



# Characteristics of cyanopindolol analogues active at the $\beta_3$ -adrenoceptor in rat ileum

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1 Cyanopindolol (CYP) is a potent antagonist at the  $\beta_3$ -adrenoceptor in rat ileum. Several analogues of CYP and pindolol were synthesized that also produced antagonist effects at the  $\beta_3$ -adrenoceptor. However, at high concentrations, these compounds appear to act as 'partial agonists'. This study was conducted to determine the structural requirements of CYP analogues necessary for antagonist activity and to examine the possibility that the agonist effects of CYP and its analogues may occur through a mechanism independent of  $\beta$ -adrenoceptor activation.

2 Analogues of CYP and pindolol were tested for antagonist activity in rat ileum in which the  $\beta_1$ - and  $\beta_2$ -adrenoceptors were blocked. Fourteen compounds were tested against (–)-isoprenaline, and four of the more potent analogues were then tested against BRL 37344. The two most potent antagonists were CYP and iodocyanopindolol. The  $pK_b$  values (negative log of equilibrium dissociation constant) obtained against (–)-isoprenaline were significantly higher than those obtained against BRL 37344, but the cause of this difference is not known.

3 Several structural requirements were determined for antagonist activity. Modification at the carbon atom alpha to the secondary amine caused the antagonist potency to fall as the level of saturation was reduced. Thus, a quaternary carbon group, such as *t*-butyl, produced the most potent antagonist. Substitution with a large moiety such as a cyclohexyl or benzyl group reduced antagonist activity, probably due to steric hindrance. Inclusion of an electron-withdrawing group, such as a cyano or ethylester moiety, alpha to the indole nitrogen, also increased the potency. Iodination of CYP and ethylesterpindolol at the 3-position of the indole ring did not increase antagonist potency. In contrast, iodination of the almost inactive analogues produced a significant increase in potency, suggesting that a beneficial electronic effect on the indole ring imparted by the iodo moiety may be able to offset partially the negative effects caused by either the steric hindrance, or lack of a quaternary carbon alpha to the secondary amine.

4 Values for pseudo- $pD_2$  were also determined by conducting cumulative concentration-response studies up to the limit of drug solubility. For nine of the compounds tested, the  $pK_b$  was significantly higher than the pseudo- $pD_2$  value.

5 The discrepancy between the  $pK_b$  and pseudo- $pD_2$  values was examined further. The agonist effects of iodocyanopindolol, the agonist with the highest potency, were not antagonized by CYP which was the most potent antagonist of (–)-isoprenaline and BRL 37344 at the  $\beta_3$ -adrenoceptor. This suggests that the agonist effects of iodoCYP were produced through a different mechanism: either via another receptor, another isoform of the rat  $\beta_3$ -adrenoceptor, or through a non-receptor-mediated effect. Pseudo- $pD_2$  values did not correlate with log P values for these compounds, indicating that their relaxant effects were not simply a function of their lipid solubility.

6 This study has highlighted several structural requirements for antagonist binding potency at the rat ileum  $\beta_3$ -adrenoceptor and should assist in the development of potent selective antagonists for this receptor.

**Keywords:** Pindolol; cyanopindolol; iodocyanopindolol;  $\beta_3$ -adrenoceptors; rat ileum; atypical receptor

## Introduction

The development and testing of BRL 35135A and its analogues in rat tissues led to the discovery of a third  $\beta$ -adrenoceptor now known as the 'atypical' or  $\beta_3$ -adrenoceptor (Arch *et al.*, 1984). Since its discovery using functional pharmacological methods, the  $\beta_3$ -adrenoceptor has also been sequenced, cloned and expressed in CHO cells (Muzzin *et al.*, 1991; Granneman *et al.*, 1991). Following the first generation of agonists for the rat  $\beta_3$ -adrenoceptor, other agonists such as CGP 12177A (Granneman & Whitty, 1991), ICI D7114 (Growcott *et al.*, 1993), SR 58611A (Landi *et al.*, 1993) and SM-11044 (Sugasawa *et al.*, 1992) have also been developed. While all these drugs have been regarded as potentially useful  $\beta_3$ -adrenoceptor agonists, their affinities and efficacies at the rat  $\beta_3$ -adrenoceptor vary considerably. Furthermore, the

pharmacological characterization of the  $\beta_3$ -adrenoceptor has been limited somewhat by the lack of a selective antagonist possessing a suitably high affinity.

Cyanopindolol (CYP) and (–)-alprenolol have been reported to act as antagonists at the  $\beta_3$ -adrenoceptors present in the rat gastrointestinal tract, with CYP having affinities ( $pK_b$ : negative log of equilibrium dissociation constant) of 7.44, 7.01 and 7.12 in gastric fundus, jejunum and colon respectively (McLaughlin & MacDonald, 1990; 1991; MacDonald *et al.*, 1994); and (–)-alprenolol having a  $pK_b$  of 8.0 in proximal colon (Landi *et al.*, 1993). Recently, we compared the effects of CYP, iodocyanopindolol (iodoCYP) and pindolol, using a well-characterized rat ileum preparation (Hoey *et al.*, 1996). The present study was conducted to extend these observations for a range of CYP analogues, so that structure-activity data could be obtained that would increase our knowledge of the requirements for antagonist binding at the rat ileum  $\beta_3$ -adrenoceptor. Cyanopindolol has also been reported to possess

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'partial agonist' activity at the  $\beta_3$ -adrenoceptor in the rat gastric fundus and ileum. However, as the agonist effects occurred at concentrations that exceed those required to antagonize  $\beta_3$ -adrenoceptor-mediated relaxation (MacLaughlin & MacDonald, 1991; Hoey *et al.*, 1996), we propose that they were mediated by an independent mechanism. This hypothesis was also tested in the present study.

## 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,  $MgCl_2 \cdot H_2O$  1.05,  $NaH_2PO_4 \cdot 2H_2O$  0.42,  $NaHCO_3$  22.6,  $CaCl_2 \cdot 2H_2O$ ; glucose 5.5, ascorbic acid 0.28,  $Na_2EDTA$  0.05). The ileum was trimmed of any fat any 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 (A D Instruments) using a Macintosh LC475 computer. The tissues were bathed in warm ( $37.0 \pm 0.5^\circ C$ ) aerated (carbogen 95%  $O_2$ ; 5%  $CO_2$ ) Tyrode solution in 25 ml water-jacketed organ baths. The preparations were suspended with a preload maintained at 5 to 10 mN for the first hour, while the Tyrode solution was exchanged every 15 min. After this equilibration period, CGP 20712A and ICI 118 551 (final bath concentration 0.1  $\mu M$ ), atropine (0.5  $\mu M$ ) and corticosteron (0.1 mM) were added to block  $\beta_1$ - and  $\beta_2$ -adrenoceptors, prevent spontaneous activity and to block extraneuronal uptake, 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. The KCl-induced contraction was stable after 10 min, allowing concentration-response curves to be generated by the cumulative addition of a test agonist. Relaxation was expressed as a percentage of the contraction produced by 40 mM KCl. 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 competing 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) using paired tissues. For experiments testing for partial agonist activity, the  $pD_2$  value was calculated as negative log concentration that produced 50% of the drugs' maximum effect. However, for some drugs it was not possible to know whether that maximum effect was reached, as concentrations above 0.1 mM were not tested due to the limits of drug solubility. Thus, the 0.1 mM value was taken as the pseudo-maximum, and values from such experiments are presented as pseudo- $pD_2$  values. For those compounds that were dissolved in dimethylsulphoxide (DMSO), the equivalent volume of solvent used to attain the highest concentration of test compound also produced some relaxation in control tissues. Thus, the data are presented after subtraction of this effect.

The following drugs 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-carbamoyl-4-hydroxyphenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)-phenoxy]-2-propanol methanesulphonate) (Ciba-Geigy, Basel, Switzerland) and ICI 118 551 (erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamino)-butan-2-ol) (Zeneca Pharmaceuticals, Cheshire, U.K.) were dissolved in DMSO. (-)-Isoprenaline (Sigma Chemical Co., St. Louis, MO, U.S.A.) was dissolved in acidified water. (-)-Alprenolol (LabKemi, Stockholm, Sweden) and BRL 37344 (sodium 4-(2-[2-hydroxy-2-(3-chlorophenyl)-ethylamino] propyl) phenoxyacetate) (SmithKline Beecham Pharmaceuticals, Middlesex, U.K.) were dissolved in demineralized water. Pindolol (Sigma Chemical Co., St Louis, MO, U.S.A.), CYP and its analogues (synthesized in our laboratory) were dissolved in DMSO at a stock concentration of  $10^{-2}$  M, and diluted further in 50% DMSO and subsequently in demineralized water.

The following novel drugs were tested (Figure 1): ethylesterpindolol, iodoethylesterpindolol, *n*-butylcyanopindolol, iodobutylcyanopindolol, allylcyanopindolol, iodoallylcyanopindolol, cyclohexylcyanopindolol, iodocyclohexylcyanopindolol, *iso*-propylcyanopindolol and benzylcyanopindolol.

Log P values for CYP and its analogues were determined by their retention time on a high performance liquid chromatography column according to the method of Haky & Young (1984).

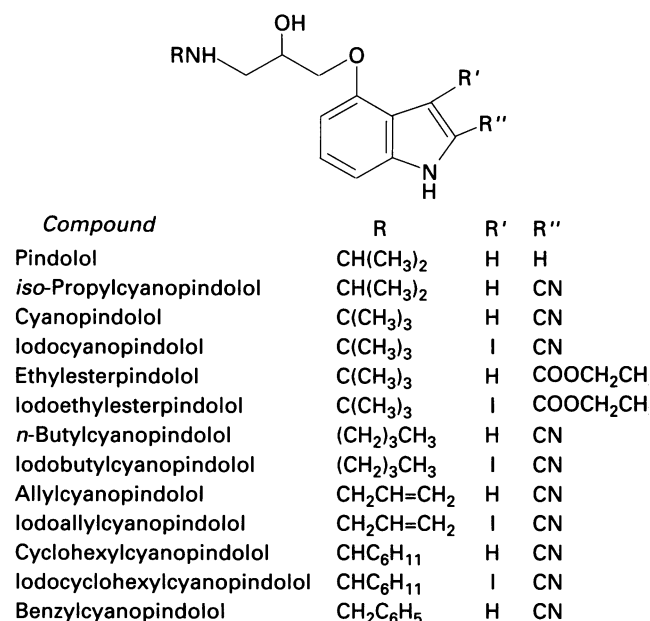
## Data analysis

Results are expressed as mean  $\pm$  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

**Table 1** Pharmacological values for compounds tested

Drug	Intrinsic activity	$pD_2$	$pK_b$ vs (-)Iso	$pK_b$ vs BRL	$pK_b$ - $pD_2$	log P
(-)-Isoprenaline	1.0	$7.76 \pm 0.14$				-0.33
BRL 37344	1.0	$8.35 \pm 0.04$				1.43
Pindolol	0	-	$6.68 \pm 0.1$			0.59
CYP	0.71	$5.28 \pm 0.26$	$7.59 \pm 0.07$	$7.20 \pm 0.22$	2.31***	1.08
Ethylesterpindolol	0.84	$6.22 \pm 0.23$	$7.36 \pm 0.09$	$6.81 \pm 0.16$	1.14***	1.61
<i>n</i> -ButylCYP	0.39	$4.81 \pm 0.26$	$6.50 \pm 0.05$		1.69***	1.43
AllylCYP	0.61	$4.96 \pm 0.21$	$6.34 \pm 0.15$		1.38	1.28
Iso-propylCYP	0.6	$5.68 \pm 0.47$	$7.19 \pm 0.12$	$6.4 \pm 0.23$	1.51**	0.94
IodoCYP	0.78	$7.00 \pm 0.26$	$7.59 \pm 0.11$	$7.21 \pm 0.14$	0.59	1.72
IodobutylCYP	0.84	$6.42 \pm 0.40$	$7.19 \pm 0.09$		0.77	2.06
IodoallylCYP	0.73	$5.66 \pm 0.38$	$6.89 \pm 0.10$		1.23**	1.85
BenzylCYP	0.58	$5.28 \pm 0.30$	$6.78 \pm 0.15$		1.50***	2.23
Iodoethylesterpindolol	0.57	$5.86 \pm 0.22$	$7.42 \pm 0.08$		1.56*	2.17
CyclohexylCYP	0.73	$4.92 \pm 0.14$	$6.45 \pm 0.17$		1.53**	1.85
IodocyclohexylCYP	0.73	$6.00 \pm 0.5$	$6.79 \pm 0.07$		0.79	2.44
(-)-Alprenolol	0.94	$4.80 \pm 0.12$	$7.22 \pm 0.09$		2.42***	2.44

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ,  $n = 4-5$  except for log P determinations. Values for ICI D7114, pindolol, CYP and iodoCYP from Hoey *et al.* (1996).



**Figure 1** Structure of pindolol, cyanopindolol and their analogues.

considered to be statistically significant. Linear regression analysis was used to determine if a significant correlation existed between  $\log P$  and  $pD_2$  values.

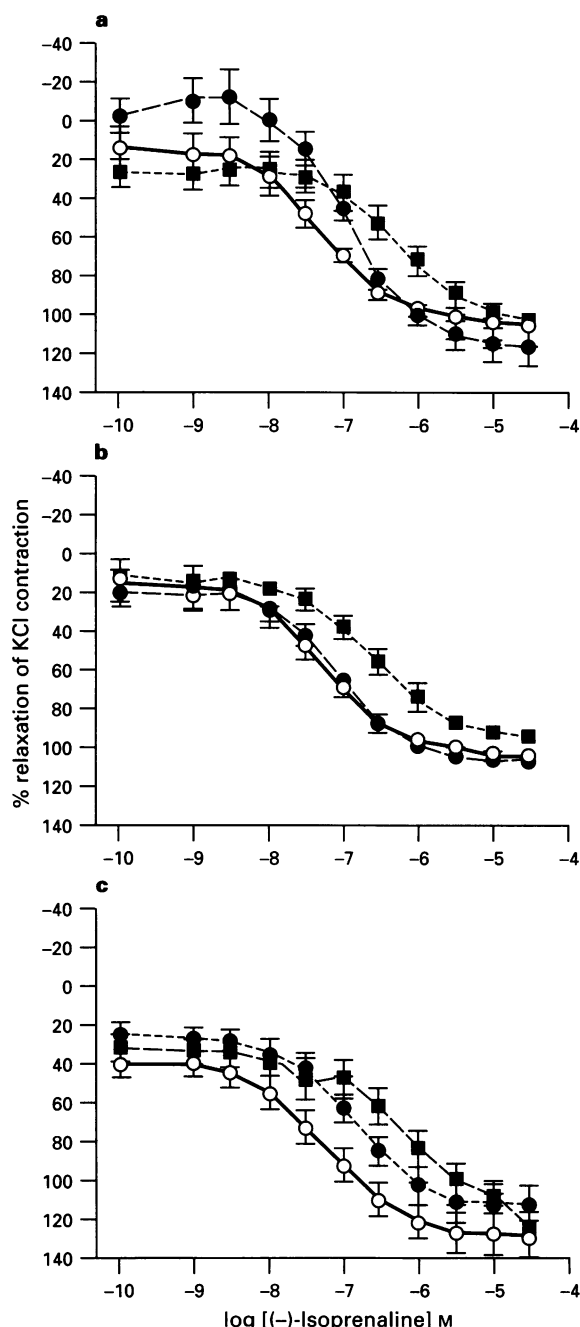
## Results

(-)-Isoprenaline produced relaxation of KCl-contracted rat ileum in which the  $\beta_1$ - and  $\beta_2$ -adrenoceptors were blocked. This effect was antagonized by CYP, its parent compound pindolol, and various analogues of these compounds (Table 1, Figure 2). The following order of potencies for antagonist effects was evident when (-)-isoprenaline was used as the agonist: iodoCYP = CYP > iodoethylesterpindolol = ethylesterpindolol > (-)-alprenolol = iodobutylCYP = *iso*-propylCYP > iodoallylCYP > iodocyclohexylCYP = benzylCYP > pindolol > *n*-butylCYP = cyclohexylCYP > allylCYP. The last three compounds all produced a concentration-ratio of two or less when tested at 0.3  $\mu$ M. Thus, these three compounds could be considered ineffective as antagonists against (-)-isoprenaline.

Several compounds that produced high  $pK_b$  values against (-)-isoprenaline were then examined at the same concentration (0.3  $\mu$ M) using BRL 37344 as a selective  $\beta_3$ -adrenoceptor agonist. The following order of potency was evident: iodoCYP = CYP > ethylesterpindolol > *iso*-propylCYP, and this did not differ from the rank order obtained using (-)-isoprenaline. However, *iso*-propylCYP produced a concentration-ratio of less than two and so could not be considered as an effective antagonist against BRL 37344.

The CYP analogues also produced some relaxation of the KCl-contracted rat ileum. The following order of potencies was evident for this effect (Figure 3): iodoCYP > iodobutylCYP > ethylesterpindolol > iodocyclohexylCYP > iodoethylesterpindolol > *iso*-propylCYP = iodoallylCYP > CYP = benzylCYP > allylCYP = cyclohexylCYP > *n*-butylCYP = (-)-alprenolol. No  $pD_2$  could be calculated for pindolol as it did not produce significant relaxation. This order was different to that observed for the antagonist effects at the  $\beta_3$ -adrenoceptor. The drug with the highest potency for relaxation (iodoCYP) was then tested in the presence of CYP (a compound with a high  $pK_b$  and low  $pD_2$ ). The relaxant effects of iodoCYP were not antagonized by CYP (Figure 4).

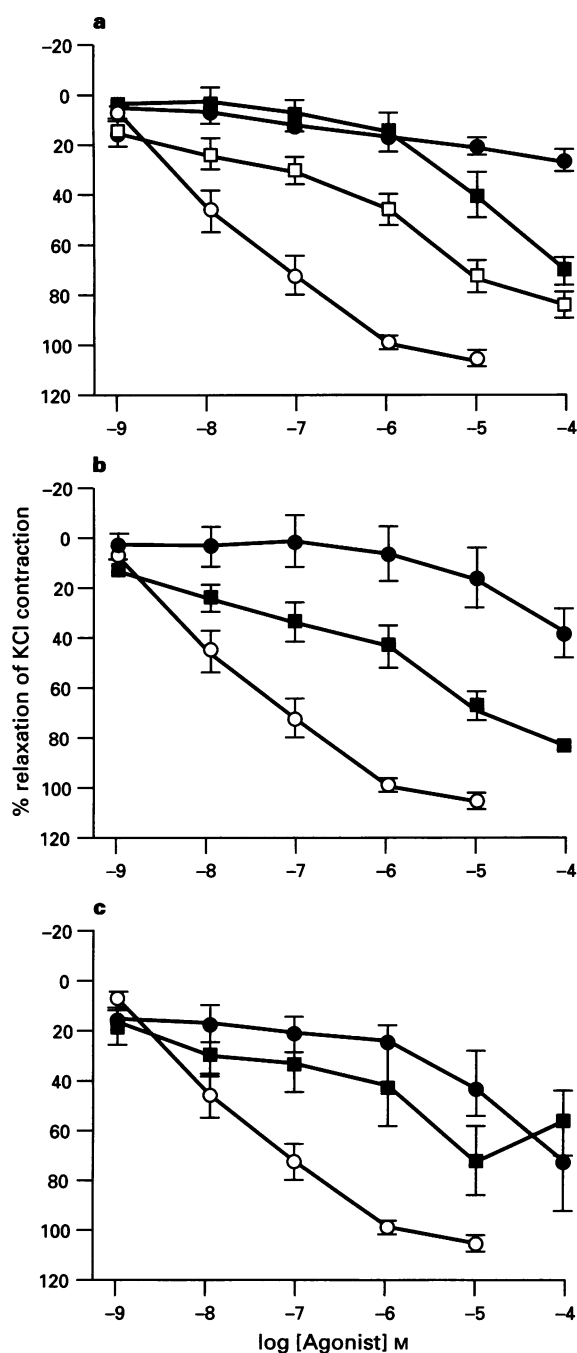
There was a statistically significant difference between the  $pD_2$  and the  $pK_b$  values (vs (-)-isoprenaline) for CYP, ethylesterpindolol, *n*-butylCYP, *iso*-propylCYP, iodoallylCYP,



**Figure 2** Concentration-response curves to (-)-isoprenaline (○) in the presence of 0.3  $\mu$ M antagonist: (a) pindolol (●), CYP (■); (b) *n*-butylCYP (●), iodobutylCYP (■); (c) cyclohexylCYP (●) iodoethylesterpindolol (■). Experiments were conducted in rat ileum precontracted with 40 mM KCl, and in the presence of 0.1  $\mu$ M CGP 20712A and ICI 118 551, 100  $\mu$ M corticosterone and 0.5  $\mu$ M atropine.  $n = 4$ .

benzylCYP, iodoethylesterpindolol, cyclohexylCYP and (-)-alprenolol. The levels of statistical significance for these differences are provided in Table 1.

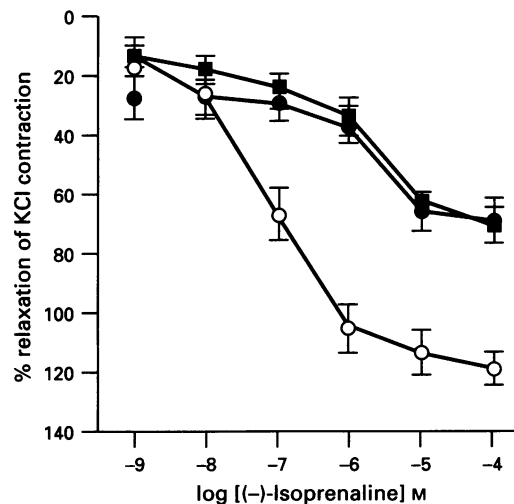
Lastly, the following order of  $\log P$  values was determined for the compounds tested (from the least to the most hydrophobic): pindolol < *iso*-propylCYP < CYP < allylCYP < *n*-butylCYP < ethylesterpindolol < iodoCYP < iodoallylCYP = cyclohexylCYP < iodobutylCYP < iodoethylesterpindolol < benzylCYP < iodocyclohexylCYP. No significant correlation was evident between the  $pD_2$  values and the  $\log P$  values obtained for CYP and its analogues.



**Figure 3** Concentration-response curve to (-)-isoprenaline (○) and test compounds: (a) pindolol (●), CYP (■), ethylesterpindolol (□); (b) *n*-butylCYP (●), iodobutylCYP (■); (c) cyclohexylCYP (●), iodoethylesterpindolol (■). Experiments were conducted in rat ileum precontracted with 40 mM KCl, in the presence of 0.1 μM CGP 20712A and ICI 118 551, 100 μM corticosterone and 0.5 μM atropine. *n* = 4 to 5.

## Discussion

Cyanopindolol and its analogues all acted as antagonists at the rat β<sub>3</sub>-adrenoceptor. However the potency of the analogues varied considerably with structural modifications at three different sites. The replacement of hydrogen with a cyano moiety at the indole 2-position (R''), changing pindolol to *iso*-propylcyanopindolol, resulted in an increase in potency from 6.68 ± 7.19 (*P* < 0.02). Thus, the presence of an electron-withdrawing group on the indole ring had a significant effect on binding affinity. Having established the importance of the 2-cyano moiety, we also investigated structural modification of the secondary amine moiety at the R position.



**Figure 4** Concentration-response curves to (-)-isoprenaline (○), iodoCYP alone (●), or iodoCYP in the presence of 0.3 μM CYP (■). Experiments were conducted in rat ileum precontracted with 40 mM KCl and in the presence of 0.1 μM CGP 20712A and ICI 118 551, 100 μM corticosterone and 0.5 μM atropine. *n* = 4.

Replacement of the *iso*-propyl moiety with a *t*-butyl group to produce CYP, resulted in a further increase in potency (*P* < 0.01). However, when the *t*-butyl moiety was replaced with a moiety of the same molecular weight, but in the form of the straight chain *n*-butyl, the antagonist activity fell to a negligible level. Similarly, the allyl amino analogue possessed negligible antagonist activity. The order of potency of compounds with the following groups at the secondary amine was: -C(CH<sub>3</sub>)<sub>3</sub> > -CH(CH<sub>3</sub>)<sub>2</sub> > -(CH<sub>2</sub>)<sub>3</sub>CH<sub>3</sub> = -CH<sub>2</sub>CH=CH<sub>2</sub>; indicating that as the level of substitution decreases adjacent to the secondary amino nitrogen, affinity is reduced. This highlights the necessity of a quaternary carbon atom, alpha to the secondary amine.

Analogous replacement of the *iso*-propyl moiety with a larger cyclohexyl moiety also caused a reduction in potency, probably due to excessive steric hindrance around the amino group. Replacement of the *iso*-propyl moiety with a benzyl moiety also reduced the potency, although this effect did not quite reach statistical significance (*P* = 0.08). The benzyl group would not be expected to sterically crowd the amino function as much as cyclohexyl, plus there is an additional methylene (-CH<sub>2</sub>) bridge between the nitrogen and the six-membered ring, allowing additional conformational flexibility.

With the establishment of CYP as the most potent antagonist developed thus far, the *t*-butyl moiety was left intact, and the cyano moiety was replaced with an ethylester group. This caused a non-significant fall (*P* = 0.08) in potency from 7.59 to 7.36. As discussed above, when comparing the potency of pindolol and *iso*-propylcyanopindolol, this suggests the importance of an electron withdrawing group at the R'' to increase potency. As both the cyano and the ethylester groups are electron withdrawing, substitution of either results in only a small change in potency, and we suggest it is unlikely that the size of this group is the primary determinant of affinity.

The iodination of CYP results in a significant increase in affinity for the β<sub>1</sub>- and β<sub>2</sub>-adrenoceptors, as well as at the propranolol-resistant (-)-[<sup>125</sup>I]-CYP binding site in rat soleus muscle (Roberts *et al.*, 1993). At the rat ileum β<sub>3</sub>-adrenoceptor, iodination resulted in a differentiation of the CYP analogues into two groups. Iodination of the almost inactive straight chain analogues, allylCYP and *n*-butylCYP, resulted in a significant increase in potency, while cyclohexylCYP also showed an increase in potency that was not significant (*P* = 0.10). In contrast, iodination of the more potent antagonists, CYP and ethylesterpindolol, had no effect on their potencies. This suggests that CYP and ethylesterpindolol are already structurally

optimized to block the rat ileum  $\beta_3$ -adrenoceptor, and therefore iodination does not produce any additional beneficial effect. The improvement in potency upon iodination of allylCYP, *n*-butylCYP and cyclohexylCYP is probably due to an electronic influence on the receptor binding characteristics of the indole ring, and may assist with interactions (e.g. pi bond stacking) of these analogues with aromatic groups present in the amino acids of the receptor. This effect must be sufficient partially to overcome the negative effects caused either by steric hindrance, or the lack of a quaternary carbon alpha to the secondary amine.

We also investigated the relaxant effects observed at high concentrations of CYP, which previously have been attributed to the drug acting as a partial agonist at the rat ileum  $\beta_3$ -adrenoceptor. The potencies and intrinsic activities of CYP analogues covered a broad range but the  $pD_2$  values were consistently lower than the  $pK_b$  values. For drugs that act as antagonists and partial agonists at the same receptor, these numbers should be roughly equal, as they are supposed to reflect 50% receptor occupancy. In fact, the  $EC_{50}$  for the relaxant effects of CYP was double that required to saturate the  $\beta_3$ -adrenoceptors, based on the  $pK_b$  for CYP determined against (-)-isoprenaline. While this observation strongly suggests that the relaxant effects are independent of the  $\beta_3$ -adrenoceptors, a common mechanism could still be argued if the  $pD_2$  values had been deceptively low because of physiological antagonism produced between the agonist and the contractile agent KCl (Roffel *et al.*, 1995). However, the observation that CYP did not antagonize the relaxant effects of iodoCYP at concentrations that did antagonize (-)-isoprenaline and BRL 37344, leads us to conclude that whereas the antagonist effects of CYP analogues are produced at the  $\beta_3$ -adrenoceptor, the relaxant effects are not.

The relaxant effect might have been caused through the activation of a sub-type of a 5-HT receptor, as it is known that pindolol can interact with 5-HT receptors (Moretti-Rojas *et al.*, 1983). It should be noted, however, that 5-HT itself causes

contraction of the rat ileum preparation rather than relaxation (Hoey *et al.*, 1996). The existence of an additional receptor does not exclude the possibility that some partial agonist effects may occur through the  $\beta_3$ -adrenoceptors, as cloned  $\beta_3$ -adrenoceptors expressed in CHO cells have been shown to be stimulated by CYP and pindolol (Blin *et al.*, 1994). It is also possible that the individual enantiomers of CYP and its analogues may act preferentially on different receptors, or that different isoforms of the  $\beta_3$ -adrenoceptor may exist (Arch, 1994). It has been recognized, for example, that (+)-pindolol acts as a partial agonist at the  $\beta_2$ -adrenoceptor (Kauman, 1989). Further studies using the individual enantiomers are in progress in our laboratories.

To examine the possibility that the partial agonist actions of the CYP analogues arise through some non-receptor-mediated mechanism, the log P values were calculated from the octanol:water partition coefficients. As all the compounds tested were of the same structural class, this provides a reliable indicator of relative lipid solubility. The log P values determined for the test compounds did not correlate with their  $pD_2$  values, indicating that lipid solubility alone is unlikely to be responsible for the relaxant effects. There may be other mechanisms through which the relaxant effects could be considered as non-receptor-mediated, and further investigations of reversibility, saturability and distribution are required to clarify this. Whatever the mechanism, more information on the relaxant effects of CYP analogues may help to dissociate these from their  $\beta_3$ -adrenoceptor effects, and could result in the development of more selective and useful antagonists.

In conclusion it appears that the relaxation of rat ileum can be produced through the activation of  $\beta_3$ -adrenoceptors, and through a second mechanism that is not yet understood. Further studies in rat ileum would benefit from the use of potent, enantiomerically pure compounds. The structural requirements for antagonist binding at the rat ileum  $\beta_3$ -adrenoceptor have been further elucidated, and these should assist in the development of potent antagonists at this site.

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