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Contributions of β-Adrenoceptor Subtypes to Responses to Isoprenaline in Rat Isolated Distal Colon

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Abstract

The effects of the non-selective β-adrenoceptor antagonist propranolol and the β_1- and β_2-adrenoceptor-selective antagonists, respectively CGP 20712A (((±)-[2-(3-carbamoyl-4-hydroxyphenoxy)-ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol hydrochloride)) and ICI 118,551 ((erythro(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol-hydrochloride)), on isoprenaline-induced inhibition of methacholine contractions in rat distal colon were investigated to determine the contributions of β-adrenoceptor subtypes to relaxation of smooth muscle.

Longitudinal segments of rat distal colon were suspended in Krebs solution at 37°C for isometric recording. The Krebs solution contained EDTA (30 μM), ascorbic acid (30 μM) and prazosin (0·1 μM) and was gassed with 95% O_2–5% CO_2. Isoprenaline produced a concentration-dependent inhibition of methacholine-induced contractions. Propranolol produced a small (4–6-fold) significant shift of the isoprenaline concentration–response curve at 0·003–0·01 μM. Larger shifts were produced by 0·3 μM (13-fold) and 1 μM (20-fold). CGP 20712A produced a small (3–5-fold) significant shift at 0·03–1 μM. ICI 118,551 produced small non-significant shifts (2–3-fold) at 0·03–1 μM. A combination of ICI 118,551 (0·3 μM) and CGP 20712A (0·1 μM) produced a 13-fold shift, a significantly greater shift than expected from the individual shifts. The shift produced by the combination of antagonists was slightly less than that produced by 1 μM propranolol (20-fold).

The effects of propranolol appear to be due mainly to a combination of β_1- and β_2-adrenoceptor blockade, although an additional action of propranolol at β_3-adrenoceptors is likely.

Pharmacological and molecular biological studies have confirmed the existence of atypical β-adrenoceptors, now referred to as β_3-adrenoceptors, characterized by low affinity of classical β-adrenoceptor antagonists and responsiveness to synthetic agonists, particularly BRL 37344 (Arch 1989; Arch & Kaumann 1993).

In rat distal colon, β-adrenoceptor-mediated relaxation to isoprenaline is poorly antagonized by the classical β-adrenoceptor antagonist propranolol. In addition, BRL 37344 is a potent agonist, suggesting that the response to isoprenaline is mediated largely via β_3-adrenoceptors (McLaughlin & MacDonald 1990). However a small component of the response to isoprenaline is sensitive to propranolol and in a previous study we examined the effects of the selective β_1- and β_2-adrenoceptor antagonists, respectively CGP 20712A (Dooley et al 1986) and ICI 118,551 (O'Donnell & Wanstall 1980), on isoprenaline-induced inhibition of methacholine contractions in an attempt to determine the contributions of different subtypes to relaxation (MacDonald & Lamont 1993). The results suggested that β_2-adrenoceptors may make a small contribution to the isoprenaline response but that β_1-adrenoceptors were absent. However we could not rule out an effect of propranolol and ICI 118,551 on β_3-adrenoceptors. We report further experiments using wider concentration ranges of antagonists in an attempt to determine the contribution from β_1-, β_2- and β_3-adrenoceptors to the response to isoprenaline.

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A preliminary account of this work has been presented to the British Pharmacological Society (McKean & MacDonald 1994).

Materials and Methods

Male Wistar rats, 200–300 g, were killed by a blow to the head and cervical dislocation. The distal 6 cm of the colon was removed and the luminal contents were carefully washed out with Krebs physiological saline solution (PSS) at room temperature. Segments (3 cm) were suspended under an initial isometric tension of 1 g in 30-mL organ baths containing warm (37°C) Krebs PSS solution, bubbled continuously with 95% O_2–5% CO_2. The composition of the Krebs PSS solution was as follows (mM): NaCl 118, $CaCl_2$ 2·5, KCl 4·7, $NaHCO_3$ 25, KH_2PO_4 1·2, $MgSO_4$ 1·2 and glucose 11·1. The Krebs PSS also contained ascorbic acid (30 μM) and EDTA (30 μM) to prevent oxidation of catecholamines and prazosin (0·1 μM) to eliminate effects of α-adrenoceptors. Tissues were allowed to equilibrate for at least 45 min before experimental procedures were begun. A concentration of methacholine capable of producing around 80% of the maximum response was chosen from preliminary methacholine concentration–response experiments. Reproducible contractions to a sub-maximal concentration of methacholine (0·3 μM) were obtained in each tissue before carrying out a concentration–response study to isoprenaline in a non-cumulative manner. Isoprenaline was added to the bath 1 min before the addition of methacholine. Up to four consecutive responses to isoprenaline were constructed with

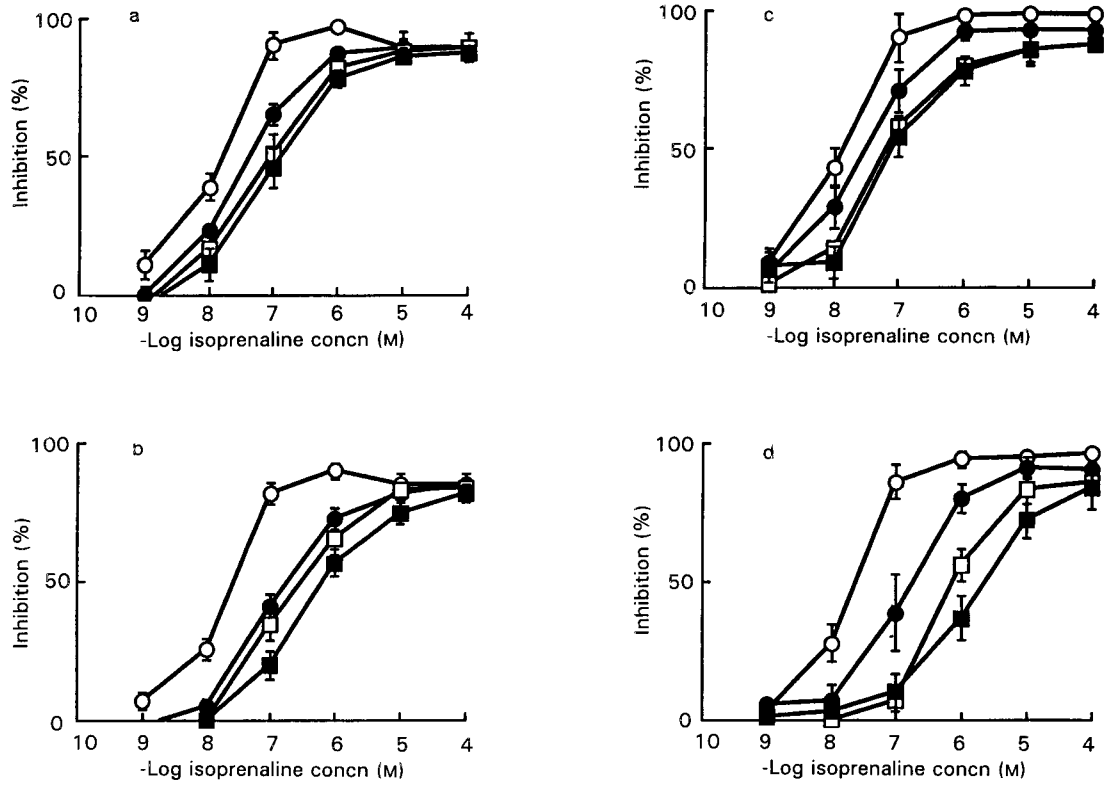

FIG. 1. Concentration-response curves to isoprenaline in rat distal colon. a and c, Time controls: first (○), second (●), third (□) and fourth (■) concentration–response curves to isoprenaline, constructed at hourly intervals. b, Propanolol: 0 μM (○), 0·003μM (●), 0·01 μM (□) and 0·03 μM (■). d, Propanolol: 0 μM (○), 0·1 μM (●), 0·3 μM (□) and 1·0 μM (■).

a 1 h interval between each. Antagonists were added at the end of the first challenge and, in some experiments, the concentration was increased after each consecutive challenge, allowing three concentrations of antagonist to be tested. Control tissues received no antagonist to estimate the magnitude of time-dependent changes.

Agonist concentration ratios (CRs) were determined from mean EC50 values (where the EC50 is the concentration required to produce 50% inhibition of the methacholine response at the concentrations examined).

Drugs

The following were dissolved in distilled water: acetyl-β-methyl choline (methacholine) chloride (Sigma, Poole, UK); CGP 20712A ((±)-[2-(3-carbamoyl-4-hydroxyphenoxy)-ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol) (Ciba-Geigy, Basle, Switzerland); ICI 118,551 (erythro-(±)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol-hydrochloride) (ICI, Macclesfield, UK); (-)-isoprenaline (+)-bitartrate (Sigma); prazosin hydrochloride (synthesized by Reckitt and Colman plc, Hull, UK); (±)-propranolol hydrochloride (Sigma).

Statistical analysis

Results are expressed as mean ± s.e. mean. Statistical significance between two data sets was tested by paired Student's *t*-test. A probability level of $P < 0.05$ was considered to be statistically significant.

Results

Isoprenaline produced a concentration-dependent inhibition of methacholine-induced contractions (Fig. 1). A time-dependent shift in the concentration–response curve to isoprenaline was observed (Fig. 1) and shifts produced by antagonists were calculated using the appropriate time control.

Propranolol produced a small (4–6-fold) significant shift of the isoprenaline curve at concentrations 0·003–0·01 μM (Fig. 1, Table 1). Larger shifts were produced by 0·3 μM (13-fold) and 1 μM (20-fold) (Fig. 1, Table 1).

CGP 20712A produced a small (3–5-fold) significant shift at concentrations 0·03–1 μM (Table 2). Lower concentrations of CGP 20712A (0·003–0·01 μM) had no significant effect.

ICI 118,551 produced small non-significant shifts (2–3-fold) at concentrations of 0·03 to 1 μM (Table 3).

A combination of ICI 118,551 (0·3 μM) and CGP 20712A (0·1 μM) produced a 13-fold shift, a significantly greater shift than expected from the individual shifts (Fig. 2, Table 4).

Table 1. Mean pD_2 values for isoprenaline in the absence and presence of propranolol.

Concn (μM)	pD_2		Shift
	Control	Propranolol	
0·003	7·3 ± 0·1	6·7 ± 0·1***	4·0
0·01	7·1 ± 0·2	6·5 ± 0·2*	4·0
0·03	6·9 ± 0·2	6·1 ± 0·2*	6·3
0·1	7·5 ± 0·2	6·8 ± 0·2*	5·0
0·3	7·2 ± 0·1	6·1 ± 0·1**	12·6
1	7·0 ± 0·2	5·7 ± 0·2**	20·0

Values are means ± s.e. mean of six observations. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Mean pD_2 values for isoprenaline in the absence and presence of CGP 20712A.

Concn (μM)	pD_2		Shift
	Control	CGP 20712A	
0·003	7·5 ± 0·2	7·0 ± 0·2	3·2
0·01	7·3 ± 0·1	6·9 ± 0·2	2·5
0·03	7·2 ± 0·1	6·7 ± 0·1*	3·2
0·1	7·1 ± 0·2	6·4 ± 0·2*	5·0
0·3	7·0 ± 0·2	6·4 ± 0·1*	4·0
1	6·9 ± 0·2	6·3 ± 0·2*	4·0

Values are means ± s.e. mean of 5–6 observations. *P < 0·05.

The shift produced by the combination of antagonists was slightly less than that produced by 1 μM propranolol (20-fold).

Discussion

The results confirm previous observations that inhibitory responses to isoprenaline are poorly antagonized by propranolol (McLaughlin & MacDonald 1990; MacDonald & Lamont 1993), although the effect of propranolol in this study was greater than seen in the previous studies (20-fold shift compared with 5-fold shift in previous studies). The response to propranolol appeared to be biphasic in that lower concentrations gave small shifts which were not concentration-dependent with larger shifts produced by the higher concentrations. The biphasic nature of the response to propranolol might indicate blockade of a small population of classical β_1- or β_2-adrenoceptors at low concentrations with an additional weak blocking activity at β_3-adrenoceptors at higher concentrations.

The β_1-adrenoceptor antagonist CGP 20712A, at concentrations greater than 0·03 μM, produced small significant shifts, not concentration-dependent, of the isoprenaline concentration–response curve. This suggests that a small contribution to the isoprenaline response mediated by β_1-adrenoceptors is removed by CGP 20712A. In a previous study CGP 20712A had no effect on the isoprenaline concentration–response curve in rat distal colon (MacDonald & Lamont 1993). However, as mentioned above, propranolol also had less effect in that study. Thus there may be some variability in the small β_1-adrenoceptor-mediated component of the response to isoprenaline.

Table 3. Mean pD_2 values for isoprenaline in the absence and presence of ICI 118,551.

Concn (μM)	pD_2		Shift
	Control	ICI 118,551	
0·003	7·5 ± 0·2	7·4 ± 0·8	1·3
0·01	7·3 ± 0·1	7·2 ± 0·1	1·3
0·03	7·2 ± 0·1	6·7 ± 0·3	3·2
0·1	7·1 ± 0·2	6·8 ± 0·1	2·0
0·3	7·0 ± 0·2	6·6 ± 0·1	2·5
1	6·9 ± 0·2	6·5 ± 0·2	2·5

Values are means ± s.e. mean of 4–6 observations. *P < 0·05; **P < 0·01; ***P < 0·001.

Table 4. Mean pD_2 values for isoprenaline in the absence and presence of CGP 20712A (0·1 μM), ICI 118,551 (0·3 μM) and a combination of both.

Antagonist	pD_2	Shift
Control	7·2 ± 0·1	—
CGP 20712A (0·1 μM)	6·7 ± 0·1*	3·2
ICI 118,551 (0·3 μM)	7·0 ± 0·2	1·6
Combination	6·1 ± 0·2*	12·6

Values are means ± s.e. mean of six observations. *P < 0·05 compared with control.

ICI 118,551 produced small non-significant shifts of the isoprenaline curve. However, a β_2-adrenoceptor component was detectable since a combination of ICI 118,551 and CGP 20712A produced a significantly greater effect than seen with CGP 20712A alone. The shifts observed with ICI 118551 (2–3-fold) were smaller than seen in a previous study (6-fold, MacDonald & Lamont 1993) and again perhaps indicate some variation in the small contribution to the isoprenaline response from classical β-adrenoceptors.

The shifts produced by the lower concentrations of propranolol (5–6-fold) are in agreement with shifts produced separately by CGP 20712A (3–5-fold) and ICI 118,551 (2–3-fold) for blockade of β_1- and β_2-adrenoceptors. The larger shifts produced by 0·3 and 1 μM propranolol (13- and 20-fold, respectively) are greater than would be expected from the effects of the antagonists separately. However, a combination of CGP 20712A and ICI 118,551 also produced a significantly greater shift than expected from the individual shifts and only slightly smaller than the shift produced by 1 μM propranolol. Thus it appears that the effect of blocking one receptor subtype may be masked to some extent by an increased contribution to the response from the other receptor; only by blocking both receptor subtypes together, either by a non-selective antagonist such as propranolol, or by a combination of subtype selective antagonists, can the full effect be seen. This confirms that both β_1- and β_2-adrenoceptors make small contributions to the response to isoprenaline and that the effect of propranolol is largely due to a combination of β_1- and β_2-adrenoceptor blockade. Ek (1985) also found β_1- and β_2-adrenoceptors in rat colon, with the β_1-adrenoceptors located on enteric neurons and the β_2-adrenoceptors located on the smooth muscle.

The shift produced by propranolol was slightly greater than that produced by the combination of antagonists and, therefore, propranolol may have an additional weak blocking activity at β_3-adrenoceptors. Propranolol has previously been shown to be a weak competitive antagonist at gut β_3-adrenoceptors in rabbit jejunum (Norman & Leathard 1990) and rat small intestine (Van Der Vliet et al 1990), although atypical β_3-adrenoceptors in guinea-pig ileum are reported to be totally resistant to propranolol (Bond & Clarke 1988). Our own previous studies in rat distal colon showed that propranolol weakly antagonized responses to BRL 37344 with an estimated pA_2 of 6·4 (McLaughlin & MacDonald 1990). This pA_2 value is in agreement with that of 6·4 reported in mouse brown adipocytes (Arch 1989) and of 6·3 in Chinese hamster ovary cells transfected with the

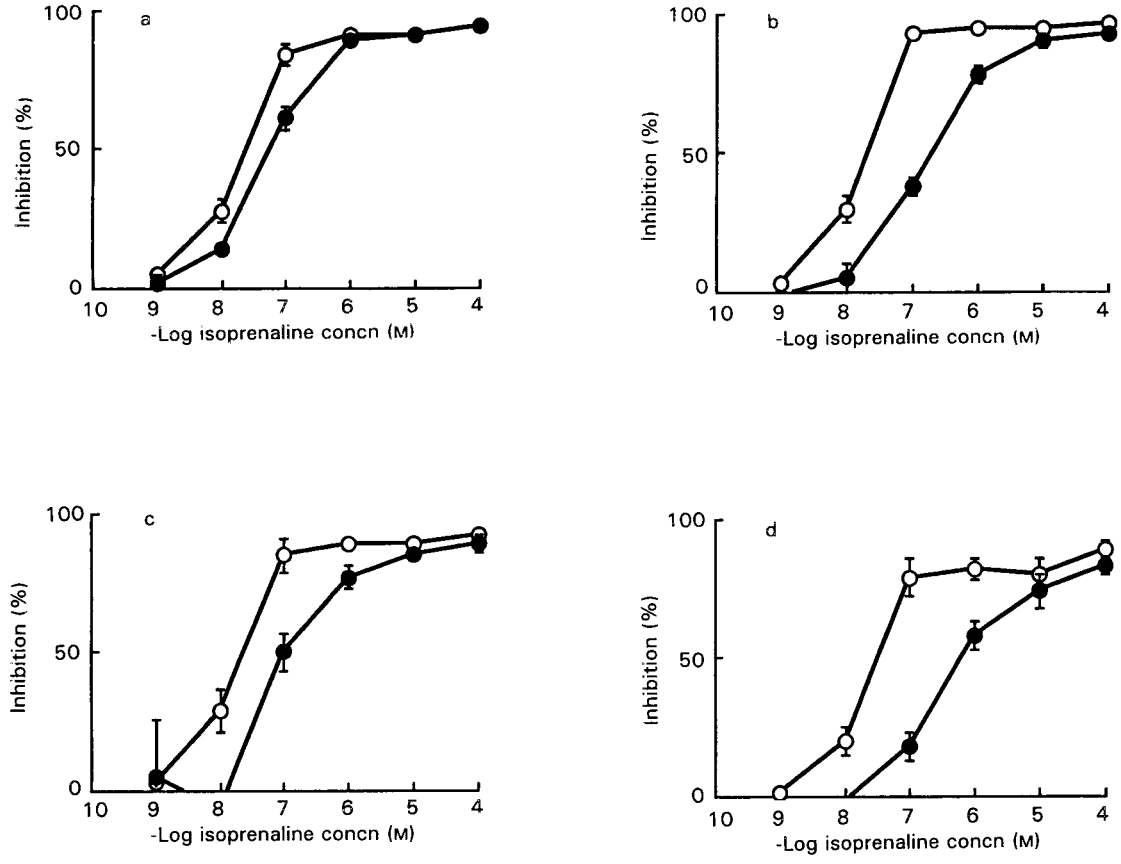

FIG. 2. Concentration–response curves to isoprenaline in rat distal colon. a, Time controls: first (○), second (●) concentration–response curves to isoprenaline, constructed 1 h apart. b, Control (○) and CGP 20712A (0·1 μM) (●). c, Control (○) and ICI 118,551 (0·3 μM) (●). d, Control (○) and combination of CGP 20712A (0·1 μM) and ICI 118,551 (0·3 μM) (●).

murine β_3-adrenoceptor gene (Blin et al 1994). Thus, it seems likely that some blocking activity at β_3-adrenoceptors might be expected at the higher concentrations of propranolol used in this study.

In conclusion, responses to isoprenaline in rat distal colon are mediated largely through β_3-adrenoceptors with small contributions to the response from both β_1- and β_2-adrenoceptors. The propranolol-sensitive component of the isoprenaline response is mainly due to a combination of β_1- and β_2-adrenoceptor blockade, although an additional weak blocking activity of propranolol at β_3-adrenoceptors is likely.

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