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HUMAN β_1- AND β_2-ADRENERGIC RECEPTOR BINDING AND MEDIATED ACCUMULATION OF cAMP IN TRANSFECTED CHINESE HAMSTER OVARY CELLS

PROFILE OF NEBIVOLOL AND KNOWN β-ADRENERGIC BLOCKERS

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Abstract—The interaction of nebivolol and its SRRR and RSSS enantiomers, and of known β-adrenergic blockers, with human β_1- and β_2-adrenergic receptors expressed separately in Chinese hamster ovary cells in culture (CHO-Huβ_1 and CHO-Huβ_2), was investigated. We studied [^{3}H]CGP-12177 binding to the intact cells and the accumulation of cAMP induced by isoproterenol. Each of the receptor subtypes displayed saturable [^{3}H]CGP-12177 binding on intact cells with sub-nanomolar affinity. The density of β_1- and β_2-adrenergic receptor sites was 1.1×10^6 receptor binding sites per CHO-Huβ_1 cell and 0.2×10^6 receptor binding sites per CHO-Huβ_2 cell, respectively. The β-adrenergic antagonists CGP 20712-A, ICI 118-551 and propranolol showed the same binding properties as β-adrenergic receptors in previously described tissues or cells. The potencies of these compounds in inhibiting β-adrenergic receptor mediated accumulation of cAMP corresponded well with their binding affinities. *d*-Nebivolol (SRRR) and nebivolol showed combined high affinity and selectivity for inhibition of β_1-adrenergic receptor coupled accumulation of cAMP in CHO-Huβ_1 cells (0.41 and 0.42 nM for *d*-nebivolol and nebivolol, respectively). *l*-Nebivolol (RSSS) was 1460 times less potent than *d*-nebivolol in CHO-Huβ_1 cells. The binding affinities of *d*-nebivolol and nebivolol for human β_1-adrenergic binding sites correlated well with their potencies in inhibiting β_1-adrenergic receptor coupled accumulation of cAMP. CHO cells transfected with human β_1- and β_2-adrenergic receptors are a valid model system for studying the interaction of compounds with human β-adrenergic receptors.

Nebivolol, the racemic mixture of the SRRR and RSSS enantiomers, is being investigated as a new anti-hypertensive agent. Clinical and *in vivo* pharmacological studies with nebivolol revealed an interesting haemodynamic profile, different from that of classical β-adrenergic antagonists. Nebivolol improves left ventricular function at rest and the negative influence of classical β-blockade on myocardial contractility during exercise [1–5]. The particular haemodynamic profile was obtained specifically with nebivolol whereas *d*-nebivolol (SRRR) showed the activities of a typical β-adrenergic receptor antagonist. The specific properties of nebivolol seem to result from the combined activities of the two enantiomers.

The biochemical mechanisms underlying the *in vivo* functional effects of nebivolol that distinguish it from other β-adrenergic antagonists are not clear. *In vitro* receptor binding studies have shown that nebivolol revealed high affinity and selectivity, and a slow dissociation for the β_1-adrenergic receptor sites in the β_1-adrenergic rabbit lung membrane preparation [6]. The β-adrenergic activity of nebivolol resided in the SRRR enantiomer (*d*-nebivolol); the RSSS enantiomer (*l*-nebivolol) had a β_1-adrenergic receptor affinity 175 times lower. β_1-Adrenergic receptor mediated accumulation of cAMP in rat neonatal cardiac cell cultures was inhibited by nebivolol and its SRRR-enantiomer. *l*-Nebivolol (RSSS) showed weak β-adrenergic receptor activity [7].

The genes for human β_1- and β_2-adrenergic receptors have been cloned and expressed in *Xenopus laevis* oocytes [8], *Escherichia coli* [9, 10] and mammalian cell lines [11, 12]. Using this technology, the interaction of drugs with human β_1- and β_2-adrenergic receptors can easily be studied. In this study, nebivolol and its enantiomers were compared to the β_1-adrenergic receptor blocker CGP 20712-A, the β_2-adrenergic receptor blocker ICI 118-551 and the non-selective β-adrenergic receptor blocker propranolol in their interaction with human β_1- and β_2-adrenergic receptors expressed in Chinese hamster ovary (CHO) cells. CHO cells transfected with cloned human β_1- and β_2-adrenergic receptor genes were obtained commercially [13]. We studied radioligand binding to intact cells using [^{3}H]CGP-12177 and isoproterenol induced accumulation of cAMP. The binding affinities of the β-adrenergic antagonists for human β_1- and β_2-adrenergic receptor sites were compared with their potencies in inhibiting β-adrenergic receptor mediated accumulation of cAMP.

MATERIALS AND METHODS

Cell culture. Chinese hamster ovary cells, expressing human β_1- and β_2-adrenergic receptors (CHO-Huβ_1 and CHO-Huβ_2), were cultivated in nutrient mixture Ham's F12 supplemented with

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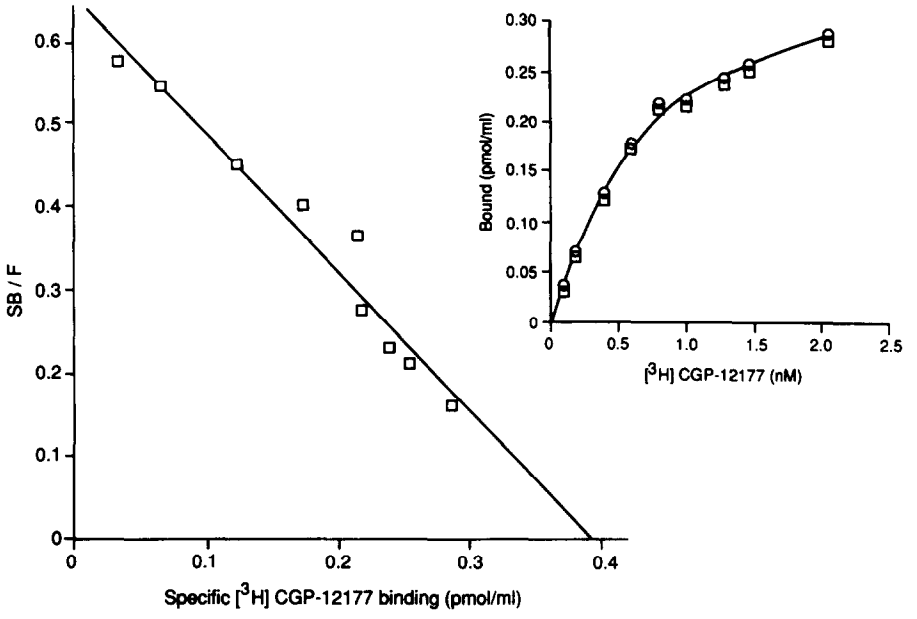

Fig. 1. Saturation binding curve (inset) and Scatchard plot of [^{3}H]CGP-12177 binding to β_1-adrenergic receptor sites on CHO-Huβ_1 cells. Binding assays were performed as described in Materials and Methods with 0.3×10^6 cells per assay. Non-specific binding was defined in the presence of 1 μM propranolol; (○) total binding, (□) specific binding. Curves were constructed using mean values of binding data from a representative experiment. SB, specific [^{3}H]CGP-12177 binding, total bound [^{3}H]CGP-12177 minus non-specifically bound. F, free [^{3}H]CGP-12177 concentration calculated as the added concentration of [^{3}H]CGP-12177 minus the total concentration bound. K_d value was given by the reciprocal value of the slope of the lines. B_{max} value was given by the interception point with the abscissa (in pmol/mL). Lines were calculated using the method of least squares. Values are presented in Table 1.

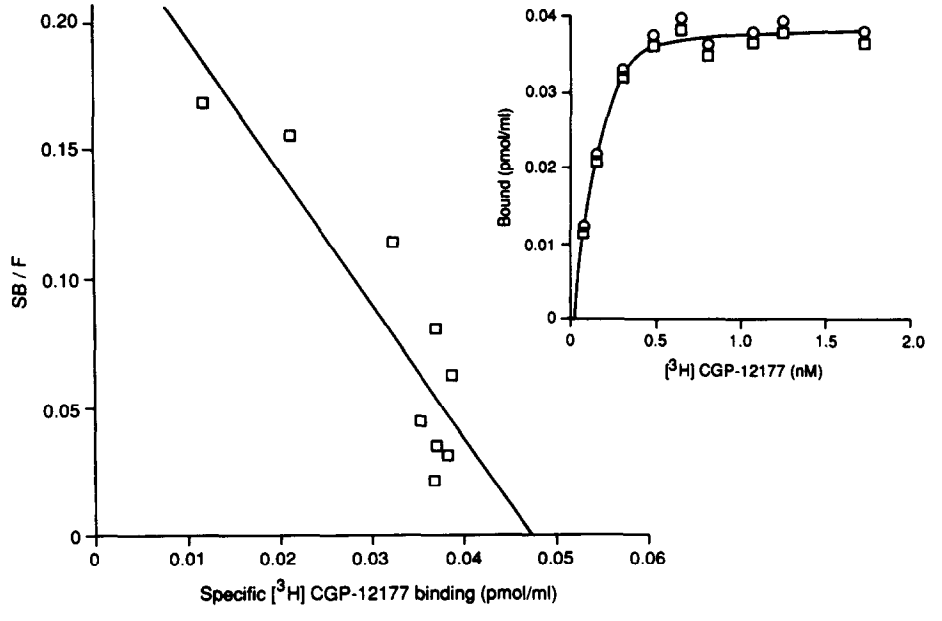

Fig. 2. Saturation binding curve (inset) and Scatchard plot of [^{3}H]CGP-12177 binding to β_2-adrenergic receptor sites on CHO-Huβ_2 cells. Binding was carried out as described in the legend to Fig. 1 with 0.2×10^6 cells per assay. (○) Total binding, (□) specific binding. Derived K_d and B_{max} values are presented in Table 1.

Table 1. K_d and B_{max} values of [^{3}H]CGP-12177 binding to human β_1- and β_2-adrenergic receptor sites on intact CHO-Huβ_1 and CHO-Huβ_2 cells

	[^{3}H]CGP-12177	
	CHO-Huβ_1	CHO-Huβ_2
K_d (nM)	0.79 ± 0.27	0.32 ± 0.15
B_{max}		
pmol/mg protein	8.00 ± 3.58	1.43 ± 0.31
pmol/10^6 cells	1.87 ± 0.51	0.37 ± 0.09
Number of receptors/intact cell	(1.1 ± 0.3) 10^6	(0.22 ± 0.06) 10^6

K_d and B_{max} values are the means ± SD of values obtained in three separate experiments.

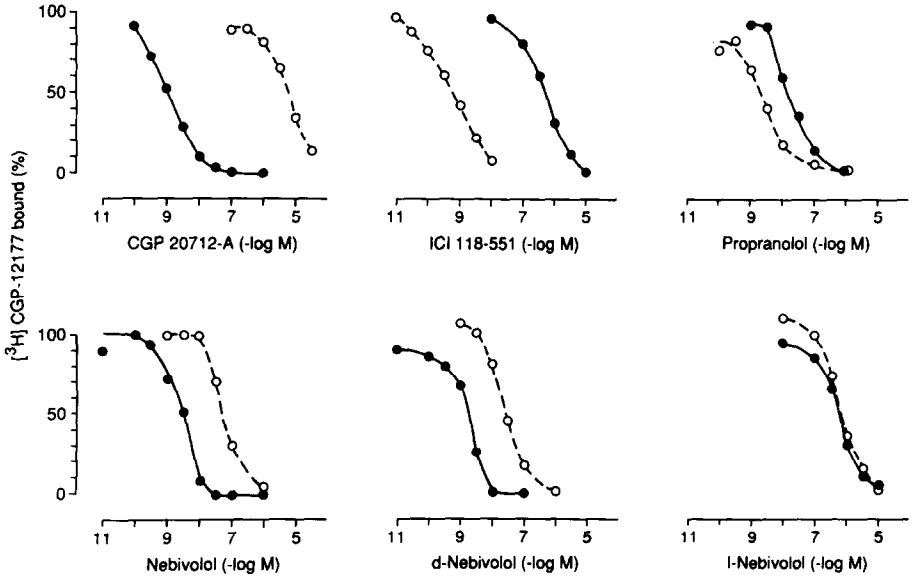

Fig. 3. Inhibition of [^{3}H]CGP-12177 binding to β_1- and β_2-adrenergic receptor sites on CHO-Huβ_1 and CHO-Huβ_2 cells by CGP 20712-A, ICI 118-551, propranolol, nebivolol, *d*-nebivolol and *l*-nebivolol. CHO-Huβ_1; total binding, 9439 ± 792 to 19,739 ± 1610 dpm/well; and non-specific binding, 401 ± 57 to 1225 ± 347 dpm/well (three separate experiments). CHO-Huβ_2; total binding, 2959 ± 281 to 7803 ± 1258 dpm/well; and non-specific binding, 148 ± 40 to 434 ± 34 dpm/well (three separate experiments). [^{3}H]CGP-12177 binding is expressed as percentage of specifically bound, total bound [^{3}H]CGP-12177 minus non-specifically bound. Curves were constructed using mean values from two separate experiments performed in quadruplicate. (●) CHO-Huβ_1, (○) CHO-Huβ_2.

2 mM glutamine, 1 mM pyruvate and 10% heat-inactivated foetal calf serum. Subcultures were made by using 0.025% trypsin in phosphate buffered salt solution. The split rate was 1 to 6. A new cell line was started from a frozen stock after the sixth subculture.

Experiments were carried out with cultures in 24-well culture plates (Nunc) with 1.0 mL medium/well (surface: 1.8 cm^2). After 4–5 days, confluent cultures 2–3 × 10^5 cells/well) were washed twice with CSS buffer (120 mM NaCl, 5.4 mM KCl, 0.8 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM glucose, 25 mM Tris–HCl, pH 7.4) and used for binding assays and accumulation of cAMP.

Binding assays to β_1- and β_2-adrenergic receptor sites on intact CHO-Huβ_1 and CHO-Huβ_2 cells. Cultures were exposed to 1.0 mL CSS buffer with 1 nM [^{3}H]CGP-12177 in the absence (total binding) or in the presence of 1 μM propranolol (nonspecific binding). The incubation was run for 30 min at 37°. The incubation was stopped by washing the cultures three times with 1.0 mL ice-cold CSS buffer. The cells were lysed by collecting them in 1.0 mL 0.2 N NaOH. For quantifying [^{3}H]CGP-12177 binding, 1.0 mL of the cell extract was mixed with 10 mL Instagel II and this mixture was counted in a Packard Tricarb liquid scintillation counter. Specific binding of [^{3}H]CGP-12177 was defined as the portion of total binding which was inhibited by 1 μM propranolol. To measure potencies of drugs in inhibiting binding, the drugs were added to the incubation mixture at six to eight concentrations between 10^{-10} and 10^{-5} M. Data were analysed graphically with inhibition curves and IC_{50} values

Table 2. Apparent equilibrium inhibition constants (K_i values) of drugs for inhibition of [^{3}H]CGP-12177 binding to human β_1- and β_2-adrenergic receptor sites on intact CHO-HUβ_1 and CHO-Huβ_2 cells, and potencies of drugs (IC_{50} values) in inhibiting accumulation of cAMP induced by 10 nM isoproterenol in CHO-Huβ_1 and CHO-Huβ_2 cells

	Mean K_i values (nM) for inhibition of [^{3}H]CGP-12177 binding		Ratio of β_2 over β_1 K_i value	Mean IC_{50} values (nM) for inhibition of isoproterenol-induced cAMP accumulation		Ratio of β_2 over β_1 IC_{50} value
	CHO-Huβ_1	CHO-Huβ_2		CHO-Huβ_1	CHO-Huβ_2	
CGP 20712A	0.60 ± 0.16	1503.0 ± 582.8	2505.0	3.9 ± 1.1	1000	256.4
ICI 118-551	198.2 ± 31.8	0.14 ± 0.00	0.00071	515 ± 115	0.28 ± 0.18	0.00054
Propranolol	7.5 ± 1.9	0.53 ± 0.12	0.07	16 ± 9	0.63 ± 0.38	0.04
Nebivolol	1.2 ± 0.5	12.2 ± 1.9	10.2	0.42 ± 0.22	89.5 ± 10.5	213
d-Nebivolol (SRRR)	0.72 ± 0.23	5.6 ± 1.7	7.8	0.41 ± 0.09	36.7 ± 8.5	89.5
l-Nebivolol (RSSS)	200.8 ± 65.5	139.4 ± 77.1	0.69	600 ± 100	750 ± 40	1.25

K_i and IC_{50} values are the means ± SD of values obtained in two to three separate experiments.

(concentration of the drug producing 50% inhibition of specific binding) were derived. K_i values were calculated according to the equation:

$$K_i = IC_{50}/(1 + C/K_d)$$

with C the concentration and K_d the equilibrium dissociation constant of the [^{3}H]ligand. To investigate concentration binding curves of [^{3}H]CGP-12177, concentrations ranging from 0.1 to 2 nM were used. Data were analysed in Scatchard plots. The line of best fit was calculated by linear regression using the method of least squares.

β-adrenergic receptor mediated accumulation of cAMP in intact CHO-Huβ$_1$ and CHO-Huβ$_2$ cells. Cultures were loaded with 2.0 μCi [^{3}H]adenine in 0.5 mL CSS buffer/well and incubated for 120 min at 37°. The cultures were washed with 1.0 mL CSS buffer and incubated in 1.0 mL CSS buffer for 20 min with 10 nM isoproterenol in the presence of 0.5 mM isobutylmethylxanthine. Basal accumulation of cAMP was measured in the absence of isoproterenol. The reaction was stopped by the addition of 0.1 mL ice-cold $HClO_4$ to a final concentration of 0.1 N. The extract was neutralized with 0.5 M KH_2PO_4/K_2HPO_4 buffer (pH 7.4) for assay of the [^{3}H]cAMP content. The latter was performed as described by Salomon *et al.* [14]. The neutralized extract was sequentially passed over Dowex 50W-X4 (200–400 mesh) and aluminum oxide columns eluted with water and 0.1 M imidazole (pH 7.4), as described previously [7]. The cAMP eluate was mixed with 10 mL Pico-aqua and the mixture was counted in a Packard Tricarb liquid scintillation counter. To measure potencies of drugs in inhibiting β-adrenergic receptor coupled accumulation of cAMP, the drugs were preincubated 15 min before 10 nM isoproterenol was added. IC_{50} values (concentration of the drug inhibiting 50% of accumulation of cAMP induced by 10 nM isoproterenol) were derived graphically. To measure the effect of the compounds on basal accumulation of cAMP, the cultures were incubated for 20 min in the presence of 10^{-9} or 10^{-6} M of the compound.

Protein content. Protein was estimated with the dye-binding assay using the Bio-Rad kit [15]. Bovine serum albumin was used as a standard.

Materials. Chinese hamster ovary (CHO) cells, deficient in dihydrofolate-reductase (DHFR) and transfected separately with cloned human β_1- and β_2-adrenergic receptor genes [13] were obtained commercially from the Centre National de Recherche Scientific (Paris, France). Nutrient mixture Ham's F12, foetal calf serum and 24-well tissue culture plates were obtained from Gibco Biocult. Laboratories (Paisley, U.K.). Dowex 50W-X4 (200–400 mesh) was from Serva (Westbury, NY, U.S.A.). Aluminum oxide was from Merck (Darmstadt, F.R.G.). [^{3}H]-CGP-12177 (34 Ci/mmol) was from Amersham (Amersham, U.K.). [^{3}H]Adenine (5 Ci/mmol) was from New England Nuclear (Dreieich, Germany). The drugs were kindly provided by the companies of origin. The stock solutions of drugs were prepared in 100% ethanol. Dilutions were made in 0.1% hydroxypropyl-β-cyclodextrin (Janssen Biotech, Olen, Belgium).

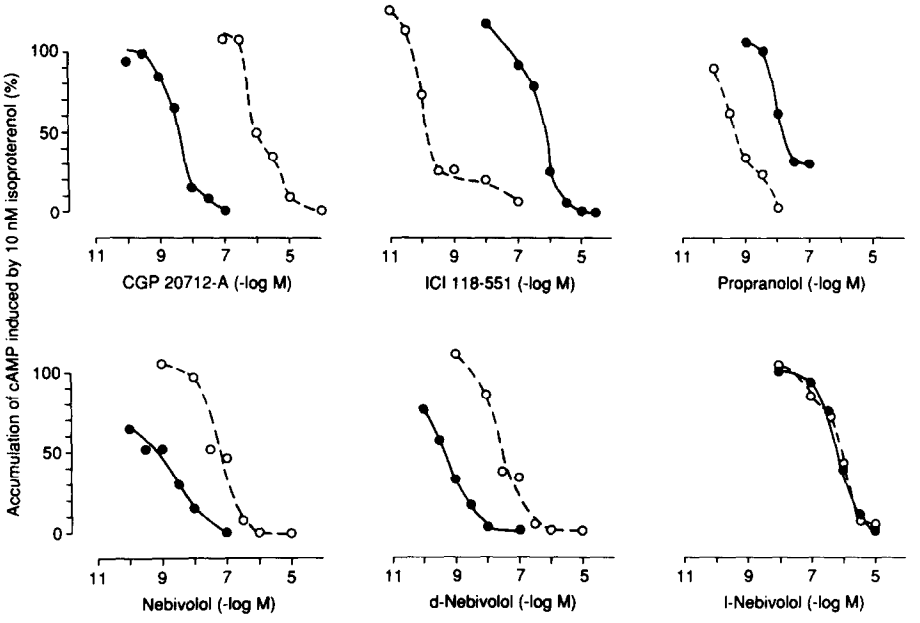

Fig. 4. Inhibition of the accumulation of cAMP induced by 10 nM isoproterenol in CHO-Huβ_1 and CHO-Huβ_2 cells by CGP 20712-A, ICI 118-551, propranolol, nebivolol, *d*-nebivolol and *l*-nebivolol. Cells were preincubated for 15 min with drug before exposure to 10 nM isoproterenol for 20 min as described in Materials and Methods. CHO-Huβ_1; basal accumulation of cAMP, 5027 ± 486 to 8241 ± 2383 dpm/well; and accumulation of cAMP induced by 10 nM isoproterenol, 46,194 ± 2908 to 86,523 ± 5976 dpm/well (three separate experiments). CHO-Huβ_2; basal accumulation of cAMP, 668 ± 221 to 4514 ± 1782 dpm/well; and accumulation of cAMP induced by 10 nM isoproterenol, 7921 ± 2677 to 39,301 ± 451 dpm/well (three separate experiments). Accumulation of cAMP is expressed as percentage of accumulation of cAMP induced specifically by 10 nM isoproterenol, obtained by subtraction of basal accumulation of cAMP. Curves were constructed using mean values from two separate experiments performed in quadruplicate. (●) CHO-Huβ_1, (○) CHO-Huβ_2.

RESULTS

Figures 1 and 2 show the saturation binding curves for the binding of [^{3}H]CGP-12177 to intact CHO-Huβ_1 and CHO-HUβ_2 cells. Scatchard analysis revealed a single population of binding sites in each of the transfected cells. K_d and B_{max} values for [^{3}H]-CGP-12177 binding are summarized in Table 1. [^{3}H]-CGP-12177 bound with sub-nanomolar affinity to β_1- and β_2-adrenergic receptor sites, the affinity to the β_2-adrenergic receptor being 2.5 times higher. The density of β_1-adrenergic receptor sites on CHO-Huβ_1 cells was 5 times higher than the density of β_2-adrenergic receptor sites on CHO-Huβ_2 cells.

A series of β-adrenergic antagonists was tested to assess the characteristics of the two receptor subtypes. Figure 3 shows the inhibition curves of CGP 20712-A, ICI 118-551, propranolol and nebivolol and its *d*-enantiomer (SRRR) and *l*-enantiomer (RSSS) on [^{3}H]CGP-12177 binding to intact CHO-Huβ_1 and CHO-Huβ_2 cells. With CHO-Huβ_1 cells, CGP 20712-A showed a monophasic inhibition curve and inhibited 50% of [^{3}H]CGP-12177 binding at 0.6 nM. *d*-Nebivolol was as potent as CGP 20712-A while nebivolol and *l*-nebivolol were two and 325 times less active. ICI 118-551 inhibited at micromolar concentrations [^{3}H]CGP-12177 binding to CHO-Huβ_1 cells. Propranolol bound to human β_1- and β_2-adrenergic receptor sites, the affinity to the β_2-adrenergic receptor in CHO-Huβ_2 cells being 14 times higher. ICI 118-551 inhibited at sub-nanomolar concentrations [^{3}H]CGP-12177 binding to CHO-Huβ_2 cells. In these transfected cells, CGP 20712-A was 10,700 times less active than ICI 118-551. *d*-Nebivolol and nebivolol were 40–87 times less potent than ICI 118-551 whereas *l*-nebivolol was still 11 times less active. The β-adrenergic receptor binding affinity and selectivity of these compounds for β_1- and β_2-adrenergic receptor sites is summarized in Table 2.

β_1- and β_2-adrenergic receptor sites were found to be functionally coupled to adenylyl cyclase in transfected CHO cells [13]. In the presence of 10 nM isoproterenol, accumulation of cAMP was increased about 10-fold in CHO-Huβ_1 as well as in CHO-Huβ_2 cells. Its inhibition by β-adrenergic blockers is shown in Fig. 4. Accumulation of cAMP induced by 10 nM isoproterenol in CHO-Huβ_1 cells was inhibited potently by nebivolol, *d*-nebivolol, CGP 20712-A and propranolol with IC_{50} values ranging from 0.41 to 16 nM (Table 2). *l*-Nebivolol and ICI 118-551 were weak inhibitors. In CHO-Huβ_2 cells, the compounds were in the following rank order for potency of inhibition of β_2-adrenergic coupled production of cAMP: ICI 118-551 > propranolol > *d*-nebivolol > nebivolol > *l*-nebivolol > CGP 20712-A. The corresponding IC_{50} values varied between

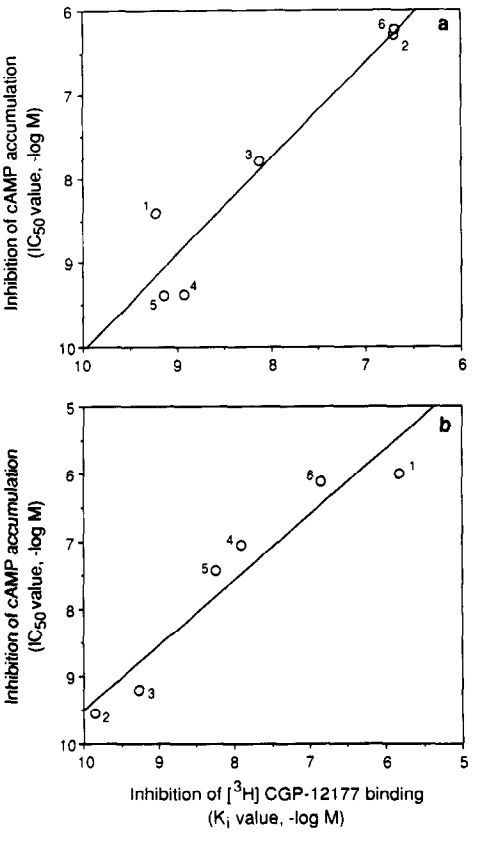

Fig. 5. (a) Correlation between K_i-values of drugs for inhibition of [^{3}H]CGP-12177 binding to human β_1-adrenergic receptor sites on intact CHO-Huβ_1 cells and potencies of drugs (IC_{50}-values) to inhibit accumulation of cAMP induced by 10 nM isoproterenol in CHO-Huβ_1 cells. Mean K_i and IC_{50}-values from Table 2 were plotted. The correlation between the K_i-values and IC_{50}-values was calculated by linear regression analysis (slope: 1.14, correlation coefficient: 0.90). (b) Correlation between K_i-values of drugs for inhibition of [^{3}H]CGP-12177 binding to human β_2-adrenergic receptor sites on intact CHO-Huβ_2 cells and potencies of drugs (IC_{50}-values) to inhibit accumulation of cAMP induced by 10 nM isoproterenol in CHO-Huβ_2 cells. Mean K_i and IC_{50}-values from Table 2 were plotted (slope: 0.97, correlation coefficient: 0.92). 1, CGP 20712-A; 2, ICI 118-551; 3, propranolol; 4, nebivolol; 5, *d*-nebivolol; 6, *l*-nebivolol.

0.28 and 1000 nM (Table 2). These compounds were also tested for their effect on the basal accumulation of cAMP in CHO-Huβ_1 and CHO-Huβ_2 cells. No increase or decrease in basal accumulation of cAMP was noted at concentrations of 10^{-9} and 10^{-6} M.

DISCUSSION

Specificity of the human β_1 and β_2-adrenergic receptors expressed in CHO cells

Receptor preparations from tissues are seldom homogenous. Most tissues contain several receptors or several subtypes of a particular receptor; the latter situation especially may pose serious problems for receptor studies. In the case of β-adrenergic receptors, there is no tissue that contains only the β_1- or β_2-subtype. Selective labeling of β_1- and β_2-adrenergic receptors in tissues can be achieved in the presence of an appropriate concentration of selective β_2- and β_1-adrenergic blockers, respectively [6]. At the cellular level, mammacarcinoma TA_3 cells are one example of a cell type which contains β_1-adrenergic receptors and no β_2-adrenergic receptors. Nevertheless, the amount of β_1-adrenergic receptors in TA_3 cells is low (1355 receptors/cell; Pauwels *et al.*, unpublished results). In contrast, cloned receptors expressed in cell lines have several major advantages: (1) one particular receptor type can be expressed in the absence of other receptor subtypes; (2) a human receptor can be studied easily and (3) a large amount of receptors can be obtained per cell. This study shows that CHO-Huβ_1 and CHO-Huβ_2 cells contain 1.1×10^6 human β_1- and 0.22×10^6 human β_2-adrenergic receptors per cell, respectively.

The binding affinities of the β-adrenergic receptor antagonists for β_1- and β_2-adrenergic receptor sites on CHO-Huβ_1 and CHO-Huβ_2 cells corresponded well with the potencies of these antagonists in inhibiting the accumulation of cAMP, induced by 10 nM isoproterenol, in these transfected CHO cells (Fig. 5). The observed affinities for the β_1-adrenergic receptor are also in good agreement with those reported for the β_1-adrenergic receptor found in rabbit lung [6] and the human β_1-adrenergic receptor expressed in *E. coli* [10, 16] and *Xenopus laevis* oocytes [8]. The affinities for the β_2-adrenergic receptor are similar to those reported for the β_2-adrenergic receptor found in rat lung [6] and the human β_2-adrenergic receptor expressed in *E. coli* [10, 16], B-82 cells [17], L-cells [18] and TP_3 cells [11]. Hence, human β_1- and β_2-adrenergic receptors expressed in CHO cells can be considered as functional β-adrenergic receptors with the same binding properties and the same ability to stimulate adenylate cyclase as β-adrenergic receptors observed in other mammalian tissues and cell lines.

The accumulation of cAMP in CHO-Huβ_1 and CHO-Huβ_2 cells was apparent in the presence of nanomolar concentrations of β-adrenergic agonists, such as noradrenaline, adrenaline, prenalterol, salbutamol [13] and isoproterenol (Fig. 4). Higher concentrations of β-adrenergic agonists were used to induce accumulation of cAMP in rat neonatal cardiac cells in culture, such as 30 nM isoproterenol and 0.3 μM noradrenaline [7]. This suggests that in CHO cells the expressed β-adrenergic receptors are efficiently coupled to a very sensitive signal transducing system.

Affinity and selectivity of nebivolol for human β_1- and β_2-adrenergic receptors expressed in CHO cells compared to that of its enantiomers

Nebivolol and its enantiomers did not affect basal accumulation of cAMP in transfected CHO cells. The cAMP data support the theory that *d*-nebivolol and nebivolol have high affinities in the blockade of β_1-adrenergic receptor sites in CHO-Huβ_1 cells. The observed affinities were 0.41 and 0.42 nM for *d*-nebivolol and nebivolol, respectively. These affinities

are 37–52 times higher than those reported for the inhibition of β_1-adrenergic receptor mediated accumulation of cAMP in neonatal cardiac cells in culture [7]. This difference in potency may be due to the use of a higher agonist concuntration in the experiments with cardiac cells.

d-Nebivolol and nebivolol showed a pronounced β_1-adrenergic selectivity inasmuch as they were 90–210 times less potent in inhibiting β_2-adrenergic receptor mediated accumulation of cAMP in CHO-Huβ_2 cells. The binding affinities of *d*-nebivolol and nebivolol for human β_1-adrenergic binding sites are close to observed affinities for the β_1-adrenergic receptor in rabbit lung [6] and in potencies in inhibiting human β_1-adrenergic receptor mediated accumulation of cAMP. The binding affinities of *d*-nebivolol and nebivolol for human β_2-adrenergic binding sites are 7 times more potent compared to the corresponding potencies in inhibiting human β_2-adrenergic receptor mediated accumulation of cAMP. Hence, the β_1-adrenergic selectivity is less pronounced (8–10 times) when the binding data are considered. Similar findings have been observed in the binding studies with the cloned human β_1- and β_2-adrenergic receptors expressed in *E. coli* [16]. Nevertheless, nebivolol is still more selective for human β_1-adrenergic receptor sites than atenolol, which is generally referred to as a selective β_1-adrenergic blocker [16].

l-Nebivolol was compared to *d*-nebivolol and found to be 280 and 1460 times less potent in human β_1-adrenergic receptor binding and mediated accumulation of cAMP in CHO-Huβ_1 cells, respectively. It cannot be excluded that this weak effect was due to the presence of residual *d*-nebivolol.

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