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Differences between the third cardiac β-adrenoceptor and the colonic β_3-adrenoceptor in the rat

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1 The heart of several species including man contains atypical β-adrenoceptors, in addition to coexisting β_1- and β_2-adrenoceptors. We now asked the question whether or not the third cardiac β-adrenoceptor is identical to the putative β_3-adrenoceptor. We compared the properties of the third cardiac β-adrenoceptor with those of β_3-adrenoceptors in isolated tissues of the rat. To study the third cardiac β-adrenoceptor we used spontaneously beating right atria, paced left atria and paced left ventricular papillary muscles. As a likely model for putative β_3-adrenoceptors we studied atypical β-adrenoceptors of the colonic longitudinal muscle precontracted with 30 mM KCl. We used β_3-adrenoceptor-selective agonists, antagonists and non-conventional partial agonists (ie high-affinity blockers of both β_1- and β_2-adrenoceptors known to exert also stimulant effects through β_3-adrenoceptors).

2 The non-conventional partial agonist (−)-CGP 12177 caused positive chronotropic effects in right atria ($pD_2 = 7.3$) and positive inotropic effects in left atria ($pD_2 = 7.5$). The stimulant effects of (−)-CGP 12177 were resistant to blockade by 200 nM–2 μM (−)-propranolol and 3 μM ICI 118551 (a β_2-selective antagonist) but antagonized by 1 μM (−)-bupranolol ($pK_B = 6.4-6.8$), 3 μM CGP 20712A (a β_1-selective antagonist) ($pK_B = 6.3-6.4$) and 6.6 μM SR 59230A (a β_3-selective antagonist, $pK_B = 5.1-5.4$).

3 The non-conventional partial agonist cyanopindolol caused positive chronotropic effects in right atria ($pD_2 = 7.7$) and positive inotropic effects in left atria ($pD_2 = 7.1$). The stimulant effects of cyanopindolol were resistant to blockade by 200 nM (−)-propranolol but antagonized by 1 μM (−)-bupranolol ($pK_B = 6.8-7.1$).

4 Neither (−)-CGP 12177 nor cyanopindolol caused stimulant effects in papillary muscles at concentrations between 0.2 nM and 20 μM.

5 In the presence of 200 nM (−)-propranolol the β_3-adrenoceptor-selective agonists BRL 37344 (6 μM), SR 58611A (6 μM), ZD 2079 (60 μM) and CL 316243 (60 μM) did not cause stimulant effects or modify the potency and efficacy of the effects of (−)-CGP 12177 in right and left atria. The combination of 2 μM (−)-propranolol and 2 μM (−)-noradrenaline did not modify the chronotropic potency and efficacy of (−)-CGP 12177 compared to the potency and efficacy in the presence of 2 μM (−)-propranolol alone.

6 (−)-CGP 12177 relaxed the colon with a pD_2 of 6.9 and a maximum effect of 55% compared to (−)-isoprenaline. The relaxant effects of (−)-CGP 12177 were resistant to blockade by 200 nM (−)-propranolol, 3 μM CGP 20712A, 3 μM ICI 118551 but blocked by 2 μM (−)-propranolol ($pK_B = 6.0$), 1 μM (−)-bupranolol ($pK_B = 6.4$) and 3 μM SR 59230A ($pK_B = 6.3$). In the presence of 200 nM (−)-propranolol, (−)-CGP 12177 (20 μM) antagonized surmountably the relaxant effects of BRL 37344 ($pK_P = 7.3$), (−)-noradrenaline ($pK_P = 7.0$); and CL 316243 ($pK_P = 7.0$).

7 Cyanopindolol in the presence of 200 nM (−)-propranolol relaxed the colon with a pD_2 of 7.0 and a maximum effect of 40% compared to (−)-isoprenaline. As expected from a partial agonist, cyanopindolol antagonized the relaxant effects of both BRL 37344 and CL 316243 with a $pK_P = 7.6$ and (−)-noradrenaline with a $pK_P = 7.4$.

8 The following β_3-adrenoceptor-selective agonists were potent colonic relaxants (pD_2 values between parentheses): BRL 37344 (9.1), ZD 2079 (7.0), CL 316243 (9.0) and SR 58611A (8.2). The relaxant effects of these agonists were only marginally affected by 200 nM (−)-propranolol, not blocked by 3 μM CGP 20712A or 3 μM ICI 118551, and blocked by SR 59230A 3 μM ($pK_B = 6.9-7.5$), 1 μM (−)-bupranolol ($pK_B = 6.2-6.4$) and 2 μM (−)-propranolol ($pK_B = 6.3-6.5$).

9 The colonic relaxation caused by the nanomolar concentrations of the β_3-adrenoceptor-selective agonists and the non-conventional partial agonists (−)-CGP 12177 and cyanopindolol and their relative resistance to blockade by antagonists with high affinity for β_1- and β_2-adrenoceptors but blockade by the β_3-adrenoceptor selective SR 59230A agree with the hypothesis that the receptors involved are β_3-adrenoceptors. On the other hand, the failure of micromolar concentrations of β_3-adrenoceptor-selective agonists to produce cardiac stimulation or affect the cardiostimulant effects of (−)-CGP 12177 is inconsistent with the hypothesis that the third cardiac β-adrenoceptor is β_3. Additionally, the selective blockade of the colonic putative β_3-adrenoceptor compared to the third cardiac β-adrenoceptor by SR 59230A, as well as the blockade of cardiac but not colonic receptors by CGP 20712A is also inconsistent with an identical putative β_3-adrenoceptor in colon and heart. We conclude that in the rat the third cardiac β-adrenoceptor is different from the colonic β_3-adrenoceptor.

Keywords: Rat atrium and colon; non-conventional partial agonists; β_3-adrenoceptor; selective agonists; antagonists; putative β_3-adrenoceptors

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Introduction

Evidence for the existence of a third cardiac β-adrenoceptor population, in addition to β_1- and β_2-adrenoceptors, is accumulating. The proposal for a third cardiac β-adrenoceptor was based on the *in vitro* cardiostimulant effects of non-conventional partial agonists (Kaumann, 1989). Non-conventional partial agonists were defined as β-adrenoceptor blocking agents that exhibit agonist effects at concentrations considerably greater than those causing blockade of β_1- and β_2-adrenoceptors (Kaumann, 1973; 1989; Kaumann & Blinks, 1980). The effects of non-conventional partial agonists are resistant to blockade by β-adrenoceptor antagonists that have high affinity for β_1- and β_2-adrenoceptors (eg (−)-propranolol) or blocked only with moderate potency (eg (−)-bupranolol, Walter *et al.*, 1984; Kaumann, 1989). The agonist effects of non-conventional partial agonists have been observed in cardiac preparations of rat, guinea-pig and cat (reviewed by Kaumann, 1989 and Arch & Kaumann, 1993), recently found in human atrium (Kaumann, 1996) and detected for the first time also *in vivo* (eg pithed rat, Malinowska & Schlicker, 1996).

Non-conventional partial agonists are agonists at adipose β_3-adrenoceptors causing lypolysis and inducing thermogenesis (reviewed by Arch & Kaumann, 1993). Agonist properties of non-conventional partial agonists have been confirmed (adenylyl cyclase stimulation) with cloned and transfected β_3-adrenoceptors (Emorine *et al.*, 1989; Granne-

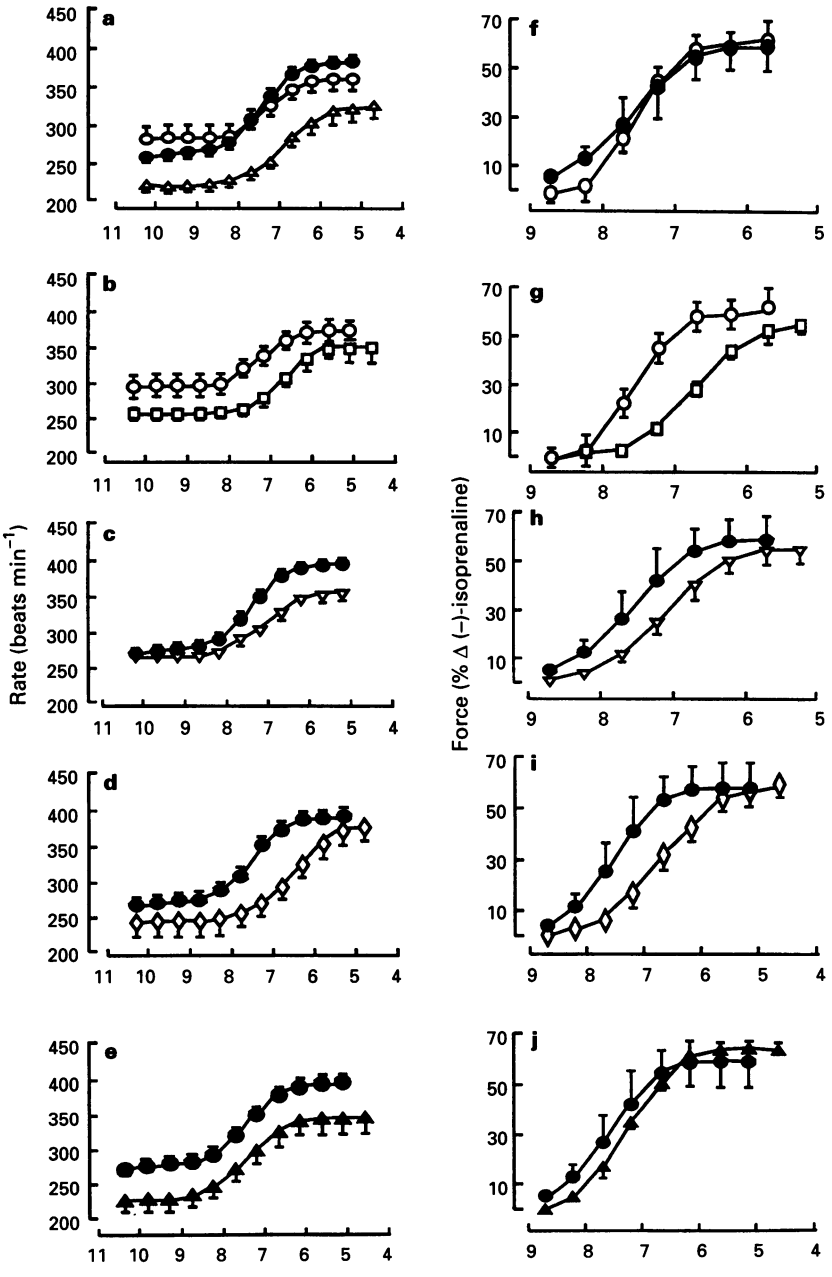

Figure 1 Comparison of the positive chronotropic and inotropic effects of (−)-CGP 12177; effects of antagonists. Data from spontaneously beating right atria (left panels) and paced left atria (right panels). Concentration-effect curves in the absence of antagonists (○, a, b, f and g), or presence of (−)-propranolol 200 nM (●, a, c–f, h–j) or (−)-propranolol 2 μM (△, a); (−)-bupranolol 1 μM (□, b and g); (−)-propranolol 200 nM + SR 59230A 6.6 μM (▽, c and h); (−)-propranolol 200 nM + CGP 20712A 3 μM (◇, d and i); (−)-propranolol 200 nM + ICI 118551 3 μM (▲, e and j). Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4-6$ tissues for each curve.

man *et al.*, 1991). Furthermore, (−)-bupranolol antagonizes effects of agonists both in adipose tissue (Languin *et al.*, 1991) and cells expressing cloned and transfected β_3-adrenoceptors (Blin *et al.*, 1993). The similarity of the agonist properties of non-conventional partial agonists in heart, adipose tissue and cells expressing cloned β_3-adrenoceptors would support the hypothesis that the third cardiac β-adrenoceptor greatly resembles the β_3-adrenoceptor (Blin *et al.*, 1994). However, two additional sets of evidence are not in line with this hypothesis. One is the notorious lack or paucity of cardiostimulant effects of a family of β_3-adrenoceptor-selective agonists that include BRL 37344 (Arch *et al.*, 1984), ZD 2079 (Pietri-Rouxel & Strosberg, 1995), SR 58611A (Bianchetti & Manara, 1990; Manara *et al.*, 1995a) and CL 316243 (Dolan *et al.*, 1994; Cohen *et al.*, 1995). The other is the failure to detect β_3-adrenoceptor mRNA in myocardial tissue, while it is consistently found in adipose tissue and gut muscle including colon (Granneman *et al.*, 1993; Krief *et al.*, 1993; Evans *et al.*, 1996).

In the present work we have asked the question whether or not the third cardiac β-adrenoceptor is the β_3-adrenoceptor. To address this question we decided to compare the properties of the third cardiac β-adrenoceptor with those of another atypical β-adrenoceptor in the same species. We chose the rat heart and colon because in this species there is both *in vitro* (Kaumann *et al.*, 1979) and *in vivo* (Malinowska & Schlicker, 1996) evidence for a third cardiac β-adrenoceptor (assessed with non-conventional partial agonists) and a colonic atypical β-adrenoceptor that greatly resembles the β_3 subtype (McLaughlin & MacDonald, 1990; reviewed in Arch & Kaumann, 1993 and Pietri-Rouxel & Strosberg, 1995). To define a β_3-adrenoceptor we followed the three criteria established by Arch & Kaumann (1993): (i) the receptor should be selectively stimulated by β_3-receptor-selective agonists, (ii) the receptor should be stimulated by non-conventional partial agonists and (iii) the receptor should be resistant to blockade by antagonists possessing only high affinity for β_1- and β_2-adrenoceptors. Accordingly we have searched for possible properties of β_3-selective agonists and non-conventional partial agonists in spontaneously beating right atria, paced left atria and left ventricular papillary muscles, as well as the longitudinal smooth muscle of the colon. We have also assessed the blocking potencies of several antagonists, including SR 59230A, shown to be selective for β_3-adrenoceptors (Manara *et al.*, 1995a). Our results suggest that atypical β-adrenoceptors of heart and colon are different and that the latter but not the former are β_3-adrenoceptors.

Methods

Isolated tissues

Experiments were carried out at 37°C on tissues of Sprague-Dawley rats of either sex (220–280 g). The rats were stunned by a blow on the head, rapidly exsanguinated and the heart and colon (a 6 cm segment, with the proximal cut 3 cm distal from the ileal-ceacal junction) rapidly removed and dissected at room temperature in oxygenated solution containing (mM): Na^+ 140, K^+ 5, Ca^{2+} 2.25, Mg^{2+} 1, Cl^- 98.5, SO_4^{2-} 1, HCO_3^- 29, HPO_4^{2-} 1, fumarate 10, pyruvate 5, L-glutamate 5, glucose 10, EDTA 0.04, ascorbate 0.2. The solution was prepared with deionized, glass-redistilled water and equilibrated with 95% O_2 and 5% CO_2.

Cardiac tissues were set up as described by Kaumann (1972) with some modifications.

Spontaneously beating atria These were set up in pairs in an isolated organ bath (50 ml) and suspended at enough resting tension for measurable force development and detection of beating rate.

Left atria These were set up in pairs and paced to contract isometrically at 2 Hz with square-wave pulses of 5 ms duration and just over threshold voltage. A length-force curve was determined, and the length of each atrium set to obtain 50% of the resting tension associated with maximum developed force.

Left ventricular papillary muscles These were paced at 2 Hz and stretched to contract at optimal length, after determination of a length-force curve.

Longitudinal muscle of the colon A partially depolarised colon preparation essentially as described previously (McLaughlin & MacDonald, 1990) with modifications was used. Briefly, two 'whole' 3 cm segments were mounted in separate organ baths with care taken not to occlude the lumen. Tissues were allowed to equilibrate for 30 min, some in the presence of β-adrenoceptor antagonists before the addition of KCl 30 mM. Tone was maintained by KCl for 30 min, the tissues washed twice and allowed to equilibrate for a further 60 min before re-addition of KCl 30 mM. β-Adrenoceptor antagonists were re-added following each wash and were incubated with tissue for at least 2 h before the addition of agonist to the organ bath. Cumulative concentration-response curves were commenced following stabilisation of KCl induced tone. Figure 6 (c and d) shows an entire experiment.

Table 1 Chronotropic and inotropic potency of (–)-CGP12177; effect of ligands

Drug	*Right atrium* pD_2	*Right atrium* Log (CR)[a]	*Left atrium* pD_2	*Left atrium* Log (CR)[a]
None	7.26±0.13		7.55±0.18	
(–)-Propranolol 200 nM	7.33±0.06*		7.54±0.06*	
(–)-Propranolol 2 μM	6.89±0.03*	0.37±0.13	ND	
(–)-Noradrenaline 2 μM + (–)-propranolol 2 μM	6.84±0.13‡	0.05±0.13	ND	
(–)-Bupranolol 1 μM	6.73±0.04**	0.53±0.14	6.70±0.07**	0.85±0.19
(–)-Bupranolol 1 μM + (–)-propranolol 200 nM	6.75±0.06††	0.58±0.08	ND	
CGP20712A 3 μM + (–)-propranolol 200 nM	6.42±0.09††	0.91±0.11	6.73±0.08††	0.81±0.10
ICI118551 2 μM + (–)-propranolol 200 nM	7.43±0.02†		7.17±0.18†	0.37±0.19
SR59230A 6.6 μM + (–)-propranolol 200 nM	7.09±0.03††	0.24±0.07	7.10±0.12††	0.44±0.13
BRL37344 6 μM + (–)-propranolol 200 nM	7.70±0.20†		7.32±0.04†	0.22±0.07
SR58611A 6 μM + (–)-propranolol 200 nM	7.56±0.11†		7.51±0.13†	
ZD2079 60 μM + (–)-propranolol 200 nM	7.48±0.09†		7.48±0.05†	
CL316243 60 μM + (–)-propranolol 200 nM	7.50±0.09†		7.55±0.06†	

For each group tissues from 4 to 6 rats were used. [a] Log (CR) was obtained by subtracting the pD_2 value in the presence of a blocker (antagonist) from the pD_2 value in the absence of the blocker (antagonist). *$P>0.05$ compared to (–)-CGP 12177. **$P<0.05$ compared to (–)-CGP 12177. †$P>0.05$ compared to (–)-CGP 12177 + 200 nM (–)-propranolol. ††$P<0.05$ compared to (–)-CGP 12177 + 200 nM (–)-propranolol. ‡$P>0.05$ compared to (–)-CGP 12177 + 2 μM (–)-propranolol.

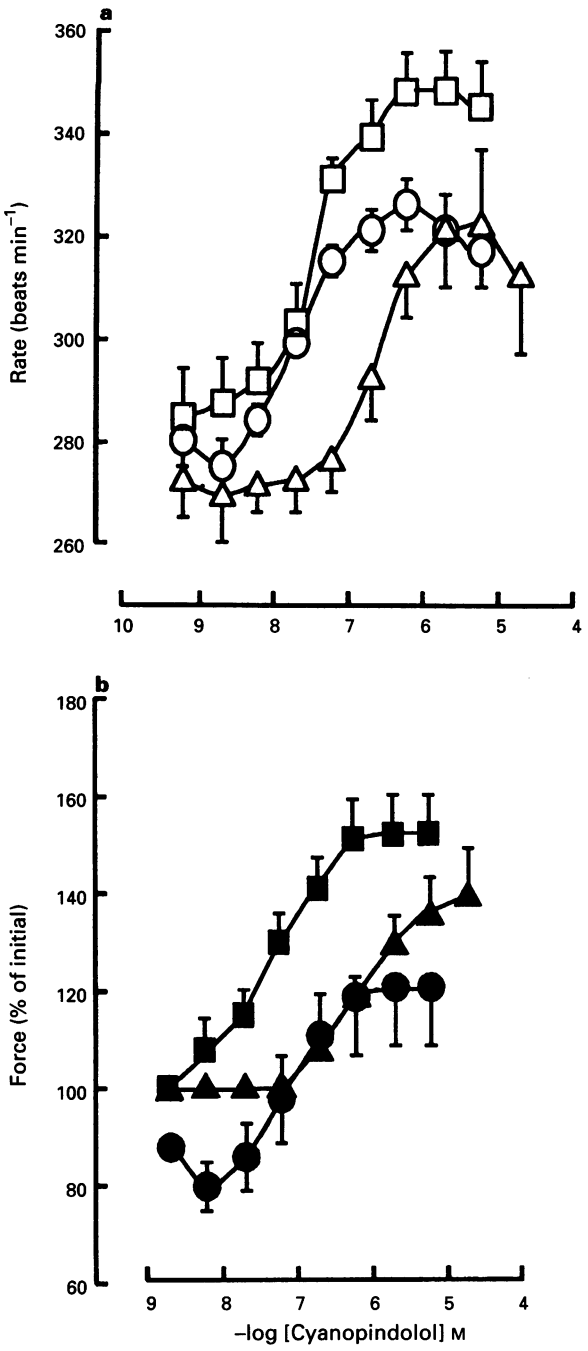

Figure 2 Positive chronotropic (a, right atria, open symbols) and positive inotropic (b, left atria, closed symbols) effects of cyanopindolol; antagonism by (−)-bupranolol. Concentration-effect curves in the absence (○, ●) and presence of 200 nM (−)-propranolol (□, ■) or 1 μM (−)-bupranolol (△, ▲). Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4$ tissues for each curve.

The rate of spontaneously beating right atria, the force of left atria and papillary muscles and the tension of colonic muscles were recorded on 8 channel Watanabe recorders.

Concentration-effect curves

All tissues were exposed to 30 μM corticosterone (to block extraneuronal uptake of amines), 3 μM cocaine (to block neuronal uptake of amines) and 1 μM phentolamine (to block α-adrenoceptors). The chronotropic and inotropic potency, as well as the colon-relaxing potency of agonists were estimated from a single cumulative concentration-effect curve determined in each tissue. Curves were determined by the sequential administration of agonist to the bath in amounts that increased the total concentration by $\frac{1}{2}$ log unit. Unless otherwise stated, all experiments were terminated by the administration of a β-adrenoceptor-saturating concentration of (−)-isoprenaline (200–600 μM). Effects of agonists on spontaneously beating right atria were measured as increases in beats min^{-1}. The positive inotropic effects of agonists on paced left atria were usually expressed as a percentage of the effect of (−)-isoprenaline, recorded at the end of the experiment. Due to the very high affinity of cyanopindolol for β_1- and β_2-adrenoceptors (K_B values at picomolar concentrations), (−)-isoprenaline failed to elicit maximum stimulant effects and was therefore not referred to as a standard in these experiments. The positive inotropic effects of cyanopindolol were therefore expressed as percentage of basal force.

Drug-receptor constants and statistics

The equilibrium dissociation constants for antagonists (K_B) K_B was estimated usually from the shift of an agonist concentration-effect curve by a single concentration of antagonist from the relation

$$K_B = [\text{antagonist}]/(\text{concentration ratio} - 1) \quad (1)$$

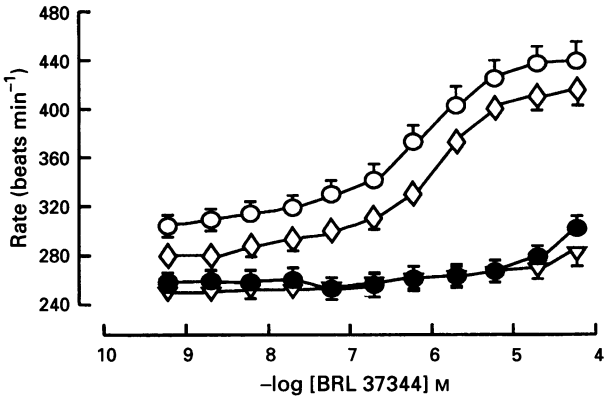

Figure 3 Positive chronotropic effects of BRL 37344 (○), antagonism by ICI 118551 50 nM (◇), (−)-propranolol 200 nM (●) and CGP 20712A 300 nM (▽). Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4–6$ tissues for each curve.

Table 2 Potency of cyanopindolol on rat atria: effects of antagonists

	Right atrium		*Left atrium*			
	n	pD_2	n	pD_2	Log $CR_{(RA)}$	Log $CR_{(LA)}$
Control	5	7.68 ± 0.14	4	7.05 ± 0.12		
(−)-Propranolol 200 nM	4	7.35 ± 0.08*	6	7.31 ± 0.15*	0.33 ± 0.16	−0.26 ± 0.19
(−)-Bupranolol 1 μM	4	6.55 ± 0.05**	4	6.19 ± 0.09**	1.13 ± 0.15	0.86 ± 0.15

RA, right atrium, LA, left atrium. *$P > 0.05$ compared to control. **$P < 0.05$ compared to control.

The error of the agonist concentration-ratio (CR), caused by an antagonist B, was estimated by use of log forms ($-\log EC_{50} = pD_2$, $-\log EC_{50}$ in the presence of antagonist $= pD_{2,B}$) as by Kaumann (1990):

$$pD_2 - pD_{2,B} \pm (\text{s.e.mean}^2 pD_2 + \text{s.e.mean}^2 pD_{2,B})^{\frac{1}{2}} \quad (2)$$

EC_{50} values of agonists were estimated in log form.

The equilibrium dissociation constants for a partial agonist (K_P) K_P was estimated as by Marano & Kaumann (1976) and Lemoine & Kaumann (1982). K_P was estimated from the slope of the plot which relates equieffective concentrations of agonist in the absence (A_2) and presence (A_3) of a partial agonist P, $A_2 = i + mA_3$, where i is the ordinate intercept. The slope m of the regression equals $m = 1 - Y_P$, where the fractional receptor occupancy Y_P by the partial agonist P is given by $[P]/([P] + K_P)$. pK_P was calculated from

$$\log(1/m - 1) = \log [P] - \log K_P \quad (3)$$

Statistics

The Mann-Whitney U-test was used to test for significant differences between groups of data. Differences at the 95% confidence interval were considered significant.

Drugs

The following drugs were gifts: (−)-CGP 12177 hydrochloride ((−)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one) and BRL 34377 ((**RR**+SS)[4-[2-[[2-(3-chlorophenyl)-2-

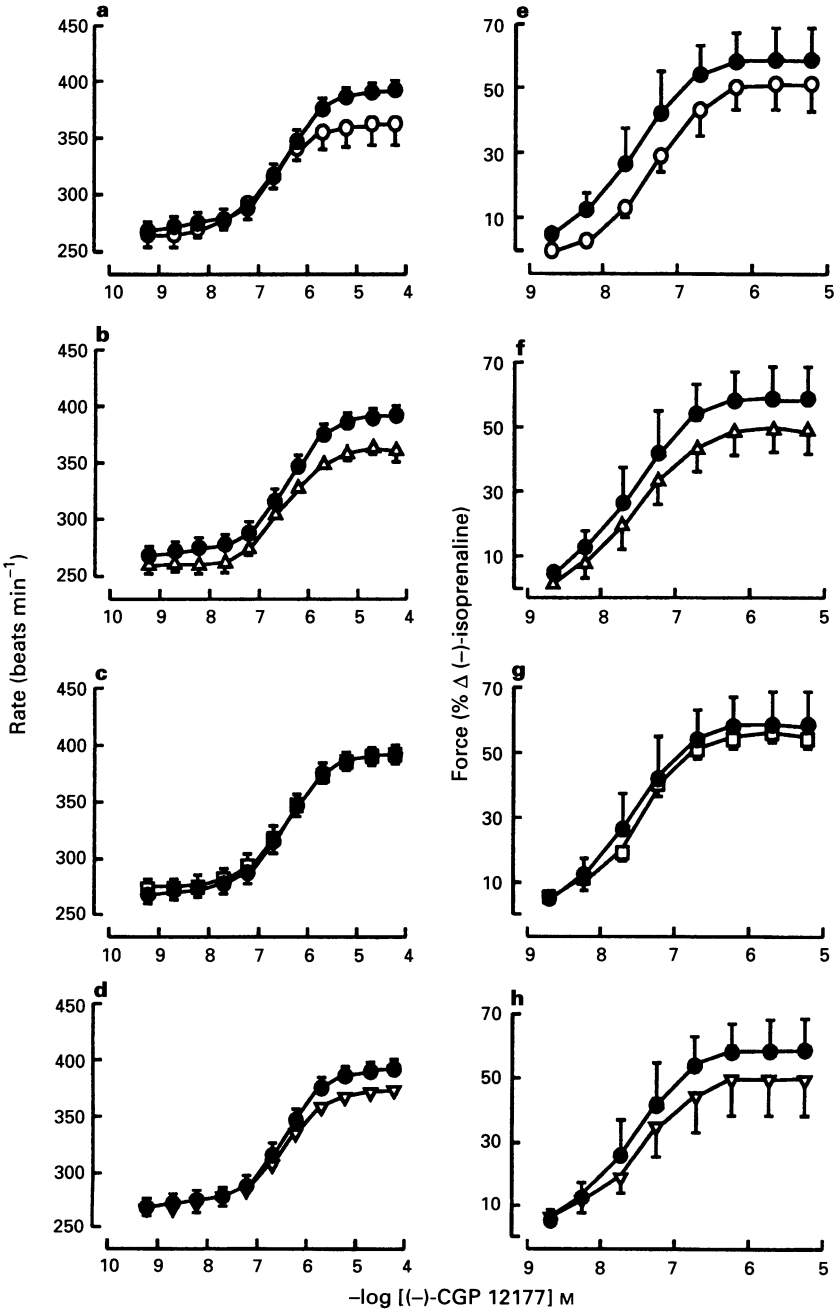

Figure 4 Positive chronotropic (a–d) and inotropic (e–h) effects of (−)-CGP 12177. Lack of influence of high agonist concentrations. All experiments were carried out in the presence of 200 nM (−)-propranolol in the absence (●) or presence of 6 μM BRL 37344 (○) (a,e), 6 μM SR 58611A (△) (b,f), 60 μM ZD 2079 (□) (c,g) or 60 μM CL 316243 (▽) (d,h). Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4-6$ tissues for each curve.

hydroxy-ethyl]amino]propyl]phenoxy]acetic acid) from Dr Lee Beeley (SmithKline Beecham, Welwyn, U.K.), (−)-bupranolol from Dr Klaus Sandrock (Sanol, Monheim, Germany), SR 58611A (ethyl{(7**S**)-7-[(2**R**)-2-(3-chlorophenyl)-2-hydroxyethylamino]-5,6,7,8-tetrahydronaphtyl2-yloxy}acetate hydrochloride) and SR 59230A (3-(2-ethylphenoxy)-1-[(1**S**)-1,2,3,4-tetrahydronaphth-1-ylamino]-2**S**-2-propanol oxalate) from Dr Luciano Manara (Sanofi, Milan, Italy), (±)-cyanopindolol from Dr Günter Engel (Sandoz, Basle, Switzerland), ZD 2079 ((±)-1-phenyl-2-(2-(4-carboxymethylphenoxy)-ethylamino)-ethan-1-ol) and ICI 118,551 (erythro-DL-1(7-methylindan - 4 - yloxy) - 3 - isopropylamino - butan - 2 - ol) (Zeneca Pharmaceuticals, Macclesfield, U.K.), CL 316243 (disodium (**R,R**)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate) (Wyeth-Ayerst Research Princeton, NJ, U.S.A.), CGP 20712A (2-hydroxy-5(2-((2-hydroxy-3-(4-((1-methyl-4-trifluoromethyl) 1H-imidazole-2-yl) - phenoxy) propyl) amino) ethoxy)-benzamide monomethane sulphonate) from Alexandra Sedlacek (Ciba-Geigy AG, Basel, Switzerland). (−)-Isoprenaline bitartrate, (−)-noradrenaline bitartrate, (−)-propranolol hydrochloride, corticosterone and phentolamine methanesulphonate were purchased from Sigma, St Louis, U.S.A. Cocaine HCl was purchased from Victorian Hospitals Association, Mulgrave, Australia.

Results

Cardiac tissues

Effect of non-conventional partial agonists and antagonists (−)-CGP 12177 caused positive chronotropic and inotropic effects on right and left atria respectively. The maximum effect of (−)-CGP 12177 with respect to (−)-iso-

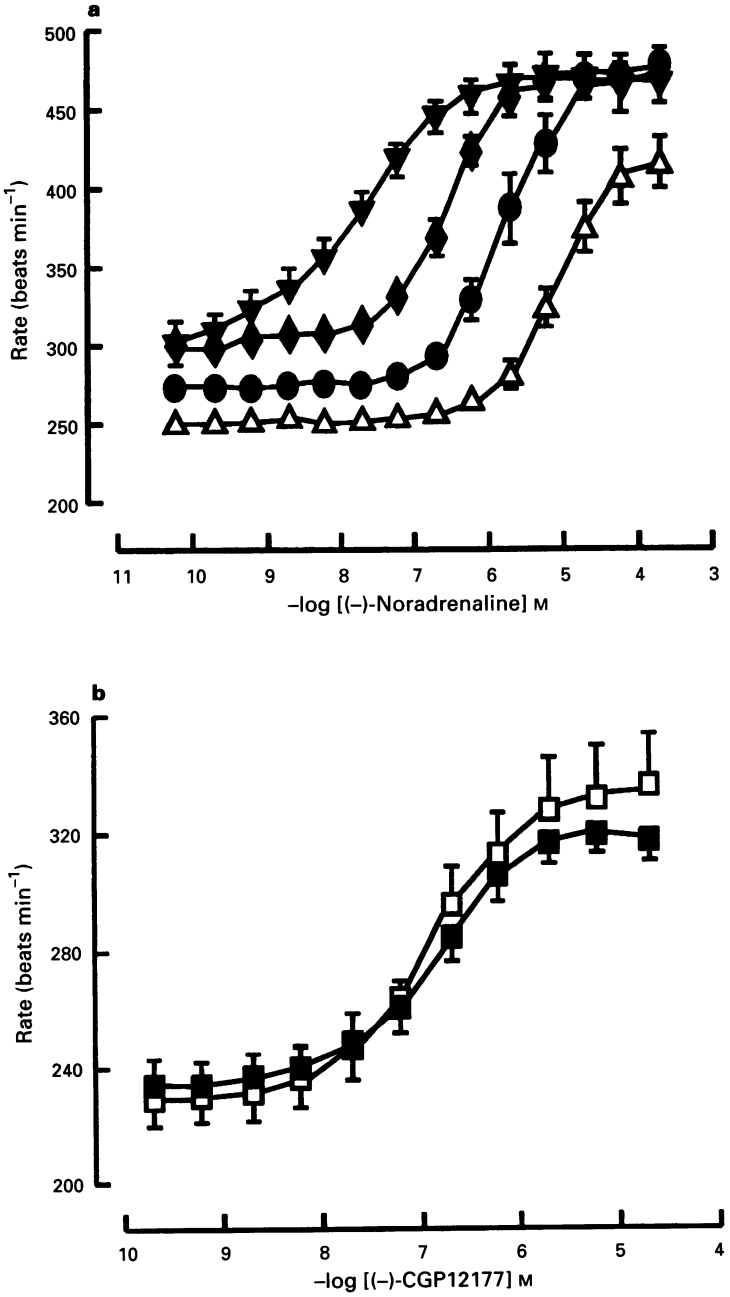

Figure 5 (a) Positive chronotropic effects of (−)-noradrenaline in the absence (▼) and presence of (−)-propranolol 20 nM (◆), 200 nM (●) and 2 μM (△). (b) Positive chronotropic effects of (−)-CGP 12177 in the presence of (−)-propranolol 2 μM without (□) and with (−)-noradrenaline 2 μM (■). Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4-6$ tissues for each curve.

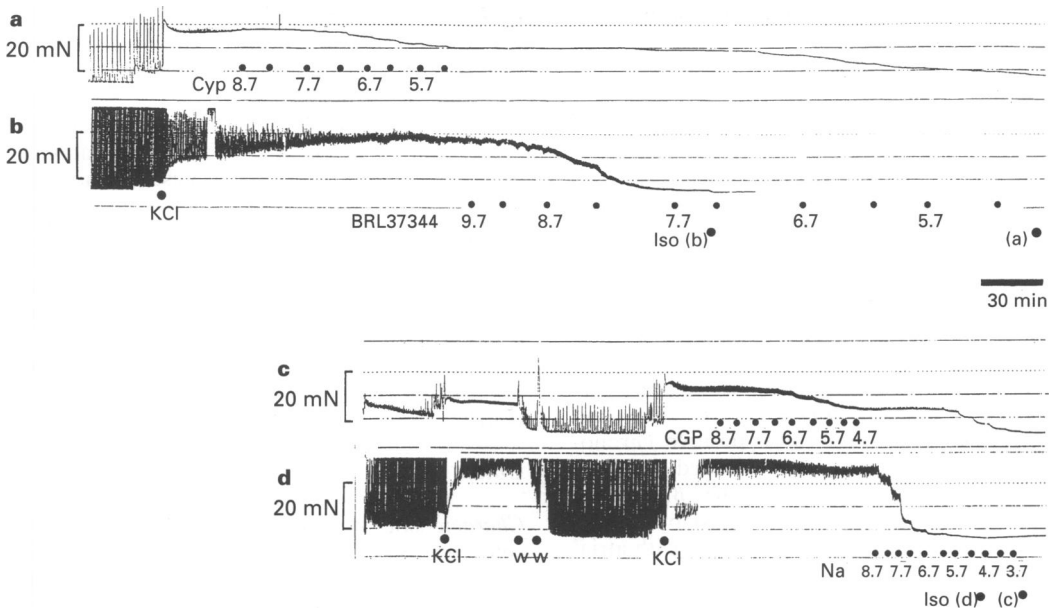

Figure 6 Representative recordings of distal (a and c) and proximal (b and d) colon segments as defined in Methods showing a part (a and b) and a whole experiment (c and d). In (a) a concentration-response curve to cyanopindolol (Cyp) was constructed. Note the relaxant effect of cyanopindolol. (b) BRL 37344 was added simultaneously to (a and b). Maximal relaxation was tested by the addition of (−)-isoprenaline (Iso) 600 μM at the points indicated. In (c) a concentration-response curve to (−)-CGP 12177 was constructed which caused concentration-dependent relaxation. (d) (−)-Noradrenaline (NA) was then added simultaneously to (c and d), followed by (−)-isoprenaline (Iso) 600 μM at the points indicated. Note the markedly slower kinetics of BRL 37344 compared to (−)-noradrenaline, cyanopindolol and (−)-CGP 12177. Dots indicate additions of drugs in half log increments (values are −log M). Shown also are additions of KCl 30 mM and change of buffer solution (wash, w). Bar = 30 min.

prenaline was 60% in both right atrium (not shown) and left atrium (Figures 1 and 4). The concentration-effect curve of (−)-CGP 12177 was not shifted significantly by 200 nM or 2 μM (−)-propranolol ($P>0.05$, Figure 1a and f); however, the higher concentration of (−)-propranolol caused some cardiodepression. The concentration-effect curves to (−)-CGP 12177 were also unaffected by 3 μM ICI 118551 ($P>0.05$, Figure 1e and j), which also caused a slight decrease in basal beating rate. (−)-Bupranolol 1 μM caused surmountable blockade of the effects of (−)-CGP 12177 in both right and left atria ($P<0.05$, Figure 1b and g). (−)-CGP 20712A 3 μM (Figure 1d and i) also caused surmountable antagonism of the effects of (−)-CGP 12177 ($P<0.05$, in the presence of 200 nM (−)-propranolol). SR 59230A 6.6 μM also caused a small amount of antagonism of the effects of (−)-CGP 12177 ($P<0.05$, in the presence of (−)-propranolol, Figure 1c and h), which was only partially surmountable on right atria, as shown previously for guinea-pig right atria (Manara *et al.*, 1996). The antagonism of the positive chronotropic effects of (−)-CGP 12177 by 1 μM (−)-bupranolol was not affected by 200 nM (−)-propranolol (not shown). The blockade of the atrial effects of (−)-CGP 12177 by antagonists is summarised in Table 1. pK_B values estimated were 6.4–6.8 for (−)-bupranolol, 6.3–6.4 for CGP 20712A and 5.1–5.4 for SR 59230A. None of the antagonists changed basal left atrial contractile force (2.4±0.1 mN, $n=27$), nor the maximum positive inotropic effects of (−)-isoprenaline (7.5±0.2 mN).

Cyanopindolol caused positive chronotropic effects (Figure 2a) with a maximum effect of around 50% with respect to (−)-CGP 12177 (compare Figures 1a–e and 2a). The positive chronotropic effects of cyanopindolol were resistant to blockade by 200 nM (−)-propranolol ($P>0.05$) but antagonized surmountably by 1 μM (−)-bupranolol ($P<0.05$, Figure 2a, Table 2).

Cyanopindolol 2 nM and 6 nM caused some depression of the force of left atria; concentrations greater than 6 nM caused concentration-dependent positive inotropic effects (Figure 2b). In the presence of 200 nM (−)-propranolol, cyanopindolol only elicited positive inotropic effects. (−)-Propranolol did not significantly change the potency of cyanopindolol but 1 μM

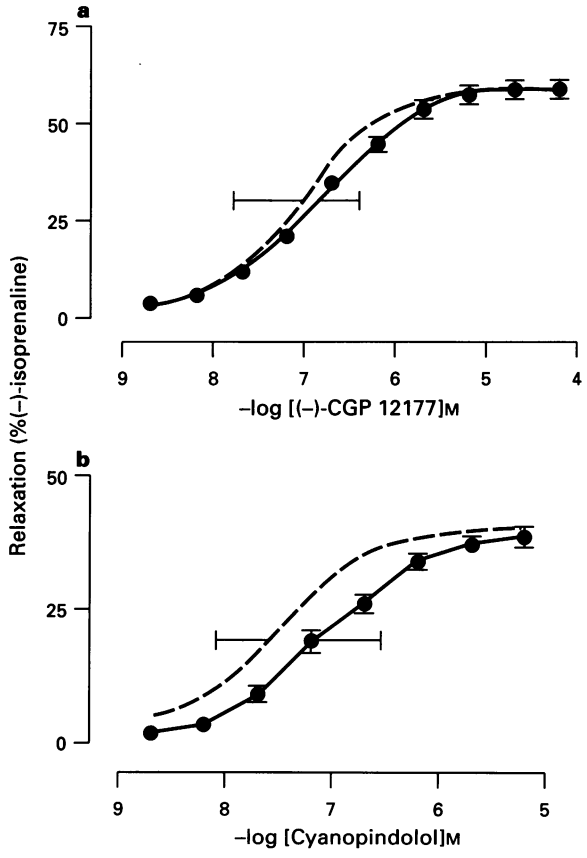

Figure 7 Relaxant responses in colon of (−)-CGP 12177 (a) and cyanopindolol (b) in the presence of 200 nM (−)-propranolol (●). Responses are expressed as a percentage of relaxation caused by a maximal concentration of (−)-isoprenaline. Also shown is receptor occupancy (broken lines) for each partial agonist. Horizontal error bars are standard deviation at the pK_p (receptor occupancy) and pD_2, vertical error bars are ±s.e.mean, $n=12$ (cyanopindolol) and 36 ((−)-CGP 12177) tissues.

(−)-bupranolol antagonized surmountably the positive inotropic effects of cyanopindolol ($P<0.05$, Figure 2b; Table 2). The antagonism of the effects of cyanopindolol yielded pK_B values of (−)-bupranolol of 7.1 for the right atrium and 6.8 for the left atrium (Table 2).

(−)-CGP 12177 (2 nM−20 μM) and cyanopindolol (0.2 nM−20 μM) did not cause positive inotropic effects in papillary muscles, either in the absence or presence of 200 nM (−)-propranolol ($n=4$ for each condition, experiments not shown).

Effects of β_3-adrenoceptor-selective agonists Cumulative concentration-effect curves did not reveal any positive chronotropic and inotropic effects of the following β_3-adrenoceptor-selective agonists (highest used concentration between parentheses): CL 316243 (60 μM), ZD 2079 (60 μM) and SR 58611A (6 μM). BRL 37344 did cause positive chronotropic effects; however, the effects were blocked by both 200 nM (−)-propranolol and 300 nM of the β_1-selective CGP 20712A, and were also marginally reduced by the β_2-selective ICI 118551, indicating a major interaction with atrial β_1-adrenoceptors (Figure 3).

To investigate whether the β_3-selective agonists caused conceivably silent occupancy of the atrial atypical β-adrenoceptor we studied the possible influence of a high agonist concentration on the effects of (−)-CGP 12177. Because we also included BRL 37344 to answer this question the experiments were carried out in the presence of 200 nM (−)-propranolol that blocks the stimulant effects of BRL 37344 but not those of (−)-CGP 12177. None of the agonists reduced significantly the positive chronotropic and inotropic effects of (−)-CGP 12177 ($P>0.05$, Figure 4; Table 1), indicating that micromolar concentrations of β_3-adrenoceptor-selective agonists did not cause significant occupancy of the atrial receptor activated by (−)-CGP 12177.

We also attempted to detect the plausible occupancy of the third atrial β-adrenoceptor by (−)-noradrenaline, the endogenous neurotransmitter. Because (−)-noradrenaline causes positive chronotropic effects in the rat right atrium mostly mediated through β_1-adrenoceptors (Kaumann, 1986) we used up to 2 μM (−)-propranolol to block these receptors (Figure 5a). We then chose 2 μM (−)-propranolol to investigate whether (−)-noradrenaline 2 μM, a concentration that caused only small tachycardia, affected the responses to (−)-CGP 12177. (−)-Noradrenaline 2 μM did not cause blockade of the effects of (−)-CGP 12177, in the presence of 2 μM (−)-propranolol (Figure 5).

Colon

Characteristics of colon preparations Two 3 cm segments of colon from each rat were used. In each case the distal portion (Figure 6a and c) displayed less frequent spontaneous contractions than the proximal portion (Figure 6b and d) both in the absence and presence of KCl 30 mM. The addition of β_3-adrenoceptor selective agonists and non-conventional β-adrenoceptor partial agonists caused a reduction of KCl-induced tone and also in the frequency of spontaneous contractions. Basal tone immediately before addition of KCl was 8.4±0.1 mN, which was raised by 30 mM KCl to 25.2±0.4 mN then reduced by (−)-isoprenaline (200−600 μM) to 6.6±0.1 mN, $n=259$. A comparison of the potency

Table 3 Potencies of agonists and effect of antagonists on rat colon

					Antagonists					
				pD_2 ((−)-propranolol		pD_2 ((−)-propranolol		pD_2 ((−)-bupranolol		pD_2 (SR59230
Agonist	n	pD_2	n	200 nM)	n	2 μM)	n	1 μM)	n	3.3 μM)
BRL37344	4	9.05±0.13	22	8.87±0.04* (0.18±0.14)[a]	6	8.33±0.05** (0.72±0.14)[a]		−		−
BRL37344+ (−)-propranolol 200 nM	22	8.87±0.04					7	8.35±0.13† (0.52±0.14)[a]	4	7.45±0.11† (1.42±0.12)[a]
(−)-Noradrenaline	12	7.23±0.09	16	7.22±0.13* (0.01±0.16)[a]	6	6.39±0.12** (0.84±0.15)[a]		−		−
(−)-Noradrenaline+ (−)-propranolol 200 nM	16	7.22±0.13						−	6	5.23±0.10† (1.99±0.16)[a]
ZD2079	4	7.02±0.14	10	7.00±0.11* (0.02±0.18)[a]		−		−		−
ZD2079+ (−)-propranolol 200 nM	10	7.00±0.11					4	6.61±0.02† (0.39±0.11)[a]	6	5.49±0.07† (1.51±0.13)[a]
CL316243	4	9.02±0.06	16	9.09±0.06* (−0.07±0.08)[a]		−		−		−
SR58611A	4	8.15±0.13	11	7.66±0.05** (0.49±0.14)[a]		−		−		−
SR58611A+ (−)-propranolol 200 nM	11	7.66±0.05		−				−	4	6.26±0.09† (1.40±0.10)[a]
(−)-CGP12177	10	6.88±0.09	36	6.82±0.05* (0.08±0.10)[a]	6	6.41±0.06** (0.47±0.11)[a]		−		−
(−)-CGP12177+ (−)-propranolol 200 nM	36	6.82±0.05					6	6.37±0.07† (0.45±0.09)[a]	6	5.95±0.14† (0.87±0.15)[a]
Cyanopindolol+ (−)-propranolol 200 nM	12	−		7.03±0.12				−		−

[a] Figures between parentheses represent Log (CR) values, obtained by subtracting the pD_2 value in the presence of an antagonist from the pD_2 value in the absence of antagonist. *$P>0.05$ compared to agonist in the absence of 200 nM (−)-propranolol. **$P<0.05$ compared to agonist in the absence of 200 nM (−)-propranolol. †$P<0.05$ compared to agonist in the presence of 200 nM (−)-propranolol.

of agonists or partial agonists showed no difference between proximal and distal segments.

Effects of non-conventional partial agonists and antagonists (−)-CGP 12177 was a potent partial agonist in the presence of 200 nM (−)-propranolol, causing relaxation with a maximal effect of 55% with respect to (−)-isoprenaline (Figures 6c and 7a). Quantitative data are summarised in Table 3.

Cyanopindolol was also a potent partial agonist in the presence of 200 nM (−)-propranolol, causing relaxation with a maximal effect of 40% with respect to (−)-isoprenaline, as shown in the representative experiment of Figure 6a and in Figure 7b.

In the presence of 200 nM (−)-propranolol, the relaxant effects of (−)-noradrenaline, BRL 37344 and CL 316243 were antagonized surmountably by 20 μM (−)-CGP 12177 and 6 μM cyanopindolol (Figures 6 and 8). pK_P values for (−)-CGP 12177 ranged from 6.96–7.32 and for cyanopindolol 7.40–7.65 (Table 4). This information was used to calculate receptor occupancy for agonist effects of (−)-CGP 12177 and cyanopindolol (Figure 7).

Effects of agonists and antagonists (−)-Noradrenaline, the β_3-adrenoceptor-selective agonists, BRL 37344, SR 58611A and ZD 2079 and the partial agonists (−)-CGP 12177 and cyanopindolol caused relaxant effects (Figures 7–9). The relaxant kinetics of the chlorine containing compounds, BRL 37344 (Figure 6a and b), CL 316243 and SR 58611A were considerably slower than those of the other agonists (for comparison with (−)-noradrenaline see Figure 6c and d).

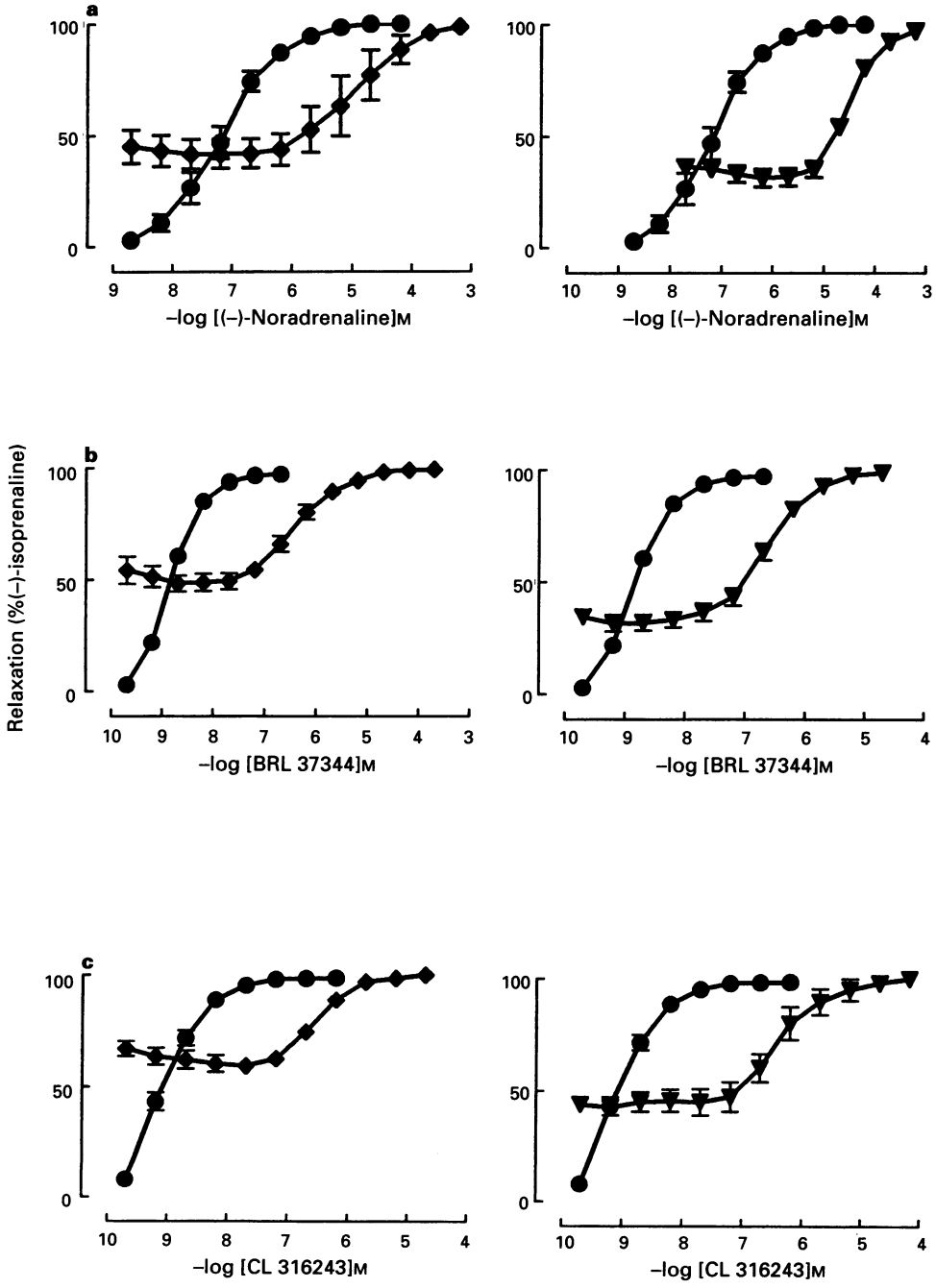

Figure 8 Relaxant responses in colon of (a) (−)-noradrenaline, (b) BRL 37344 and (c) CL 316243 in the presence of 200 nM (−)-propranolol (●) and in the presence of 200 nM (−)-propranolol + 20 μM (−)-CGP 12177 (◆) or 200 nM (−)-propranolol + 6 μM cyanopindolol (▼). Responses are expressed as a percentage of relaxation caused by a maximal concentration of (−)-isoprenaline. Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 3-22$ tissues for each curve.

Table 4 Dissociation constants of partial agonists on rat colon

Partial agonist	*Condition*	n	pK_p
(-)-CGP12177	BRL 37344 + (-)-propranolol 200 nM	6	7.32±0.33
	CL 316243 + (-)-propranolol 200 nM	6	6.97±0.11
	(-)-Noradrenaline + (-)-propranolol 200 nM	6	6.96±0.12
Cyanopindolol	BRL 37344 + (-)-propranolol 200 nM	7	7.56±0.12
	CL 316243 + (-)-propranolol 200 nM	3	7.65±0.44
	(-)-Noradrenaline + (-)-propranolol 200 nM	6	7.40±0.14

Agonist effects were resistant to blockade by 200 nM (−)-propranolol ($P>0.05$), with the exception of the effects of SR 58611A which were slightly antagonized ($P=0.01$, Figure 9, Table 3). However, 2 μM (−)-propranolol, and the other antagonists 3.3 μM SR 59230A or 1 μM (−)-bupranolol both in the presence of 200 nM (−)-propranolol caused significant, surmountable antagonism ($P<0.05$, Figure 9, Table 3). pK_B values were 6.0–6.5 for (−)-propranolol, 6.4–7.5 for (−)-bupranolol and 6.4–7.5 for SR 59230A. In the presence of 200 nM (−)-propranolol, 3 μM CGP 20712A or 3 μM ICI 118551 did not cause blockade of the relaxant effects of BRL 37344 (not shown, $P>0.05$, Table 3). Table 5 provides a summary of affinity values for antagonists at β-adrenoceptor subtypes.

Discussion

General comments

We have confirmed the existence of a third population of β-adrenoceptors in rat sinoatrial node (Kaumann *et al.*, 1979) and also provided evidence for its existence in the left atrium but not in the rat left ventricular papillary muscle. Our data obtained from the rat colon strongly support the hypothesis that the receptors mediating relaxation evoked by a variety of agonists including (−)-noradrenaline are identical to the cloned β_3-adrenoceptor. Comparison of the third atrial β-adrenoceptor (for rat atrial β_1- and β_2-adrenoceptors see Kaumann, 1986) and colonic putative β_3-adrenoceptor reveals fundamental differences and some similarities. For an effect to be mediated through β_3-adrenoceptors we used the three criteria of Arch & Kaumann (1993): (i) stimulation by β_3-selective agonists, (ii) stimulation by non-conventional partial agonists and (iii) resistance to blockade by antagonists possessing only high affinity for β_1- and β_2-adrenoceptors. We now add another criterium: (iv) blockade by β_3-selective antagonists. All four criteria were fulfilled for the agonist-evoked relaxation of rat colon. The important criteria (i) and (iv) were not fulfilled for the rat atrium. We therefore propose that the third cardiac β-adrenoceptor population is different from the population of colonic putative β_3-adrenoceptors.

Properties of the third cardiac β-adrenoceptor

The evidence for a third β-adrenoceptor in rat heart consists so far in cardiostimulation by non-conventional partial agonists and resistance to blockade by antagonists with high affinity for β_1- and β_2-adrenoceptors. The non-conventional partial agonists used are pindolol analogues, including carazolol, (Kaumann *et al.*, 1979; Kaumann, 1989), (±)-CGP 12177 and cyanopindolol used *in vivo* by Malinowska & Schlicker (1996) and (−)-CGP 12177 and cyanopindolol used *in vitro* (present paper). The atrial cardiostimulant effects of (−)-CGP 12177 and cyanopindolol were resistant to (−)-propranolol 200 nM, a concentration that caused an over 2 log units rightward shift of the chronotropic concentration-effect curve of (−)-noradrenaline through blockade of β_1-adrenoceptors (Figure 5 this paper, see also Kaumann, 1986). As seen *in vivo* by Malinowska & Schlicker (1996) we observed that both (−)-bupranolol and CGP 20712A caused surmountable antagonism of the cardiostimulant effects of (−)-CGP 12177; (−)-bupranolol also blocked the cardiostimulant effects of cyanopindolol. Antagonism of the positive inotropic effects of (−)-CGP 12177 by (−)-bupranolol has also been observed in human atrium (Kaumann, 1996), suggesting but not proving that the third receptor population of human and rat atrium are species homologues of the same receptor.

We have now shown that micromolar concentrations of agonists selective for β_3-adrenoceptors neither cause cardiostimulant effects nor cause significant occupancy of the third cardiac β-adrenoceptor in rat atria. The cardiostimulant effects of BRL 37344 were mostly mediated through β_1-adrenoceptors marginally through β_2-adrenoceptors as demonstrated by marked blockade with β_1-selective CGP 20712A 300 nM, a concentration which does not interact with the third cardiac β-adrenoceptor. Some increase in heart rate with BRL 37344 in the pithed rat was observed by Oriowo *et al.* (1994) and Cohen *et al.* (1995). Malinowska & Schlicker (1996) also observed that BRL 37344 caused tachycardia in the pithed rat that was blocked by a combination of CGP 20712A and ICI 118551 at concentrations that selectively blocked β_1- and β_2-adrenoceptors. These authors observed that ZD 2079 and CL 316243 elicited small tachycardia mediated through β_1- and β_2-adrenoceptors but we failed to observe any stimulant effect on isolated atria with up to 60 μM of the two agonists. Dolan *et al.* (1994) also showed that CL 316243 only causes marginal tachycardia in guinea-pig isolated right atrium.

Our observations and those of Malinowska & Schlicker (1996) that β_3-adrenoceptor-selective agonists fail to activate and occupy the third cardiac β-adrenoceptor population are inconsistent with the hypothesis that the cardiac receptors are β_3-adrenoceptors. Furthermore, the failure of the β_3-selective SR 59230A to produce potent antagonism of the stimulant effects of (−)-CGP 12177 in both right and left atria adds support to the hypothesis that the third cardiac β-adrenoceptor is distinct from the β_3-adrenoceptor.

Is there a third ventricular β-adrenoceptor

The lack of stimulant effects of (−)-CGP 12177 and cyanopindolol in papillary muscles does not necessarily rule out the existence of a third β-adrenoceptor in rat ventricle. The cardiostimulant effects of (−)-CGP 12177 observed by us in rat atria have a lower maximal effect with respect to (−)-isoprenaline than equivalent effects in feline atria (Kaumann, 1983; 1989) in which the maximal effect is also larger than in feline ventricle. This raises the possibility for a third β-adrenoceptor population in rat ventricle that is less tightly coupled to effector and/or has a lower receptor density than in atria. Future autoradiography studies may resolve this issue. In general, the properties of the third rat cardiac β-adrenoceptor population characterized by Malinowska & Schlicker *in vivo* and by us *in vitro* agree with the properties of a third cardiac β-adrenoceptor of other species (Kaumann, 1989; Arch & Kaumann, 1993), including man (Kaumann, 1996).

Preliminary evidence, obtained from human isolated ventricular preparations, suggests the existence of a receptor with β_3-adrenoceptor characteristics (Gauthier *et al.*, 1995). These receptors mediate both shortening of the action potential and negative inotropic effects with nanomolar concentrations of BRL 37344 ($IC_{50}=2$ nM). The effects were not blocked by nadolol (10 μM) but antagonized by 1 μM bupranolol ($K_B\sim7.7$) SR 58611A was also a potent inotropic depressant ($IC_{50}=10$ nM). The high relaxant potencies of both BRL 37344 and SR 58611A observed by Gauthier *et al.* (1995) on human ventricle resemble those observed by us on rat colon as would be expected from species homologues of the same

receptor. We did not observe, however, cardiodepressant effects of BRL 37344 and SR 58611A in rat atria, which, together with other discussed arguments, makes the existence of atrial β_3-adrenoceptors unlikely. The existence of rat ventricular β_3-adrenoceptors mediating cardiodepressant effects remains to be investigated, although the lack of the corresponding cardiac mRNA (Evans *et al.*, 1996) seems to preclude their existence in this species.

Properties of the putative β_3-adrenoceptor of colon

All four criteria (see above) for an effect to be mediated through β_3-adrenoceptors were fulfilled. (i) We found that the β_3-selective agonists BRL 37344, CL 316243, ZD 2079 and SR 58611A were potent relaxants of the rat colon which is in line with some previous data (BRL 37344, McLaughlin & MacDonald, 1990; CL 316243, cited in Manara *et al.*, 1995b;

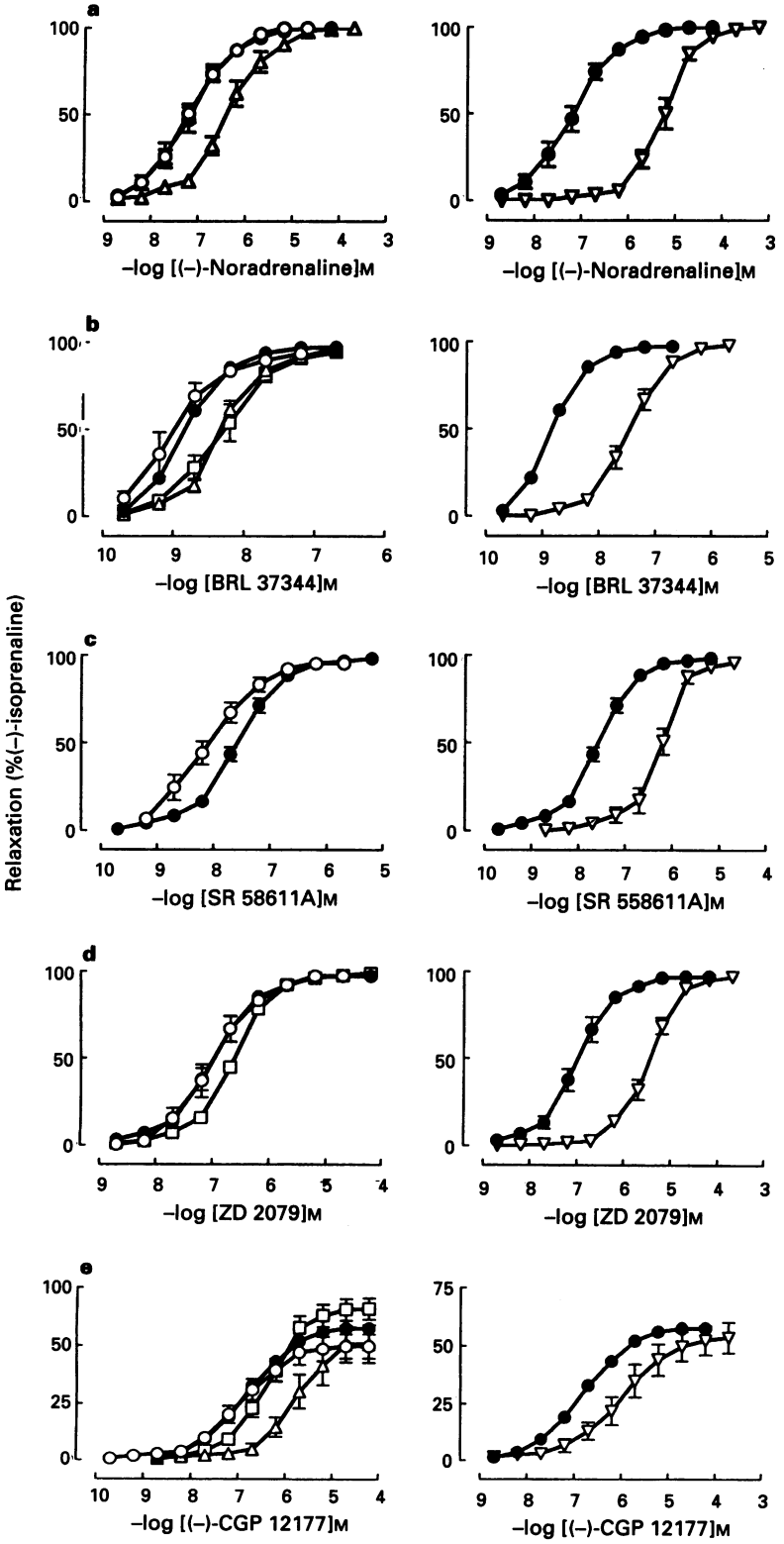

Figure 9 Relaxant responses in colon of (a) (−)-noradrenaline, (b) BRL 37344, (c) SR 58611A, (d) ZD 2079 and (e) (−)-CGP 12177 in the absence (○) or presence of 200 nM (−)-propranolol (●), 2 μM (−)-propranolol (△), 200 nM (−)-propranolol + 1 μM (−)-bupranolol (□) or 200 nM (−)-propranolol + 3.3 μM SR 59230A (▽). Responses are expressed as a percentage of the maximal response to (−)-isoprenaline. Values shown are mean ± s.e.mean (vertical lines) where larger than symbol size, $n = 4-36$ tissues for each curve.

Table 5 Affinity values[a] of five β-adrenoceptor (AR) antagonists at β-AR subtypes

Antagonist	*β_1-AR*	*β_2-AR*	*Third cardiac β-AR*[b]	*β_3-AR*[b]	*Selectivity*
(−)-Propranolol	8.5[c]	8.9[c]	< 5.7	6.0–6.5	$\beta_2 > \beta_1 > > \beta_3$ > third cardiac β
(−)-Bupranolol	9.1[d]	9.7[d]	6.4–6.8	6.2–6.4	$\beta_2 > \beta_1 > >$ third cardiac $\beta \geqslant \beta_3$
CGP20712A	9.6[e]	5.4[e]	6.3–6.4	< 5.5	$\beta_1 > > \beta_2$, third cardiac $\beta = \beta_3$
ICI118551	7.2[f]	9.3[f]	< 5.5	< 5.5	$\beta_2 > > \beta_1$ > third cardiac $\beta = \beta_3$
SR 59230A	–	–	5.1–5.4	6.4–7.5	$\beta_3 > >$ rat heart atypical β

[a] Data represent log equilibrium dissociation constants (pK_B) obtained from antagonism. [b] Present study. [c] Gille *et al.* (1985). [d] Lemoine & Kaumann (1983). [e] Lemoine & Kaumann (1991). [f] Bilski *et al.* (1983).

SR 58611A, Bianchetti & Manara, 1990). We also show that (−)-noradrenaline is a potent relaxant. (ii) (−)-CGP 12177 and cyanopindolol were potent relaxants and their concentration-effect curves matched the corresponding fractional receptor occupancies, as expected from classical partial agonists (Figure 7). (iii) The effects of all agonists were virtually resistant to blockade by (−)-propranolol 200 nM, a concentration that causes 99% β_1-adrenoceptor occupancy, as can be estimated from the antagonism of the positive chronotropic effects of (−)-noradrenaline of Figure 5. The relaxant effects of agonists were also resistant to blockade by 3 μM of each of the β_1-selective antagonist CGP 20712A and the β_2-selective antagonist ICI 118551. (iv) The relaxant effects of agonists were blocked by the β_3-selective antagonist SR 59230A (Manara *et al.*, 1995a) and to a minor extent by (−)-bupranolol and (−)-propranolol.

Our results disagree with those of McLaughlin & MacDonald (1990) who obtained data for the rat colon with BRL 37344 and (−)-noradrenaline showing that the relaxant potencies of these agonists were approximately 100 times lower than those found in the present work. Although McLaughlin & MacDonald (1990) described some blocking effects of cyanopindolol they failed to detect relaxing effects by this agent. The high relaxant potencies observed by us suggest that under our conditions receptor-effector coupling is considerably more optimal than under the conditions of McLaughlin & MacDonald (1990), thereby revealing the relaxant effects of cyanopindolol (and (−)-CGP 12177). We do not know the reason for these marked differences, that are especially puzzling to us because we adopted a variant of the method of McLaughlin & MacDonald (1990).

Similarities between the third cardiac β-adrenoceptor and putative β_3-adrenoceptor of colon

Atrium and colon share the property that both (−)-CGP 12177 and cyanopindolol cause agonist effects resistant to blockade by 200 nM (−)-propranolol in both systems. We have also recently found that the β_1-selective agonist (−)-RO363 (McPherson *et al.*, 1984) has agonist effects on the human atrial third cardiac β-adrenoceptor, on the putative β_3-adrenoceptor of both rat colon and guinea-pig ileum as well as on the cloned and transfected human β_3-adrenoceptor (Molenaar, Sarsero, Arch, Kelly, Henson & Kaumann, unpublished observations).

Receptor differences between heart and colon

Differences between the third cardiac β-adrenoceptor greatly outweigh similarities between the third cardiac β-adrenoceptor and the putative β_3-adrenoceptor of rat colon. (i) β_3-selective agonists and even (−)-noradrenaline are effective colonic relaxants at nanomolar concentrations but are ineffective at micromolar concentrations at the third cardiac β-adrenoceptor. (ii) The β_3-selective antagonist SR 59230A (Manara *et al.*, 1995a) causes blockade of agonist-evoked colonic relaxation but hardly blocks the third cardiac β-adrenoceptor. (iii) CGP 20712A 3 μM causes some antagonism of the cardiostimulant effects of (−)-CGP 12177 but fails to block agonist-evoked colonic relaxation. These differences strongly suggest that the relevant receptors are distinct in the two systems. The blockade of the atrial effects by the β_1-selective antagonist CGP 20712A is, however, not evidence for an interaction with β_1-adrenoceptors because the affinity of rat sinoatrial β_1-adrenoceptors ($pA_2 = 9.44$, Kaumann, 1986) is 1000 times higher than for the putative third cardiac β-adrenoceptor activated by (−)-CGP 12177.

Comparison with cloned β_3-adrenoceptors

There is consensus in the literature that β_3-adrenoceptors are expressed in the colon of rat and man (Granneman *et al.*, 1991; 1993; Krief *et al.*, 1993; Evans *et al.*, 1996) but not in myocardial cells of man (Krief *et al.*, 1993; Berkowitz *et al.*, 1995) and rat (Evans *et al.*, 1996). These studies, based on the detection of β_3-adrenoceptor mRNA, agree with the concept emerging from our present work that the third cardiac β-adrenoceptor is distinct from the colonic putative β_3-adrenoceptor identical to the cloned β_3-adrenoceptor.

β_3-selective agonists relax the colon at concentrations 3 to 5 orders of magnitude lower than those that fail to interact with the third cardiac β-adrenoceptor. This impressive difference would strongly indicate that the relevant receptor populations in heart and colon are different. However, the actual binding affinity of β_3-selective agonists is considerably lower than the corresponding relaxant potencies on colon. For example, the equilibrium dissociation constants obtained from binding of BRL 37344 and CL 316243 to cloned, transfected rat β_3-adrenoceptors are only 0.15 and 1 μM, respectively (Dolan *et al.*, 1994). Furthermore, the EC_{50} (Kact) for adenylyl cyclase stimulation of rat β_3-adrenoceptors transfected into CHO cells is 80 nM for BRL 37344 but only 5.8 μM for noradrenaline (Granneman *et al.*, 1991). Extrapolation of these low affinity estimates to the putative β_3-adrenoceptor of rat colon would suggest quite tight receptor-effector coupling for activation by these agonists (ie spare receptors) because relaxant effects can already be detected at subnanomolar concentrations for BRL 37344 and CL 316243 and nanomolar concentrations of (−)-noradrenaline (Figures 8 and 9). Although the binding affinity for cloned transfected β_3-adrenoceptors of agonists such as BRL 37344 and CL 316243 is considerably lower than their relaxant potencies, the concentrations causing half maximal β_3-adrenoceptor occupancy are still one to two orders of magnitude lower than the corresponding concentrations that fail to interact with the third cardiac β-adrenoceptor.

Conclusions

We conclude that the third cardiac β-adrenoceptor and putative β_3-adrenoceptor that mediate relaxation of longitudinal muscle of colon in the rat are different. The potent cardiostimulant and colonic relaxant effects of (−)-CGP 12177 found in this study opens the possibility for the use of this compound as a radioactive marker of the receptors of both tissues for a possible biochemical verification and further characterization of the two distinct receptor populations.

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