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Affinity and Selectivity of β-Adrenoceptor Antagonists In Vitro

A. Wellstein, D. Palm, and *G. G. Belz

Zentrum der Pharmakologie, Klinikum der J. W. Goethe-Universität Frankfurt,
**Institut für kardiovaskuläre Therapie, Wiesbaden, F.R.G.*

Summary: The potency order of the catecholamines (−)-isoprenaline (Iso), (−)-noradrenaline (NA), and (−)-adrenaline (Adr) in competition for radiolabelled sites is used for their pharmacological classification. It is shown that the radioligand ^{3}H-CGP 12177 exclusively labels β_1-adrenoceptors in rat salivary gland membranes (Iso > NA > Adr), and β_2-adrenoceptors in rat reticulocytes (Iso > Adr ≥ NA). These models are then used to derive the subtype-selectivity of the classical β-adrenoceptor antagonists (±)-propranolol (prop; twofold β_2-selective) and (±)-atenolol (aten; 35-fold β_1-selective), as well as of the newer antagonists (±)-betaxolol and (±)-bisoprolol (betax and biso; 35-fold and 75-fold β_1-selective, respectively). The ligand with the highest selectivity is ICI 118,551 (ICI), with a 300-fold β_2-subtype selectivity. For comparison with antagonistic effects in humans at given plasma concentrations, the equilibrium dissociation constants of the ligands are measured in the presence of native human plasma and yield values for the relative selectively labelled subtype in the mean (K_i-values in nmol/l): prop: 20, aten: 250, biso: 24, betax: 23, and ICI: 2.5. **Key Words:** β-Adrenoceptor — Atenolol — Betaxolol — Bisoprolol — ICI 118,551 — Propranolol — Rat reticulocytes — Rat salivary gland.

In classical pharmacology, the use of well-defined agonists is a very important tool for defining receptor-mediated effects and for differentiation of α- and β-adrenoceptor–mediated effects in the adrenergic system (1). Furthermore, this approach has, in addition, allowed Lands and his co-workers (2,3) to define β_1- and β_2-adrenoceptor–mediated effects. The use of antagonistic drugs has in parallel been an equivalent tool for defining drug receptors (4–6). Antagonist radioligand binding studies have refined the investigation of drug receptors further, far beyond the adrenergic system (7). They have increased our understanding of the action of antagonistic, as well as agonistic, drugs also by use of the more sophisticated technique of agonist radioligand binding studies (8). The recent use of subtype-selective radioligands is a step toward further improvement of the receptor-binding approach (9–11).

The comparison of results from the receptor-binding technique with those from classical pharmacological and biochemical studies of drug effects has given support to the reliability of results derived by receptor binding (9,10,12–15). In recent studies in our laboratory, it was possible to use receptor-binding studies for the prediction of the effects of the β-adrenoceptor antagonists atenolol and propranolol in humans (16,17). Using this approach, it is not only possible to predict the overall β-adrenoceptor antagonism in humans, but also to derive the antagonism versus β_1- and β_2-subtype effects in the case of selective ligands.

Thus, it was the aim of the present study to investigate the in vitro effects of antagonists of different subtype selectivity in receptor-binding studies. The studies were carried out using tissues with predominant populations of either β_1-adrenoceptors (rat salivary gland) or β_2-adrenoceptors (rat reticulocytes). For obvious reasons, this approach makes the measurement of subtype selectivity more reliable than studies carried out in tissues with mixed portions of receptor subtypes: One should expect monophasic curves using each of the selective tissues, rather than more complicated biphasic curves, in the case of mixed subpopulations present in a single preparation. The studies were performed in the presence of human plasma to allow for an extrapolation of effects to humans at given plasma concentrations of the drugs investigated.

Address correspondence and reprint requests to Dr. A. Wellstein at Zentrum der Pharmakologie, Klinikum der J. W. Goethe-Universität, Theodor-Stern-Kai 7, D-6000 Frankfurt, Federal Republic of Germany.

METHODS

Receptor-binding studies

β-Adrenoceptor–containing membranes were prepared from rat salivary glands or rat reticulocytes, as described earlier (17). In brief, salivary glands were removed, homogenized in 310 mosmol/l of sodium phosphate buffer (pH 7.4), and washed three times in this buffer by centrifugation (20,000 g for 15 min). 100 μl of the final suspension of 1 g of gland per 10 ml of buffer was used in the assay. Reticulocyte-rich blood was obtained on the 7th day after a 3-day treatment of rats with 40 mg/kg/day of 1-acetyl-2-phenylhydrazide (Schuchardt, München, F.R.G.; 18). Cells were washed in the aforementioned buffer, lysed in hypotonic buffer (17 mosmol/l), and membranes were washed free of haemoglobin, as described by Wiemer et al. (8). Then 50 μl of the final suspension of membranes in the original volume of blood was used in the assay. The incubation volume of 300 μl contained membranes (50 or 100 μl; 100–250 μg of protein), 20 μl of the radioligand ^{3}H-CGP 12177 (Amersham-Buchler; Braunschweig, F.R.G.; 30–40 Ci/mmol; 19) in the concentrations indicated in the figures, 30 μl of competing ligand of buffer and additional buffer or native human plasma to make up the volume. All drugs were dissolved in 310 mosmol/l of sodium phosphate buffer. After 60 min of incubation at 25°C, the whole volume was filtrated through glass fibre filters (AP 15 Millipore; Dreieich, F.R.G.), using a multifold filtration device. After washing the filters with additional buffer (twice with 10 ml), retained radioactivity on the filters was detected in a liquid scintillation counter. Nonspecific binding was calculated from the total binding isotherm and amounted to $<5\%$ at concentrations close to the K_d-value of the radioligand. Samples at each concentration of ligand were run in triplicate. The figures show total binding (mean ± SEM, if larger than the size of the symbols).

Calculations

Estimation of parameters was performed by the GIP-program (8,20) for nonlinear, least-squares curve-fitting (21) using the following equation for competitive antagonists (5,16,17):

$$B = B_{max} \times L/[L + K_d \times (1 + i/K_i)] + nsb \times L$$

where B is bound radioligand at the respective concentration L, with a maximal binding capacity B_{max}, an equilibrium dissociation constant K_d, and nonspecific binding nsb. The concentration of inhibitory (nonlabelled) ligand is i, if present, with the respective equilibrium dissociation constant K_i.

K_i-values for the respective experiment are depicted in the figures. Each experiment was run with 3 to 6 independent preparations. Selectivity ratios give the mean value from these experiments.

The drugs used were (−)-adrenaline bitartrate (Fluka AG, Buchs, Switzerland); (±)-atenolol hydrochloride, (±)-ICI 118,551, and (±)-propranolol hydrochloride (ICI-Pharma, Plankstadt, F.R.G.); (±)-betaxolol hydrochloride (Beiersdorf, Hamburg, F.R.G.); (±)-bisoprolol hemifumarate (Merck, Darmstadt, F.R.G.); ^{3}H-CGP 12177, which is 4-(3-*t*-butylamino-2-hydroxypropyl)-5,7-^{3}H-benzimidazol-2one (specific activity 30–40 Ci/mmol; Amersham-Buchler, Braunschweig, F.R.G.); (−)-isoprenaline hydrochloride (Cilag-Chemie, Alsbach-Hähnlein, F.R.G.); and (−)-noradrenaline bitartrate (Serva, Heidelberg, F.R.G.). Other drugs and substances used were supplied by Merck (Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

As mentioned in the introduction, the pharmacological characteristics of a system should be characterised by the potency ratio of well-defined agonists (1–3). Figure 1 shows that in the two systems used, (−)-isoprenaline is the most potent of the catecholamines in competition for the binding of the radiolabel ^{3}H-CGP 12177. This indicates that, indeed, in both systems, β-adrenoceptors are labelled. The similar potency ratio of (−)-noradrenaline and (−)-adrenaline in rat salivary

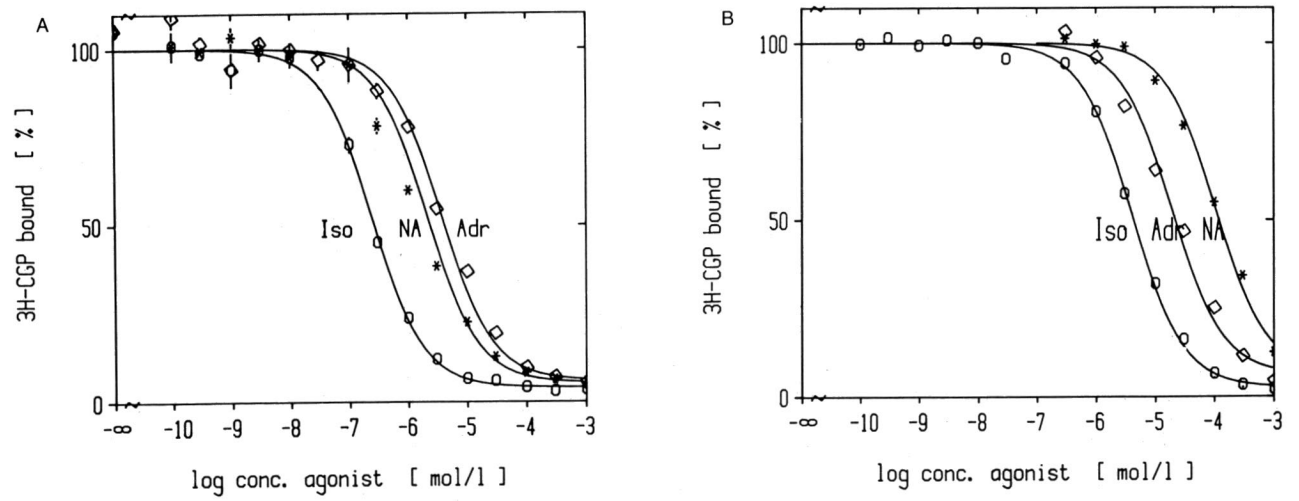

FIG. 1. Competition of catecholamines with ^{3}H-CGP 12177 binding in rat salivary gland (**A**) and rat reticulocyte (**B**) membranes. As can be seen, (−)-isoprenaline (Iso) is the most potent of the catecholamines in both preparations. In salivary glands (−)-noradrenaline (NA) and (−)-adrenaline (Adr) are equipotent, whereas in reticulocytes (−)-adrenaline is much more potent than (−)-noradrenaline. This indicates that in salivary glands, β_1-adrenoceptors are labelled by the radioligand, whereas in reticulocytes the β_2-subtype predominates.

gland indicates that β_1-adrenoceptors are predominantly present in this preparation. The much higher potency of (−)-adrenaline versus (−)-noradrenaline in rat reticulocyte membranes shows that β_2-adrenoceptors are labelled in this system (8,12,22,23). It should be stressed that the radioligand does not show subtype selectivity (17).

The identical steepness of the agonist competition isotherms (slope unity) is in favor of >95% of the receptors belonging to either of the two subtypes. Otherwise, one should have expected at least a clearly biphasic isotherm for (−)-noradrenaline, since this ligand shows a clearly separate affinity for β_1- and β_2-adrenoceptors. Thus, on the basis of the first figure, with the use of a well-established approach, one can delineate the receptor type under investigation.

One further aspect merits comment: the agonist competition curves show only a simple ligand/receptor interaction, but no high- and low-affinity state. This is due to the assay procedure employed, in which a high sodium concentration in the absence of magnesium was used (see Methods section, 8).

In Figure 2, the competition isotherm of (±)-propranolol is shown. The racemic mixture was investigated, since in clinical investigations and therapy the racemate is used. The equilibrium dissociation constants between 10 and 20 nmol/l appear 5- to 10-fold too high compared with data from literature, which gives values in the low nanomolar range (12–14, 22–25) [for review, see Broadley (26)]. This apparent discrepancy is due to the presence of human plasma in the assay. As shown earlier (27), plasma protein binding of (±)-propranolol amounts to ~80%. Thus, the free drug concentration is reduced by this portion, resulting in apparent lower affinity of the drug for its receptor.

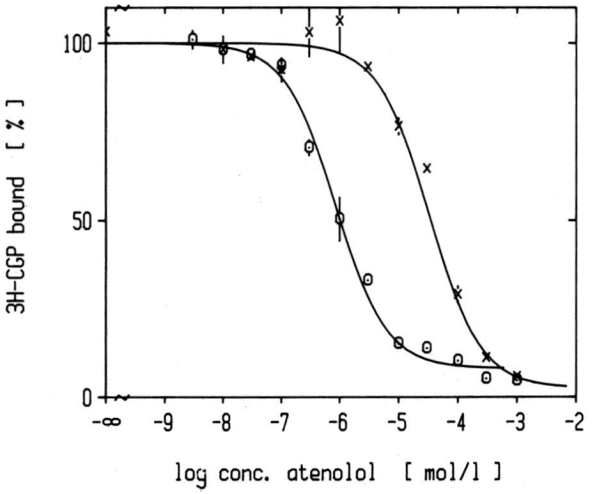

FIG. 3. Competition of (±)-atenolol with ^{3}H-CGP 12177 binding in rat salivary glands (o) and rat reticulocytes (x) in the presence of 2/3 vol/vol of native human plasma. A β_1-subtype selectivity of 35-fold prevails in the mean. K_i-values from the experiment presented: 0.27 (β_1) and 9.4 μmol/l (β_2).

In studies with healthy volunteers, as well as using data from the literature (28), we have shown that antagonistic effects of propranolol in humans can be predicted by the use of this apparent equilibrium dissociation constant in association with the respective plasma concentrations (16,17). Thus, this rather unfamiliar approach in receptor-binding studies, i.e., the use of a racemic mixture of a drug and the presence of human plasma, can be a good rationale in the context of clinical studies.

The small β_2-subtype selectivity (twofold) of pro-

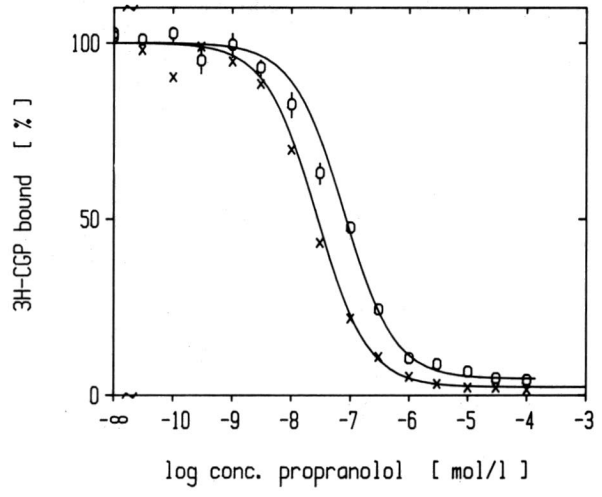

FIG. 2. Competition of (±)-propranolol with ^{3}H-CGP 12177 binding in rat salivary glands (o) and rat reticulocytes (x) in the presence of 2/3 vol/vol of native human plasma. In the mean, a small β_2-subtype selectivity of twofold prevails. K_i-values from the experiment presented: 25 (β_1) and 11 nmol/l (β_2).

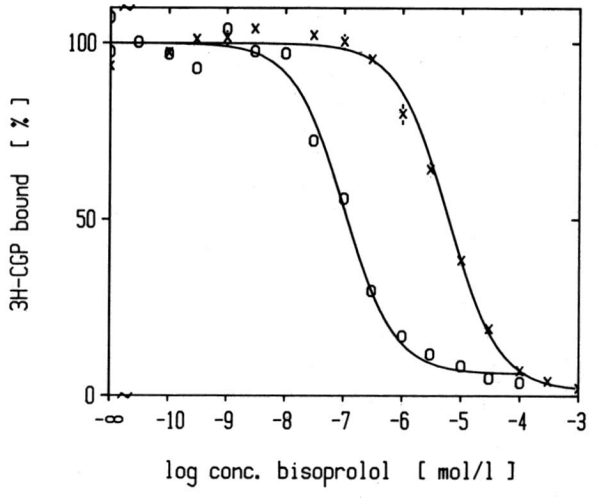

FIG. 4. Competition of (±)-bisoprolol with ^{3}H-CGP 12177 binding in rat salivary glands (o) and rat reticulocytes (x) in the presence of 2/3 vol/vol of native human plasma. A β_1-subtype selectivity of 75-fold prevails in the mean. K_i-values from the experiment presented: 24 (β_1) and 1,945 nmol/l (β_2).

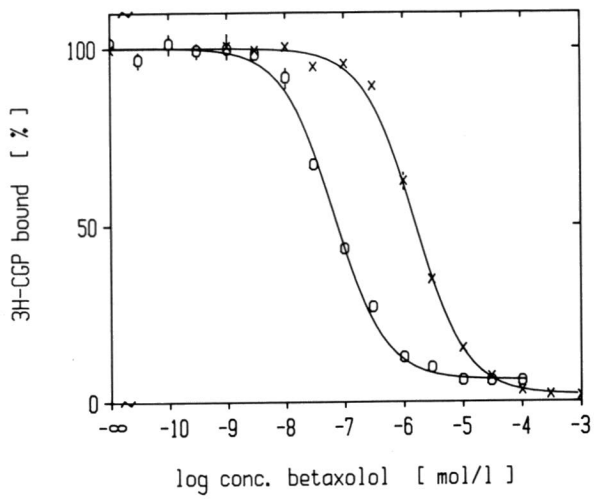

FIG. 5. Competition of (±)-betaxolol with ^{3}H-CGP 12177 binding in rat salivary glands (o) and rat reticulocytes (x) in the presence of 2/3 vol/vol of native human plasma. A β_1-subtype selectivity of 35-fold prevails in the mean. K_i-values from the experiment presented: 23 (β_1) and 790 nmol/l (β_2).

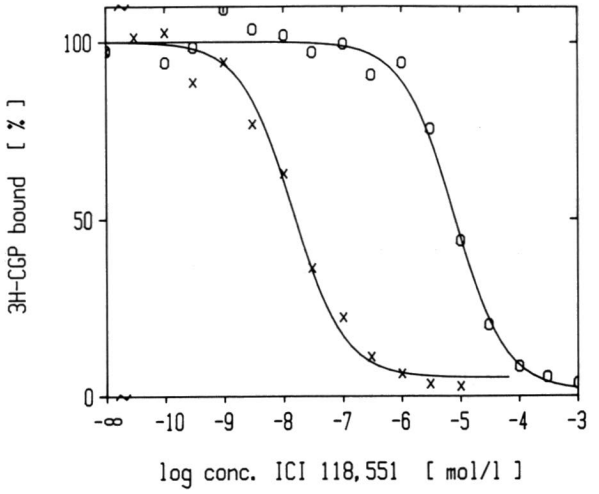

FIG. 6. Competition of (±)-ICI 118,551 with ^{3}H-CGP 12177 binding in rat salivary glands (o) and rat reticulocytes (x) in the presence of 2/3 vol/vol of native human plasma. A pronounced β_2-subtype selectivity of 300-fold prevails in the mean. K_i-values from the experiment presented: 1,380 (β_1) and 2.7 nmol/l (β_2).

pranolol seems of minor importance, but is in agreement with data from the literature (13). In contrast to the rather nonselective (±)-propranolol, (±)-atenolol, (±)-betaxolol, and (±)-bisoprolol are clearly β_1-subtype–selective drugs, with the latter displaying the greatest subtype selectivity (Figs. 3–5).

The equilibrium dissociation constant as well as the selectivity ratio of (±)-atenolol are in good agreement with findings from other authors (12,14,29) [for review see Broadley (26)]. The best support for the reliability of this in vitro result is the comparison with drug effects in humans. As shown earlier, the reduction of exercise tachycardia in humans can be predicted by the use of both the equilibrium dissociation constant at β_1-adrenoceptor in vitro and plasma concentrations of (±)-atenolol (16). Recent clinical investigations in our laboratory have confirmed this prediction (unpublished observations).

While (±)-betaxolol (Fig. 5) shows a similar subtype selectivity as (±)-atenolol (Fig. 3), (±)-bisoprolol (Fig. 4) shows a twofold higher selectivity for β_1-adrenoceptors. The selectivity ratio and the equilibrium dissociation constants of bisoprolol agree with in vitro results from other authors (9,11). Unpublished studies from our laboratory and studies in humans have given strong support to this high β_1-selectivity in vitro.

Finally, the β_2-selective ligand ICI 118,551 was investigated. For this ligand, various authors (10,30) have reported from in vitro studies in different tissues an identical subtype selectivity to those presented in this paper (Fig. 6), although O'Donnell and Wanstall (31) have reported only a 54-fold selectivity. Results from studies in humans (32,33), however, confirm the very high selectivity expected for this investigational drug.

In conclusion, results from receptor-binding studies have proved to be a valuable tool in our qualitative and quantitative understanding of drug effects. Furthermore, they allow for reliable extrapolation into the clinical situation, and may thus lend important support to the rational comparison of different drugs from one class. Once the affinity ratio of antagonists for the subpopulations (e.g., β_1/β_2) are known, it should be possible to predict plasma concentrations (and finally the dose) at which a clinically relevant subtype selectivity is lost due to the dosage regimen.

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