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β-Adrenoceptor Subtype Selective Antagonism in Humans and in Vitro—A Comparison of pA_2 and K_i Values of Propranolol, Atenolol, and Bisoprolol

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Summary: We have compared the rather nonselective β-adrenoceptor antagonist propranolol and the β_1-subtype selective antagonists atenolol and bisoprolol in humans and in in vitro receptor binding studies. In receptor binding studies in rat salivary and reticulocyte membranes, propranolol showed a very small (about twofold) β_2-adrenoceptor selectivity, whereas atenolol showed a 35-fold and bisoprolol a 75-fold β_1-adrenoceptor subtype selectivity. After administration of 240 mg of propranolol, 100 mg of bisoprolol, and 200 mg of atenolol (single oral dose) to healthy male volunteers (N = 6 in each group), isoprenaline dose–response curves at different time intervals were run. They showed rightward shifts, which were in agreement with the concentrations of antagonist present in plasma of the volunteers, if these concentrations were expressed as multifolds of the K_i values in the receptor binding studies. Deviations from the direct relation between antagonist in plasma and rightward shift of the isoprenaline dose–response curves could be explained by the subtype selectivity of the different antagonists used. **Key Words:** Bisoprolol—Propranolol—Atenolol—β-Adrenoceptors—pA_2 values—K_i values.

The aim of our study was to compare results from in vitro receptor binding studies at β-adrenoceptors from rat tissues with antagonistic effects of β-blockers of different subtype selectivity in humans. Using isoprenaline dose–response curves in humans after administration of single doses of the β-blockers propranolol, atenolol, and bisoprolol, we hoped to get a quantitative measure of antagonist present at the receptor site in humans. In addition, we tried to construct Schild plots (1) by comparing the rightward shifts in the isoprenaline dose–response curves with the concentrations of the antagonists present in plasma samples obtained in parallel. One would expect that such Schild plots in humans should give K_i values (or pA_2 values) in agreement with results from in vitro experiments (binding or functional studies), if the following assumptions hold true.

1. The antagonist concentration in plasma is in equilibrium with that at functionally active β-adrenoceptors in humans.
2. The antagonist concentration in plasma is detectable as the *free* concentration which appears as effective concentration at the β-adrenoceptors in humans.

METHODS

Receptor binding studies were run as described earlier (2, 3) using a β_1- and a β_2-adrenoceptor preparation from rat salivary gland and reticulocyte membranes, respectively. As a radioligand (−)[^{3}H]CGP 12177 (4-(3-t-butylamino-2-hydroxypropoxy)-[5,7-^{3}H]benzimidazol-2-one) from Amersham–Buchler (Braunschweig, F.R.G.; specific activity 50 Ci/mmol) was used.

Volunteers (n = 6 in each group) received either propranolol (240 mg), bisoprolol (100 mg), atenolol (200 mg), or placebo as a single oral dose after giving their written informed consent. Thereafter, isoprenaline dose–response curves were constructed at time points 0, 3, 6, 9, 12, 24, 36, 48, 60, 72, and 84 h. Plasma was sampled in parallel. Isoprenaline was infused between 0.1 and maximally 256 μg/min for 3 min at twofold increasing dose steps until at least 25 beats/min of increase in heart rate was observed. Heart rate, blood pressure, peripheral resistance, and inotropy (QS_2c) were monitored noninvasively using impedance cardiography and systolic time intervals.

From the rightward shift of the dose–response curves, Schild plots were constructed using the concentration of antagonist detected from the receptor assay (2, 3).

RESULTS AND DISCUSSION

From receptor binding studies, a β_1-adrenoceptor subtype selectivity of propranolol (0.5-fold), bisoprolol (75-fold), and atenolol (35-fold) was delineated, which was in good agreement with results reported in literature (e.g., Refs. 4 and 5) in both receptor binding and functional studies. As shown in Fig. 1, 3 h after administration of the different antagonists, the initial free concentrations

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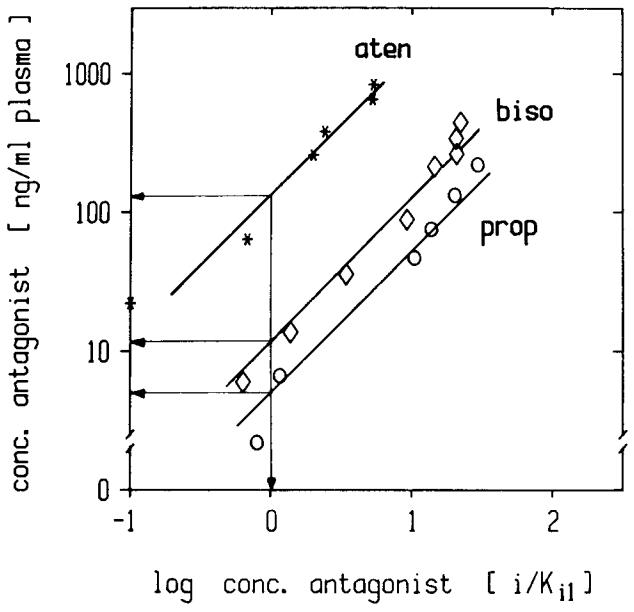

FIG. 1. Comparison of effect equivalents (multifolds of the K_i value) and plasma concentrations of different antagonists after administration to healthy volunteers. **Abscissa:** The respective antagonist concentration in plasma is expressed in multifolds of the K_i value at β_1-adrenoceptors (free concentration only). **Ordinate:** The scale used is ng/ml (total plasma concentration). The data points for atenolol (aten), bisoprolol (biso), and propranolol (prop) were derived after administration of 200, 100, and 240 mg once orally, respectively, at time points 3, 6, 9, 12, 24, 36, 48, and 60 h (from right top corner to left bottom corner). Atenolol ran below the detection limit after 36 h, bisoprolol after 60 h, and propranolol after 36 h. The arrows indicate the respective apparent K_i value expressed as total plasma concentration in ng/ml.

in plasma expressed in the conventional way as ng/ml were 1000 for atenolol, 300 for bisoprolol, and 250 for propranolol.

If one gives these concentrations in effect equivalents (the way they were actually measured in the receptor assay used), one can delineate for this time point the following:

At β_1-adrenoceptors, 240 mg of propranolol should induce a 97% blockade (= 40-fold of the K_i value), 200 mg of atenolol a 85% blockade (sixfold of the K_i value), and 100 mg of bisoprolol a 96% blockade (30-fold of the K_i value). In addition, one may read from this figure at which time point after administration of the respective antagonist the plasma concentration falls below the respective K_i concentration and thus 50% of β-adrenoceptor blockade: For propranolol and atenolol the data point at 24 h and for bisoprolol at 48 h approach this concentration and should thus be an indicator of the half time of effect.

The rightward shifts of the isoprenaline dose–response curves (not shown) reflected this picture in the case of propranolol inasmuch as, independent of parameter used (heart rate, blood pressure, or peripheral resistance), a direct linear relation with a slope of 1 was seen when plotting the concentration of antagonist on the abscissa (as done in Fig. 1) and dose ratio (1) as a measure of the rightward shift on the ordinate (Schild plot) (1).

For the selective antagonists bisoprolol and atenolol, however, only for the parameter QS_2c—as a measure of inotropy—a slope of 1 in the Schild plot was seen and a decreasing slope (0.9–0.6) for systolic blood pressure > heart rate > diastolic blood pressure > peripheral resistance. This finding may be interpreted in terms of subtype-selective blockade of the isoprenaline response in humans by the different levels of receptor blockade achieved with propranolol in comparison to those observed for bisoprolol and atenolol. The differing sensitivity of the cardiovascular parameters towards blockade by the β_1-subtype-selective antagonists indicates differing proportions of β_2-adrenoceptors participating in the isoprenaline response.

In conclusion, the free concentration of β-adrenoceptor antagonists in plasma of humans as detectable by a subtype-selective receptor assay indicates that the plasma compartment is in equilibrium with the effect compartment (β-adrenoceptors). Moreover, in vitro receptor interaction yielding K_i values in receptor binding studies predicts the in vivo antagonistic effects and thus give Schild plots (and pA_2 values) in humans identical with those in vitro.

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