British Journal of Pharmacology (1996) 117, 712–716

Atypical responses of rat ileum to pindolol, cyanopindolol and iodocyanopindolol

*Andrew Hoey, *Clifford Jackson, *Graham Pegg & [1],†Martin Sillence

Tropical Beef Centre: A joint venture between *Central Queensland University, †CSIRO Division of Tropical Animal Production, and Queensland Department of Primary Industries, Rockhampton, Queensland, 4702, Australia

1 Pindolol, cyanopindolol (CYP) and iodocyanopindolol (IodoCYP) have been reported to act either as antagonists, agonists or partial agonists at the β_3-adrenoceptor in different preparations. A comprehensive investigation has not yet been described with these compounds tested in one tissue from one species. This study was conducted to delineate the pharmacological effects of pindolol, CYP and IodoCYP and to provide data on their affinities at the predominant β-adrenoceptor in rat ileum.

2 The β-adrenoceptors present in rat ileum were characterized in the presence of CGP 20712A and ICI 118 551, atropine and corticosterone, with (−)-isoprenaline used as an agonist. The role of the β_1 and β_2-adrenoceptors was determined by the omission of either CGP 20712A, ICI 118 551, or both, from the buffers. Conversely, the effectiveness of the β_1- and β_2-adrenoceptor blockade was examined by use of the β_1-adrenoceptor-selective agonist, RO 363 and the β_2-adrenoceptor-selective agonist, salbutamol.

3 There was no evidence for the presence of functional β_1-adrenoceptors, and no strong evidence that β_2-adrenoceptor stimulation contributed to the relaxant effects of (−)-isoprenaline. (−)-Phenylephrine did not produce relaxation of the tissue and 5-hydroxytryptamine produced contraction.

4 The β_3-adrenoceptor-selective agonist, BRL 37344 and (−)-isoprenaline were potent full agonists (pD_2 8.35±0.04 and 7.76±0.14 respectively), whereas ICI D7114 was less potent (pseudo pD_2 6.92±0.15). These results indicate that the predominant functional β-adrenoceptors in rat ileum are β_3-adrenoceptors.

5 Partial agonist effects were produced by CYP (pD_2 5.28±0.26) and IodoCYP (pD_2 7.0±0.26), but not pindolol. All three compounds antagonized the effects of (−)-isoprenaline with pK_b values of 6.68±0.10, 7.59±0.07 and 7.59±0.11 for pindolol, CYP and IodoCYP respectively. Likewise, CYP and IodoCYP antagonized the effects of BRL 37344 with pK_b values of 7.20±0.22 and 7.21±0.14 respectively. This study provides the first functional data on the effects of IodoCYP, the ligand with the highest known affinity for the β_3-adrenoceptor, at the characterized rat ileum β_3-adrenoceptor.

6 In conclusion, whereas pK_b values suggest that CYP and IodoCYP have a similar affinity for the β_3-adrenoceptor in rat ileum, the higher potency of IodoCYP suggests that it promotes a greater coupling efficiency, or that its partial agonist effects are produced through a site other than the β_3-adrenoceptor. The similar pK_b values for CYP and IodoCYP at the β_3-adrenoceptor contrast with their order of known affinities at the β_1- and β_2-adrenoceptors, where IodoCYP is far more potent than CYP. This provides evidence of further differences in the characteristics of the β_3-adrenoceptors compared to the β_1- and β_2-adrenoceptors. Finally, the utility of IodoCYP as a β_3-adrenoceptor antagonist would appear to be limited because of the greater magnitude of partial agonist effects that it produces.

Keywords: Pindolol; cyanopindolol; iodocyanopindolol; β_3-adrenoceptors; rat ileum; atypical receptor

Introduction

The radioligand ^{125}Iodocyanopindolol (^{125}IodoCYP) has been widely used for the characterization of β-adrenoceptors. It shows a high affinity (pK 10.4 to 10.6) for β_1- and β_2-adrenoceptors (Engel *et al.*, 1981) with approximately a two fold higher affinity for β_2-adrenoceptors over β_1-adrenoceptors (Neve *et al.*, 1986). At the β_3-adrenoceptor cloned from rat brown adipose tissue and expressed in Chinese hamster ovary (CHO) cells, ^{125}IodoCYP exhibits a pK of 8.9 (Muzzin *et al.*, 1991), which is substantially lower than that observed at the β_1- and β_2-adrenoceptor, but which is still higher than any other β_3-adrenoceptor ligand. Recently (−)-IodoCYP has been reported to act as a potent full agonist of lipolysis in the garden doormouse, exhibiting a potency (pD_2 9.4) similar to that observed with BRL 37344 (pD_2 9.2; Carpene *et al.*, 1994), and close to the pK of ^{125}IodoCYP for the rat cloned β_3–adrenoceptor. However, when tested in rat adipose tissue, (±)-^{125}IodoCYP has been reported to act as a partial agonist only, with a comparatively low potency (pD_2 7.0; Engel *et al.*, 1981). Thus the pharmacological effects of IodoCYP between tissues and species do not appear to be consistent.

Likewise, the non-iodinated compound cyanopindolol (CYP) also acts as an antagonist at β_1- and β_2-adrenoceptors (Meunier & Labrie, 1982), and has since been shown to act as an antagonist at the β_3-adrenoceptors found in rat gastric fundus, jejenum and colon (McLaughin & MacDonald, 1990; 1991; MacDonald *et al.*, 1994). Cyanopindolol has also been shown to possess some partial agonist activity in rat gastric fundus at a concentration that exceeds that required to produce antagonism of β_3-agonist-mediated relaxation (McLaughin & MacDonald, 1991).

The difference between the agonist, partial agonist and antagonist effects of IodoCYP and its analogues between species and tissues prompted us to compare the effects of these compounds in functional studies limited to one tissue from one species, in order to delineate more clearly their pharmacological actions.

[1] Author for correspondence at: Tropical Beef Centre, PO Box 5545, Rockhampton Mail Centre, Queensland, 4702, Australia.

Methods

Rats of either sex (150 to 250 g) were stunned by a blow to the head and exsanguinated. Approximately 20 cm of ileum, proximal to the caecum, was removed and placed in cold Tyrode solution (in mM: NaCl 136.9, KCl 5.4, $MgCl_2.H_2O$ 1.05, $NaH_2PO_4.2H_2O$ 0.42, $NaHCO_3$ 22.6, $CaCl_2.2H_2O$ 1.8, glucose 5.5, ascorbic acid 0.28, Na_2EDTA 0.05). The ileum was trimmed of any fat and cut into strips approximately 25 mm long, which were then cleared of their contents by gentle flushing with Tyrode solution. A small stainless steel hook was placed in one end to connect the tissue to the tissue holder, and a silk thread at the other end connected the tissue to a force transducer (Grass FT03). Force of contraction was recorded via a MacLab system (AD Instruments) by a Macintosh LC475 computer. The tissues were bathed in warm (37 ± 0.5°C) aerated (95% O_2; 5% CO_2) Tyrode solution in 25 ml water-jacketed organ baths. The preparations were suspended with a preload of 5 mN which was maintained for the first hour, while the Tyrode solution was exchanged every 15 min. After this equilibration period, atropine (final bath concentration 0.5 μM) and corticosterone (0.1 mM) were added to prevent spontaneous activity, and to block extraneuronal uptake. Unless otherwise indicated, CGP 20712A (0.1 μM) and ICI 118 551 (0.1 μM) were added to block β_1- and β_2-adrenoceptors respectively. These drugs were allowed to equilibrate for 20 min before the tissues were contracted by the addition of 4.0 M KCl, to give a final concentration of 40 mM KCl in the organ baths. After 10 min, the contraction was stable and a concentration-response curve was generated by the cumulative addition of a test compound. In experiments conducted to investigate antagonist activity, one concentration of test compound was added 10 min after the KCl, and was allowed to equilibrate with the tissue for a further 30 min before a concentration-response curve to an agonist was generated. For these experiments, concentration-response curves to agonists were also obtained 40 min after KCl addition.

Because repeated exposure of the tissue to agonists produced a rightward shift in the concentration-response curves, only a single curve was generated from each tissue. Values for pK_b were calculated by the dose-ratio method (Mackay, 1978) in paired tissues.

The following drugs and solvents were used: atropine (Sigma Chemical Co. St Louis, MO, U.S.A.) was dissolved in ethanol; corticosterone (Sigma Chemical Co., St Louis, MO, U.S.A.), CGP 20712A (1-[2-((3-carbamyl-4-hydroxy)-phenoxy)-ethylamino]-3-[4-(1-methyl- 4-trifluoro-methyl - 2 -imidazolyl)-phenoxy]-propan-2-ol; Ciba-Geigy, Basel, Switzerland) and ICI 118 551 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamino)-butan-2-0l; Imperial Chemical Industries, Cheshire, U.K.) were dissolved in dimethylsulphoxide (DMSO). (−)-Isoprenaline, (−)-phenylephrine, 5-hydroxytryptamine (5-HT) (all from Sigma Chemical Co., St Louis, MO, U.S.A.), salbutamol (Riker Laboratories, California, U.S.A.) and RO363 ((I) - 1- (3,4 - dimethoxy - phenethylamino) - 3 -(3,4-dihydroxy-phenoxy)-2-propanol oxalate) (gift from C. Raper, Victorian College of Pharmacy, Australia) were dissolved in acidified water. BRL 37344 (4-[2-[(2-hydroxy-2-(3-chlorophenyl)ethyl)-amino] - propyl] - phenoxyacetic acid; SmithKline Beecham Pharmaceuticals, Middlesex, U.K.) and ICI D7114 ((5)-4-[2-hydroxy -3-phenoxy-propylamino-ethoxy]-N-(2-methoxyethyl) -phenoxyacetamide) (Imperial Chemical Industries, Cheshire, U.K.) were dissolved in demineralized water. Pindolol (Sigma Chemical Co., St Louis, MO, U.S.A.), cyanopindolol and iodocyanopindolol (synthesized in our laboratory) were dissolved in DMSO at a stock solution of 10^{-2} M and diluted further in 50% DMSO and subsequently in demineralized water.

Data analysis

Results are expressed as means ± s.e.mean of the number (n) of experiments. Statistical significance between two data sets was tested by Student's t test. A probability level of $P<0.05$ was considered to be statistically significant.

Results

The rat ileum was characterized to examine the role of the different β-adrenoceptors that may be present. Tissues were relaxed with the non-selective β-adrenoceptor agonist (−)-isoprenaline, in the absence of CGP 20712A and ICI 118 551, or in the presence of 0.1 μM of each antagonist, either alone or in combination (Figure 1). These concentrations of β_1- and β_2-adrenoceptor-selective antagonists were calculated to be sufficient to block 98% and 96% of the β_1- and β_2-adrenoceptors respectively in the absence of agonist, according to the formula: % occupancy $= ([B]/[B]+K_d)$; where [B] is the concentration of antagonist and K_d is the equilibrium dissociation constant. Values for K_d were obtained from Marullo *et al.* (1989). Neither antagonist caused a rightward shift in the concentration-response curve to (−)-isoprenaline. CGP 20712A alone did not alter the slope of the curve, whereas ICI 118 551, alone or in combination with CGP 20712A, produced a steeper curve.

To confirm that the relaxation produced by (−)-isoprenaline was not due to β_1- or β_2-adrenoceptor activation, and that the antagonists were present in sufficient concentrations to block any β_1- and β_2-adrenoceptors in the tissue, concentration-response studies to β_1- and β_2-adrenoceptor-selective agonists were conducted in the presence of CGP 20712A and ICI 118 551. RO 363, an agonist which has a high affinity for β_1-adrenoceptors (pD_2 8.05 in guinea-pig right atria; McPherson *et al.*, 1984), and which exhibits approximately 400 fold selectivity for this subtype over β_2-adrenoceptors, was used. However, in rat ileum, RO 363 was significantly less potent than (−)-isoprenaline, and did not produce complete relaxation even at the highest concentration tested (10 μM; Figure 2). Similarly, salbutamol was used as a β_2-adrenoceptor-selective agonist (pD_2 7.4 for guinea-pig trachea; Nials *et al.*, 1993). At the highest concentration tested (10 μM), only 40% relaxation was observed. (−)-Phenylephrine and 5-hydroxytryptamine (5-HT) were also tested: (−)-phenylephrine did not produce any relaxation, and 5-HT produced further contraction.

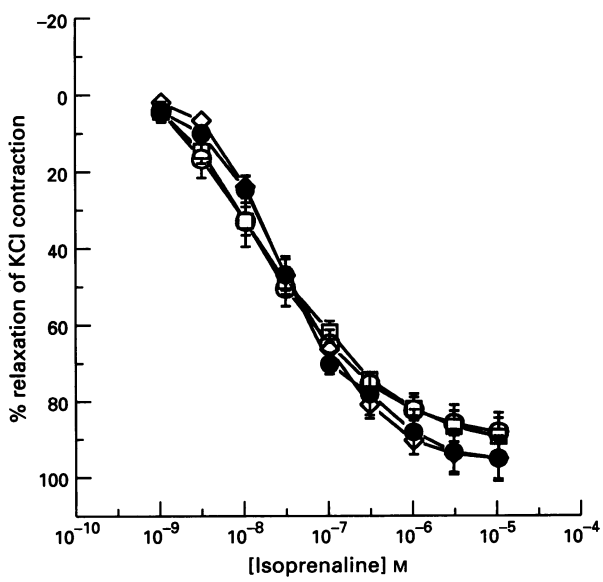

Figure 1 Concentration-response curves to (−)-isoprenaline in rat ileum precontracted with 40 mM KCl. Experiments were conducted in the absence of CGP 20712A and ICI 118 551 (○), in the presence of 0.1 μM CGP 20712A (□), 0.1 μM ICI 118 551 (◇), or 0.1 μM CGP 20712A and ICI 118 551 (●). Atropine (0.5 μM) and corticosterone (100 μM) were present in all preparations. $n=4$ to 5.

Different responses to β_3-agonists have been reported in different areas of the rat alimentary canal (McLaughlin & MacDonald, 1990; 1991; MacDonald *et al.*, 1994). However, Figure 2 shows that there was no difference in the response to (−)-isoprenaline between the first preparation of ileum removed 20 cm proximal to the caecum, and the last preparation removed immediately proximal to the caecum.

The β_3-agonists, BRL 37344 and ICI D7114, were examined for their relaxant effects on the rat ileum. BRL 37344 acted as a full agonist (pD_2 8.35±0.04), and was significantly more potent than (−)-isoprenaline (pD_2 7.76±0.14; $P<0.01$). In contrast, ICI D7114 did not elicit complete relaxation at the highest concentration tested (10^{-4} M) and produced a pseudo pD_2 6.92±0.15 which was lower ($P<0.002$) than the pD_2 of (−)-isoprenaline (Figure 3).

Pindolol and CYP have been shown to produce partial agonist effects in CHO cells that express mouse and human β_3-adrenoceptors. Therefore the agonist effects of pindolol, CYP and IodoCYP were examined in our rat ileum preparation (Figure 4). Pindolol did not produce relaxation that was significantly different from that seen in the control tissues treated with solvent only. CYP behaved as a partial agonist, producing significant relaxation at concentrations above 1 μM (pD_2 5.28±0.26; Table 1). IodoCYP produced a concentration-dependent relaxation with a significant effect seen at concentrations above 0.01 μM (pD_2 7.0±0.26). The equivalent volume of solvent used for the highest concentration of test compound produced some relaxation in control tissues, and so the data are presented after subtraction of this effect.

Pindolol, CYP and IodoCYP were tested for their ability to antagonize relaxation caused by the β_3-adrenoceptor agonists, (−)-isoprenaline and BRL 37344. A single concentration of antagonist (0.3 μM) was chosen that was insufficient to cause a large relaxation by itself (as judged from the partial agonist effects described above). Thus, pK_b values were calculated by the dose-ratio method. Pindolol was a comparatively weak antagonist, causing only a small shift of the isoprenaline concentration-response curve, resulting in an estimated pK_b of only 6.68±0.10 (Table 1). CYP and IodoCYP both caused significant antagonism of the effects of (−)-isoprenaline, with pK_b values of 7.59±0.07 and 7.59±0.11 respectively (Figure 5). Similarly, both CYP and IodoCYP also antagonized the relaxation produced by BRL 37344, with pK_b values of 7.20±0.22 and 7.21±0.14 respectively (data not shown).

There was a significant difference between the pD_2 and the pK_b values for CYP ($P<0.05$), with a similar trend apparent for IodoCYP ($P=0.08$). No pD_2 values could be calculated for pindolol as the drug did not produce significant relaxation.

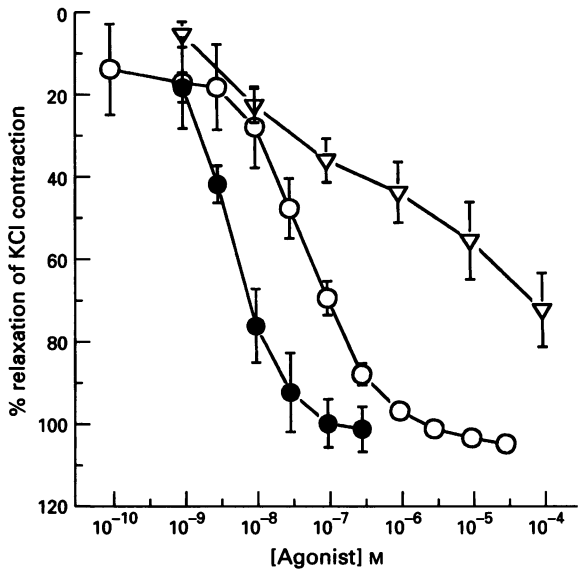

Figure 3 Concentration-response curves to various β_3-agonists in rat ileum precontracted with 40 mM KCl. (−)-Isoprenaline (○) and BRL 37344 (●) were both potent full agonists, whereas ICI D7114 (▽) was only a partial agonist with a lower potency. CGP 20712A and ICI 118 551 (0.1 μM), atropine (0.5 μM) and corticosterone (100 μM) were present in all preparations. $n=4$.

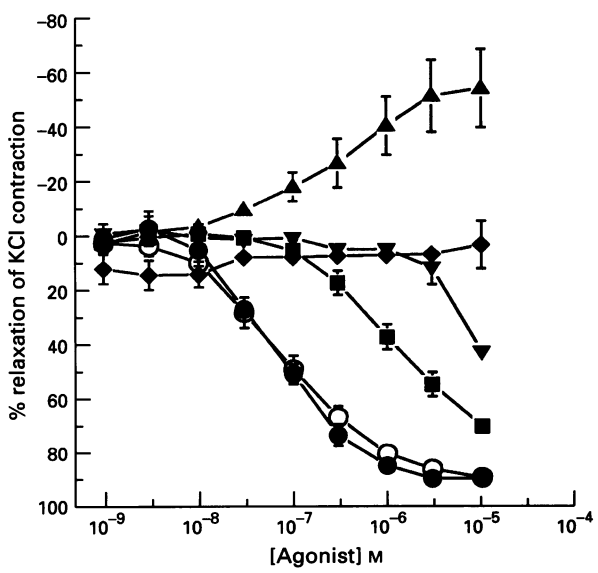

Figure 2 Concentration-response curves for various compounds in rat ileum precontracted with 40 mM KCl. (−)-Isoprenaline produced almost identical relaxation in ileum obtained 20 cm proximal to the caecum (○) and immediately proximal to the caecum (●). Relaxation was also induced by the β_1-selective agonist, RO363 (■), and the β_2-selective agonist salbutamol (▼). The α-agonist (−)-phenylephrine (◆) did not cause relaxation, and 5-HT (▲) caused contraction. CGP 20712A and ICI 118 551 (0.1 μM), atropine (0.5 μM) and corticosterone (100 μM) were present in all preparations. $n=4$.

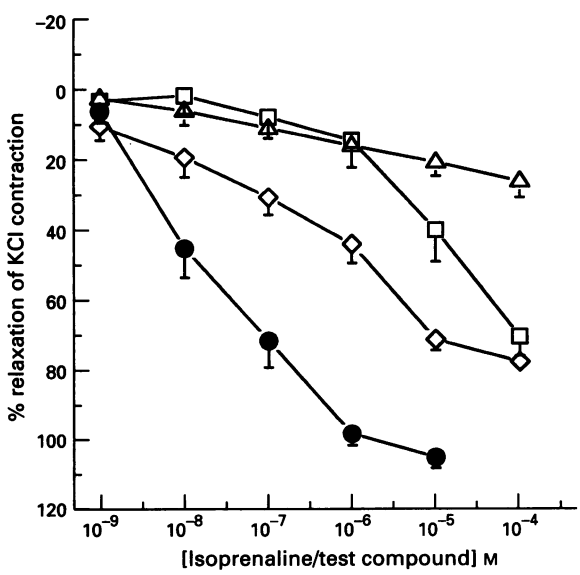

Figure 4 Concentration-response curves to (−)-isoprenaline and pindolol analogues in rat ileum precontracted with 40 mM KCl. Relaxation was induced by (−)-isoprenaline (●), and with descending potencies by iodocyanopindolol (◇) and cyanopindolol (□). Pindolol (△) did not cause relaxation significantly different from that produced by the solvent (DMSO). CGP 20712A and ICI 118 551 (0.1 μM), atropine (0.5 μM) and corticosterone (100 μM) were present in all preparations. $n=4$ to 5.

Table 1 Characterization of pindolol, cyanopindolol and iodocyanopindolol in rat ileum

Drug	Intrinsic activity	pD_2	pK_b vs (−)-Iso	pK_b vs BRL 37344	pK_{bIso}-pD_2
Pindolol	0		6.68 ± 0.10	ND	
CYP	0.71 ± 0.02	5.28 ± 0.26	7.59 ± 0.07	7.20 ± 0.22	$P<0.05$
IodoCYP	0.78 ± 0.02	6.92 ± 0.26	7.59 ± 0.11	7.21 ± 0.14	$P=0.08$

ND not determined; $n=4$ to 6.

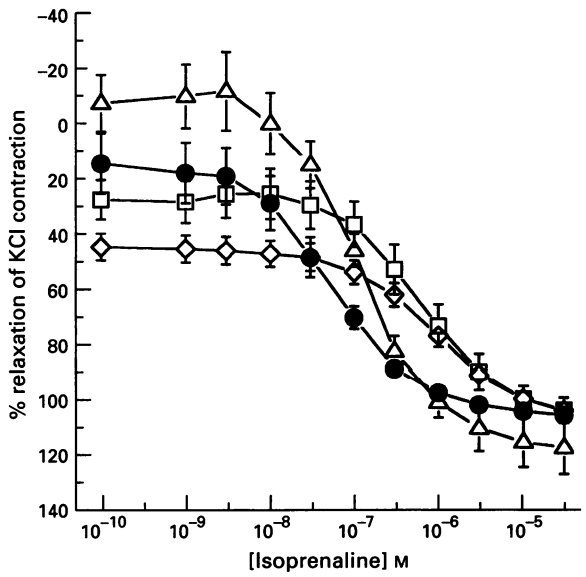

Figure 5 Concentration-response curves to (−)-isoprenaline (●) in rat ileum precontracted with 40 mM KCl, in the presence of pindolol (△), cyanopindolol (□) and iodocyanopindolol (◇). CGP 20712A and ICI 118551 (0.1 μM), atropine (0.5 μM) and corticosterone (100 μM) were present in all preparations. $n=4$ to 5.

Discussion

The present study confirmed that the rat ileum possesses functional β_3-adrenoceptors which predominate in mediating catecholamine-induced relaxation. There was no evidence of a role for β_1-adrenoceptors, based on the lack of a change in the (−)-isoprenaline concentration-response curve in the presence or absence of CGP 20712A. It is possible that the β_2-adrenoceptors do play a minor role in relaxation, as shown by the small difference in the slope of the concentration-response curve in the presence and absence of ICI 118 551. The effectiveness of blockade of the β_1- and β_2-adrenoceptors was shown by the fact that high concentrations of RO 363 and salbutamol were required to produce relaxation in the presence of CGP 20712A and ICI 118 551. An α-adrenoceptor agonist caused neither contraction or relaxation. Because the effects of pindolol and its analogues were being tested, and because pindolol may interact with the 5-HT receptor (Moretti-Rojas *et al.*, 1983), the effects of 5-HT on the ileum preparation were also examined. 5-HT did not cause any relaxation, but instead caused contraction. Throughout the region of the ileum used in our experiments, there was no difference in the relaxation produced by (−)-isoprenaline, indicating that it was appropriate for eight sequential tissue preparations from this region to be utilized.

The observation that BRL 37344 was a potent full agonist in rat ileum agrees with reports in rat distal colon, gastric fundus and jejenum (McLaughin & MacDonald, 1990; 1991; MacDonald *et al.*, 1994), but differs slightly from the observations of Growcott *et al.* (1993) who reported that BRL 37344 was only a partial agonist in rat ileum (intrinsic activity, 0.71). However, Growcott *et al.* (1993) found BRL 37344 to be nine times more potent than (−)-isoprenaline; an observation which compares favourably with the six fold difference observed in the present study. ICI D7114 did not elicit complete relaxation at the highest concentration tested in rat ileum in the present study, and acted as an antagonist against BRL 37344 (pK_b 7.30 ± 0.18; $n=5$, results not shown). This result may also be compared with that of Growcott *et al.* (1993) and MacDonald & Lamont (1993) who determined the pK_b of ICI D7114 against BRL 37344 and (−)-isoprenaline to be 6.7 and 7.29 respectively. Having completed a full characterization of the rat ileum preparation in our laboratory which produced results similar to those reported elsewhere in the literature, we started testing other compounds.

Partial agonist effects of pindolol on β_3-adrenoceptors were not evident in the rat ileum preparation. This is in contrast to the effect of pindolol in cloned mouse and human β_3-adrenoceptors where it acts as a partial agonist with a pD_2 of 6.0 and 6.8 respectively (Blin *et al.*, 1994). The lack of partial agonist effects of pindolol in rat ileum may indicate either species differences between β_3-adrenoceptors in the rat, mouse and human, or improved receptor expression or coupling efficiency for cloned receptors in CHO cells (Cohen *et al.*, 1995). In contrast to pindolol, both CYP and IodoCYP produced partial agonist effects in rat ileum. This was expected, as CYP acts as a partial agonist at the cloned human and rat β_3-adrenoceptor with a pD_2 of 6.76 and 6.92 respectively (Blin *et al.*, 1994; Cohen *et al.*, 1995). Furthermore, CYP and IodoCYP were found to act as partial agonists in stimulating lipolysis in rat adipose tissue (Engel *et al.*, 1981). CYP has been reported to act as a partial agonist in rat gastric fundus (McLaughlin & MacDonald, 1991), although partial agonist effects have not been observed in other rat gastrointestinal tract preparations where CYP has been tested for its effects (McLaughin & MacDonald, 1990; 1991; MacDonald *et al.*, 1994). When tested for partial agonist activity, IodoCYP was distinctly more potent than CYP. This suggests that either IodoCYP promotes a greater efficiency of coupling than CYP, or that IodoCYP and CYP may produce partial agonist effects at a site other than the characterized β_3-adrenoceptor. This second possibility is substantiated by the finding that pD_2 values for both compounds were lower than their pK_b values. This difference was statistically significant for CYP, and narrowly missed significance for IodoCYP.

The results described in this paper show a disparity between the pK_b value obtained for IodoCYP in the rat ileum (pK_b 7.59) and that obtained with cloned rat β_3-adrenoceptors expressed in CHO cells (pK 8.9; Muzzin *et al.*, 1991). However, it is not clear why this difference occurs. The possibility of the IodoCYP used in the present study being defective can be excluded as the same material produced a pK of 11.7 against β_2-adrenoceptors in our hands in a separate study (data not shown). Another possibility is that this difference may be due to the altered environment of the cloned β_3-adrenoceptors in CHO cells, although K_d values reported for cloned β_1- and β_2-

adrenoceptors match those reported in mammalian cells very closely (Blin *et al.*, 1994). We believe, therefore, that this discrepancy warrants further investigation.

It has previously been shown that the pK values for ^{125}IodoCYP obtained from binding studies are significantly lower at the rat β_3-adrenoceptor (pK 8.9; Muzzin *et al.*, 1991) than at the rat β_1- and β_2-adrenoceptors (pK 10.9 and 10.6 respectively; Brown *et al.*, 1992). This exemplifies the difference between the β_3-adrenoceptors and the β_1- and β_2-adrenoceptors. A further difference between the β_3-adrenoceptors and the β_1- and β_2-adrenoceptors is now apparent from the results of the present study. Cyanopindolol normally displays a large increase in affinity for the β_1- and β_2-adrenoceptors when it is iodinated to produce IodoCYP. However, we now show that this improvement in affinity by iodination is not apparent at the rat ileum β_3-adrenoceptor, with the pK_b for CYP being identical to that of IodoCYP, regardless of the agonist used. Interestingly, the pK_b values of both CYP and IodoCYP were consistently lower when BRL 37344 was used as an agonist than when (−)-isoprenaline was used. This was unexpected, as the pK_b should be independent of the agonist used (Mackay, 1978). Nevertheless, similar results have been obtained previously (Stock & Sudera, 1988; McLaughlin & MacDonald, 1990). Binding studies conducted in CHO cells with CYP and cold IodoCYP to determine their pK values, would provide useful information to confirm this lack of difference in affinities between CYP and IodoCYP at the β_3-adrenoceptor.

The results of this study provide the first data on the functional pharmacological effects and drug affinities of IodoCYP at a clearly characterized β_3-adrenoceptor, and provide further evidence of differences between the β_3-adrenoceptors and the β_1- and β_2-adrenoceptors. Furthermore, even though IodoCYP is the ligand with the highest known affinity for the β_3-adrenoceptor, in functional studies its affinity is not significantly different from that of CYP. Indeed, because IodoCYP produces partial agonist effects at lower concentrations than CYP, its value as a β_3-adrenoceptor antagonist is limited.

References

BLIN, N., CAMOIN, L., MAIGRET, B. & STROSBERG, A.D. (1994). Structural and conformational features determining selective signal transduction in the β_3-adrenergic receptor. *Mol. Pharmacol.*, **44,** 1094–1104.

BROWN, L., DIGHTON, N.M., BALS, S., SÖHLMANN, W., ZERKOWSKI, H-R., MICHEL, M.C. & BRODDE, O-E. (1992). Spare receptors for β-adrenoceptor-mediated positive inotropic effects of catecholamines in the human heart. *J. Cardiovasc. Pharmacol.*, **19,** 222–232.

CARPENE, C., AMBID, L. & LAFONTAN, M. (1994). Predominance of β_3-adrenergic component in catecholamine activation of lipolysis in garden dormouse adipocytes. *Am. J. Physiol.*, **266,** R896–904.

COHEN, M.L., GRANNEMAN, J.G., CHAUDHRY, A., SCHENCK, W., CUSHING, D.J. & PALKOWITZ, A.D. (1995). Is the 'atypical' β-receptor in the rat stomach fundus the rat β_3 receptor? *J. Pharmacol. Exp. Ther.*, **272,** 446–451.

ENGEL, G., HOYER, D., BERTHOLD, R. & WAGNER, H. (1981). (±)[^{125}Iodo]cyanopindolol, a new ligand for β-adrenoceptors: identification and quantitation of subclasses of β-adrenoceptors in guinea-pig. *Naunyn-Schmied. Arch. Pharmacol.*, **317,** 277–285.

GROWCOTT, J.W., HOLLOWAY, B., GREEN, M. & WILSON, C. (1993). Zeneca ZD7114 acts as an antagonist at β_3-adrenoceptors in rat isolated ileum. *Br. J. Pharmacol.*, **110,** 1375–1380.

MACDONALD, A., FORBES, I.J., GALLACHER, D., HEEPS, G. & MCLAUGHLIN, D.P. (1994). Adrenoceptors mediating relaxation to catecholamines in rat isolated jejunum. *Br. J. Pharmacol.*, **112,** 576–578.

MACDONALD, A. & LAMONT, M. (1993). Effects of selective antagonism of β-adrenoceptor sub-types on responses to isoprenaline in rat distal colon *in vitro*. *Br. J. Pharmacol.*, **110,** 1551–1555.

MACKAY, D. (1978). How should values of pA_2 and affinity constants for pharmacological competitive antagonists be estimated? *J. Pharm. Pharmacol.*, **30,** 312–313.

MARULLO, S., DELAVIER-KLUTCHO, C., GUILLET, J-G., CHARBIT, A., STROSBERG, A.D. & EMORINE, L.J. (1989). Expression of human β1 and β2 adrenergic receptors in *E. coli* as a new tool for ligand screening. *Biotechnology*, **7,** 923–927.

MCLAUGHLIN, D.P. & MACDONALD, A. (1990). Evidence for the existence of 'atypical' β-adrenoceptors (β_3-adrenoceptors) mediating relaxation in the rat distal colon *in vitro*. *Br. J. Pharmacol.*, **101,** 569–574.

MCLAUGHLIN, D.P. & MACDONALD, A. (1991). Characterization of catecholamine-mediated relaxations in rat isolated gastric fundus: evidence for an atypical β-adrenoceptor. *Br. J. Pharmacol.*, **103,** 1351–1356.

MCPHERSON, G.A., MALTA, E., MOLENAR, P. & RAPER, C. (1984). The affinity and efficacy of the selective β_1-adrenoceptor stimulant RO363 at β_1- and β_2-adrenoceptor sites. *Br. J. Pharmacol.*, **82,** 897–904.

MEUNIER, H. & LABRIE, F. (1982). Specificity of the β_2-adrenergic receptor stimulating cAMP accumulation in the intermediate lobe of the rat pituitary gland. *Eur. J. Pharmacol.*, **81,** 411–420.

MORETTI-ROJAS, I., EZRAILSON, E.G., BIRNBUMER, L., ENTMAN, M.L. & GARBER, A.J. (1983). Serotonergic and adrenergic regulation of skeletal muscle metabolism in the rat. *J. Biol. Chem.*, **258,** 12499–12508.

MUZZIN, P., REVILLI, J-P., KUHNE, F., GOCAYNE, J.D., MCCOMBIE, W.R., VENTER, J.C., GIACOBINO, J-P. & FRASER, C.M. (1991). An adipose tissue-specific β-adrenergic receptor: molecular cloning and down-regulation in obesity. *J. Biol. Chem.*, **266,** 24053–24058.

NIALS, A.T., SUMNER, M.J., JOHNSON, M. & COLEMAN, R.A. (1993). Investigations into factors determining the duration of action of the β_2-adrenoceptor agonist, salmeterol. *Br. J. Pharmacol.*, **108,** 507–515.

NEVE, K.A., MCGONIGLE, P. & MOLINOFF, P.B. (1986). Quantitative analysis of the selectivity of radioligands for subtypes of beta adrenergic receptors. *J. Pharmacol. Exp. Ther.*, **238,** 46–53.

STOCK, M.J. & SUDERA, D.K. (1988). Schild analysis of β-adrenoceptor antagonists inhibition of brown adipocyte respiratory responses to novel β-adrenoceptor agonists. *Br. J. Pharmacol.*, **95,** 741P.

(Received August 8, 1995
Revised October 19, 1995
Accepted October 24, 1995)