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

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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 (β2-antagonist, 10⁻⁶ to 10⁻⁵ M) and SR 58894A (β3-antagonist, 10⁻⁷ to 10⁻⁵ M) caused a rightward shift of the concentration-relaxation curve for isoprenaline, but CGP-20712A (β1-antagonist, 10⁻⁹ to 10⁻⁷ M) and ICI-118551 (β2-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 β3-subtype.

Keywords: β-Adrenoceptor, β1-Adrenoceptor, Monkey, Detrusor, Bladder

In humans, a number of functional and molecular biological studies have confirmed that β3-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-adrenergic receptor activation has important functional effects on urine storage (7). This raises the possibility that β3-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 β1-adrenoceptor in cats (8) and guinea pigs (9), but mainly via the β2-adrenoceptor in rabbits (10). In contrast, it has been confirmed that β3-adrenoceptor agonists strongly relax the canine, rat and ferret detrusors (11–13), although the β3-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).

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-
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; NEC San-ai, Tokyo). A venous catheter was inserted into the left femoral vein (PE-50) for drug injection.

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 pD₂ value, which is the negative logarithm of the EC₅₀ value, was calculated for each agonist from its concentration-relaxation curve. The pA₂ 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 (Nikkken Kagaku, Tokyo); (−)-isoprenaline (+)-bitartrate, proteratol hydrochloride, (−)-noradrenaline bitartrate, (−)-adrenaline (+)-bitartrate, salbutamol hemisulphate, hydrocortisone 21-hemisuccinate, desmethylylimipramine 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-methyldindan-4-yl)oxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI-118551 hydrochloride) (Funakoshi, Tokyo); xameterol hemifumarate (Tocris, Ballwin, MO, USA); phenolamine 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-hydroxy-3-4-(1-methyl-4-trifluoromethyl)1H-imidazole-2-yl)-phenoxy)propyl)amino) ethoxy)-benzamide monomethane sulphonate (CGP-20712A), 3-2 allylphenoxy)-1-[1S]-1,2,3,4-tetrahydro-1-naphth-1-ylamino)2S)-2-propanol hydrochloride (SR 5889A) and buprenolol 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 CaCl₂, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 1.2 mM KH₂PO₄ and 11.1 mM glucose (pH 7.4). For the in vivo study, (−)-isoprenaline hydrochloride 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. pD₂ values for β-adrenoceptor agonists in monkey detrusor

|                         | n  | pD₂     | 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  | 5.15 ± 0.13 | 84.2 ± 4.2            |
| Procaterol              | 6  | 5.35 ± 0.11 | 88.5 ± 5.1            |
| Salbutamol              | 4  | 5.15 ± 0.13 | 84.2 ± 4.2            |
| 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 pD₂ value was not determined).
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 \(\beta_1\)-adrenoceptor agonists (CGP-12177 and BRL 37344) proved more potent as relaxants than either the \(\beta_1\)-adrenoceptor agonists (dobutamine and xamoterol) or the \(\beta_2\)-adrenoceptor agonists (procaterol and salbutamol) (Fig. 4). The pD\(_2\) values and maximal percentage relaxations are shown for all the \(\beta\)-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 \(\beta\)-adrenoceptor antagonists on the relaxation induced by isoprenaline in the monkey detrusor**

In the isolated detrusor, neither a selective \(\beta_1\)-adrenoceptor antagonist, CGP-20712A, nor a selective \(\beta_2\)-adrenoceptor antagonist, ICI-118551, had any effect on the relaxation induced by isoprenaline (Fig. 5: a and b). A non-selective \(\beta\)-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

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**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.

**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).
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 β₂-adrenoceptor antagonist SR 58894A produced a rightward shift of the concentration-response curve for isoprenaline without altering the maximal response. The pA₂ 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 β₁-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 β₁-adrenoceptor subtype present in the human urinary bladder has functional characteristics not of β₁-or β₂-adrenoceptors but mainly of β₁-adrenoceptors (4–6). Thus, stimulation of the β₁-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 β₁-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 β₁-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 β₁-adrenoceptor agonists using monkey detrusor strips in vitro. β₁-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 β₁-adrenoceptor-mediated responses is isoprenaline > noradrenaline > adrenaline for β₁- and β₂-adrenoceptors, but isoprenaline > adrenaline > noradrenaline for β₁-adrenoceptors (15, 16). In the present study, the rank order obtained was isoprenaline > noradrenaline > adrenaline, suggesting the existence of β₁- and/or β₂-adrenoceptors in the monkey detrusor. When we examined the relaxing effects of selective agonists for the β₁-adrenoceptor subtypes, the rank order of potencies obtained was CGP-12177 > BRL 37344 (both β₂-adrenoceptor agonists) > dobutamine (β₁-adrenoceptor agonist), salbutamol, procarterol (both β₂-adrenoceptor agonists) > xamoterol (β₂-adrenoceptor agonist). This indicated that it is the β₁-adrenoceptor that functionally predominates in the relaxation of the monkey detrusor. CGP-12177 has been reported to be a partial agonist for the β₁-adrenoceptor and an antagonist for β₁/β₂-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 β₁-adrenoceptor antagonists against the isoprenaline-induced relaxation of the isolated monkey detrusor. Neither CGP-20712A, a selective β₁-adrenoceptor antagonist, nor ICI-118551, a selective β₂-adrenoceptor antagonist, had any effect on the concentration-response curve for isoprenaline at the concentrations (10⁻⁸ – 10⁻⁶ M) at which they show selectivity for β₁ or β₂-subtypes. Bupranolol, a non-selective β-adrenoceptor antagonist, antagonized the isoprenaline-induced relaxation of the monkey detrusor.
The pA₂ value obtained for bupropranolol was 7.00 and the slope of the Schild plot (0.87) was not significantly different from unity. Bupropranolol has been shown to exhibit β₂-adrenoceptor antagonistic activity at high concentrations (µM), in addition to the β₁- and β₂-adrenoceptor antagonistic activities it shows at lower concentrations (nM) (18). These results suggest that neither β₁- nor β₂-adrenoceptors play an important functional role in the relaxation of the monkey detrusor. An additional experiment using a selective β₁-adrenoceptor antagonist, SR 58945A (19), supported this idea. In the presence of CGP-20712A (10⁻⁷ M) and ICI-118551 (10⁻⁷ M), SR 58945A effectively antagonized the isoprenaline-induced relaxation of the monkey detrusor, and the slope of Schild plot for SR 58945A (0.94) was not significantly different from unity. This result suggests that the relaxation of the monkey detrusor induced by isoprenaline is produced by β₁-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 β₁-adrenoceptor. There are many reports of β₁-adrenoceptors coexisting with other subtypes of β-adrenoceptors in urinary bladders: for example, β₁- and β₂-adrenoceptors in dog (12), β₂- and β₃-adrenoceptors in rat (12), and β₁- 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 β₁-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 β₁-adrenoceptor. Consequently, we conclude that the monkey is a very good animal for investigations of the function of the β₁-adrenoceptor in the urinary bladder.

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