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Characterization of β-adrenoceptor subtypes in the ferret urinary bladder in vitro and in vivo

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Abstract

In the present study, the β-adrenoceptor subtypes distributed in the detrusor of the ferret were investigated in functional experiments in vitro and in vivo using a variety of β-adrenoceptor agonists and antagonists. All the β-adrenoceptor agonists tested relaxed the isolated detrusor strip, the rank order of potency being (±)-(*R**,*R**)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]phenoxy]-acetic acid sodium (BRL 37344A) > (±)-4-(3-*t*-butylamino-2-hydroxypropoxy) benzimidazol-2-one (CGP-12177A), isoprenaline and (*R*,*R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243) > dobutamine and procaterol. In antagonist experiment, 3-(2-allylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydro-naphth-1-ylamino]-(2*S*)-2-propanol hydrochloride (SR 58894A), but neither 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulphonate (CGP-20712A) nor erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI-118,551), caused a rightward shift of the concentration-relaxation curve for isoprenaline. In in vivo experiments, isoprenaline and CL 316,243 each reduced bladder pressure in a dose-dependent manner. CL 316,243 was the only drug that did not produce any significant influences on blood pressure and heart rate at doses that reduced bladder pressure. The present functional study provides the first evidence that relaxation of the ferret detrusor by β-adrenoceptor activation is mediated mainly via the β_3-adrenoceptor, as in the human detrusor.

Keywords: β-Adrenoceptor, subtype; (Ferret); Detrusor; Cardiovascular system

1. Introduction

A number of functional and molecular biological studies have confirmed that β_3-adrenoceptors exist in a variety of mammalian tissues, including adipocytes (Arch, 1989), trachea (Webber and Stock, 1992), heart (Kaumann and Molenaar, 1997), gut (De Ponti et al., 1995) and urinary tract (Tomiyama et al., 1998; Yamazaki et al., 1998). Recently, Evans et al. (1999) reported that in the mouse, there are two types of β_3-adrenoceptor mRNAs, namely, β_{3a}- and β_{3b}-adrenoceptor mRNAs. In addition, the existence of a putative β_4-adrenoceptor in mammalian heart (Molenaar et al., 1997) and adipocytes (Galitzky et al., 1997) has been proposed, although Kompa and Summers (1999) questioned whether the putative β_4-adrenoceptor exists as a separate entity or whether it is an alternative affinity state of a known receptor. In the human, it has been confirmed that β_3-adrenoceptors play important functional roles in adipocytes (Lönnqvist et al., 1993), gut (De Ponti et al., 1996; Bardou et al., 1998) and urinary bladder (Igawa et al., 1998, 1999; Takeda et al., 1999). Consequently, it is thought that β_3-adrenoceptor agonists may be effective in the treatment of irritable bowel syndrome, some urinary dysfunctions, and some kinds of obesity.

Sympathetically mediated β-adrenoceptor activation has important functional effects on urine storage in the human urinary bladder (Andersson, 1999). Thus, it is thought that stimulation of the β_3-adrenoceptor in the human detrusor may be effective in the treatment of such urinary bladder dysfunctions as frequent urination and incontinence. However, the β-adrenoceptor subtypes mediating relaxation of

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the mammalian detrusor differ significantly among species. For example, the relaxation response to adrenergic stimulation of the detrusor is mediated mainly via the β_1-adrenoceptor in guinea-pigs (Li et al., 1992), but mainly via the β_2-adrenoceptor in rabbits (Yamazaki et al., 1998). In contrast, it has been confirmed that β_3-adrenoceptor agonists strongly relax both the canine and rat detrusors (Seguchi et al., 1998; Yamazaki et al., 1998), although the β_2-adrenoceptor is also concerned in the relaxation response of the rat detrusor to β-adrenoceptor stimulation. A few years ago, it was reported that functional β_3-adrenoceptors are distributed in the ferret trachea (Webber and Stock, 1992). In the light of this finding, we decided to carry out a functional analysis of the β-adrenoceptor subtypes present in the ferret detrusor to determine whether the ferret might be a useful animal for investigations of bladder function.

2. Methods

2.1. Animals

This study was conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical. Male ferrets (1.0–1.8 kg, from Charles River Japan, Yokohama, Japan) were used. They were maintained in a 12-h light–dark cycle with free access to water and standard laboratory food until the day of the experiment.

2.2. Tissue preparation and in vitro experimental protocol

Ferrets were anaesthetized with sodium pentobarbital (30 mg/kg, i.v.) 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, Japan) and changes in muscle tension were measured and recorded on a pen-writing oscillograph (Rectigraph 8K; Sanei, Tokyo, Japan). 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 data for 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, an α-adrenoceptor antagonist.

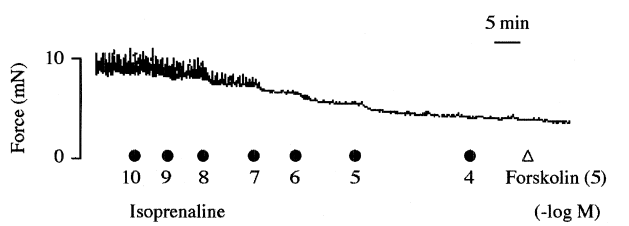

Fig. 1. Representative recording of the effect of isoprenaline on resting tension in ferret detrusor preparation.

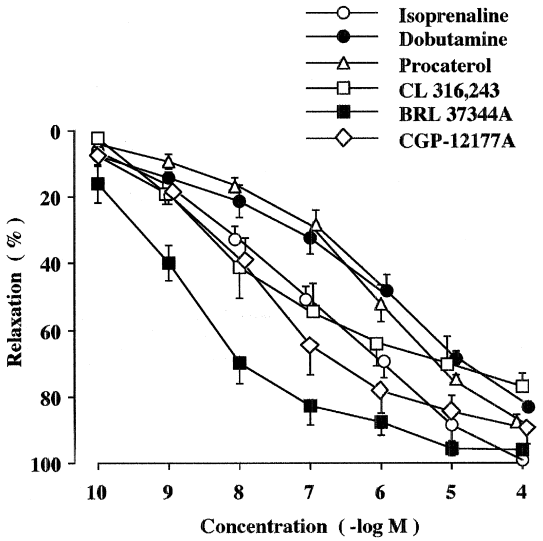

Fig. 2. Effects of β-adrenoceptor agonists on resting tension in ferret detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Data represent the mean ± S.E. of 6–9 experiments.

2.3. In vivo experimental protocol

Ferrets were anaesthetized with sodium pentobarbital (30 mg/kg, i.v.), and the midline abdomen was incised. The ureter on each side was ligated and a polyethylene catheter (PE-50; Nihon Becton Dickinson, Tokyo, Japan) was inserted into the ureter above the ligature. Urine was drained out through this catheter. After the urethra had

Table 1
Relaxing potencies of β-AR agonist in ferret detrusor

	n	pD_2	Maximal relaxation (%)
Isoprenaline	8	7.07 ± 0.29	98.9 ± 1.1
Dobutamine	9	6.47 ± 0.28	83.1 ± 1.6
Procaterol	8	6.42 ± 0.18	87.6 ± 2.1
CL 316,243	6	7.79 ± 0.29	77.2 ± 4.2
BRL 37344A	6	8.72 ± 0.23	96.2 ± 1.8
CGP-12177A	6	7.74 ± 0.08	89.3 ± 2.2

The value represent mean ± S.E.

been ligated, a polyethylene catheter (PE-50; Nihon Becton Dickinson) was inserted into the urinary bladder via the top of the bladder dome and connected through a three-way connector to a pressure transducer (P23XL; Gould, Valley View, OH, USA) and syringe filled with warmed saline. The initial bladder pressure was adjusted to 10 cm H_2O by instillation of warmed saline (37°C) in 0.5 ml increments. An arterial catheter was inserted into the left carotid artery (size 6; Hibiki, Tokyo, Japan) and connected to a pressure transducer (P23XL; Gould) for the measurement of blood pressure. Heart rate was measured via a tachometer (1321; NEC Sanei, Tokyo, Japan) connected to the transducer amplifier (1829; NEC Sanei). Bladder pressure, blood pressure and heart rate were recorded continuously on a rectigraph (Recti-Horiz-8K; NEC Sanei). A venous catheter was inserted into the left femoral vein (PE-50; Nihon Becton Dickinson) for drug injection.

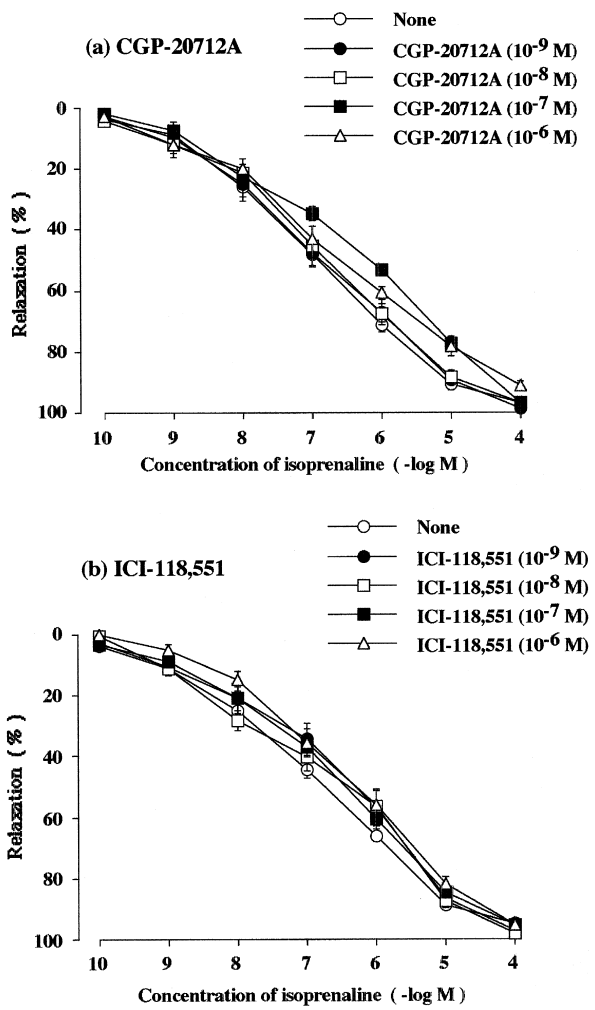

Fig. 3. Effects of CGP-20712A (a) and ICI-118,551 (b) on isoprenaline-induced relaxation in ferret detrusor preparations. Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Data represent the mean ± S.E. of 5–10 experiments.

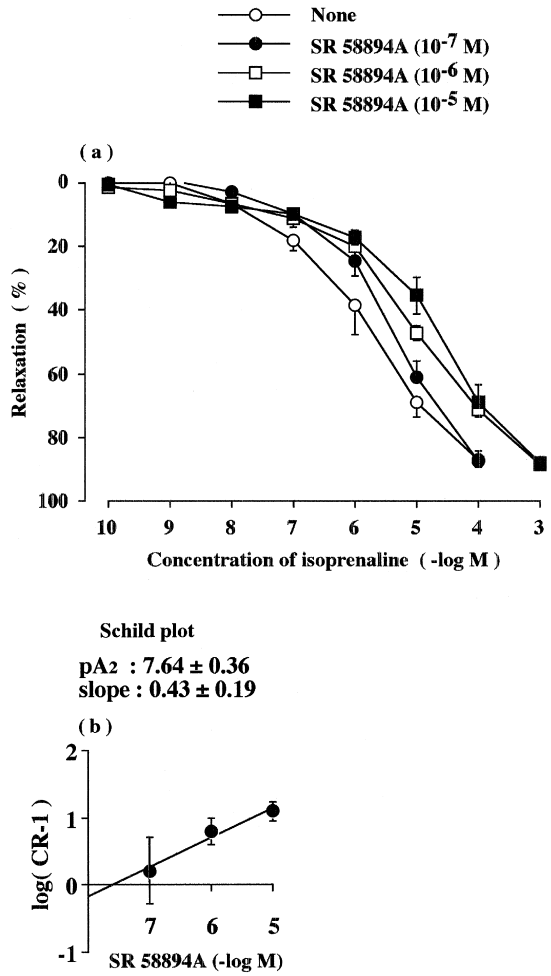

Fig. 4. Effect of SR 58894A on isoprenaline-induced relaxation in ferret detrusor preparations (a). Each value is expressed as a percentage of the response to forskolin (10^{-5} M). Data represent the mean ± S.E. of 5–6 experiments. Schild plot for the inhibition produced by SR 58894A (b).

2.4. Analysis of data

The results are expressed as mean ± standard error of the mean (S.E.). The relaxing effect of each agonist on smooth muscle preparations was expressed as a percentage of the resting tension, a range of doses of the agonist being used. A 100% relaxation of the isolated detrusor was taken as the maximal relaxation induced by 10^{-5} M forskolin. 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 (1959), 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% values for bladder pressure, blood pressure and heart rate were, for each parameter, taken as the level before administration of a given agonist. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. A proba-

bility level less than 0.05 was accepted as significant. The JMP Statistics and Graphics Guide (version 3.1; SAS Institute, Cary, NC, USA) was used as the resource text for the statistical analysis.

2.5. Drugs

The following drugs were used: (−)-isoprenaline hydrochloride (Nikken Kagaku, Tokyo, Japan), procaterol hydrochloride (Sigma, St. Louis, MO, USA), (±)dobutamine hydrochloride, (±)-(R^*,R^*)-[4-[2-[[2(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]phenoxy] acetic acid sodium (BRL 37344A), (±)-4-(3-*t*-butylamino-2-hydroxypropoxy) benzimidazol-2-one hydrochloride ((±)-CGP-12177A hydrochloride), erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI-118,551 hydrochloride) (Funakoshi, Tokyo, Japan), phentolamine mesylate (Ciba-Geigy, Basel, Switzerland) and dimethyl sulphoxide (DMSO) (Nacalai tesque, Kyoto, Japan). (*R*,*R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243), 2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)-phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulphonate (CGP-20712A) and 3-(2-allylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydro-naphth-1-ylamino]-(2*S*)-2-propanol hydrochloride (SR 58894A) were synthesized in our laboratories (Kissei, Hotaka, Japan). The Krebs solution was of the following composition (mM): NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, $KH_2PO_4$1.2, and glucose 11.1 (pH 7.4). For the in vitro study, forskolin was dissolved in 100% DMSO; the other drugs in distilled water. The reported concentrations are the calculated final concentrations in the bath solution. For the in vivo study, all drugs were dissolved in saline. The solutions were prepared on the day of the experiment and kept in dark vessels to minimize light-induced degradation.

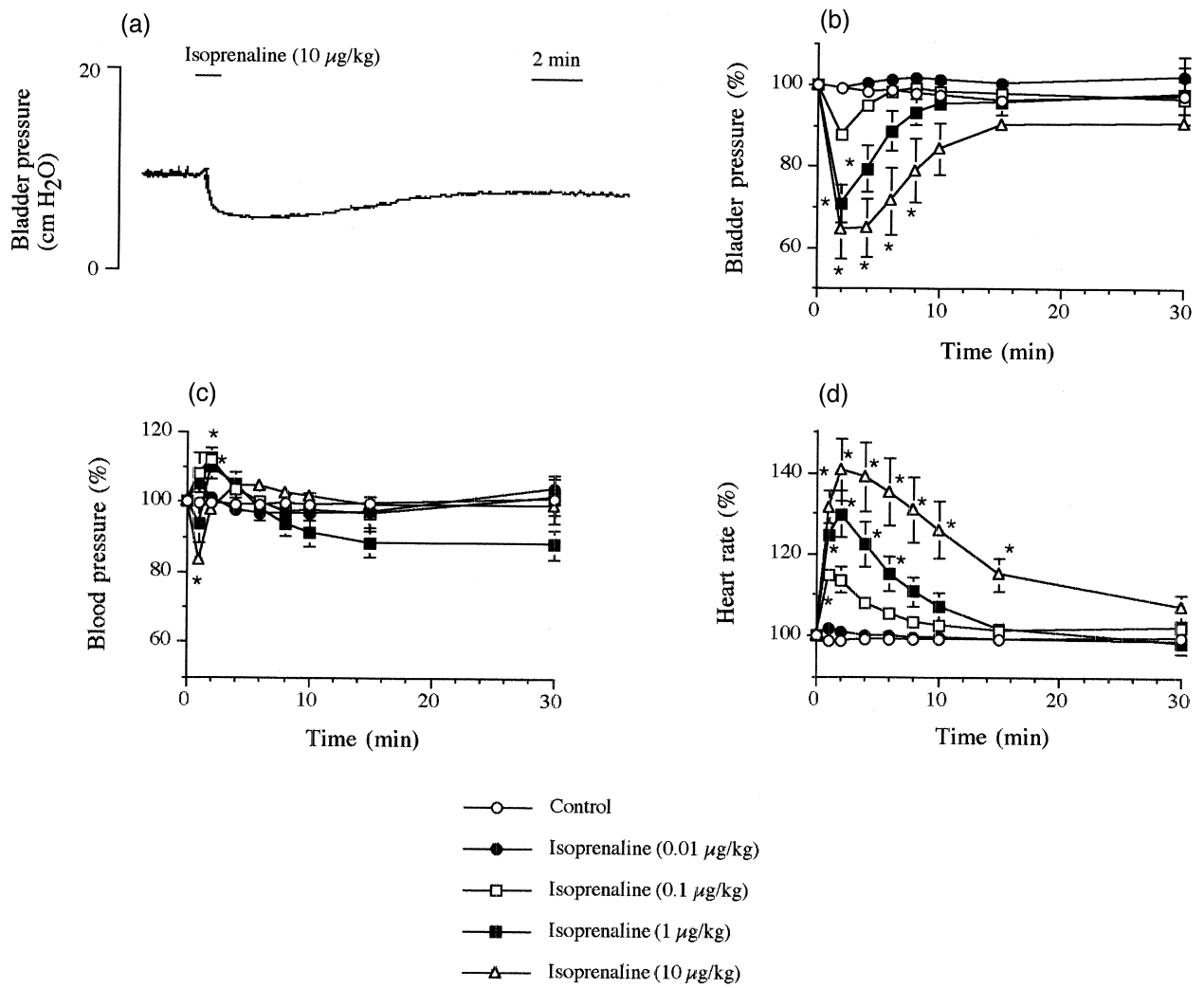

Fig. 5. Representative recording of effect of isoprenaline (10 μg/kg, i.v.) on bladder pressure in anaesthetized ferret (a). Effects of isoprenaline on bladder pressure (b), blood pressure (c) and heart rate (d) in anaesthetized ferret. Each point represents the mean ± S.E. of 4–5 experiments. * Indicates significant difference from control ($P < 0.05$).

3. Results

3.1. β-Adrenoceptor agonist activity in the ferret detrusor

A definite relaxation of the ferret detrusor preparation was produced by forskolin (10^{-5} M), an adenylyl cyclase activator, the tension decreasing to 59 ± 2% of the initial value. Isoprenaline, a nonselective β-adrenoceptor agonist, produced relaxation in a concentration-dependent manner (Fig. 1). A selective β_3-adrenoceptor agonist, BRL 37344A, was the most potent relaxant of all the β-adrenoceptor agonists tested (Fig. 2). Another β_3-adrenoceptor agonist, CL 316,243, and a nonconventional partial β_3-adrenoceptor agonist, CGP-12177A, were almost as potent as isoprenaline at producing relaxation. The potency of these two drugs was about one-hundredth that of BRL 37344A. A selective β_1-adrenoceptor agonist, dobutamine, and a selective β_2-adrenoceptor agonist, procaterol, had weaker relaxing effects. The rank order for the relaxing potencies of these β-adrenoceptor agonists in the ferret detrusor was BRL 37344A > CGP-12177A, isoprenaline and CL 316,243 > dobutamine and procaterol. The pD_2 values and maximal percentage relaxation for all the β-adrenoceptor agonists tested are shown in Table 1.

3.2. Effects of β-adrenoceptor antagonists on the relaxation induced by isoprenaline in the ferret detrusor

In the ferret detrusor, a selective β_1-adrenoceptor antagonist, CGP-20712A, only slightly affected the relaxation induced by isoprenaline (Fig. 3a), in spite of a selective β_2-adrenoceptor antagonist, ICI-118,551, had no effect on it (Fig. 3b). In the presence of CGP-20712A (10^{-7} M) and ICI-118,551 (10^{-7} M), a β_3-adrenoceptor antagonist, SR 58894A, produced a rightward shift of the concentration–

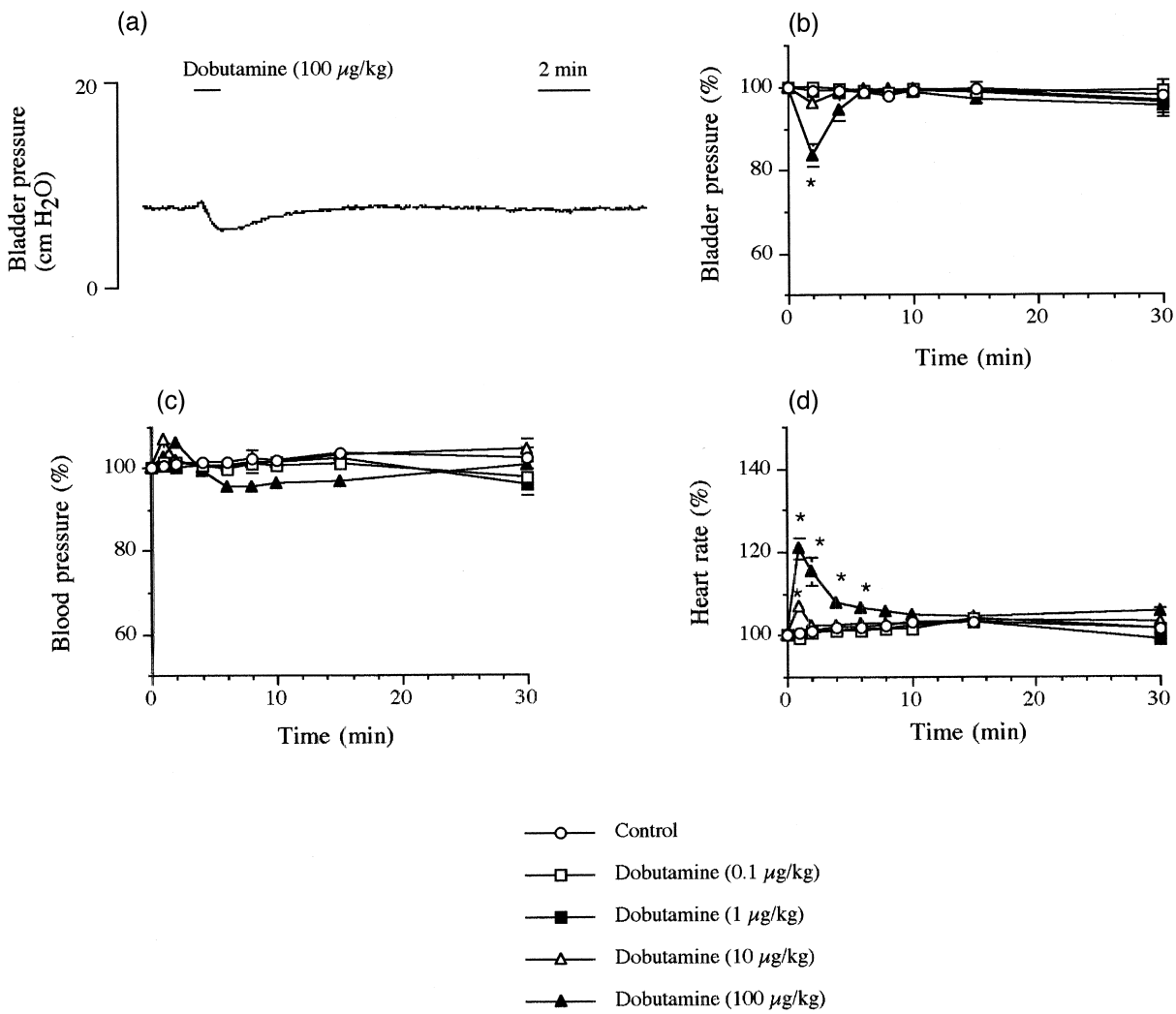

Fig. 6. Representative recording of effect of dobutamine (100 μg/kg, i.v.) on bladder pressure in anaesthetized ferret (a). Effects of dobutamine on bladder pressure (b), blood pressure (c) and heart rate (d) in anaesthetized ferret. Each point represents the mean ± S.E. of 5 experiments. * Indicates significant difference from control ($P < 0.05$).

response curve for isoprenaline without altering the maximal response (pA_2 7.64 ± 0.36, slope 0.43 ± 0.19: Fig. 4).

3.3. β-Adrenoceptor agonist activity in the anaesthetized ferret

Injection of vehicle (saline, 1 ml/kg, i.v.) had no effect on bladder pressure in anaesthetized ferrets. In contrast, isoprenaline (0.1–10 μg/kg, i.v.) reduced bladder pressure in a dose-dependent manner (Fig. 5a,b). The maximal reduction was observed 2 min after the injection of isoprenaline, the bladder pressure being reduced to 70.9 ± 4.7% and 64.3 ± 7.0% of the resting pressure by 1 and 10 μg/kg of isoprenaline, respectively. Recovery had occured within 15 min after isoprenaline administration. The maximum dose of dobutamine used (100 μg/kg, i.v.) reduced bladder pressure significantly to 83.7 ± 2.7% of the resting pressure (Fig. 6a,b). Procaterol significantly reduced bladder pressure (to 93.5 ± 2.2% of the resting pressure) only at 6 min after the injection of 1 μg/kg but there was no dose-dependency (Fig. 7b). CL 316,243 produced a reduction in bladder pressure (Fig. 8a) that was dose-dependent (0.01–1 μg/kg, i.v.) (Fig. 8b). The relaxant activity was maintained for at least 30 min after the injection of 0.1 and 1 μg/kg of CL 316,243. Bladder pressure was reduced to 82.4 ± 5.1% and 61.0 ± 4.6% of the resting pressure by 0.1 and 1 μg/kg of this drug, respectively.

Concerning their cardiovascular effects, injection of vehicle produced no changes in either blood pressure or heart rate in the ferret. Mean blood pressure responded dose-dependently to isoprenaline. The middle doses (0.1 and 1 μg/kg, i.v.) increased blood pressure, while the highest dose (10 μg/kg) decreased it transiently (Fig. 5c). Ferrets receiving 1 μg/kg of isoprenaline showed a biphasic change in blood pressure, a rise followed by a slight fall. Isoprenaline produced a dose-dependent increase in heart rate (Fig. 5d). The maximal response was seen 2 min after isoprenaline injection, the heart rate being increased to 114.7 ± 2.0%, 124.7 ± 2.2% and 131.5 ± 3.9% of the

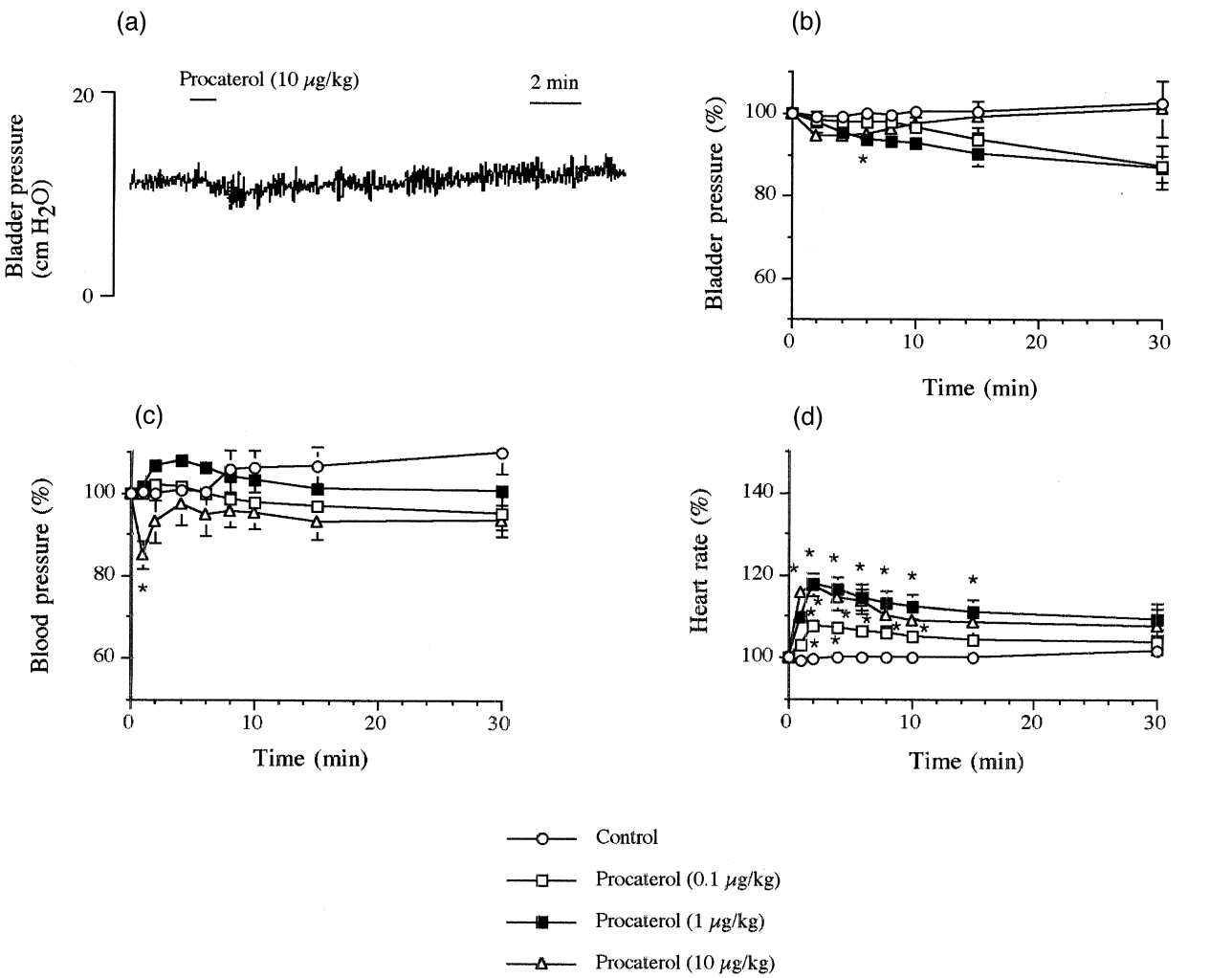

Fig. 7. Representative recording of effect of procaterol (10 μg/kg, i.v.) on bladder pressure in anaesthetized ferret (a). Effects of procaterol on bladder pressure (b), blood pressure (c) and heart rate (d) in anaesthetized ferret. Each point represents the mean ± S.E. of 4–5 experiments. * Indicates significant difference from control ($P < 0.05$).

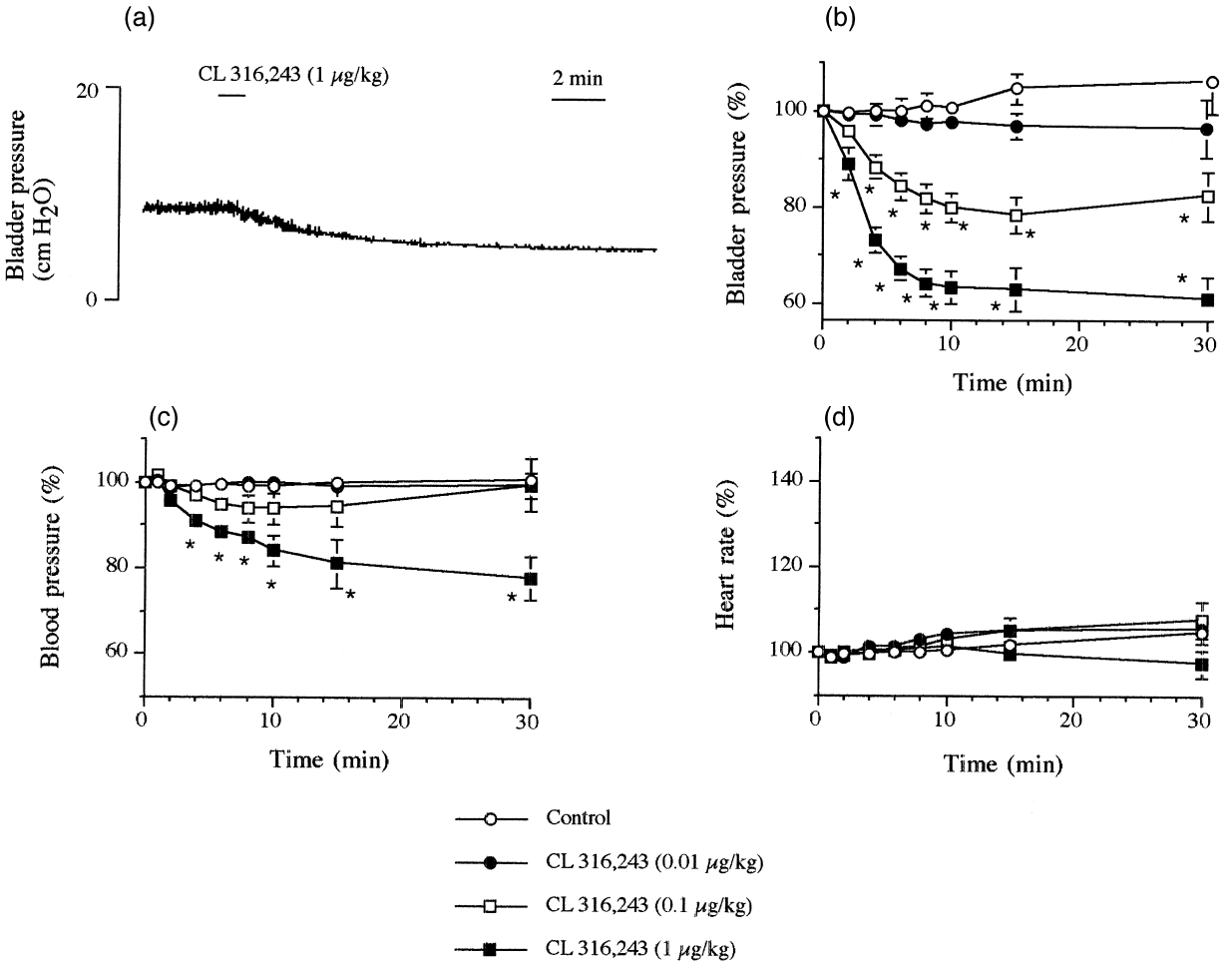

Fig. 8. Representative recording of effect of CL 316,243 (1 μg/kg, i.v.) on bladder pressure in anaesthetized ferret (a). Effects of CL 316,243 on bladder pressure (b), blood pressure (c) and heart rate (d) in anaesthetized ferret. Each point represents the mean ± S.E. of 4 experiments. * Indicates significant difference from control ($P < 0.05$).

resting value by 0.1, 1 and 10 μg/kg of isoprenaline, respectively. Dobutamine did not alter blood pressure even at its highest dose (Fig. 6c) but it increased heart rate dose-dependently, the heart rate being increased to 106.8 ± 0.8% and 120.9 ± 2.7% of the resting value by 10 and 100 μg/kg, respectively (Fig. 6d). At its highest dose (10 μg/kg, i.v.), procaterol produced a transient decrease in blood pressure (Fig. 7c), the maximal reduction being observed at 1 min after the injection. Procaterol increased heart rate dose-dependently, the increases being to 107.5 ± 1.0%, 117.7 ± 2.8% and 117.2 ± 2.4% of the resting value with 0.1, 1 and 10 μg/kg of procaterol, respectively (Fig. 7d). The highest dose of CL 316,243 (1 μg/kg, i.v.) decreased blood pressure but did not alter heart rate (Fig. 8c,d).

4. Discussion

In the present study, we carried out a functional analysis of the β-adrenoceptor subtypes present in the ferret urinary bladder to determine whether the ferret might be a useful experimental animal for investigating bladder function.

In our first experiment, we evaluated the relaxing effects of several β-adrenoceptor agonists on the ferret detrusor in vitro. Prior to the agonist experiment, we tested the relaxing effect of forskolin, an adenylyl cyclase activator, on ferret detrusor strips. At a concentration of 10^{-5} M, forskolin decreased the basal tone of the preparation, indicating that cAMP cascades play a very important role in the relaxation of the ferret detrusor. It is well known that β-adrenoceptor agonists act through an accumulation of intracellular cAMP after binding to a β-adrenoceptor coupled with a Gs type of G-protein, which in turn activates adenylyl cyclase. In this study, all of the β-adrenoceptor agonists tested relaxed the detrusor but they differed significantly from each other in their potency. The β_3-adrenoceptor agonists, BRL 37344 and CL 316,243, and the nonconventional partial β_3-adrenoceptor agonist, CGP-12177A, had much higher potencies than either the β_1-adrenoceptor agonist, dobutamine, or the β_2-adrenoceptor agonist, procaterol. Thus, we confirmed the functional

predominance of the β_3-adrenoceptor in the relaxation of the ferret detrusor. A comparison of the efficacies of β_3-adrenoceptor agonists in the ferret and human detrusors reveals that the maximal relaxing effects of both CL 316,243 and BRL 37344A on the ferret detrusor were larger than those on the human detrusor (Igawa et al., 1999). The maximal relaxation of detrusor caused by CGP-12177A were 90% for ferret, 30% for human, 60% for rabbit, 40% for rat and 80% for dog, respectively (Igawa et al., 1999; Yamazaki et al., 1998). Same difference was observed between human and rat colon. BRL 37344 cause complete relaxation of rat colon (Oriowo et al., 1996; Kaumann and Molenaar, 1996), whereas it had no effect on human colon (De Ponti et al., 1996; Bardou et al., 1998). The effect of CGP-12177, which reported a partial β_3-adrenoceptor agonist, also differed among species. Moreover, the pD_2 value for BRL 37344A differed between ferret (8.72) and human (6.42) detrusor (Igawa et al., 1999). On this basis, though both ferret and human detrusors predominantly relaxed by β_3-adrenoceptor agonists, it seems that a species difference in functional β_3-adrenoceptors may exist between human and ferret detrusors. As for the effect of CGP-12177A on the ferret detrusor, there is a possibility that it might be mediated via the putative β_4-adrenoceptor (Molenaar et al., 1997) in addition to the β_3-adrenoceptor (Bylund et al., 1994). Further functional and molecular biological studies will be needed to resolve this point.

In our second experiment, we investigated the activities of several β-adrenoceptor antagonists against the isoprenaline-induced relaxation of the isolated ferret detrusor. CGP-20712A, a selective β_1-adrenoceptor antagonist, exerted an antagonistic effect on the isoprenaline-induced relaxation but only at higher concentrations (10^{-7}–10^{-6} M). ICI-118,551, a selective β_2-adrenoceptor antagonist, did not affect the concentration–response curve for isoprenaline. These results suggest that neither β_1- nor β_2-adrenoceptors play an important functional role in the relaxation of the ferret detrusor. An additional experiment using a selective β_3-adrenoceptor antagonist, SR 58894A (Manara et al., 1996), confirmed this idea. In the presence of 10^{-7} M CGP-20712A and 10^{-7} M ICI-118,551, SR 58894A effectively counteracted the isoprenaline-induced relaxation of the ferret detrusor. However, the slope for SR 58894A obtained from the Schild plot was 0.43 ± 0.19, which is significantly different from unity. This phenomenon has also been observed in the human detrusor (slope 0.68, Igawa et al., 1999). Thus, it seems likely that while relaxation of both the ferret and human detrusors is mediated mainly via the β_3-adrenoceptor, either atypical β-adrenoceptors or the putative β_4-adrenoceptor also plays a part.

From these in vitro experiments using β-adrenoceptor agonists and antagonists, it is suggested that the relaxation of the ferret detrusor is mediated mainly by the β_3-adrenoceptor.

And then, in the third experiment, we attempted to clarify the effects of β-adrenoceptor agonists on bladder pressure, blood pressure and heart rate in the anaesthetized ferret. We used CL 316,243 as a β_3-adrenoceptor agonist in this experiment, because it has an excellent β_3-adrenoceptor selectivity so far reported (Bloom et al., 1992). Both isoprenaline and CL 316,243 reduced bladder pressure dose-dependently, while procaterol only slightly affected it. Moreover, CL 316,243 had a long-lasting effect on bladder pressure with respect to isolrenaline. This would be caused by the difference of a half-life between CL 316,243 and isoprenaline. In contrast, only the maximal dose of dobutamine (100 μg/kg) reduced bladder pressure. In our preliminary experiments on rat tissues: (i) dobutamine produced an increase in the spontaneous rate of beating of the isolated atrium (β_1-adrenoceptor-mediated response) at concentrations over 10^{-8} M, the pD_2 value being 7.0 and (ii) it produced an inhibition of spontaneous contractions in the pregnant uterus (β_2-adrenoceptor-mediated response) and in the proximal colon (β_3-adrenoceptor-mediated response), the pD_2 values being 6.6 and 6.7, respectively. As the β_1-adrenoceptor selectivity of dobutamine is not great enough to enable us reliably to distinguish the β_1-adrenoceptor from the other β-adrenoceptors, we cannot exclude a contribution of the β_1-adrenoceptor to reductions in bladder pressure in the ferret. The above data support the conclusion that the reduction in bladder pressure in the ferret resulted from a relaxation of the detrusor mediated largely by β_3-adrenoceptor stimulation.

In the present in vivo study, only the maximal dose of CL 316,243 (1 μg/kg) produced a fall in blood pressure. We found in our preliminary experiments that CL 316,243 (10^{-10}–10^{-4} M) did not relax the 20-mM KCl-induced contraction in isolated ferret aorta and portal vein preparations (data not shown). So, the induced fall in blood pressure might be the result of dilatation of microvessels in the skin (Wilson and Warren, 1993).

CL 316,243 had no stimulating effect on heart rate in the present study. It has been reported that all of the β-adrenoceptor subtypes (β_1-, β_2-, β_3- and putative β_4-adrenoceptors) exist in the ferret heart (Lowe et al., 1998). On the other hand, there is an apparently contrary report indicating that β_3-adrenoceptor agonists lack positive or negative inotropic effects on the ferret heart (Gauthier et al., 1999). We have observed that CL 316,243 (10^{-10}–10^{-4} M) does not produce a chronotropic effect on the isolated atria of the ferret (data not shown). On the basis of these data, it is suggested that selective β_3-adrenoceptor agonists can reduce bladder pressure without affecting either blood pressure or heart rate in the ferret. Furthermore, in humans only a low level of β_3-adrenoceptor mRNA is present in the heart (Krief et al., 1993; Berkowitz et al., 1995) and probably in blood vessels.

The present in vitro functional study has clearly demonstrated that relaxation of the ferret detrusor by β-adrenoc-

eptor agonists is mediated mainly via the β_3-adrenoceptor, as in the human detrusor. From this, we infer that the ferret may be an ideal animal for investigations of the function of the β_3-adrenoceptor in the detrusor. Moreover, because a selective β_3-adrenoceptor agonist only slightly affected the cardiovascular system, such agonists have the potential to be useful therapeutic agents for such urinary dysfunctions as frequent urination and urinary incontinence.

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