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Characterization of β-Adrenoceptor Subtype in Bladder Smooth Muscle in Cynomolgus Monkey

Hiroo Takeda[1,*], Yoshinobu Yamazaki[1], Masuo Akahane[1], Satoshi Akahane[1], Hiroshi Miyata[1], Yasuhiko Igawa[2] and Osamu Nishizawa[2]

[1]*Division of Discovery Research, Central Research Laboratory, Kissei Pharmaceutical Co., Ltd., 4365-1, Hotaka, Nagano 399-8304, Japan*
[2]*Department of Urology, Shinshu University School of Medicine, 3-1-1, Asahi, Matsumoto 390-8621, Japan*

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ABSTRACT—We first investigated the relaxations of the urinary bladder induced by β-adrenoceptor agonists in anesthetized cynomolgus monkeys and then employed a variety of β-adrenoceptor agonists and antagonists in vitro to identify the β-adrenoceptor subtype responsible for the relaxation (using isolated monkey detrusors). Isoprenaline reduced bladder pressure in a dose-dependent manner. Isoprenaline, noradrenaline and adrenaline each produced a concentration-dependent relaxation of isolated detrusor strips, the rank order of relaxing potencies being isoprenaline > noradrenaline > adrenaline. Subtype-selective β-adrenoceptor agonists also relaxed isolated detrusor strips, the rank order of potencies being CGP-12177 > BRL 37344 > dobutamine, salbutamol, procaterol > xamoterol. In the antagonist experiment, bupranolol (β-antagonist, 10^{-6} to 10^{-5} M) and SR 58894A (β_3-antagonist, 10^{-7} to 10^{-5} M) caused a rightward shift of the concentration-relaxation curve for isoprenaline, but CGP-20712A (β_1-antagonist, 10^{-9} to 10^{-7} M) and ICI-118551 (β_2-antagonist, 10^{-9} to 10^{-7} M) did not. The present functional study provides the first evidence that relaxation of the monkey detrusor by β-adrenoceptor activation is mediated via the β_3-subtype.

Keywords: β-Adrenoceptor, β_3-Adrenoceptor, Monkey, Detrusor, Bladder

In humans, a number of functional and molecular biological studies have confirmed that β_3-adrenoceptors play important functional roles in adipocytes (1), gut (2, 3) and urinary bladder (4 – 6). In the urinary bladder, activity is mainly regulated by the parasympathetic and sympathetic nervous systems and sympathetically mediated β-adrenoceptor activation has important functional effects on urine storage (7). This raises the possibility that β_3-adrenoceptor stimulation in the urinary bladder may be effective in the treatment of such dysfunctions as frequent urination and incontinence. However, there are marked species differences in the receptor subtypes mediating relaxation of the mammalian detrusor. For example, such relaxation occurs mainly via the β_1-adrenoceptor in cats (8) and guinea pigs (9), but mainly via the β_2-adrenoceptor in rabbits (10). In contrast, it has been confirmed that β_3-adrenoceptor agonists strongly relax the canine, rat and ferret detrusors (11 – 13), although the β_2-adrenoceptor is also involved in the rat. In the present study, in a search for an appropriate animal model for bladder function in humans, we pharmacologically characterized the β-adrenoceptors present in the cynomolgus monkey urinary bladder using selective β-adrenergic reagents (agonists and antagonists).

*Corresponding author. FAX: +81-263-82-8827
E-mail: hiroo_takeda@pharm.kissei.co.jp

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 cynomolgus monkeys (2 – 5 kg; Toyota Tsusho Corporation, Tokyo) were housed individually at a stable temperature and humidity and under a 12-h light-dark cycle, and they had free access to water and standard laboratory food until the day of the experiment.

In vivo experimental protocol

Monkeys were initially anesthetized with ketamine (10 mg/kg, intramuscular). Then, after tracheal intubation, they were connected to a respirator (SN-480-5; Shinano Seisakusyo, Tokyo: 10 ml/kg, 20 strokes/min) and anes-

thetized with enflurane. After a midline abdominal incision, the ureter on each side was ligated and a polyethylene catheter (PE-50; Nihon Becton Dickinson, Tokyo) was inserted above the ligature for the drainage of urine. After the urethra had been ligated, a polyethylene catheter (PE-50) was inserted into the urinary bladder via the top of the bladder dome and connected through a three-way connector to both a pressure transducer (P23XL-1, Nihon Becton Dickinson) and a syringe filled with warmed saline. The initial bladder pressure was adjusted to about 7 cmH_2O by instillation of warmed saline (37°C) in 5-ml increments. Bladder pressure was recorded continuously on a rectigraph (Recti-Horiz-8K; NEC San-ei, Tokyo). A venous catheter was inserted into the left femoral vein (PE-50) for drug injection.

Tissue preparation and in vitro experimental protocol

Monkeys were anesthetized with ketamine (10 mg/kg, intramuscular) and sacrificed by rapid exsanguination. After isolation of the urinary bladder, the fat and mucosa were removed. Then, a detrusor strip approximately 10 × 3 mm was taken and suspended in a 10-ml organ bath containing Krebs solution. The preparations were allowed to equilibrate for 60 min after the establishment of an initial resting tension of 8 mN. The bath solution was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force-displacement transducer (SB-1T; Nihon-Kohden, Tokyo) and changes in muscle tension were measured and recorded on a pen-writing oscillograph (Rectigraph 8K; Sanei, Tokyo). After the basal tone had stabilized, concentration-response curves for various β-adrenoceptor agonists were obtained by the cumulative addition of one agonist to the bathing fluid. In experiments examining the effects of β-adrenoceptor antagonists, tissues were exposed to the appropriate antagonist for 30 min prior to the collection of the data needed for the construction of an isoprenaline concentration-response curve. Only one agonist concentration-response curve was generated per preparation. All experiments were conducted in the presence of 10^{-6} M phentolamine (to block α-adrenoceptors), 10^{-7} M desmethylimipramine and 5×10^{-6} M hydrocortisone (to block neuronal and extraneuronal uptake of catecholamines).

Analyses of data

Results are expressed as the mean ± standard error of the mean (S.E.M.). The relaxing effect of each agonist on smooth muscle preparations is expressed by giving the percentage of the resting tension seen with a range of doses of the agonist. The maximal relaxation induced by 10^{-5} M forskolin was taken as a 100% relaxation of the isolated detrusor. The pD_2 value, which is the negative logarithm of the EC_{50} value, was calculated for each agonist from its concentration-relaxation curve. The pA_2 value for each antagonist, as defined by Arunlakshana and Schild (14), was obtained from a linear regression analysis of a plot of values for log(Concentration ratio (CR) − 1) vs the negative logarithm of the antagonist concentration. The 100% value for bladder pressure was taken as the level before administration of a given test compound. Statistical analysis was performed using the unpaired Student's *t*-test, a one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison test or the Tukey-Kramer test. A probability level less than 0.05 was accepted as significant. The JMP Statistics and Graphics Guide (version 3.1; SAS Institute, Inc., Cary, NC, USA) or SAS/STAT (version 6.12, SAS Institute, Inc.) was used as the resource text for the statistical analyses.

Drugs

The following drugs were used: (−)-isoprenaline hydrochloride (Nikken Kagaku, Tokyo); (−)-isoprenaline (+)-bitartrate, procaterol hydrochloride, (−)-noradrenaline bitartrate, (−)-adrenaline (+)-bitartrate, salbutamol hemisulphate, hydrocortisone 21-hemisuccinate, desmethylimipramine hydrochloride (Sigma Chemical, St. Louis, MO, USA); (±)-dobutamine hydrochloride, (±)-4-(3-*tert*-butylamino-2-hydroxypropoxy) benzimidazol-2-one hydrochloride ((±)-CGP-12177 hydrochloride), erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI-118551 hydrochloride) (Funakoshi, Tokyo); xamoterol hemifumarate (Tocris, Ballwin, MO, USA); phentolamine mesylate (Ciba-Geigy, Basel, Switzerland); ketamine hydrochloride (Sankyo, Tokyo); enflurane (Dainippon Seiyaku, Osaka); and dimethyl sulphoxide (DMSO) (Nacalai Tesque, Kyoto). (*R,R*)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]phenoxy]-acetic acid (BRL 37344), 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1H-imidazole-2-yl)-phenoxy)propyl)amino) ethoxy)-benzamide monomethane sulphonate (CGP-20712A), 3-(2-allylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2*S*)-2-propanol hydrochloride (SR 58894A) and bupranolol were synthesized in our laboratories (Kissei, Hotaka). The Krebs solution was of the following composition: 118.1 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4 and 11.1 mM glucose (pH 7.4). For the in vivo study, (−)-isoprenaline hydrochrolide was dissolved in saline. For the in vitro study, forskolin was dissolved in 100% DMSO and the other drugs were dissolved in distilled water. The reported concentrations are the calculated final concentrations in the bath solution. The solutions were prepared on the day of the experiment and kept in dark vessels to minimize light-induced degradation.

RESULTS

Isoprenaline activity in the anesthetized monkey

Injection of vehicle (saline, 0.1 ml/kg, intravenous) had no effect on bladder pressure, whereas isoprenaline (0.1 to 100 μg/kg, intravenous) reduced it in a dose-dependent manner (Fig. 1: a and b). The maximal reduction was observed 2 min after the injection of isoprenaline, the bladder pressure being reduced significantly to 71.4 ± 5.0% and 66.3 ± 5.3% of the resting pressure by 10 and 100 μg /kg of isoprenaline, respectively. Recovery had occurred within 20 min after isoprenaline administration. At 20 min after 100 μg/kg of isoprenaline administration, the bladder pressure was recovered to 105.6 ± 8.0% of the resting pressure.

β-Adrenoceptor agonist activity in the monkey detrusor

A definite relaxation of the isolated detrusor preparation was produced by forskolin (10^{-5} M), an adenylyl cyclase activator, the tension decreasing by 79 ± 1% of the initial value. The relaxing potencies of isoprenaline, noradrenaline and adrenaline were studied and the results are shown in Table 1, Fig. 2 and Fig. 3. Each of these catecholamines

Table 1. pD_2 values for β-adrenoceptor agonists in monkey detrusor

	n	pD_2	Maximal relaxation (%)
Isoprenaline	6	7.62 ± 0.13	97.3 ± 1.4
Noradrenaline	5	6.83 ± 0.15	96.3 ± 1.7
Adrenaline	7	5.98 ± 0.26	92.9 ± 2.4
Dobutamine	6	5.53 ± 0.08	83.1 ± 4.2
Xamoterol	6	n.d.	17.7 ± 5.5
Procaterol	6	5.15 ± 0.13	84.2 ± 4.2
Salbutamol	4	5.35 ± 0.11	88.5 ± 5.1
BRL 37344	7	6.04 ± 0.18	66.2 ± 5.6
CGP-12177	7	6.60 ± 0.19	81.7 ± 3.0

Results are expressed as the mean ± S.E.M. "Maximal relaxation" is expressed as a percentage of the relaxation response to 10^{-5} M forskolin. n.d.: not determined (the effect had not reached a maximum at a concentration of 10^{-4} M, and the pD_2 value was not determined).

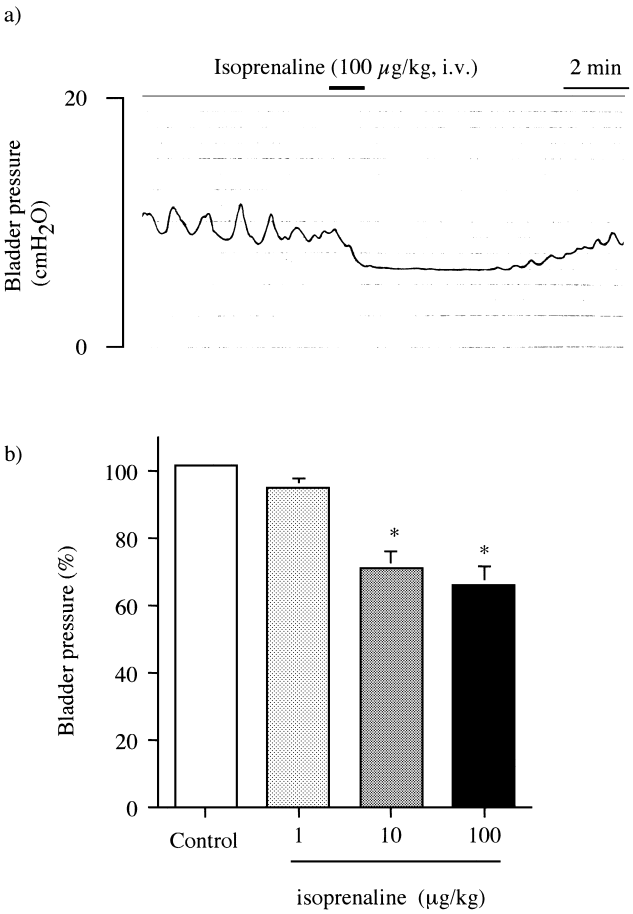

Fig. 1. Effect of isoprenaline on bladder pressure in anesthetized monkey. a: Representative recording. b: Summary of the effects. Each column represents the mean ± S.E.M. of 8 experiments. *P<0.05 vs control.

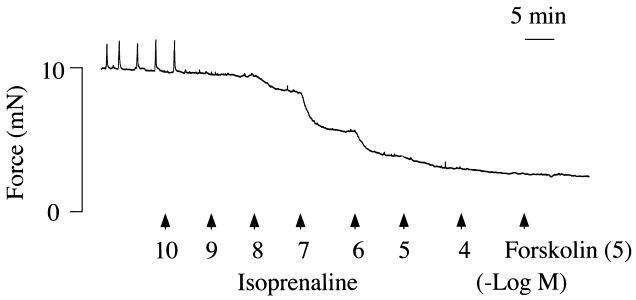

Fig. 2. Representative recording of effect of isoprenaline on resting tension in monkey detrusor preparation.

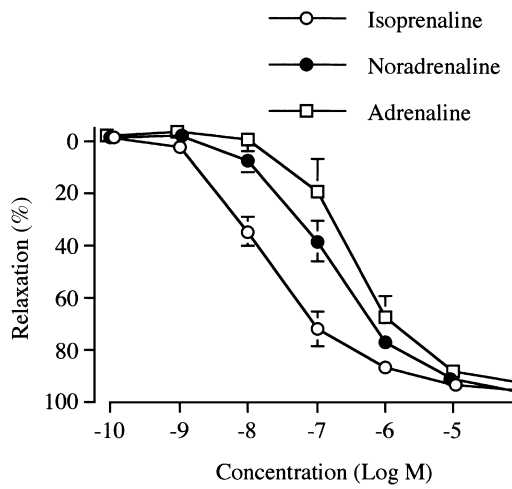

Fig. 3. Effects of isoprenaline, noradrenaline and adrenaline on resting tension in monkey detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Each point represents the mean ± S.E.M. of 5 – 7 experiments.

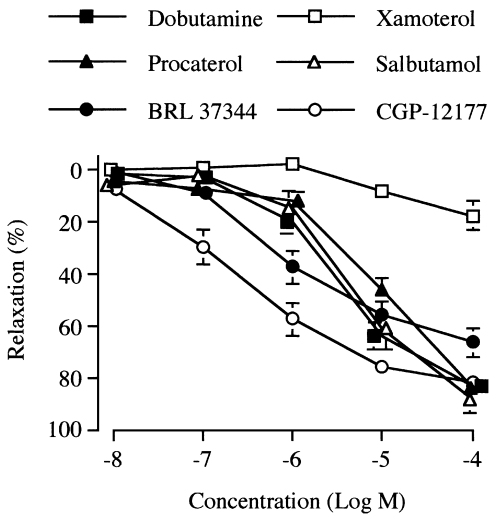

Fig. 4. Effects of dobutamine, xamoterol, procaterol, salbutamol, BRL 37344 and CGP-12177 on resting tension in monkey detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Each point represents the mean ± S.E.M. of 4 – 7 experiments.

produced a concentration-dependent relaxation of the detrusor strip. The rank order of their relaxing potencies was isoprenaline > noradrenaline > adrenaline, the pD_2 values being 7.62, 6.83 and 5.98, respectively (significantly different from each other, $P<0.05$).

Among the agonists tested, the selective β_3-adrenoceptor agonists (CGP-12177 and BRL 37344) proved more potent as relaxants than either the β_1-adrenoceptor agonists (dobutamine and xamoterol) or the β_2-adrenoceptor agonists (procaterol and salbutamol) (Fig. 4). The pD_2 values and maximal percentage relaxations are shown for all the β-adrenoceptor agonists tested in Table 1. The pD_2 value of CGP-12177 was significantly greater ($P<0.01$) from those of procaterol, salbutamol and dobutamine.

Effects of β-adrenoceptor antagonists on the relaxation induced by isoprenaline in the monkey detrusor

In the isolated detrusor, neither a selective β_1-adrenoceptor antagonist, CGP-20712A, nor a selective β_2-adrenoceptor antagonist, ICI-118551, had any effect on the relaxation induced by isoprenaline (Fig. 5: a and b). A non-selective β-adrenoceptor antagonist, bupranolol, caused a rightward shift of the concentration-response curve for isoprenaline in a dose-dependent manner. The pA_2 value was 7.00 ± 0.14 and the slope of Schild plot was

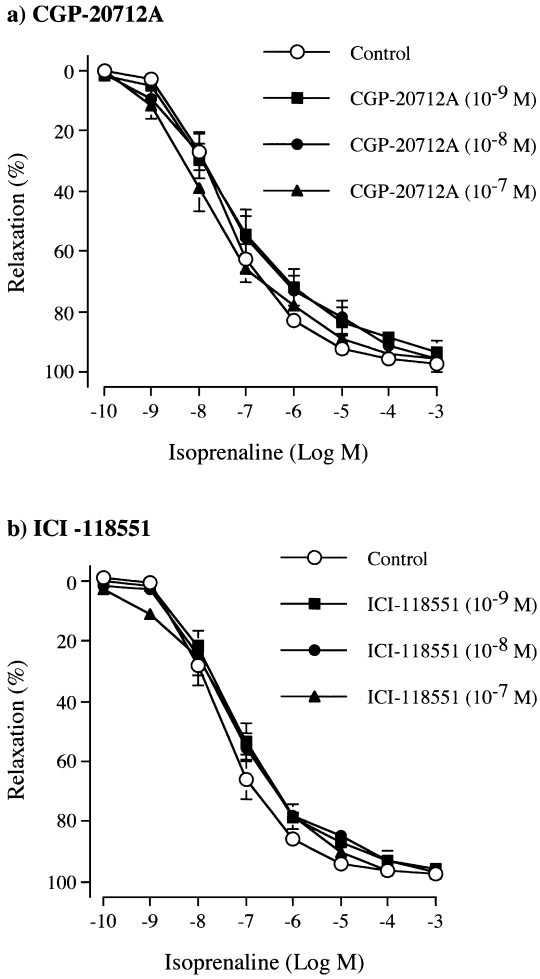

Fig. 5. Effects of CGP-20712A (a) and ICI-118551 (b) on isoprenaline-induced relaxation in monkey detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Each point represents the mean ± S.E.M. of 5 – 6 experiments.

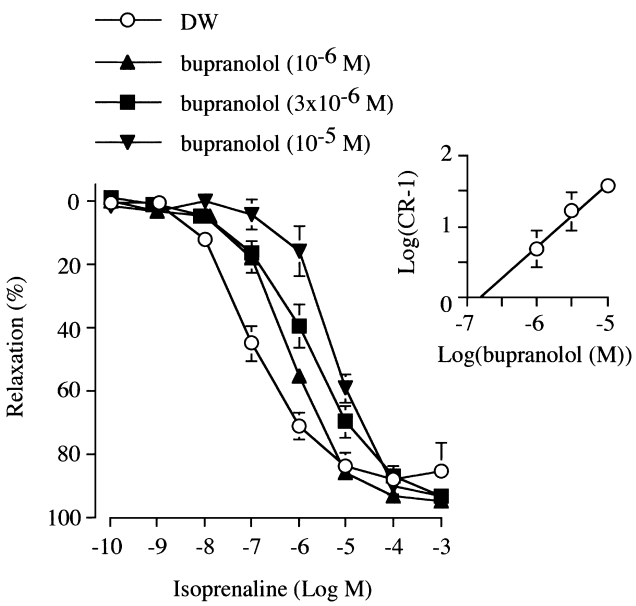

Fig. 6. Effect of bupranolol on isoprenaline-induced relaxation in monkey detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Each point represents the mean ± S.E.M. of 4 – 10 experiments. Inset: Schild plot for the inhibition produced by bupranolol (pA_2 value, 7.00 ± 0.14; slope, 0.87 ± 0.25).

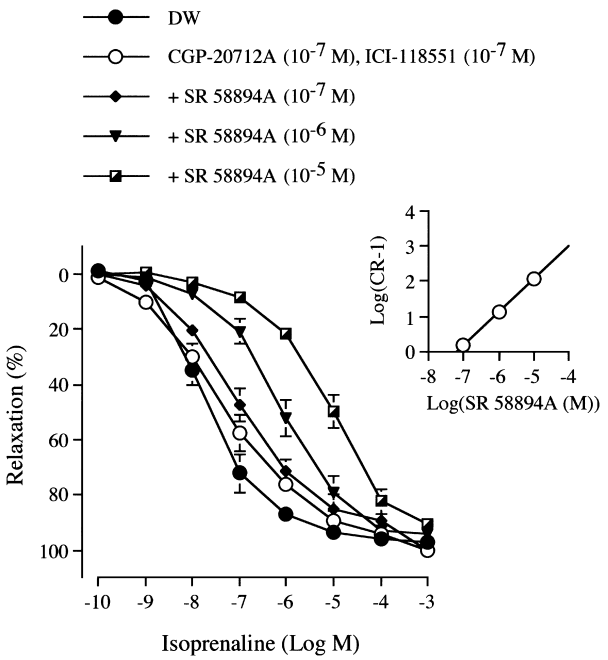

Fig. 7. Effect of SR 58894A on isoprenaline-induced relaxation in monkey detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Each point represents the mean ± S.E.M. of 6 experiments. Inset: Schild plot for the inhibition produced by SR 58894A (pA_2 value, 7.19 ± 0.15; slope, 0.94 ± 0.17).

0.87 ± 0.25 (not significantly different from unity) (Fig. 6). In the presence of CGP-20712A (10^{-7} M) and ICI-118551 (10^{-7} M), the β_3-adrenoceptor antagonist SR 58894A produced a rightward shift of the concentration-response curve for isoprenaline without altering the maximal response. The pA_2 value was 7.19 ± 0.15 and the slope of Schild plot was 0.94 ± 0.17 (not significantly different from unity) (Fig. 7).

DISCUSSION

This study is the first to show that a β_3-adrenoceptor is functionally dominant in the relaxation of the monkey urinary bladder.

First, we confirmed that isoprenaline, a non-selective β-adrenoceptor agonist, reduced bladder pressure in a dose dependent-manner in the anesthetized monkey. Sympathetically mediated β-adrenoceptor activation has important functional effects on urine storage in the human urinary bladder (7). Recently, it was reported that the β-adrenoceptor subtype present in the human urinary bladder has functional characteristics not of β_1- or β_2-adrenoceptors but mainly of β_3-adrenoceptors (4 – 6). Thus, stimulation of the β_3-adrenoceptor in the human detrusor has the potential to be effective in the treatment of such urinary bladder dysfunctions as frequent urination and incontinence. Although the β-adrenoceptor subtypes mediating relaxation of the mammalian detrusor are known to differ significantly among species, no previous report has identified the β-adrenoceptor subtypes present in the monkey urinary bladder. In our second and third experiments, we therefore carried out an in vitro functional analysis of the β-adrenoceptor subtype(s) present in this tissue to determine whether the monkey might be a useful experimental animal for investigating human bladder function.

The second experiment involved an evaluation of the relaxing effects of several β-adrenoceptor agonists using monkey detrusor strips in vitro. β-Adrenoceptors are integral membrane proteins belonging to the Gs type of G protein-coupled receptors, and agonists produce an accumulation of intracellular adenosine 3',5'-cyclic monophosphate (cyclic AMP). Prior to the agonist experiment proper, we confirmed that 10^{-5} M forskolin, an adenylyl cyclase activator, decreased the basal tone of the preparation, indicating that cyclic AMP cascades play a very important role in the relaxation of the monkey detrusor.

The rank order of potencies for catecholamines producing β-adrenoceptor-mediated responses is isoprenaline > noradrenaline > adrenaline for β_1- and β_3-adrenoceptors, but isoprenaline > adrenaline > noradrenaline for β_2-adrenoceptors (15, 16). In the present study, the rank order obtained was isoprenaline > noradrenaline > adrenaline, suggesting the existence of β_1- and/or β_3-adrenoceptors in the monkey detrusor. When we examined the relaxing effects of selective agonists for the β-adrenoceptor subtypes, the rank order of potencies obtained was CGP-12177 > BRL 37344 (both β_3-adrenoceptor agonists) > dobutamine (β_1-adrenoceptor agonist), salbutamol, procaterol (both β_2-adrenoceptor agonists) > xamoterol (β_1-adrenoceptor agonist). This indicated that it is the β_3-adrenoceptor that functionally predominates in the relaxation of the monkey detrusor. CGP-12177 has been reported to be a partial agonist for the β_3-adrenoceptor and an antagonist for β_1/β_2-adrenoceptors (17). The maximal relaxations induced by CGP-12177 and BRL 37344 were, respectively, 82% and 66% (at 10^{-4} M) of the maximal relaxation induced by forskolin (10^{-5} M) in the monkey detrusor. Interestingly, in human detrusor strips CGP-12177 and BRL 37344 are both partial relaxants (5), as they were in our experiment.

In the third experiment, we investigated the activities of several β-adrenoceptor antagonists against the isoprenaline-induced relaxation of the isolated monkey detrusor. Neither CGP-20712A, a selective β_1-adrenoceptor antagonist, nor ICI-118551, a selective β_2-adrenoceptor antagonist, had any effect on the concentration-response curve for isoprenaline at the concentrations ($10^{-9} – 10^{-7}$ M) at which they show selectivity for β_1- or β_2-subtypes. Bupranolol, a non-selective β-adrenoceptor antagonist, antagonized the isoprenaline-induced relaxation of the monkey detrusor.

The pA_2 value obtained for bupranolol was 7.00 and the slope of the Schild plot (0.87) was not significantly different from unity. Bupranolol has been shown to exhibit β_3-adrenoceptor antagonistic activity at high concentrations (μM), in addition to the β_1- and β_2-adrenoceptor antagonistic activities it shows at lower concentrations (nM) (18). These results suggest that neither β_1- nor β_2-adrenoceptors play an important functional role in the relaxation of the monkey detrusor. An additional experiment using a selective β_3-adrenoceptor antagonist, SR 58894A (19), supported this idea. In the presence of CGP-20712A (10^{-7} M) and ICI-118551 (10^{-7} M), SR 58894A effectively antagonized the isoprenaline-induced relaxation of the monkey detrusor, and the slope of Schild plot for SR 58894A (0.94) was not significantly different from unity. This result suggests that the relaxation of the monkey detrusor induced by isoprenaline is produced by β_3-adrenoceptor activation, effectively supporting the conclusion we provisionally reached on the basis of the order of agonist potencies and that of antagonist affinities. It is therefore concluded that the relaxation of the monkey detrusor is mediated almost entirely via the β_3-adrenoceptor. There are many reports of β_3-adrenoceptors coexisting with other subtypes of β-adrenoceptors in urinary bladders: for example, β_1- and β_3-adrenoceptors in dog (12), β_2- and β_3-adrenoceptors in rat (12), and β_3- and an atypical β-adrenoceptor in both ferret (13) and human (5). So far, the monkey is the only instance of detrusor relaxation being mediated by the β_3-adrenoceptor alone (although we cannot entirely exclude a very small contribution by another subtype).

The present functional study has clearly demonstrated that relaxation of the monkey detrusor by β-adrenoceptor agonists is mediated via the β_3-adrenoceptor. Consequently, we conclude that the monkey is a very good animal for investigations of the function of the β_3-adrenoceptor in the urinary bladder.

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