 (C) on tension and cAMP levels in high K^+ pre-contracted detrusor muscle. Relaxation responses are expressed as percent relative to maximal relaxation induced by papaverine (10^{-4} M). cAMP levels are expressed as percent increase of control. The basal level of cAMP was 407±21 fmol/100 μl in dialysate. Values are means±S.E.M. of 8 experiments.

Thus, this β-adrenoceptor agonists-induced relaxation was accompanied by a concentration-dependent increase in cAMP level (Fig. 3). In addition, there was a significant linear relationship between relaxation rate and cAMP level for isoproterenol ($P<0.01$), clenbuterol ($P<0.01$), and FR165101 ($P<0.01$). However, in high K^+ pre-contracted detrusor muscle, isoproterenol, clenbuterol and FR165101 relaxed detrusor muscle in a concentration dependent manner, while cAMP production reached a maximum at concentration of 10^{-7} M (Fig. 4). The decrease in force was significantly correlated with the increase in cAMP level within the concentrations from 10^{-9} to 10^{-7} M for each of the β-adrenoceptor agonists: isoproterenol ($P<0.01$), clenbuterol ($P<0.01$) and FR165101 ($P<0.01$). However, a significant correlation between the two was not found at concentrations from 10^{-7} to 10^{-4} M for each of the β-adrenoceptor agonists: isoproterenol ($P>0.05$), clenbuterol ($P>0.05$) and FR165101 ($P>0.05$). Although the relaxant response advanced at concentrations of more than 10^{-7} M, β-adrenoceptor agonists (10^{-6} to 10^{-4} M) did not produce a further increase in cAMP levels.

3.4. Effects of adenylyl cyclase inhibitor and K^+ channel inhibitors

We examined the effect of adenylyl cyclase inhibitor and K^+ channel inhibitors on β-adrenoceptor agonists-mediated relaxation. Fig. 5 shows effects of SQ22536 on isoproterenol-induced cAMP elevation. In non-contracted and pre-contracted detrusor strips treated with SQ22536 (3×10^{-4} M), isoproterenol (10^{-5} M) caused no significant change in cAMP level. Thus, SQ22536 (3×10^{-4} M) was shown to suppress significantly the increase in detrusor tissue cAMP content produced by isoproterenol (10^{-5} M). In non-contracted detrusor muscle, pretreatment of detrusor strips by SQ22536 (3×10^{-4} M) markedly inhibited relaxation induced by isoproterenol. However, pretreatment of detrusor muscle by either charybdotoxin (10^{-7} M) or iberiotoxin (10^{-7} M) did not inhibit relaxation induced by isopreterenol (Fig. 6-A). The maximal relaxation produced by isoproterenol was 93.3±4% in control, 38.3±8.3% in the SQ22536-treated group, 95±3.9% in the charybdotoxin-treated group, and 93.3±3.8% in the iberiotoxin-treated group, respectively. Glibenclamide failed to attenuate the isoproterenol-induced relaxation at the concentration of 10^{-5} M (data not shown). These inhibitors also showed similar effects on clenbuterol and FR165101-induced relaxation (Fig. 6-B,C). Therefore, only SQ22536 significantly inhibited β-adrenoceptor agonists-induced relaxation in non-contracted detrusor muscle.

On the other hand, in high K^+ pre-contracted detrusor muscle, SQ22536 had a small inhibitory effect on isoproterenol-induced relaxation, while charybdotoxin and iberiotoxin markedly suppressed the relaxation produced by isoproterenol (Fig. 7-A). The maximal relaxation produced by isoproterenol was 83.1±2.5% in control, 73.3±2.4% in the SQ22536-treated group, 45.5±2.3% in the charybdotoxin-treated group, and 40.8±3.3% in the iberiotoxin-treated group, respectively. Glibenclamide failed to attenuate the isoproterenol-induced relaxation at the concentration of 10^{-5} M (data not shown). As for the effects of the above inhibitors on clenbuterol and FR165101-induced relaxation, the same results were obtained (Fig. 7-B,C). Thus, in high K^+ pre-contracted

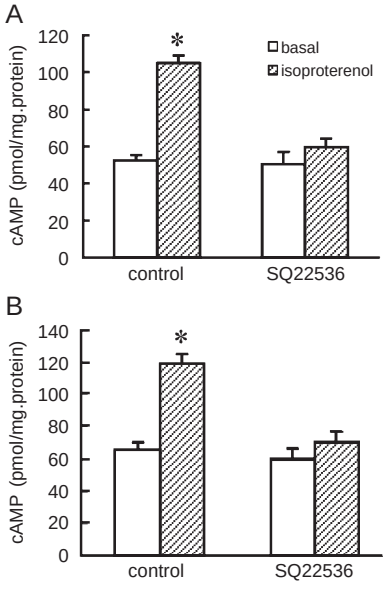

Fig. 5. Effects of SQ22536 on cAMP content induced by isoproterenol in non-contracted tissue (A) and pre-contracted tissue (B). Values are means± S.E.M. of 5 experiments. *Significantly different from basal ($P<0.05$).

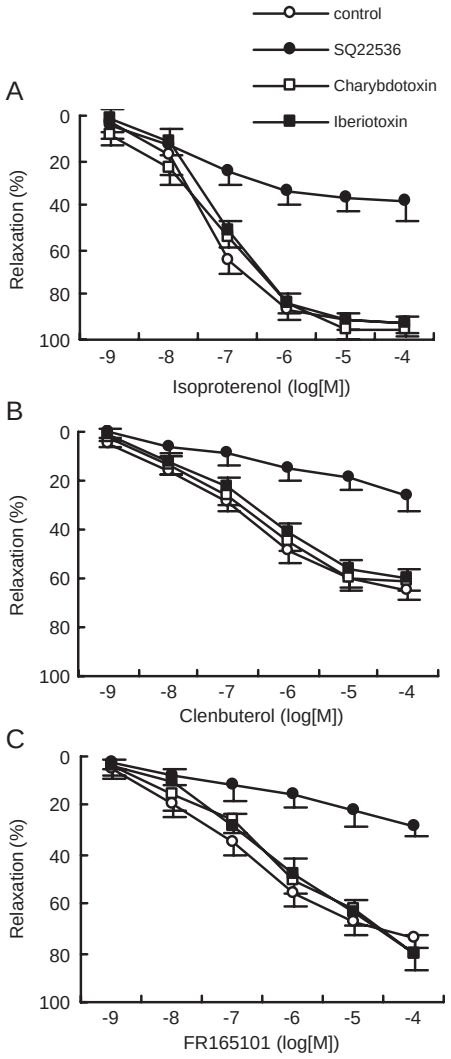

Fig. 6. Effects of SQ22536 (3×10^{-4} M), charybdotoxin (10^{-7} M) and iberiotoxin (10^{-7} M) on relaxation of non-contracted detrusor muscle induced by isoproterenol (A), clenbuterol (B) and FR165101 (C). Data are expressed as percent relative to stable tension of 10 mN. Pretreatment of SQ22536 caused statistically significant effects compared to control ($P<0.05$). Values are means±S.E.M. of 6 experiments.

detrusor muscle, K_{Ca} channel inhibitors suppressed β-adrenoceptor agonist-induced relaxation more than adenylyl cyclase inhibitor, SQ22536.

4. Discussion

The present study investigated the mechanisms by which β-adrenoceptor agonists relax bladder smooth muscle (detrusor muscle) in the rat, particularly focusing on cAMP-dependent and -independent pathways.

First, we examined the relative potencies with which β-adrenoceptor agonists relaxed the rat detrusor muscle. The rank order for the relaxant effect of these β-adrenoceptor agonists was isoproterenol>FR165101 (β_3-adrenoceptor selective agonist)>clenbuterol (β_2-adrenoceptor selective agonist). However, dobutamine (supposedly β_1-adrenoceptor selective agonist) produced no significant relaxation at concentrations from 10^{-9} to 10^{-5} M, indicating that the β_1-adrenoceptor is of little functional importance in the relaxation of rat detrusor. In accordance with our study, Yamazaki et al. (1998) also suggested that in the rat detrusor muscle, β-adrenoceptor agonist-induced relaxation is mediated mainly via both β_2- and β_3-adrenoceptor subtypes.

In the present study, we used a microdialysis technique to evaluate the change in intracellular cAMP level in response to β-adrenoceptor agonists because this technique enables us to measure tension and cAMP levels on the same detrusor tissue. Since a microdialysis method detects extracellular

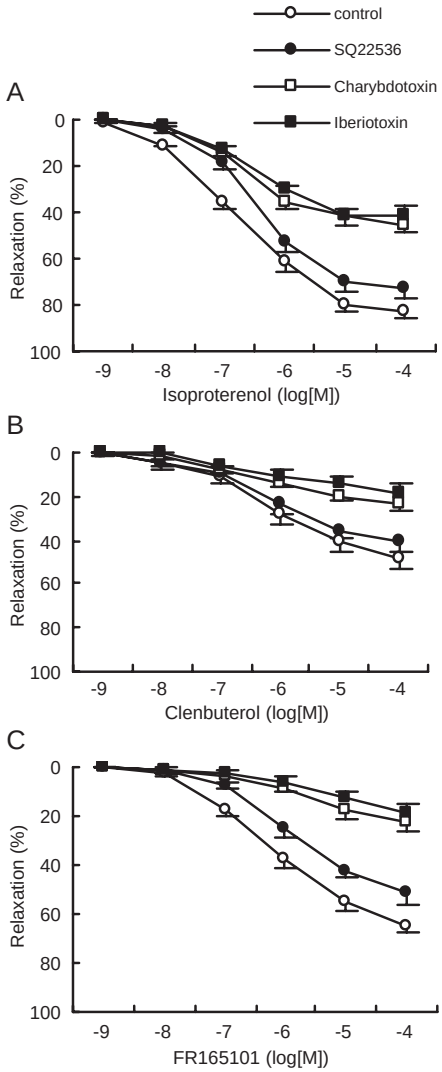

Fig. 7. Effects of SQ22536 (3×10^{-4} M), charybdotoxin (10^{-7} M) and iberiotoxin (10^{-7} M) on relaxation of high K^+ pre-contracted detrusor muscle induced by isoproterenol (A), clenbuterol (B) and FR165101 (C). Data are expressed as percent relative to steady-state tension after precontraction. Pretreatment of charybdotoxin and iberiotoxin caused statistically significant effects compared to control ($P<0.05$). Values are means±S.E.M. of 6 experiments.

cAMP, this may question the feasibility of measuring the intracellular cAMP level by microdialysis. However, most cells that produce cAMP are known to release or leak small amounts of cAMP into the extracellular space, and the rate of egress of cAMP has been found to be proportional to its intracellular concentration in both cultured cells and tissue slices (Barber and Butcher, 1983; Stoof and Kebabian, 1981; Lazareno et al., 1985; Egawa et al., 1988). In this respect, the present study has shown that both dialysate cAMP concentration and tissue cAMP content show approximately the same changes in response to β-adrenoceptor agonists. It is, therefore, substantiated that the changes in dialysate cAMP concentration can be utilized as an index of changes in its intracellular level.

Thus, using microdialysis, we were able to evaluate the exact relationship between β-adrenoceptor agonist-induced relaxation and cAMP production.

In non-contracted isolated detrusor muscle, isoproterenol, clenbuterol and FR165101 were shown to cause a concentration-dependent decrease in basal tone of the detrusor strips. This β-adrenoceptor agonist-induced relaxation was accompanied by a concentration-dependent increase in cAMP level. Furthermore, SQ22536, an adenylyl cyclase inhibitor, markedly suppressed relaxation caused by these β-adrenoceptor agonists. Since SQ22536 (300 μM) abolished the isoproterenol-induced rise in cAMP in detrusor tissue, these results suggest that β-adrenoceptor-mediated relaxation can be attributed exclusively to cAMP-dependent mechanism in non-contracted detrusor.

However, in high K^+ pre-contracted detrusor muscle, we could not find a dose–response effect of β-adrenoceptor agonists (isoproterenol, clenbuterol and FR165101) on cAMP production. It is clear from the measurements of tension and cAMP level on the same detrusor strip that although relaxation advanced with an increase in β-adrenoceptor agonist concentration, the cAMP production reached a plateau at concentrations of more than 10^{-7} M. In addition, the present study showed that SQ22536 had only a small inhibitory effect on β-adrenoceptor agonists-induced relaxation in high K^+ pre-contracted detrusor muscle. This result together with a discrepancy between relaxant effect and cAMP level indicate that β-adrenoceptor agonist-induced relaxation of contracted detrusor may be at least partly independent of cAMP levels.

Recently, the traditional view of cAMP's role has been challenged. It has been demonstrated that in airway smooth muscle, SQ22536 that inhibits the increase in cAMP content had no influence on relaxation elicited by cholera toxin (an activator of Gs-protein) or β-adrenoceptor selective agonist (Tanaka et al., 2003; Koike et al., 2004). In another study, it was shown that the PKA inhibitors failed to antagonize isoprenaline-induced vasodilation in rat mesenteric microvessels (Czyborra et al., 2002). These studies provide evidence that in various types of smooth muscle, cAMP-independent mechanisms are involved in β-adrenoceptor-mediated relaxation.

The exact mechanisms mediating cAMP-independent relaxation remain unclear. However, an alternative pathway would be activation of potassium (K^+) channels, since many studies (Scornik et al., 1993; Kume et al., 1993; Clapp et al., 1998; Jackson et al., 1993; Hein and Kuo, 1999) demonstrated that different types of K^+ channels were associated with cAMP-independent relaxation. In the present study, we showed that pretreatment of detrusor muscle by either charybdotoxin or iberiotoxin markedly inhibited relaxation induced by β-adrenoceptor agonists (isoproterenol, clenbuterol and FR165101), whereas glibenclamide failed to attenuate the relaxation (data not shown). Thus, the above results suggest calcium-activated K^+ channels (BK_{Ca} channels) are involved in the relaxant effects of β-adrenoceptor agonists on pre-contracted detrusor muscle.

Since we used high K^+ solution (40 mM K^+) to produce pre-contraction, this may effect Ca^{2+}-activated K^+ channels. Depolarizing concentrations of extracellular potassium activate voltage-dependent Ca^{2+} channels. Consequently, cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]i$) increases, leading to detrusor muscle contraction. Simultaneously with contraction, the increase in $[Ca^{2+}]i$ may open Ca^{2+}-activated K^+ channels that leads to muscle relaxation. However, this relaxant effect may be inhibited because high concentration of extracellular K^+ raises the equilibrium potential for K^+. Thus, pre-contraction produced by high K^+ can be sustained. Under the above conditions, BK_{Ca} channels may be activated to some extent. It is assumed that BK_{Ca} channels are activated further by cAMP-independent mechanisms when β-adrenoceptor agonists induce relaxation of pre-contracted detrusor muscle.

The present study also showed that in pre-contracted detrusor muscle, the cAMP production determined from both dialysate concentration and tissue content did not increase with an increase in β-adrenoceptor agonist concentration and reached a plateau at concentrations of more than 10^{-7} M. It is not likely that high extracellular K^+ (40 mM) or the resultant increase in ($[Ca^{2+}]i$ has any effect on adenylyl cyclase activity. Thus, further investigation is required to clarify the underlying mechanisms.

It can not be determined from this study whether BK_{Ca} channels are activated exclusively by cAMP-independent mechanisms. Several studies (Hein and Kuo, 1999; Kume et al., 1993; Tanaka et al., 2003) have proposed direct as well as Gs-mediated, adenylyl cyclase-independent mechanisms are responsible for the activation of BK_{Ca} channels. On the other hand, Kobayashi et al. (2000) showed that the isoproterenol-induced relaxation of guinea-pig bladder smooth muscle was mainly mediated by facilitation of BK_{Ca} channels subsequent to the activation of the cAMP/protein kinase A pathway. If BK_{Ca} channels are activated solely by this cAMP-dependent pathway, an adenylyl cyclase inhibitor (SQ22536) would suppress the isoproterenol-induced relaxation to the same extent as a BK_{Ca} channel inhibitor (iberiotoxin). However, our results showed that in high K^+ pre-contracted detrusor muscle, the

inhibitory effect of SQ22536 on relaxation elicited by isoproterenol was much smaller than iberiotoxin, suggesting that in rat detrusor muscle BK_{Ca} channels may be activated mainly by means independent of cAMP formation. Thus, the consequence of activation of protein kinase A seems to differ among species.

The present study indicates that in non-contracted detrusor muscle, relaxation mediated through β-adrenoceptors is achieved solely by cAMP-dependent mechanism while in KCl pre-contracted detrusor muscle, both cAMP-dependent mechanism and cAMP-independent mechanism via BK_{Ca} channels may be involved in β-adrenergic relaxation. Thus, the contribution of the two mechanisms seems to be dependent on the condition of the detrusor muscle.

The relaxing effect of β-adrenoceptor agonists was well demonstrated in the absence of pre-contraction (Igawa et al., 1999; Yamazaki et al., 1998), suggesting that the basal tone of detrusor muscle produced by mechanical stretch can be reduced by β-adrenoceptor agonists. This may reflect a physiological role of β-adrenoceptors during bladder filling because the bladder wall is mechanically stretched as urine is stored in the bladder. Therefore, it seems likely that the cAMP-dependent mechanism plays an important role in producing β-adrenoceptor-mediated relaxation of detrusor during the storage phase.

Most in vitro studies investigating the relaxing effect of β-adrenoceptor agonists use pharmacological agents to produce pre-contraction in the muscle strips. Although these pharmacological constrictors may have influence on the signal transduction pathway involved in β-adrenoceptor-mediated relaxation, pre-contraction seems to represent partly a pathologic state of detrusor muscle. In normal condition, detrusor muscle never contracts during bladder filling. If detrusor shows an involuntary contraction during the storage phase, this is a pathologic condition called "detrusor overactibity" that leads to the symptoms of frequency, urgency and urge incontinence. Therefore, from this clinical point of view, it is also important to elucidate the mechanisms by which β-adrenoceptor agonists relax the contracted detrusor muscle. When detrusor muscle is contracted, this study shows that β-adrenoceptor agonist-induced relaxation is not only attributable to the classical cAMP pathways, and that as a cAMP-independent mechanism, activation of BK_{Ca} channels also mediates relaxation of detrusor muscle. Finally, clinical implication of this study is that on the basis of cAMP-dependent and -independent mechanisms, β-adrenoceptor agonists may be effective for the treatment of overactive bladder.

References

Barber, R., Butcher, R.W., 1983. The egress of cyclic AMP from metazoan cells. Adv. Cycl. Nucleotide Res. 15, 119–138.

Clapp, L.H., Turcato, S., Baloch, M., 1998. Evidence that Ca^{2+}-activated K^+ channels play a major role in mediating the vascular effects of iloplost and cicaprost. Eur. J. Pharmacol. 356, 215–224.

Czyborra, P., Saxe, M., Fetscher, C., Meyer, Zu., Heringdorf, D., Herzig, S., Jakobs, K.H., Michel, M.C., Bischoff, A., 2002. Transient relaxation of rat mesenteric microvessels by ceramides. Br. J. Pharmacol. 135, 417–426.

Egawa, M., Hoebel, B.G., Stone, E.A., 1988. Use of microdialysis to measure brain noradrenergic receptor function in vivo. Brain Res. 458, 303–308.

Fujimura, T., Tamura, K., Tsutsumi, T., Yamamoto, T., Nakamura, K., Koibuchi, Y., Kobayashi, M., Ymaguchi, O., 1999. Expression and possible function role of the β3-adrenoceptor in human and rat detrusor muscle. J. Urol. 161, 680–685.

Gilman, A.G., 1987. G proteins: transducers of receptor-generated signals. Ann. Rev. Biochem. 56, 615–649.

Hein, T.W., Kuo, L., 1999. cAMP-independent dilation of coronary arterioles to adenosine: role of nitric oxide, G proteins, and K(ATP) channels. Circ. Res. 85, 634–642.

Horinouchi, T., Koike, K., 2002. Cyclic AMP-independent relaxation mediated by beta3-adrenoceptors on guinea pig gastrointestine. Eur. J. Pharmacol. 442, 137–146.

Igawa, Y., Yamazaki, Y., Takeda, H., Hayakawa, K., Akahane, M., Ajisawa, Y., Yoneyama, T., Nishizawa, O., Andersson, K.E., 1999. Functional and molecular biological evidence for a possible β3-adrenoceptor in the human detrusor muscle. Br. J. Pharmacol. 126, 819–825.

Jackson, W.F., Konig, A., Dambacher, T., Busse, R., 1993. Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. Am. J. Physiol. 264, H238–H243.

Kobayashi, H., Adachi-Akahane, S., Nagao, T., 2000. Involvement of BKCa channels in the relaxation of detrusor muscle via β-adrenoceptors. Eur. J. Pharmacol. 404, 231–238.

Koike, K., Yamashita, Y., Horinouchi, T., Yamaki, F., Tanaka, Y., 2004. cAMP-independent mechanism is significantly involved in β2-adrenoceptor-mediated tracheal relaxation. Eur. J. Pharmacol. 492, 65–70.

Kume, H., Hall, I.P., Washabau, R.J., Takagi, K., Kotlikoff, M.I., 1993. β-adrenergic agonists regulate K_{Ca} channels in airway smooth muscle by cAMP-dependent and -independent mechanisms. J. Clin. Invest. 93, 371–379.

Lazareno, S., Marriott, D.B., Nahorski, S.R., 1985. Differential effect of selective and nonselective neuroleptics on intracellular and extracellular cyclic AMP accumulation in rat striatal slices. Brain Res. 361, 91–98.

Murray, K.J., 1990. Cyclic AMP and mechanisms of vasodilation. Pharmacol. Ther. 47, 329–345.

Nomiya, M., Yamaguchi, O., 2003. A quantitative analysis of mRNA expression of α1 and β-adrenoceptor in human and rat detrusor muscle. J. Urol. 170, 649–653.

Scornik, F.S., Codina, J., Birnbaumer, L., Toro, L., 1993. Modulation of coronary smooth muscle K_{Ca} channels by Gsα independent of phosphorylation by protein kinase A. Am. J. Physiol. 265, H1460–H1465.

Stoof, J.C., Kebabian, J.W., 1981. Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 294, 366–368.

Tanaka, Y., Yamashita, Y., Yamaki, F., Horinouchi, T., Shigenobu, K., Koike, K., 2003. Evidence for the significant role for Gs-triggered mechanism unrelated to the activation of adenylyl cyclase in cyclic AMP-independent relaxant response of tracheal smooth muscle from the guinea pig. Naunyn-Schmiedeberg's Arch. Pharmacol. 368, 437–441.

Yamaguchi, O., 2002. β3-adrenoceptors in human detrusor muscle. Urology 59 (Suppl 5A), 25–29.

Yamamoto, H., Takakura, S., Yamamoto, T., Satoh, H., Higaki, M., Tomoi, M., Shimomura, K., 1997. FR149175, a β3-adrenoceptor-selective agonist, is a possible therapeutic agent for non-insulin-dependent diabetes mellitus. Jpn. J. Pharmacol. 74, 109.

Yamazaki, Y., Takeda, H., Akahane, M., Igawa, Y., Nishizawa, O., Ajisawa, Y., 1998. Species differences in the distribution of β-adrenoceptor subtypes in bladder smooth muscle. Br. J. Pharmacol. 124, 593–599.