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Characterization of β-adrenoceptors in urinary bladder: comparison between rat and rabbit

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1 β-Adrenoceptor subtypes in rat and rabbit urinary bladder were investigated in functional experiments by use of several agonists and antagonists.

2 All agonists tested produced concentration-dependent relaxation, but the relative potencies varied between both species: BRL 37344 (pD_2:8.0) > isoprenaline (7.3) > adrenaline (6.7) = noradrenaline (6.6) in rat bladder, and isoprenaline (8.7) = adrenaline (8.5) > noradrenaline (7.7) = BRL 37344 (7.4) in rabbit bladder.

3 The relaxation response to isoprenaline in rat bladder was relatively resistant to propranolol and ICI 118551, and the slopes of Schild plot for both antagonists were different from unity. The apparent pK_B values estimated by single concentrations of propranolol (1, 10 μM) and ICI 118551(10 μM) were 6.6 and 5.4, respectively.

4 On the other hand, the relaxation response to isoprenaline in rabbit bladder was antagonized by lower concentrations (1 nM – 100 nM) of propranolol and ICI 118551 in a competitive manner, resulting in pA_2 values of 8.7 and 8.6, respectively.

5 These results suggest species-heterogeneity of β-adrenoceptors in urinary bladder; β_3 and β_2 subtypes in rat and β_2 subtype in rabbit.

Keywords: Rat bladder; rabbit bladder; relaxation; β_2-adrenoceptor; β_3-adrenoceptor

Introduction

β-adrenoceptors have been subdivided into β_1, β_2 and β_3 subtypes by pharmacological methods and in molecular cloning studies (Arch & Kaumann, 1993; Emorine *et al*., 1994). The third β-adrenoceptor subtype, termed β_3, was originally identified in adipocytes (Wilson *et al*., 1984), but now β_3-adrenoceptors have been found in many smooth muscles such as rat colon (McLaughlin & MacDonald, 1990), rat ileum (Growcott *et al*., 1993) and rat oesophageal smooth muscle (Lezama *et al*., 1996). The β_3-adrenoceptors are characterized by low affinity for classical β-adrenoceptor antagonists and by high affinity for a novel class of β_3-adrenoceptor agonists such as BRL 37344 (Strosberg & Pietri-Rouxel, 1996).

In urinary bladder β-adrenoceptors are involved in the relaxation induced by catecholamines (Anderson, 1993; Hoyle & Burnstock, 1993), but little information regarding the β-adrenoceptor subtype(s) in bladder has been obtained. In the present study, we characterized the β-adrenoceptors of rat and rabbit urinary bladders.

Methods

Male Wistar rats (250 – 480 g) were killed by a blow on the head and cervical dislocation. Japanese white rabbits (2.9 – 3.6 kg) were killed by injection of a high dose of sodium pentobarbitone (50 mg kg^{-1}, i.v.). Urinary bladder strips from rats and rabbits were isolated and were mounted vertically in an organ bath containing 10 ml of Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, $MgCl_2$ 1.2, $CaCl_2$ 2.6, $NaHCO_3$ 24.9, NaH_2PO_4 1.2 and glucose 11.1. The bathing medium was maintained at 37°C, pH 7.4 and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. The Krebs-Henseleit solution contained desmethylimipramine (0.1 μM), deoxycorticosterone (5 μM) and phentolamine (10 μM) to block neuronal and extraneuronal uptake of catecholamines and to block α-adrenoceptors. An initial tension of 0.5 g in rat bladder and 1.0 g in rabbit bladder was applied and the responses were recorded isometrically with force-displacement transducer. All preparations were equilibrated for at least 60 min before the start of the experiments. In order to obtain relaxant responses, the bladder strips were contracted with KCl (50 mM) which gave approximately 60 – 70% of the maximal contraction. The amplitudes of maximal contractions induced by KCl in rat and rabbit bladders were 3.1 ± 0.18 g (n = 30) and 3.9 ± 0.22 g (n = 30), respectively. Increasing concentrations of the β-agonists were added cumulatively in 0.5 log unit increments into the bath to produce concentration – response curves. However, in the case of rat bladder BRL 37344 was added in 1.0 log unit intervals because of the slowly developing response. When antagonists were tested, cumulative concentration – response curves for isoprenaline were obtained twice from the same strips at an interval of 1 h. Since the reproducibility of the concentration – response curve for isoprenaline had been confirmed in preliminary experiments, antagonists were added 30 min before the construction of a second concentration – response curve. Affinity of the antagonist was expressed as pA_2 value when the slope of Schild plot was not different from unity. The pA_2 value was estimated according to Arunlakshana & Schild (1959). Briefly, the concentration of isoprenaline necessary to give a half-maximal relaxation in the presence of different concentrations of the antagonists was divided by the concentration giving a half-maximal response in the control, to determine the agonist concentration-ratio (CR). Data were plotted as the −log antagonist concentration (M) vs the log (CR-1), and pA_2 values were calculated from Schild plots along mean slope and 95% confidence limits (95% CL), and straight lines were drawn by least square linear regression. As propranolol or ICI 118551 itself caused relaxation at a high concentration (100 μM) in rat bladder, we did not test the effect of 100 μM of either of the antagonists in rat bladder. When the slope of the Schild plot was significantly different from unity, distinct pK_B values were estimated from the antagonist induced by one or two different concentrations of antagonism by the concentration-ratio method (Furchgott, 1972).

Experimental values are given as a mean ± s.e.mean or means with 95% confidence limits. Results were analysed by

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unpaired Student's *t* test and a probability of less than 0.05 was considered significant.

The following drugs were used: isoprenaline hydrochloride, desmethylimipramine hydrochloride (Sigma, St. Louis, U.S.A.); noradrenaline bitartrate, adrenaline bitartrate (Wako Pure Chemicals, Osaka, Japan); BRL 37344((±)-(**R***,**R***)-[4-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]phenoxy]-acetic acid sodium) (Research Biochemicals International, Natic, U.S.A.); propranolol hydrochloride (Zeneca, Osaka, Japan); ICI 118551 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride) (Cambridge Research Biochemicals, Cheshire, U.K.); phentolamine mesylate (Ciba, Basel, Switzerland) and deoxycorticosterone acetate (Nacalai tesque, Kyoto, Japan).

Results

Isoprenaline, adrenaline, noradrenaline and BRL 37344 relaxed concentration-dependently the urinary bladder strips of rat and rabbit, which had been precontracted with 50 mM KCl (Figure 1a and b). The agonist potencies to produce relaxation were different between species. In rat bladder, pD_2 values were 8.0±0.09 ($n=7$) for BRL 37344, 7.3±0.10 ($n=9$) for isoprenaline, 6.7±0.11 ($n=7$) for adrenaline and 6.6±0.05 ($n=7$) for noradrenaline, while in rabbit bladder, pD_2 values were 8.7±0.11 ($n=10$) for isoprenaline, 8.5±0.13 ($n=7$) for adrenaline, 7.7±0.12 ($n=7$) for noradrenaline and 7.4±0.07 ($n=6$) for BRL 37344. Therefore, the rank order of potencies for agonists which were normalized against isoprenaline was BRL 37344 (5.0) > isoprenaline (1.0) > adrenaline (0.25) = noradrenaline (0.20) in rat bladder, and isoprenaline (1.0) = adrenaline (0.63) > noradrenaline (0.10) = BRL 37344(0.05). Maximal relaxation response obtained with BRL 37344 in rat bladder was significantly lower than that activated with isoprenaline, but the intrinsic activities of noradrenaline and adrenaline were not significantly different from that of isoprenaline (relative intrinsic activities of agonists to isoprenaline were 1.02 for noradrenaline, 0.95 for adrenaline and 0.86 for BRL 37344) (Figure 1a). On the other hand, in rabbit bladder maximal responses to the three agonists were not significantly different from that to isoprenaline (relative intrinsic activities of agonists to isoprenaline were 1.05 for adrenaline, 1.01 for BRL 37344 and 0.94 for noradrenaline) (Figure 1b).

Figure 2 shows the representative traces of BRL 37344-induced relaxations in rat and rabbit urinary bladders. The responses to BRL 37344 were concentration-dependent, but the time course of the relaxation-response was slow in rat bladder compared with rabbit bladder. Other agonists showed fast responses in both tissues (data not shown).

The relaxation responses to isoprenaline in both species were inhibited by classical β-adrenoceptor antagonists propranolol (β_1-and β_2-antagonist) and ICI 118551 (selective β_2-antagonist). However, higher concentrations of propranolol and ICI 118551 were necessary to evoke significant inhibition in rat bladder

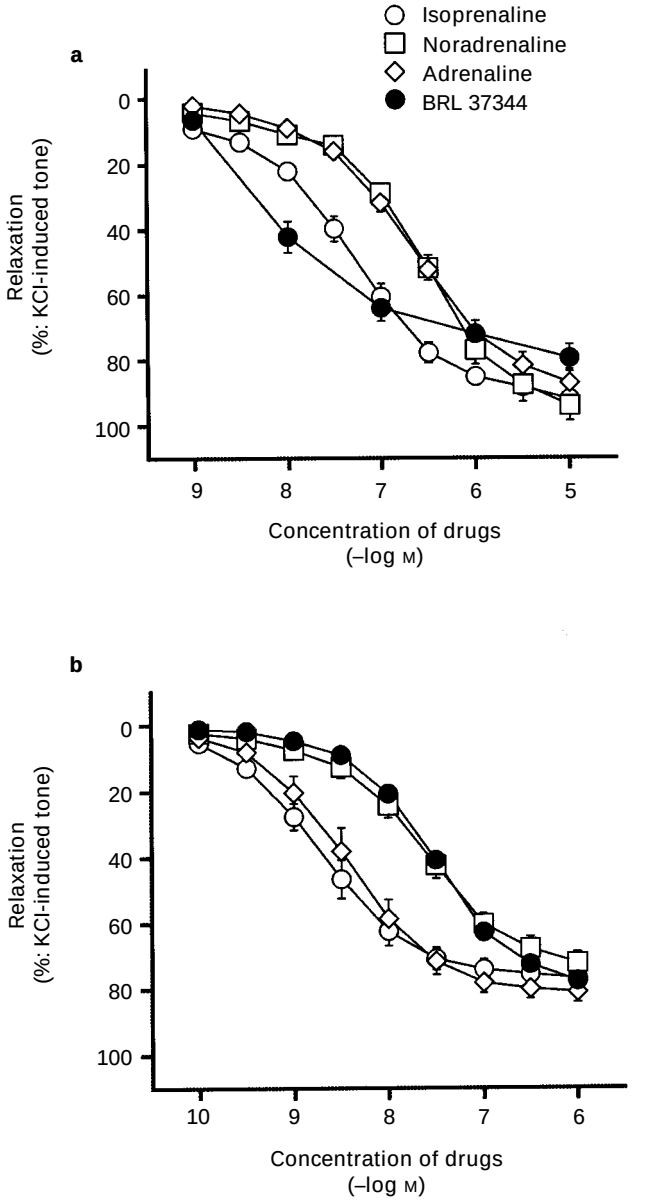

Figure 1 Relaxation response curves for isoprenaline, noradrenaline, adrenaline and BRL 37344 in rat bladder (a) and rabbit bladder (b). KCl-induced contraction is taken as 100%. Each value is the mean of 6–10 experiments; vertical lines show s.e.mean.

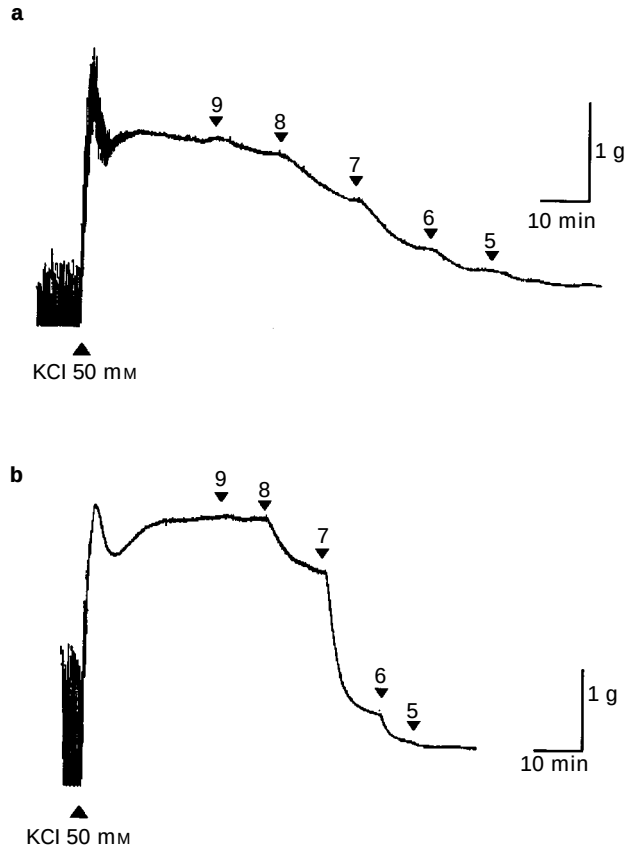

Figure 2 Typical traces showing the relaxation responses to BRL 37344 on KCl (50 mM)-induced contractile response in the rat isolated bladder (a) and rabbit bladder (b). Concentrations of BRL 37344 added in the organ bath are shown as −log(M).

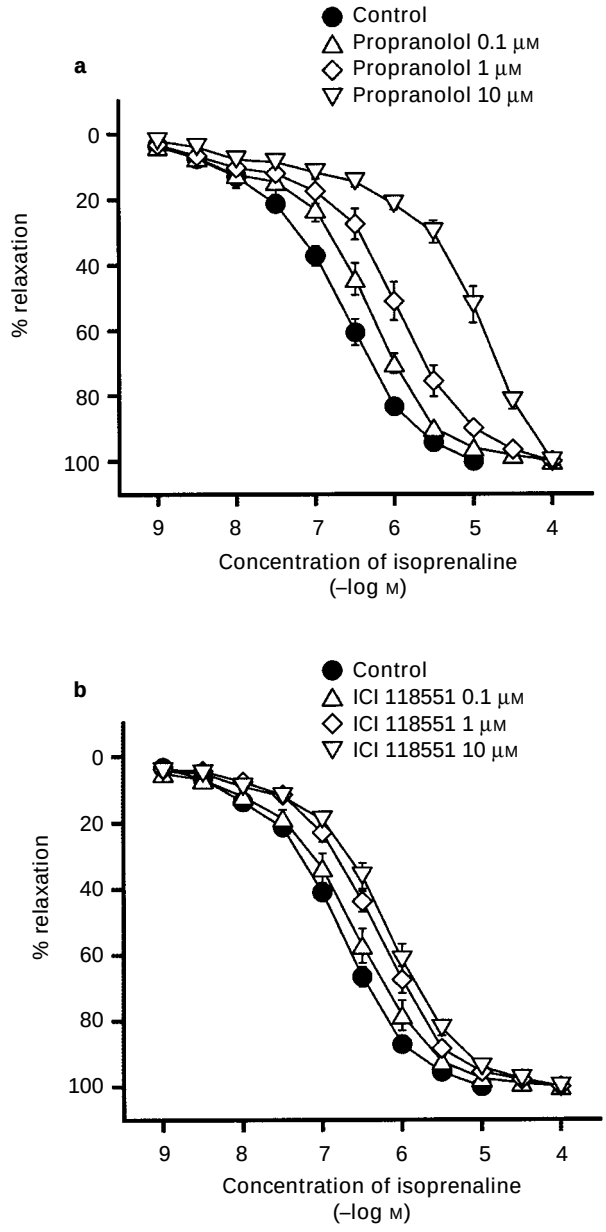

Figure 3 Inhibitory effects of propranolol (a) and ICI 118551 (b) on the relaxation response to isoprenaline in rat bladder. The maximal relaxation induced by isoprenaline is taken as 100%. Each value is the mean of 5 experiments; vertical lines show s.e.mean.

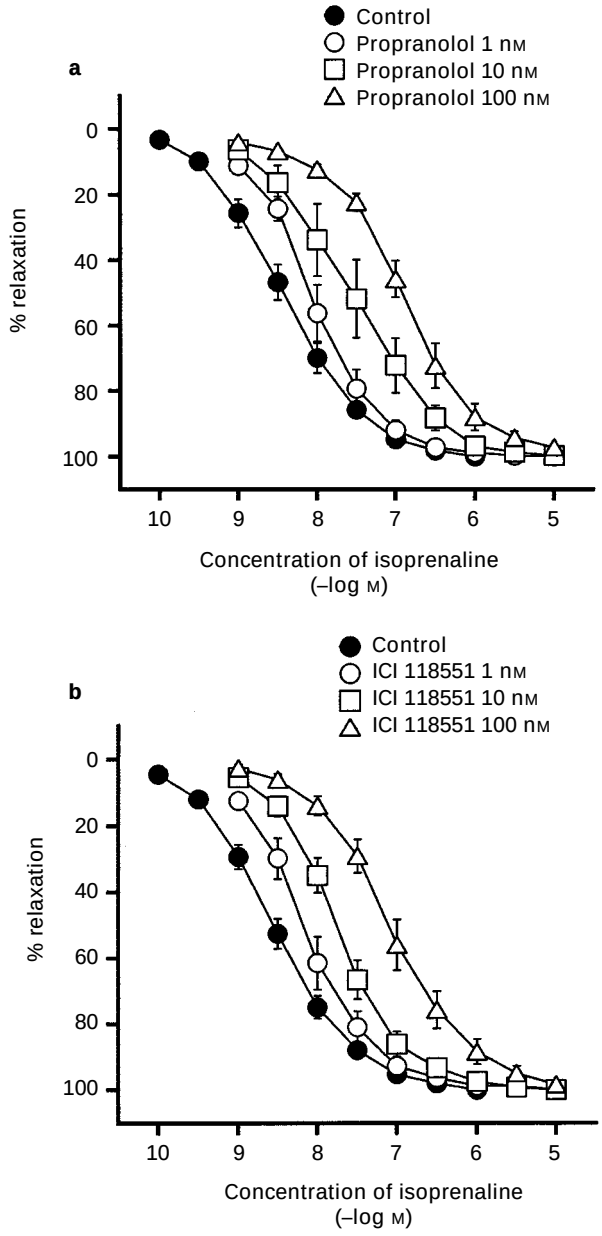

Figure 4 Inhibitory effects of propranolol (a) and ICI 118551 (b) on the relaxation response to isoprenaline in rabbit bladder. The maximal relaxation induced by isoprenaline is taken as 100%. Each value is the mean of 6–7 experiments; vertical lines show s.e.mean.

(Figure 3), compared with rabbit bladder (Figure 4). In the rat bladder the slopes of Schild plot for both antagonists were significantly different from unity (Figure 5, Table 1), so the occurence of two different receptors was suggested. An apparent pK_B value for propranolol was calculated from the antagonism by single concentrations of propranolol (1 and 10 μM) (Furchgott, 1972), giving a value of 6.6 ± 0.06 ($n = 10$) (Table 1). An apparent pK_B value was calculated with 10 μM ICI 118551, giving a value of 5.4 ± 0.08 ($n = 5$) (Table 1). On the other hand, in rabbit bladder the slopes of the Schild plots for both antagonists were not significantly different from unity (Figure 5, Table 1), suggesting the involvement of a single receptor. The pA_2 values for propranolol and ICI 118551 were 8.7 ± 0.13 ($n = 18$) and 8.6 ± 0.14 ($n = 20$), respectively (Table 1).

Discussion

The present study clearly shows the species-heterogeneity of β-adrenoceptors mediating the relaxation response in urinary bladders. The β-adrenoceptors of rat urinary bladder are highly sensitive to BRL 37344, a selective β_3-adrenceptor agonist (Howe, 1993), in contrast to those of rabbit urinary bladder. The agonist order of potency in rat bladder was BRL 37344 (5.0) > isoprenaline (1.0) > adrenaline (0.25) = noradrenaline (0.20), which is in agreement with the order in the tissues where β_3-adrenoceptor responses have been observed (MacDonald *et al.*, 1994).

Although the potencies of agonists and the order clearly show the presence of β_3-adrenoceptor in rat bladder, the results obtained from the experiments with antagonists are controversial. The slopes of Schild plots for propranolol and ICI 118551 against the isoprenaline response were significantly different from unity, suggesting the possible involvement of more than one subtype of β-adrenoceptor. Slight inhibition by the low concentration (100 nM) of propranolol and ICI 118551 indicates the minor involvement of β_2-adrenoceptor in the relaxation to isoprenaline. However, the pK_B values estimated from higher concentrations of propranolol and ICI 118551 suggest that the relaxation is mediated through β-adrenocep-

Table 1 Antagonism by propranolol and ICI 118551 of the relaxation responses to isoprenaline in rat bladder and rabbit bladder

	pA_2 or pK_B[a]			
	Propranolol		*ICI 118551*	
Rat bladder	7.3±0.20 6.6±0.06[a]	(0.63, 0.45–0.80)	6.8±0.41 5.4±0.08[a]	(0.21, 0.05–0.37)
Rabbit bladder	8.7±0.13	(0.97, 0.73–1.22)	8.6±0.14	(0.98, 0.70–1.25)

For pA_2 values slope and 95% CL are shown in parentheses. [a]Since slope factors in the Schild plot were significantly different from unity, pK_B values were estimated from the inhibitory effects of propranolol (1, 10 μM) or ICI 118551 (10 μM), according to the method proposed by Furchgott (1972).

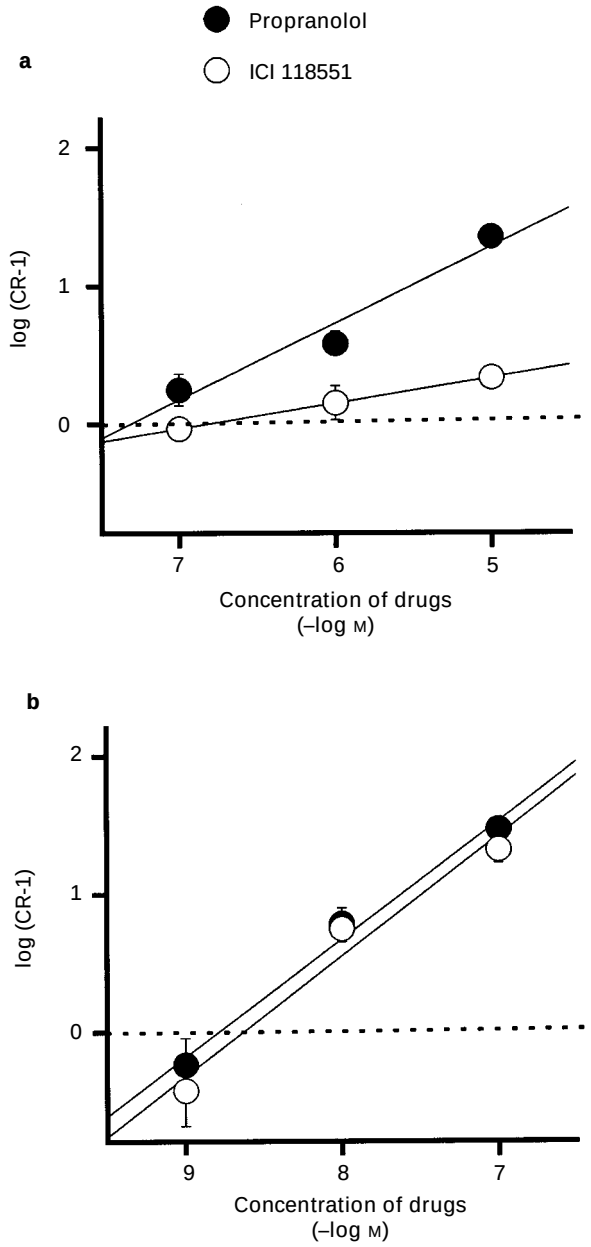

Figure 5 Schild plots for inhibition of isoprenaline-induced relaxation by propranolol and ICI 118551 in rat bladder (a) and rabbit bladder (b). Each point is the mean of data obtained from 5 to 7 preparations and vertical lines show the s.e.mean. For pA_2 values and slopes see Table 1.

tors resistant to such antagonists in rat bladder. These data with antagonists show the predominant involvement of β_3-adrenoceptors in rat bladder and are in agreement with the data with agonists mentioned above. The involvement of more than one type of β-adrenoceptors has been demonstrated in rat oesophageal muscularis mucosae (De Bore *et al*., 1993), colon (Bianchetti & Manara, 1990) and gastric fundus (Lefebvre *et al*., 1984).

In contrast, the relaxation response to isoprenaline of rabbit bladder was inhibited by low concentrations of propranolol and ICI 118551 in a simple competitive manner. Since the order of agonist potency and the antagonist affinities are well consistent with those obtained for β_2-adrenoceptors (Wilson *et al*., 1984; Strosberg & Pietri-Rouxel, 1996), it is concluded that the relaxation in rabbit bladder is mainly mediated via the β_2 subtype. Levin *et al*. (1988) demonstrated the existence of β_2-adrenoceptors in rabbit bladder with a receptor binding experiment.

The potencies of isoprenaline, noradrenaline and adrenaline in rat bladder were weaker than those in rabbit bladder. Such low potencies of β-agonists in rat bladder are in agreement with recent data obtained by Nishimoto *et al*. (1995), and Emorine *et al*. (1994) have suggested that atypical low potencies of reference β-agonists are characteristic for β_3-adrenoceptors. Furthermore, the time course of the relaxation response to BRL 37344 was slow in rat urinary bladder compared with rabbit urinary bladder. Slow development of relaxation responses mediated through β_3-adrenoceptors was described by Growcott *et al*. (1993) and Kaumann & Molenaar (1996). These features detected in rat bladder suggest the predominant involvement of β_3-adrenoceptors in the relaxation responses to β-agonists tested.

Recently, the presence of an additional β-adrenoceptor subtype (β_4) has been proposed in mammalian hearts (Kaumann, 1997). The β_4-adrenoceptor is resistant to blockade by classical β-antagonists such as propranolol, like β_3-adrenoceptors, but insensitive to selective β_3-agonists. Since BRL 37344, selective β_3-adrenoceptor agonist, caused a relaxation response in the rat, the involvement of β_4-adrenoceptors in the responses of rat bladder seems to be negligible.

Maggi & Meli (1982) previously observed in *in vivo* experiments with rats that the spontaneous contractions of bladder were inhibited by isoprenaline dose-dependently and this effect was antagonized by propranolol. Hence, they demonstrated a β_2-adrenoceptor-mediated modulation of bladder contraction of rats. This may be somewhat conflicting with the present results. However, in their study propranolol could not antagonize the effect induced by high doses of isoprenaline. It is interesting to note that β_2-adrenoceptors are more sensitive to isoprenaline than β_3-adrenoceptors (present study, Emorine *et al*., 1994) and that not only β_3- but also β_2-adrenoceptors participate in the relaxation response to isoprenaline of rat urinary bladder (see above discussion). Thus, it can be postulated that functional responses in some tissues are markedly affected by the differences in the subtypes coexisting and in their sensitivities to the agonists and antagonists used (Oshita *et al*., 1993).

In conclusion, the present study provides functional evidence that β-adrenoceptors of urinary bladder differ between species and that the β_3 subtype is predominant in the rat, whereas the β_2 subtype is in the rabbit.

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