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Short communication

LK 204–545, a highly selective β_1-adrenoceptor antagonist at human β-adrenoceptors

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Abstract

LK 204–545 ((±)-1-(2-(3-(2-cyano-4-(2-cyclopropyl-methoxy-ethoxy)phenoxy)-2-hydroxy-propyl-amino)-ethyl)-3-(4-hydroxy-phenyl) urea), an antagonist that possesses high β_1-/β_2-selectivity in the rat, and a range of cardio-selective and non-selective β-adrenoceptor antagonists were examined to compare their radioligand binding affinities for human β_1-, β_2- and β_3-adrenoceptors transfected into CHO cells. LK 204–545 and CGP 20712A displayed the highest β_1-/β_2- (~1800 and ~650, respectively) and β_1-/β_3-selectivity (~17 000 and ~2200, respectively) at human β-adrenoceptors with LK 204–545 being ~2.75-fold more β_1-/β_2-selective and ~8-fold β_1-/β_3-selective than CGP 20712A. The high potency of LK 204–545 at transfected human β_1-adrenoceptors and in functional models of rat β_1-adrenoceptors together with its high selectivity, identify it as a useful ligand for studying β_1-adrenoceptors and suggest that it may be the preferred ligand for human β-adrenoceptor studies.

Keywords: β-Adrenoceptors antagonists; β_1-/β_2-Adrenoceptor selectivity; Binding affinity; Functional potency

1. Introduction

CGP 20712A (Table 1) has become the 'gold standard' for selective β_1-adrenoceptor antagonists and numerous studies have utilised its β_1-adrenoceptor-specific nature (Dooley et al., 1986). Other highly selective β_1-adrenoceptor antagonists have been identified, for example LK 204–545 (Table 1) (Berthold and Louis, 1984; Milavec-Krizman et al., 1985). This compound possesses similar β_1-/β_2-selectivities in the rat and guinea pig to CGP 20712A but little is known about the pharmacology at the three human β-adrenoceptors. We have determined the affinities of CGP 20712A and LK 204–545 and a range of other cardio-selective and non-selective β-adrenoceptor antagonists for human β_1-, β_2- and β_3-adrenoceptors utilising the radioligand (−)-[^{125}I]iodocyanopindolol (ICYP) and three Chinese hamster ovary (CHO) cell lines transfected with human β_1-, β_2- or β_3-adrenoceptors. We have also confirmed the functional potency of the antagonists for the rat β_1-, β_2- and β_3-adrenoceptors in studies utilising isolated tissue preparations.

2. Methods

The functional potencies of the antagonists for inhibiting (−)-isoprenaline-induced: chronotropic effects in isolated atria (β_1-adrenoceptor mediated) and relaxation of tracheal ring preparations precontracted with 1 μM carbachol (β_2-adrenoceptor mediated) were determined. Tissues were taken from male and female Sprague–Dawley rats (200–250 g) according to our method described previously (Tung et al., 1993). The antagonist was added at least 30 min after the first control concentration–response curve was completed and allowed to equilibrate for 15 min before the next concentration response curve was established. Lipolysis studies were conducted as described by Wilson (1984). Isolated epididymal white adipocytes (120–150 mg tissue ml^{-1}) were incubated in triplicate for 60 min at 37°C in modified Krebs bicarbonate buffer

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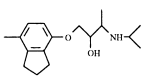
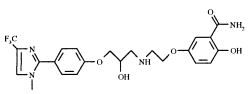
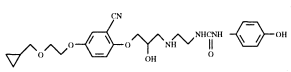
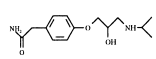
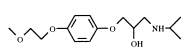
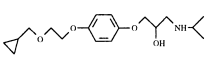
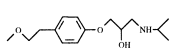
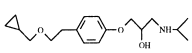
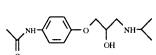
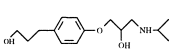
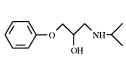
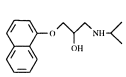
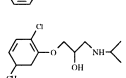
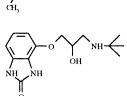
Table 1
Comparison of binding affinities for human β_1- and β_2-adrenoceptors and functional pontencies at rat β_1- and β_2-adrenoceptors

compound	structure	Human Binding			Rat Functional		
					atria	trachea	
		β_1-	β_2-	β_1-/β_2-	β_1-	β_2-	β_1-/β_2-
		pK_i	pK_i	*selectivity*	pA_2	pA_2	*selectivity*
ICI 118-551		7.38 ± 0.06	9.22 ± 0.21	0.01	6.92 ± 0.13	8.34 ± 0.11	0.04
CGP 20712A		8.48 ± 0.19	5.67 ± 0.10	646	8.52 ± 0.15	4.40 ± 0.11	13183
LK 204-545		8.52 ± 0.12	5.27 ± 0.08	1778	8.53 ± 0.08	4.73 ± 0.17	6310
atenolol		6.88 ± 0.11	5.55 ± 0.03	21	7.30 ± 0.12	5.91 ± 0.30	25
H87/07		7.00 ± 0.01	5.10 ± 0.09	79	7.37 ± 0.09	5.97 ± 0.19	25
cicloprolol		7.97 ± 0.03	6.21 ± 0.26	58	7.73 ± 0.07	5.44 ± 0.10	195
metoprolol		7.65 ± 0.13	6.29 ± 0.08	23	7.60 ± 0.17	6.43 ± 0.13	15
betaxolol		8.75 ± 0.11	7.15 ± 0.09	40	8.06 ± 0.15	6.38 ± 0.13	48
practolol		6.78 ± 0.05	5.17 ± 0.16	41	6.85 ± 0.06	5.76 ± 0.08	12
NIHP[a]		7.07 ± 0.14	5.98 ± 0.01	12	7.13 ± 0.10	6.58 ± 0.34	4
NIP[b]		8.42 ± 0.10	7.46 ± 0.18	9	8.09 ± 0.14	7.47 ± 0.14	4
propranolol		8.89 ± 0.11	9.20 ± 0.20	0.49	8.40 ± 0.32	8.13 ± 0.27	2
bupranolol		9.04 ± 0.10	9.09 ± 0.15	0.89	9.30 ± 0.15	8.44 ± 0.20	7
CGP12177		9.35 ± 0.28	9.44 ± 0.27	0.81	9.56 ± 0.18	8.42 ± 0.10	14

[a] *N*-isopropyl-4-hydroxypropylphenoxypropanolamine.
[b] *N*-isopropyl-phenoxypropanolamine.

supplemented with 3% BSA in the presence of increasing concentrations of BRL 35135 (β_3-agonist; Cawthorne et al.) and antagonist where appropriate to obtain a concentration–response curve. After centrifugation aliquots of supernatant were removed for estimation of glycerol content, determined by enzymatic assay in which the oxidation of glycerol and concomitant production of NADH + was followed spectrophotometrically at a wavelength of 340 nm (Garland and Randle, 1962). In all functional studies, at least three concentrations of each antagonist were examined to verify the antagonist potency. Concentration–response curves were expressed as a percentage of the maximum response by the agonist and plotted against the negative log (−log) molar concentration of agonist ([agonist]) and computer-fitted using the sigmoidal fit function of the graphical package Origin (Version 3.01; Micro Cal Origin, Micro Cal Software, USA). The −log [agonist that yielded 50% of the maximal response (i.e., EC_{50})] gave the pD_2 value for the agonist (Van Rossum et al., 1963) and potency of the antagonist (pA_2) was calcu-

lated according to the equation of MacKay (1978). Values given represent mean ± s.e.m. of 3–5 individual experiments.

Radioligand binding studies were conducted to determine the compounds affinities for human β-adrenoceptors. Three lines of CHO cells transfected with human β_1-, β_2- or β_3-adrenoceptors were kindly provided by the Institut Cochin de Genetique Moleculaire, Paris, France. Binding studies with CHO cell membranes were conducted as described by Blin et al. (1993). Cells were thawed as required and suspended in Hank's balanced salt solution supplemented with 1 mM ascorbic acid, pH 7.4 at 200–500 μg protein ml^{-1} for all studies. Aliquots of cells were incubated with 100 pM (−)-[^{125}I]-ICYP in the absence or presence of competitor, in a 200 μl final volume of buffer, for 45 min at 37°C in the dark. Saturation studies were performed with 0.5–500 pM [^{125}I]-ICYP for the β_1- and β_2-adrenoceptor cell lines and 1–3000 pM for the β_3-adrenoceptor cell line. Non-specific binding was determined in the presence of 2 μM (±)-propranolol for the β_1- and β_2-adrenoceptor cell lines and 100 μM (±)-bupranolol for the β_3-adrenoceptor cell line (Blin et al., 1993; Gros et al., 1998).

Binding data were analysed using the iterative curve fitting programs EBDA Version 4.0 which incorporates LIGAND Version 4.0 (Munson and Rodbard, 1980; McPherson, 1983). Inhibition constant (K_i) (drug inhibition studies) and binding density (B_{max}) values, are shown as mean ± s.e.m. of individual analyses of binding isotherms using LIGAND. Pseudo Hill coefficient (n_H) and IC_{50} values were obtained from analysis of binding data using the sigmoidal fit of the EBDA program. Values represent mean ± s.e.m. of 3–9 individual experiments.

The correlation of the human pK_is with the rat pA_2s for the β_1- and β_2-adrenoceptor subtypes was examined using the linear regression function in the computer program Minitab For Windows 32 Bit (Release 10.5 Xtra, Minitab, PA, USA). This package performed all necessary statistical tests and calculated the number of compounds (n), correlation coefficient (r^2), probability (P), standard deviation (s) and Fisher statistic (F) values. A $P < 0.05$ was considered to establish a statistically significant relationship between the parameters or combination of parameters examined. Due to the small number of compounds examined, the correlation between rat and human β_3-adrenoceptors was not examined. (−)-Isoprenaline, propranolol, ATP, NAD, glycerokinase and glycerodehydrogenase were purchased from Sigma (St. Louis, MO, USA). Collagenase (type II) was from Boehringer Mannheim (Sydney, Australia). ICYP was from Amersham (Buckinghamshire, UK) and BSA (fraction V) from Commonwealth Serum Laboratories (Melbourne, Australia). The following compounds were kindly donated: ICI 118–551 from ICI Pharmaceuticals (UK), CGP 20712A from Ciba-Geigy (Basel, Switzerland), BRL 35135 from SmithKline Beecham Pharmaceuticals (Surrey, UK) and H87/07 from Astra Pharmaceuticals (Brussels, Belgium). LK 204–545 ((±)-1-(2-(3-(2-cyano-4-(2-cyclopropylmethoxy-ethoxy)phenoxy)-2-hydroxy-propylamino)-ethyl)-3-(4-hydroxy-phenyl) urea), atenolol, cicloprolol, metoprolol, *N*-isopropyl-4-hydroxypropyl-phenoxypropanolamine, *N*-isopropyl-phenoxypropanol-amine and bupranolol were synthesised in our laboratory by Dr. D. Iakovidis. All compounds are enantiomeric mixtures unless otherwise stated and were checked by TLC, HPLC, NMR and mass spectroscopy and their physical characteristics were consistent with their chemical structures. All other chemicals were of reagent grade from BDH Chemicals (Kilsyth, Australia).

3. Results

The functional potency of the compounds (Table 1) was determined for inhibiting: (i) (−)-isoprenaline-stimulated β_1-adrenoceptor-mediated chronotropic effects in isolated spontaneously beating rat atria; and (ii) (−)-isoprenaline-stimulated β_2-adrenoceptor-mediated relaxation of rat tracheal chain preparations previously contracted with 1 μM carbachol. These studies allowed us to determine the potency of each of the compounds for rat β_1- and β_2-adrenoceptors. CGP 12177, bupranolol, LK 204–545, CGP 20712A and propranolol were the most potent compounds for rat β_1-adrenoceptors (pA_2 values > 8.10,; Table 1), while bupranolol, CGP 12177, ICI 118–551 and propranolol were the most potent compounds for rat β_2-adrenoceptors (pA_2 values ≥ 8.13; Table 1). In addition, we examined the potency and selectivity of the compounds for rat β_3-adrenoceptors by determining their ability to inhibit BRL 35135 (Table 2; Cawthorne et al., 1992) induced white fat lipolysis in the rat. Bupranolol and propranolol were the most potent β_3-adrenoceptor antagonists studied (pA_2 values ≥ 5.92; Table 2). It was difficult to determine the β_3-adrenoceptor pA_2 for CGP 12177 as it acted as a partial agonist in this system.

The dissociation constants (K_d) and maximal density of binding sites (B_{max}) of ICYP for the transfected human β_1-, β_2- and β_3-adrenoceptor subtypes were determined by saturation binding experiments. For human β_1-adrenoceptors, the K_d was 4.99 ± 0.48 pM and the B_{max} was 7127 ± 265 fmol mg $protein^{-1}$; for the β_2-adrenoceptor the K_d was 8.00 ± 1.10 pM and the B_{max} was 3914 ± 583 fmol mg $protein^{-1}$; and for the β_3-adrenoceptor the K_d was 313 ± 93.8 pM and the B_{max} was 2325 ± 322 fmol mg $protein^{-1}$. For all cell lines, Scatchard analysis gave a straight line ($n_H \approx 1.0$) consistent with the presence of a single β-adrenoceptor subtype.

Inhibition binding studies were used to determine the binding affinities of the compounds for the human β_1-, β_2- and β_3-adrenoceptors. The pK_is of the compounds for displacing ICYP binding from the human β-adrenoceptors are given in Tables 1 and 2. As expected, the compounds

Table 2
Comparison of binding affinities for human β_3-adrenoceptors and functional potencies at rat β_3-adrenoceptors

Compound	Human binding		Rat functional	
Antagonists	β_3-pK_i	β_1-/β_3-selectivity	β_3-pA_2	β_1-/β_3-selectivity
ICI 118–551	5.83 ± 0.02	35	5.10 ± 0.10	66
CGP 20712A	5.14 ± 0.13	2188	< 4	> 33 113
LK 204–545	4.29 ± 0.05	16 982	< 4	> 33 884
Atenolol	3.63 ± 0.23	1778	4.94 ± 0.14	229
Propranolol	6.28 ± 0.16	407	5.92 ± 0.24	302
Bupranolol	7.28 ± 0.14	58	6.98 ± 0.24	209
CGP12177	7.26 ± 0.03	123	partial agonist	partial agonist

displaced the binding from a single binding site population in each of the cell lines (Hill slopes, $n_H \cong 1.0$). CGP 12177, bupranolol, propranolol, LK 204–545, CGP 20712A and *N*-isopropylphenoxypropanolamine displayed the highest affinities for human β_1-adrenoceptors, while CGP 12177, propranolol, ICI 118–551 and bupranolol had the highest affinities for human β_2-adrenoceptors and bupranolol, CGP 12177 and propranolol had the highest affinities for human β_3-adrenoceptors.

The β_1-/β_2- and β_1-/β_3-selectivity ratios for rat and human β-adrenoceptors are also shown in Tables 1 and 2. By far the most β_1-selective compounds in the rat and human were LK 204–545 and CGP 20712A. Interestingly, β_1-selectivity is noticeably lower for a number of compounds at human β-adrenoceptors, particularly CGP 20712A (Tables 1 and 2). LK 204–545, however, remained highly β_1-selective at human receptors (β_1-/β_2-selectivity = ~ 1800 and β_1-/β_3-selectivity = ~ 17 000) and was ~ 2.75-fold more β_1-/β_2-selective and ~ 8-fold more β_1-/β_3-selective than CGP 20712A (Tables 1 and 2).

4. Discussion

The affinities of the compounds confirm that in the rat, as in the guinea pig (Milavec-Krizman et al., 1985; Dooley et al., 1986), LK 204–545 and CGP 20712A are potent and highly selective β_1-adrenoceptor antagonists (β_1-/β_2-selectivity ratios ranging from ~ 6300 to 13 200 and β_1-/β_3-selectivities in excess of 33 000; Tables 1 and 2); propranolol, bupranolol, practolol, *N*-isopropyl-4-hydroxypropylphenoxypropanolamine and *N*-isopropyl-phenoxypropanolamine were relatively non-selective (β_1-/β_2-selectivity ratios ranging from 2–7; Table 1); ICI 118–551 was relatively β_2-selective (β_1-/β_2-selectivity ratio = 0.04; Table 1); while all the other compounds tested were relatively β_1-selective (β_1-/β_2-selectivity ratios ranging from 12 to 195). None of the compounds examined were β_3-selective.

In our CHO cells transfected with the human β_1- and β_2-adrenoceptors, the binding affinities of atenolol, metoprolol, betaxolol and practolol correlate with previously published β_1- ($P = 0.03$) and β_2-adrenoceptor ($P = 0.03$) binding affinities in human lung tissue (Engel, 1981). Similarly, the binding affinities of ICI 118–551, CGP 20712A, propranolol, bupranolol and CGP 12177 for human β_1-, β_2- and β_3-adrenoceptors correlate with their affinities at human β_1- ($P = 0.04$), β_2- ($P = 0.01$) and β_3-adrenoceptors ($P = 0.04$) as determined by Blin et al. (1993) in the same transfected CHO cell system. The binding affinities of all the compounds for human β_1-adrenoceptors were similar to their potencies determined in rat atria ($pK_i^{(human\beta_1\text{-}adrenoceptor)} = -0.33 + 0.97 \times pA_2^{(rat\beta_1\text{-}adrenoceptor)}$; $n = 14$; $r^2 = 0.85$; $F = 69.83$; $P < 0.0001$) as were the human β_2-adrenoceptor affinities and the potencies determined in rat trachea ($pK_i^{(human\beta_2\text{-}adrenoceptor)} = -0.42 + 1.11 \times pA_2^{(rat\beta_2\text{-}adrenoceptor)}$; $n = 14$; $r^2 = 0.80$; $F = 47.65$; $P < 0.0001$).

LK 204–545 showed the highest β_1-adrenoceptor selectivity (β_1-/β_2- and β_1-/β_3-selectivity ratios = 1800 and ~ 17 000, respectively; Tables 1 and 2) for transfected human β-adrenoceptors and was the most potent of the β_1-selective compounds tested. CGP 20712A displayed much lower β_1-selectivity for transfected human receptors (~ 15–20-fold lower than in the rat functional studies) which resulted from a ~ 19-fold higher affinity at human β_2- and a > 13-fold higher affinity at human β_3-adrenoceptors compared to the rat functional studies (Tables 1 and 2). By contrast, H87/07, metoprolol, practolol, *N*-isopropyl-4-hydroxypropylphenoxypropanol-amine and *N*-isopropyl-phenoxypropanolamine displayed 1.5–8-fold higher β_1-/β_2-selectivity at human receptors compared to the rat (Table 1), while ICI 118–551 was slightly more β_2-selective at human β_1- and β_2-adrenoceptors (β_1-/β_2-selectivity ratios = 0.04 in rat and 0.01 in man; Table 1) while having similar β_1-/β_3-selectivities in both species (Tables 1 and 2). The other compounds tested displayed similar or slightly higher β_1-selectivities in the rat studies.

The data suggests, as has been reported for β_3-adrenoceptors (Liggett, 1992; Blin et al., 1994) differences in ligand recognition may also exist between the two species for β_1- and β_2-adrenoceptors. Conformation of the species differences, however, requires a comparison of the binding properties of a larger number of compounds in cell lines

transfected with each of the human and rat β-adrenoceptor subtypes. The high potency and β_1-specificity of LK 204–545 in both species contrasts with the lower specificity of CGP 20712A for transfected β_1-adrenoceptors and make it a satisfactory reference compound when a highly β_1-selective agent is required and suggest that it may be the preferred agent when studying human β-adrenoceptor subtypes.

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