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β-Adrenoceptor subtypes in the ureteral smooth muscle of rats, rabbits and dogs

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Abstract

We investigated the β-adrenoceptor subtypes mediating ureteral relaxation in rats, rabbits and dogs. The relaxing effects of β-adrenoceptor agonists were evaluated on KCl-induced ureteral contractions. The rank order of potency of the catecholamines tested was isoprenaline > noradrenaline > adrenaline in rat ureter; isoprenaline > adrenaline > noradrenaline in rabbit ureter; only isoprenaline was effective in canine tissues. The β_1-adrenoceptor agonist, dobutamine, produced relaxation of rat ureter. The β_2-adrenoceptor agonist, procaterol, produced more significant relaxation of rabbit ureter than did dobutamine. CL-316243 [(*R*,*R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarboxylate] and CGP-12177A [(±)[4-[3[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one hydrochloride], β_3-adrenoceptor agonists, were more effective in relaxing canine ureter than were dobutamine and procaterol. Isoprenaline-induced relaxation was antagonized by a β_1-adrenoceptor antagonist, CGP-20712A [2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulphonate], in rats and by a β_2-adrenoceptor antagonist, ICI-118,551 [(±)-1-[(2,3-dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride], in rabbits. The non-selective β-adrenoceptor antagonist, bupranolol, antagonized isoprenaline-induced relaxation in all species tested. In conclusion, β-adrenoceptor agonists may relax ureter by stimulating mainly β_1-adrenoceptors in rats, β_2-adrenoceptors in rabbits and mainly β_3-adrenoceptors in dogs.

Keywords: β-Adrenoceptor subtype; β_3-Adrenoceptor; Ureter; Smooth muscle relaxation; Species difference

1. Introduction

The sympathetic and parasympathetic divisions of the autonomic nervous system both play at least a modulating role in ureteral motility (Schulman, 1974), though ureteral peristalsis can occur in the absence of any innervation (Melick et al., 1961). With regard to adrenoceptor-mediated responses in ureteral smooth muscle, Deane (1967) found that ureteral motor function is stimulated by α-adrenoceptor agonists but is depressed by β-adrenoceptor agonists. In the 30 years since the subdivision of β-adrenoceptors into β_1- and β_2-adrenoceptors by Lands et al. (1967), there have been a number of reports indicating that the relaxation of the ureter induced by β-adrenoceptor agonists may be mediated mainly through the β_2-adrenoceptor subtype in rabbits (Morita et al., 1986), pigs (Hernández et al., 1992) and dogs (Stower et al., 1986; Morita et al., 1994).

Subsequently, a β_3-adrenoceptor, an additional β-adrenoceptor subtype, was identified by Emorine et al. (1989), and smooth muscle tissues from human colon and gallbladder have been found to express β_3-adrenoceptor mRNA (Berkowitz et al., 1995). However, it is unclear whether β_3-adrenoceptors are present in the smooth muscle of the mammalian ureter.

The purpose of this study was to characterize the functional β-adrenoceptor subtypes mediating relaxation of the rat, rabbit and canine ureter by comparing the potencies of a number of catecholamines, and of β-adrenoceptor agonists selective for each β-adrenoceptor subtype, with special attention being paid to the presence or absence of the β_3-adrenoceptor subtype. In addition, we examined the

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interaction between selective β-adrenoceptor antagonists and the isoprenaline-induced relaxation with the aim of determining more precisely the β-adrenoceptor subtypes distributed in the ureter in the above species.

2. Materials and methods

2.1. Animals

This study was conducted according to the guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical and conformed with current Japanese Law. Male Japanese White rabbits (2.0–3.5 kg, from SLC, Hamamatsu, Japan), male Sprague–Dawley rats (200–380 g, also from SLC) and mongrel dogs of either sex (7–14 kg, from Nagoya Labo-Service, Nagoya, Japan) were used. They were maintained under 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 experimental protocol

Rabbits were anaesthetized with urethane (1.5 g kg^{-1}, s.c.), rats and dogs with sodium pentobarbital (30 mg kg^{-1}, i.v.). The animals were then killed by rapid exsanguination and the ureters were removed. After removal of fat, blood vessels and mucosa, the whole ureter was cut into approximately 20-mm segments. For a given experiment, one of these segments was suspended longitudinally in a 10-ml organ bath containing Krebs solution (composition in mM: NaCl 118.1, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, glucose 11.1) which was maintained at 37°C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide. The tissue response to various manoeuvres was measured by means of an isometric force-transducer (SB-1T, Nihon-Kohden, Tokyo, Japan) connected to a polygraph (360, NEC-San-ei, Tokyo, Japan). An initial resting tension of 0.5 g was placed on the ureteral segment and it was allowed to equilibrate for 1 h. The ureteral segments did not show any spontaneous contractions. The actions of catecholamines and β-adrenoceptor agonists were evaluated by testing their effects upon an 80-mM KCl-induced tonic contraction, which is a maximal or a submaximal contraction induced by KCl in tissues from each of the three species (preliminary determination, data not shown). A high potassium bathing solution was prepared by adding 80 mM KCl. This was done by adding 0.2 ml of a 4 M KCl stock solution to the organ bath. After the tonic contraction stabilized, drugs were added in 0.5 log increments every 2.5 min. A concentration–response curve for each β-adrenoceptor agonist was made either in the presence or in

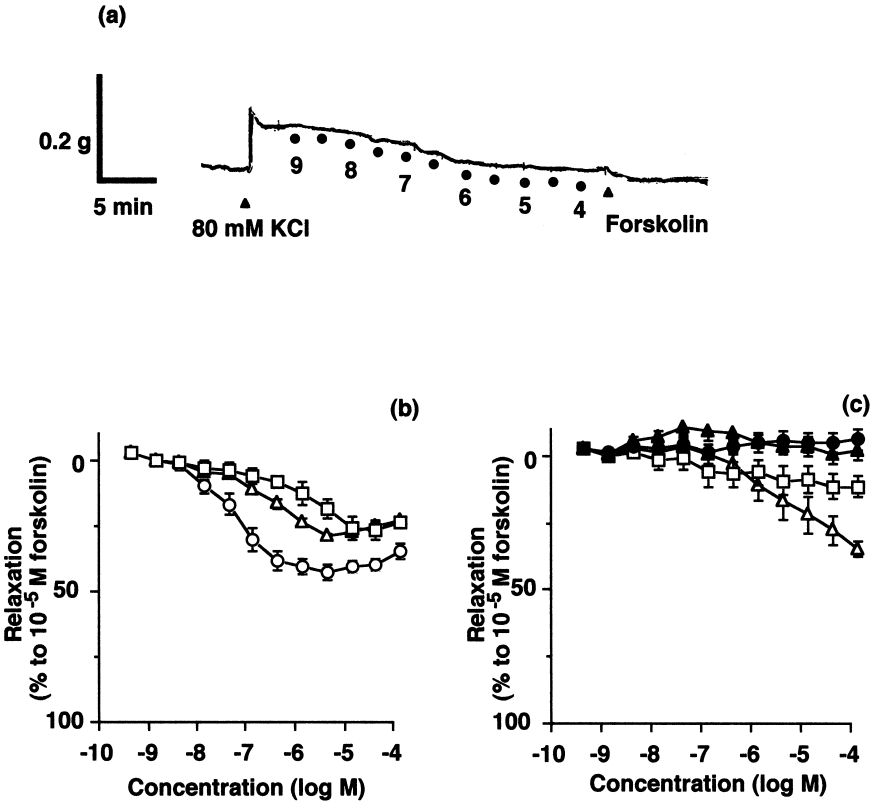

Fig. 1. Effects of β-adrenoceptor agonists on the KCl-induced ureteral contraction in the rat. Typical tracing of the isoprenaline-induced relaxation (a). Concentrations of isoprenaline are shown as −log (M). Concentration–response curves (b) for isoprenaline (○), noradrenaline (△) and adrenaline (□), and (c) for dobutamine (△), procaterol (□), CL-316243 (●) and CGP-12177A (▲). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of 6–10 experiments.

Table 1
The pD_2 value, comparative potency and intrinsic activity of catecholamines and β-adrenoceptor agonists in the ureteral smooth muscle of rats, rabbits and dogs

Agonist	Rat			Rabbit			Dog		
	pD_2 [a]	C.P.[b]	I.A.[c]	pD_2 [a]	C.P.[b]	I.A.[c]	pD_2 [a]	C.P.[b]	I.A.[c]
Isoprenaline	7.36 ± 0.14	7.51 ± 0.16	48.80 ± 2.26	7.24 ± 0.12	7.55 ± 0.17	59.20 ± 2.48	7.30 ± 0.13	7.52 ± 0.16	53.22 ± 4.14
Noradrenaline	6.83 ± 0.11	6.50 ± 0.12	31.81 ± 0.99	5.61 ± 0.12	5.67 ± 0.15	45.87 ± 4.28	4 >	4 >	19.63 ± 4.08
Adrenaline	5.96 ± 0.18	5.64 ± 0.24	30.99 ± 3.76	6.51 ± 0.08	6.67 ± 0.15	51.50 ± 5.31	4 >	4 >	10.53 ± 4.22
Dobutamine	5.62 ± 0.24	5.57 ± 0.26	38.14 ± 3.33	4.78 ± 0.09	5.00 ± 0.16	47.62 ± 2.44	5.01 ± 0.27	5.08 ± 0.36	27.10 ± 7.56
Procaterol	4 >	4 >		7.83 ± 0.15	8.10 ± 0.23	51.24 ± 2.77	5.55 ± 0.34	5.25 ± 0.27	32.86 ± 6.23
CL-316243	4 >	4 >		4 >	4 >		7.57 ± 0.11	7.76 ± 0.11	51.12 ± 1.11
CGP-12177A	4 >	4 >		4 >	4 >		6.60 ± 0.33	6.45 ± 0.41	32.18 ± 4.04

Data are expressed as the mean ± S.E.M. of 5–10 separate experiments.
[a] pD_2 = The negative logarithm of the molar concentration required to produce 50% of the maximal relaxation elicited by each drug.
[b] C.P. = Comparative potency (the negative logarithm of the molar concentration required to produce 20% of the 10^{-5} M forskolin-induced relaxation).
[c] I.A. = Intrinsic activity (10^{-5} M forskolin-induced relaxation = 100).

the absence of one concentration of an antagonist (pre-incubated for 30 min) in separate preparations. The bath solution contained 10^{-6} M phentolamine to block the α-adrenoceptor-mediated contractile responses of the ureter.

2.3. Drugs

The following drugs were used: (−)-adrenaline bitartrate, (−)-noradrenaline bitartrate, (−)-isoprenaline bitartrate, procaterol hydrochloride, phentolamine methanesul-

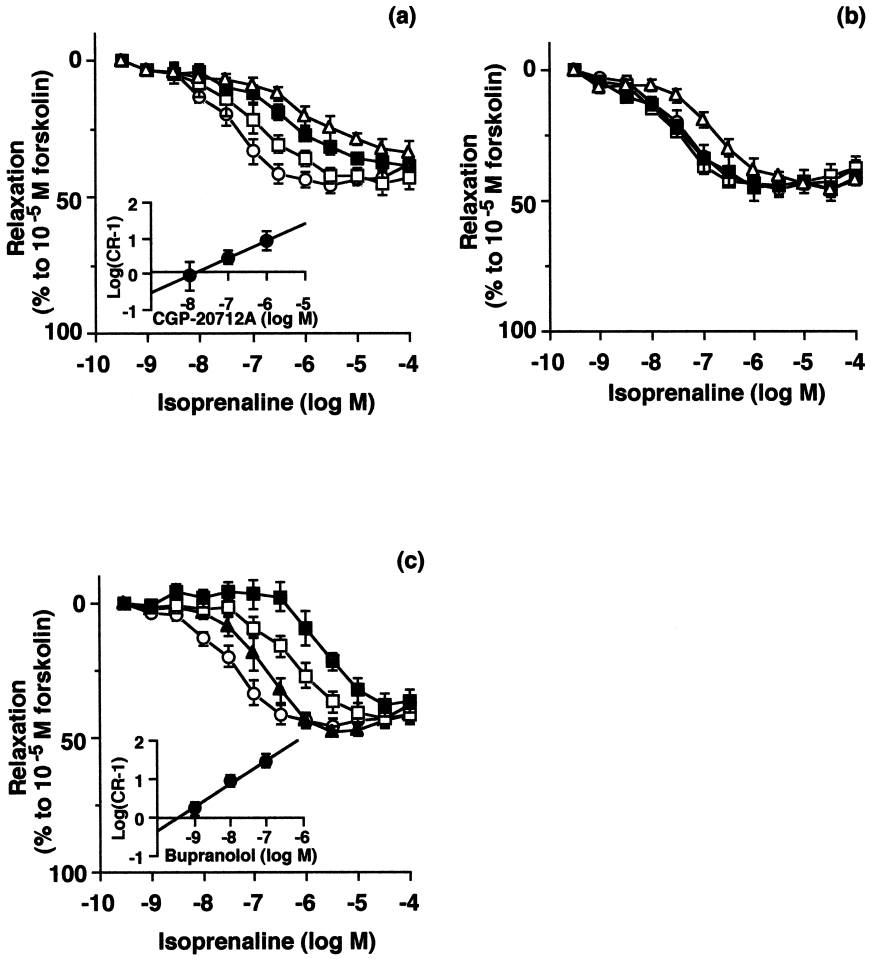

Fig. 2. Antagonism by CGP-20712A (a), ICI-118,551 (b) and bupranolol (c) of the isoprenaline-induced relaxation of the rat ureter. Control (○), 10^{-9} M (▲), 10^{-8} M (□), 10^{-7} M (■) and 10^{-6} M (△). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of 5–10 experiments. The inset shows the corresponding Schild plot.

Table 2
Antagonism between β-adrenocepter antagonists and the relaxation induced by isoprenaline in rat, rabbit or dog ureter

Antagonist	Rat		Rabbit		Dog	
	pA_2	Slope	pA_2	Slope	pA_2	Slope
CGP-20712A	7.93 ± 0.34	0.48 ± 0.20[a]	< 5		< 5	
ICI-118,551	< 5		8.51 ± 0.18	0.84 ± 0.29	< 5	
Bupranolol	9.54 ± 0.16	0.61 ± 0.11[a]	8.64 ± 0.13	0.75 ± 0.18	7.83 ± 0.18	0.58 ± 0.13[a]

Data are expressed as the mean ± S.E.M. of 5–10 separate experiments.
The pA_2 and slope value were obtained from the concentration–response curves shown in Fig. 2,4 and 6.
[a]Significantly different from unity.

fonate, (Sigma, St. Louis, MO, USA), dobutamine hydrochloride, (±) CGP-12177A hydrochloride ((±)[4-[3[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one hydrochloride), ICI-118,551 ((±)-1-[(2, 3-dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride) (Funakoshi, Tokyo, Japan), dimethyl sulfoxide (DMSO) (Nacalai tesque, Kyoto, Japan) and forskolin (Wako, Osaka, Japan). (±) Bupranolol hydrochloride, CL-316243 ((*R*,*R*)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]-1,3-benzodioxole-2,2-dicarbo-xylate) and CGP-20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl)phenoxy)propyl)amino)ethoxy)-benzamide monomethane sulphonate) were synthesized in our laboratory (Kissei, Hokata, Japan). Forskolin was dissolved in 100% DMSO and the other drugs in distilled water. We used dobutamine as a β_1-adrenoceptor agonist (Williams and Bishop, 1981), procaterol as a β_2-adrenoceptor agonist (Yamashita et al., 1978), and CL-316243 (Largis et al., 1994) and CGP-12177A (Langin et al., 1991; Kaumann and Molenaar, 1996) as β_3-adrenoceptor agonists. We also used CGP-20712A as a β_1-adrenoceptor antagonist (Kau-

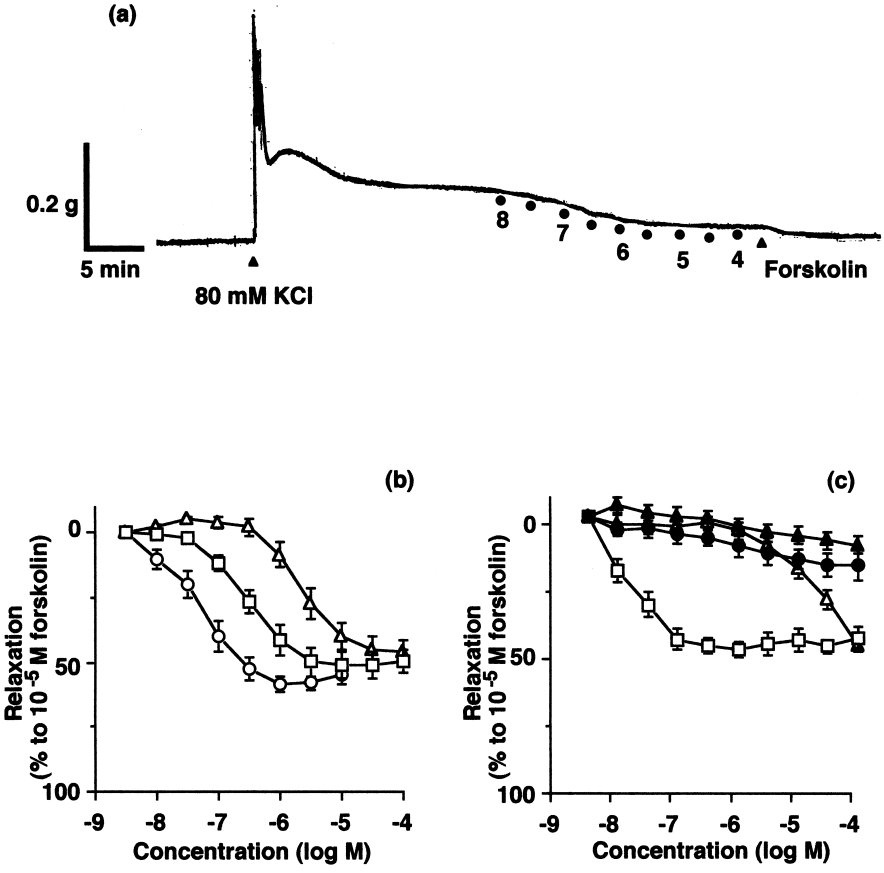

Fig. 3. Effects of β-adrenoceptor agonists on the KCl-induced contraction of the rabbit ureter. Typical tracing of the isoprenaline-induced relaxation (a). Concentrations of isoprenaline are shown as −log (M). Concentration–response curves (b) for isoprenaline (○), noradrenaline (△) and adrenaline (□), and (c) for dobutamine (△), procaterol (□), CL-316243 (●) and CGP-12177A (▲). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of five experiments.

mann, 1986), ICI-118,551 as a β_2-adrenoceptor antagonist (Bilski et al., 1983) and bupranolol as a non-selective β-adrenoceptor antagonist (Langin et al., 1991; Blin et al., 1994; Koike et al., 1995).

2.4. Data analysis

Drug-induced ureteral relaxation is expressed as a percentage of the maximal response to 10^{-5} M forskolin. The pD_2 value was taken as the negative logarithm of the molar EC_{50} value (which in turn was determined as the molar concentration required to produce 50% of the maximal relaxation elicited by each drug). The KCl-induced contraction of the ureter showed a slight progressive decrease in tone of about 20% during the course of the experiment when the same volume of distilled water (vehicle) was added to the organ bath instead of the drug solution every 2.5 min. The data were corrected by reference to the responses of the vehicle-treated control at each point (time). We calculated the corrected relaxing response to a drug by subtracting the decline in the vehicle-treated control from the relaxing response to a drug. Intrinsic activity values were calculated as the ratio between the maximal relaxation for each agent and that elicited by 10^{-5} M forskolin (added at the end of each experiment). Comparative potency was also taken as the negative logarithm of the EC_{20} value, which was determined as the molar concentration required to produce 20% of the 10^{-5} M forskolin-induced relaxation. The parallelism of each concentration–response curve in the absence and in the presence of antagonists was tested. The pA_2 value for each antagonist, as defined by Arunlakshana and Schild (1959), was obtained from a linear regression plot of the mean values of log(CR-1), where CR is the concentration ratio of the agonist in the presence or absence of antagonist, vs. the negative logarithm of the antagonist concentration. All results are expressed as means ± standard error of the mean (S.E.M.). Statistical differences between two data sets were assessed by using Student's *t*-test at a 5% level of significance.

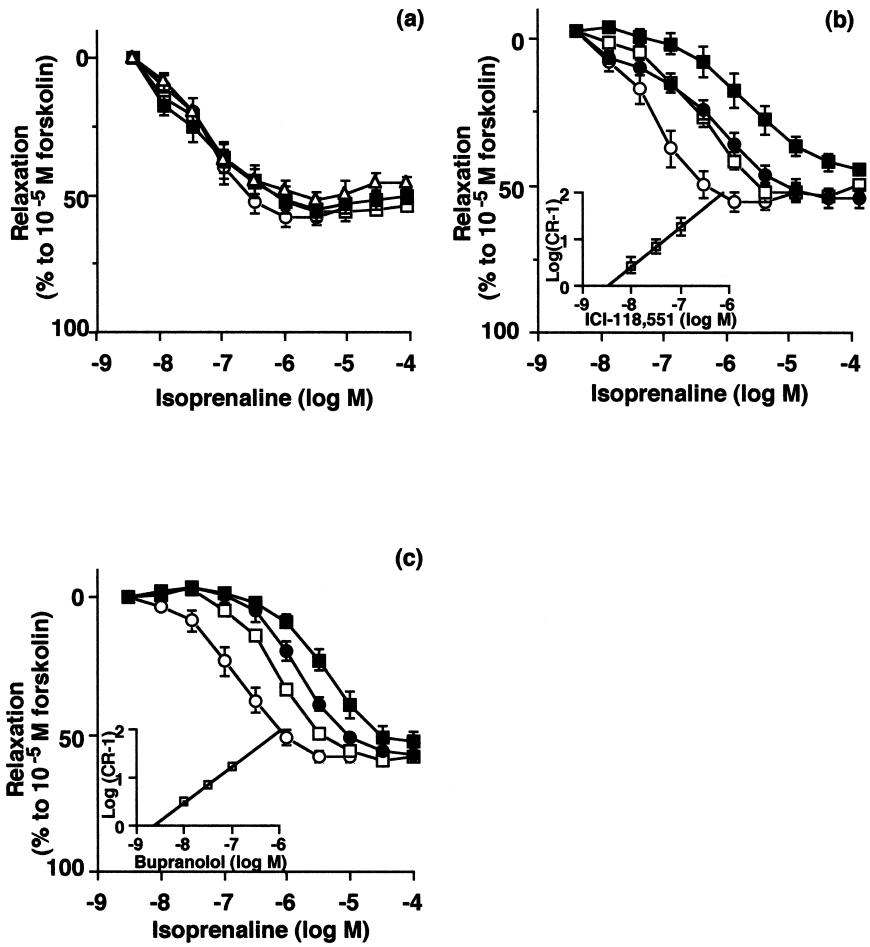

Fig. 4. Antagonism by CGP-20712A (a), ICI-118,551 (b) and bupranolol (c) of the isoprenaline-induced relaxation of the rabbit ureter. Control (○), 10^{-8} M (□), 3×10^{-8} M (●), 10^{-7} M (■) and 10^{-6} M (△). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of 5–10 experiments. The inset shows the corresponding Schild plot.

3. Results

3.1. Pharmacological studies with the rat ureter

The three catecholamines (isoprenaline, noradrenaline and adrenaline) all relaxed the KCl-induced contraction of the rat ureter in a concentration-dependent manner (Fig. 1a and b). The pD_2 value and intrinsic activity for each agonist are shown in Table 1. The maximal relaxation produced by noradrenaline and adrenaline was about 60% of that induced by isoprenaline. In terms of potency, the rank order was isoprenaline > noradrenaline > adrenaline. The relaxing effects of β-adrenoceptor agonists on the rat ureter are shown in Fig. 1c and Table 1. Higher concentrations of dobutamine, a β_1-adrenoceptor agonist (i.e., over 10^{-6} M), also produced relaxation of the ureter, the maximal relaxation being comparable to that produced by isoprenaline. The β_2-adrenoceptor-selective agonist procaterol and the β_3-adrenoceptor-selective agonists CL-316243 and CGP-12177A had little or no effect on the rat ureter. Both a selective β_1-adrenoceptor antagonist CGP-20712A (10^{-8} M to 10^{-6} M) and a non-selective β-adrenoceptor antagonist bupranolol (10^{-9} M to 10^{-7} M) caused a parallel rightward shift of the concentration–response curve for the isoprenaline-induced relaxation of the rat ureter (Fig. 2a and c), their pA_2 values being 7.93 ± 0.34 and 9.54 ± 0.16, respectively (Table 2). As indicated in Table 2, the slope of the Schild plot was 0.48 ± 0.20 for the former and 0.61 ± 0.11 for the latter antagonist; both of these values were significantly different from unity ($P < 0.05$). The concentration–response curve for isoprenaline was shifted to the right only in the presence of the highest concentration (10^{-6} M) of the selective β_2-adrenoceptor antagonist ICI-118,551 (Fig. 2b).

3.2. Pharmacological studies with the rabbit ureter

All three catecholamines tested produced concentration-dependent relaxation of the rabbit ureter (Fig. 3a and b). In terms of potency, the rank order was isoprenaline > adrenaline > noradrenaline (Fig. 3b, Table 1). With respect to the maximal relaxation they induced, the various catecholamines did not differ significantly from each other. The β_2-adrenoceptor-selective agonist procaterol was about six times more potent than isoprenaline in relaxing the ureter (Fig. 3c, Table 1). At higher concentrations (over 3×10^{-6} M), dobutamine also produced

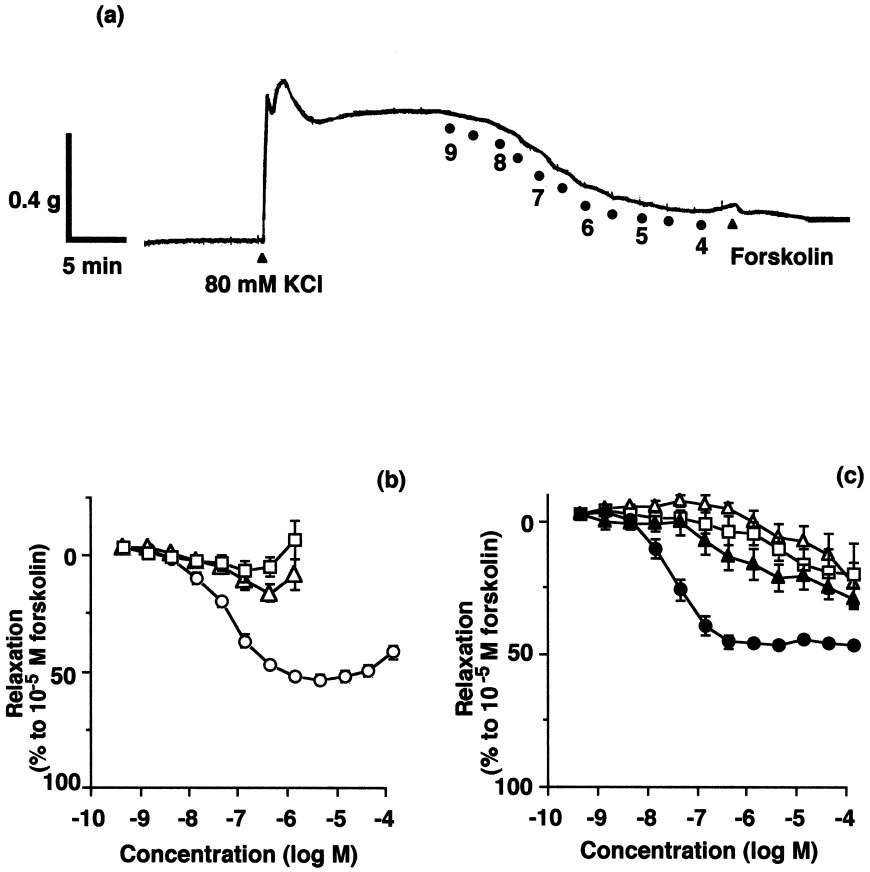

Fig. 5. Effects of β-adrenoceptor agonists on the KCl-induced contraction of the dog ureter. Typical tracing of the isoprenaline-induced relaxation (a). Concentrations of isoprenaline are shown as $-\log$ (M). Concentration–response curves (b) for isoprenaline (○), noradrenaline (△) and adrenaline (□), and (c) for dobutamine (△), procaterol (□), CL-316243 (●) and CGP-12177A (▲). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of 6–8 experiments.

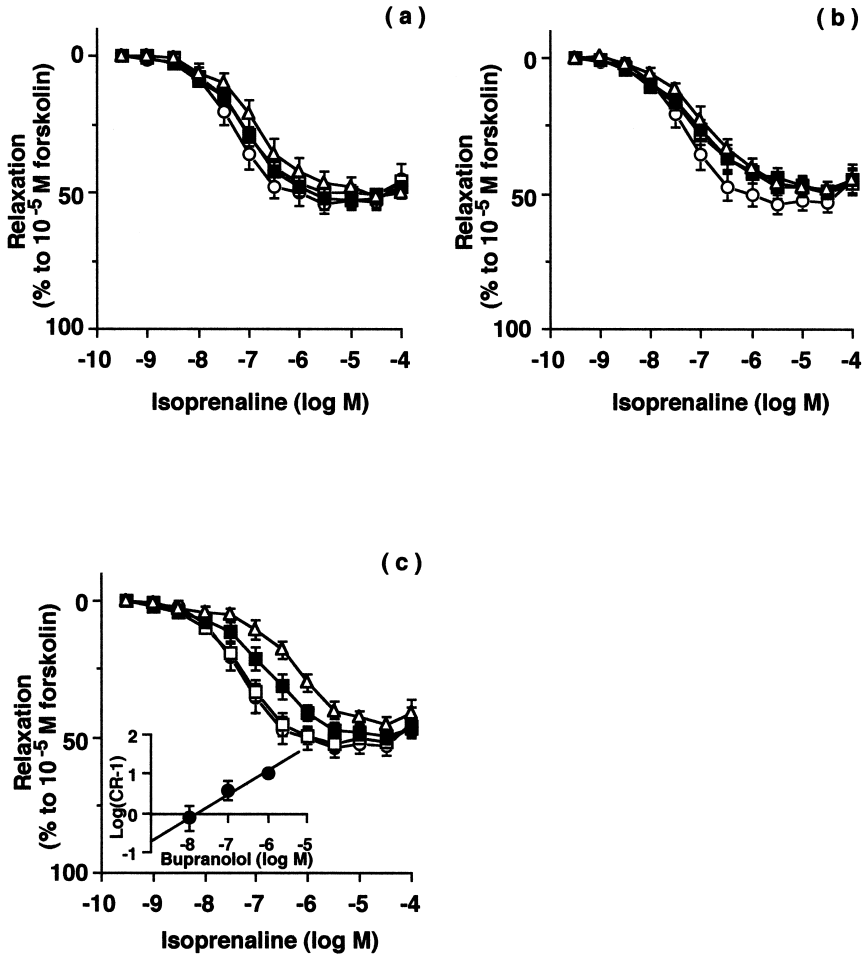

Fig. 6. Antagonism by CGP-20712A (a), ICI-118,551 (b) and bupranolol (c) of the isoprenaline-induced relaxation of the canine ureter. Control (○), 10^{-8} M (□), 10^{-7} M (■) and 10^{-6} M (△). Each value is expressed as a percentage of the response to 10^{-5} M forskolin. Data represent the mean ± S.E.M. of 7–10 experiments. The inset shows the corresponding Schild plot.

relaxation of the ureter (Fig. 3c). Neither CL-316243 nor CGP-12177A had any significant effect on the ureter. Both ICI-118,551 (10^{-9} M to 10^{-7} M) and bupranolol (10^{-9} M to 10^{-7} M) caused a parallel rightward shift of the concentration–response curve for the isoprenaline-induced relaxation of the rabbit ureter (Fig. 4b and c), their respective pA_2 values being 8.51 ± 0.18 (slope, 0.84 ± 0.29) and 8.64 ± 0.13 (slope, 0.75 ± 0.18) (Table 2). The concentration–response curve for isoprenaline was not influenced by CGP-20712A (Fig. 4a).

3.3. Pharmacological studies with the canine ureter

Of the three catecholamines tested, only isoprenaline produced significant relaxation of the canine ureter (Fig. 5a and b, Table 1). The noradrenaline-induced relaxation of the ureter was very small, in fact only about 37% of that induced by isoprenaline. At concentrations from 10^{-9} M to 3×10^{-7} M, adrenaline had no effect on the KCl-induced contraction of the ureter, but at 10^{-6} M it elicited a further contraction (Fig. 5b). The β_3-adrenoceptor-selective agonist CL-316243 was as potent as isoprenaline in relaxing the canine ureter (Fig. 5c). Another β_3-adrenoceptor agonist, CGP-12177A, also produced a concentration-dependent relaxation of the ureter, but the maximal relaxation was only about 60% of that induced by isoprenaline (Table 1). As shown in Fig. 6c, at higher concentrations (10^{-8} M to 10^{-6} M) the non-selective β-adrenoceptor antagonist bupranolol caused a parallel rightward shift of the concentration–response curve for the isoprenaline-induced relaxation of the canine ureter. The pA_2 value for bupranolol was 7.83 ± 0.18 (slope, 0.58 ± 0.13; $P < 0.05$ vs. unity, Table 2). The isoprenaline-induced relaxation of the canine ureter was not influenced by CGP-20712A or ICI-118,551 at concentrations up to 10^{-6} M (Fig. 6a and b).

4. Discussion

There have been no studies of the β-adrenoceptor subtypes present in the rat ureter, though the functional existence of β-adrenoceptors has been shown by many authors

(Ancill et al., 1972; Maggi et al., 1987). In our first experiment, the relaxing activity of three different catecholamines was examined to characterize the β-adrenoceptor subtypes present in the rat ureter. The rank order for their relaxing potency was isoprenaline > noradrenaline > adrenaline. Lands et al. (1967) classified β-adrenoceptors into β_1- and β_2-adrenoceptor subtypes by comparing the activity of catecholamines (both endogenous and synthetic). They established that in terms of potency the rank order is isoprenaline > noradrenaline > adrenaline for β_1-adrenoceptors and isoprenaline > adrenaline > noradrenaline for β_2-adrenoceptors. Recently, the β_3-adrenoceptor, an additional β-adrenoceptor subtype, was identified (Emorine et al., 1989). In terms of potency, the rank order in tissues containing β_3-adrenoceptors is isoprenaline > noradrenaline > adrenaline (Emorine et al., 1989; McLaughlin and MacDonald, 1990). To judge from these criteria, the results obtained here might seem to suggest the existence of β_1 and/or β_3-adrenoceptor subtypes in the rat ureter. However, in the agonist study, the β_1-adrenoceptor agonist dobutamine relaxed the rat ureter in a concentration-dependent manner, but neither of the selective β_3-adrenoceptor agonists (CL-316243 or CGP-12177A) produced relaxation. These results, together with the data indicating that the β_2-adrenoceptor-selective agonist procaterol had no effect, strongly suggest the predominant distribution of the β_1-adrenoceptor subtype in rat ureteral smooth muscle.

This was further confirmed by the experiment with β-antagonists. The pA_2 values for CGP-20712A (a β_1-adrenoceptor-selective antagonist) and bupranolol (a non-selective β-adrenoceptor antagonist) were 7.93 ± 0.34 and 9.54 ± 0.16, respectively. These values are almost the same as those reported by Kaumann (1986) for CGP-20712A and by Lemoine and Kaumann (1983) for bupranolol in rat atria. The slope values obtained here for CGP-20712A (0.48 ± 0.20) and bupranolol (0.61 ± 0.11) were, however, significantly different from unity ($P < 0.05$ in each case), suggesting the coexistence of β-adrenoceptor subtypes other than β_1-adrenoceptors. It has been recognized that the coexistence of two or more receptor subtypes, each of which can bind with both an agonist and an antagonist, results in a slope value in the Schild plot of less than 1.0 in competition experiments (Kenakin, 1993). As the existence of β_2- and β_3-adrenoceptor subtypes is clearly excluded by our results with both selective agonists and antagonists, the possible existence of an atypical β-adrenoceptor that is not sensitive to β_3-adrenoceptor agonists needs to be considered. Nevertheless, from the present functional study, we can conclude that rat ureteral smooth muscle contains mainly the β_1-adrenoceptor subtype.

A variety of functional experiments have indicated that β-adrenoceptors are present in rabbit ureteral smooth muscle. Morita et al. (1986) demonstrated the predominant distribution of the β_2-adrenoceptor subtype in the rabbit ureter by the use of the β_1-adrenoceptor agonist dobutamine and the β_2-adrenoceptor agonist terbutaline. In the present study, we tried to determine which functional β-adrenoceptor subtypes are present in the rabbit ureter by using a broader range of subtype-specific-β-adrenoceptor agonists and antagonists, with special attention being paid to the β_3-adrenoceptor. In the first experiment, adrenaline produced a more potent relaxation of the rabbit ureter than noradrenaline, a finding that would be expected in a tissue containing β_2-adrenoceptors, to judge from the report by Lands et al. (1967). In addition, the selective β_2-adrenoceptor agonist procaterol was the most effective of the agonists used in relaxing the rabbit ureter. Although the β_1-adrenoceptor agonist dobutamine also produced relaxation of the ureter, the effective concentration (over 3×10^{-6} M) was high enough for it to induce both β_1- and β_2-adrenoceptor-mediated responses in the rabbit ureter. Indeed, we observed in preliminary experiments that at 10^{-6} M dobutamine significantly inhibited contraction of the isolated pregnant rat uterus (a β_2-adrenoceptor-mediated response). This being so, it seems likely that the relaxation of the rabbit ureter induced by dobutamine results mainly from β_2-adrenoceptor stimulation. Furthermore, neither CL-316243 nor CGP-12177A, β_3-adrenoceptor-selective agonists, had any relaxing effects on the ureter. Thus, our results are suggestive of a predominant distribution of the β_2-adrenoceptor subtype in rabbit ureteral smooth muscle.

The antagonist study provided additional evidence for the presence of the β_2-adrenoceptor in the rabbit ureter. The concentration–response curve for the action of isoprenaline in relaxing the rabbit ureter was not influenced by the β_1-adrenoceptor-selective antagonist CGP-20712A, but it was competitively antagonized by the β_2-adrenoceptor-selective antagonist ICI-118,551 and by the non-selective β-adrenoceptor antagonist bupranolol. The pA_2 values for ICI-118,551 (8.51 ± 0.18) and bupranolol (8.64 ± 0.13) were almost the same as those reported for uterine (Bilski et al., 1983) and tracheal (Lemoine and Kaumann, 1983) β_2-adrenoceptors. Thus, we conclude from the present experiments that the predominant functional β-adrenoceptor subtype distributed in rabbit ureteral smooth muscle is almost certainly the β_2-adrenoceptor subtype.

Among the three catecholamines tested, only isoprenaline produced a significant relaxation of the canine ureter. Noradrenaline produced only a slight relaxation of the ureter, while adrenaline actually contracted the ureter at 10^{-6} M even when the ureter had been pretreated with 10^{-6} M phentolamine to block the α-adrenoceptor-mediated response (Morita et al., 1994). As mentioned above, in terms of the potency with which they produce β_3-adrenoceptor-mediated responses, the rank order is isoprenaline > noradrenaline > adrenaline. This rank order was found in the present experiments; however, noradrenaline and adrenaline were very weak in relaxing the canine ureter. This may suggest the existence of a species-specific differ-

ence in β_3-adrenoceptors (Pietri-Rouxel and Strosberg, 1995). Further experiments are clearly needed to confirm that the predominant β-adrenoceptor is indeed the β_3-adrenoceptor subtype.

In the agonist study, the β_3-adrenoceptor-selective agonist CL-316243 and the non-selective agonist isoprenaline were the most potent in relaxing the canine ureter. Another β_3-adrenoceptor-selective agonist, CGP-12177A, also produced relaxation of the ureter, but its potency was about one-tenth that of CL-316243 and its intrinsic activity was quite low. Other authors have reported that CGP-12177A behaves as a partial agonist towards β_3-adrenoceptors and as an antagonist towards both β_1- and β_2-adrenoceptors in several tissues (Langin et al., 1991; Kaumann and Molenaar, 1996). On this basis, it would seem that the relaxation of the canine ureter induced by CGP-12177A, although smaller than that induced by CL-316243, most likely resulted from the stimulation of β_3-adrenoceptors. Interestingly, higher concentrations (over 10^{-6} M) of dobutamine and procaterol also relaxed the canine ureter. We have studied the effects of both drugs on the spontaneous contractions of the isolated colon in rats (β_3-adrenoceptor-mediated response) and obtained EC_{50} values of 3.1×10^{-7} M and 3.4×10^{-7} M, respectively (unpublished data). This suggests that the relaxation of the canine ureter by higher concentrations of these drugs might be due, at least in part, to their stimulating effects on β_3-adrenoceptors in the ureteral smooth muscle.

The participation of β_1- and/or β_2-adrenoceptors in the relaxation of the ureter induced by dobutamine and procaterol was excluded by the results of the antagonist study. The relaxing effect of isoprenaline on the canine ureter was not antagonized by either CGP-20712A or ICI-118,551 at 10^{-7} M, at which concentration they occupy virtually all β_1- or β_2-adrenoceptors, respectively (De Ponti et al., 1996). The non-selective β-adrenoceptor antagonist bupranolol produced an apparent antagonism of the isoprenaline-induced relaxation of the canine ureter with a pA_2 value of 7.83 ± 0.18. The interpretation of this result in terms of an action on β_3-adrenoceptors is consistent with reports that a high concentration of bupranolol antagonizes β_3-adrenoceptor-mediated responses (Langin et al., 1991; Blin et al., 1994; Koike et al., 1995). However, the slope of the Schild plot (0.58 ± 0.13) was significantly different from unity. Consequently, the coexistence of another β-adrenoceptor subtype, for example, an atypical β-adrenoceptor, needs to be taken into consideration, as suggested above for the β-adrenoceptor subtypes in the rat ureter. Nevertheless, the data presented here in respect of the canine ureter appear to satisfy the three criteria that need to be satisfied to identify a β_3-adrenoceptor (Arch and Kaumann, 1993). These are: (1) stimulation by β_3-adrenoceptor-selective agonists; (2) stimulation by non-conventional partial agonists; and (3) resistance to blockade by antagonists possessing high affinity for β_1- and β_2-adrenoceptors only.

In conclusion, we demonstrated marked species differences in the distribution of β-adrenoceptor subtypes in mammalian ureteral smooth muscle and provided the first functional evidence for the existence of β_3-adrenoceptors in the canine ureter. Taken together with existing data, our results suggest that relaxation of the ureter by adrenergic agonists is mediated mainly via β_1-adrenoceptors in rats, via β_2-adrenoceptors in rabbits and mainly via β_3-adrenoceptors in dogs.

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