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Reduction of exercise tachycardia in man after propranolol, atenolol and bisoprolol in comparison to beta-adrenoceptor occupancy

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In a double blind, placebo controlled study, propranolol (240 mg), atenolol (200 mg) or bisoprolol (100 mg) were administered as a single oral dose to groups of 6 healthy male volunteers. Exercise tachycardia was monitored for 84 hours after administration of the drugs to monitor beta blockade in vivo. *Plasma samples drawn in parallel with these effects were used to detect $beta_1$- or $beta_2$-adrenoceptor occupancy in two subtype selective* in vitro *receptor binding assays. Reduction of exercise tachycardia parallels $beta_1$-adrenoceptor occupancy. Furthermore, at comparable $beta_1$-adrenoceptor occupancy, less $beta_2$-adrenoceptor occupancy was observed after bisoprolol than after atenolol. The latter finding is in agreement with the two-fold higher $beta_1/beta_2$-selectivity ratio of bisoprolol (75-fold) versus atenolol (35-fold). It is concluded, that beta blockade observed via the reduction of exercise tachycardia can be delineated from the* in vitro *occupancy of $beta_1$-adrenoceptors by an antagonist present in plasma samples.*

Introduction

In earlier studies[1], we have shown that the time course of reduction of exercise tachycardia (RET) after a single oral dose of propranolol is reflected by the antagonist concentration present in plasma samples at each time point. Furthermore, receptor occupancy detected by in vitro incubation of plasma samples with a beta-adrenoceptor preparation[2], allows for a prediction of the relative extent of beta-adrenoceptor antagonistic effects in man. This approach was also valid to monitor effects after penbutolol[3] and also in *i.v.* cumulative studies comparing propranolol and bufuralol[4]. In addition, the inhibitory effects on RET during 72 hours of steady state concentrations via transdermal delivery of bupranolol were directly reflected by inhibition of radioligand binding at beta-adrenoceptors *in vitro*[5]. This allows for a rational comparison of beta blockers of the rather non-selective type.

These findings in man are in accordance with *ex vivo* receptor binding studies *in rats*[6] after cumulative administration of propranolol or atenolol. It has been shown in these investigations, that the occupancy of beta-adrenoceptors in different tissues can be predicted from the presence of an antagonist in the plasma compartment of rats. In addition, studies in man have shown a parallel decline of the right shift of isoprenaline dose–response curves and plasma concentrations of propranolol in man[7].

Besides the standard drugs propranolol and atenolol[8,9], the recently introduced drug bisoprolol[10] was used in the present studies. For the latter a high selectivity has been shown *in vitro*[11–13]. In man, elimination half-lives from the plasma compartment of 10 hours for bisoprolol[14], and 6 hours for atenolol[8,15] were reported earlier. Assuming effective doses of 5 mg for bisoprolol[16,17], we expected from the theoretical model described earlier[1], that antagonist effects should prevail for more than 50 hours after a 100 mg dose of bisoprolol. After 240 mg of propranolol[1] and 200 mg of atenolol[8,9], 48 hours were shown to be sufficient for a complete decline of antagonism. Based on these considerations, we decided to monitor the volunteers for 84 hours with respect to effects and plasma sampling.

The aims of our study were thus, to find out whether the $beta_1$-subtype or $beta_2$-subtype selective assay of beta-adrenoceptor occupancy *in vitro*

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can predict the antagonistic effects observed from the reduction of exercise tachycardia in man. Furthermore, it was of interest to see, to what extent the subtype selectivity of different beta blockers being compared is represented by the *in vitro* assay of *ex vivo* plasma samples. The intention was to contribute a rational basis for a comparison of different drugs of one class by the application and combination of *in vitro* and *in vivo* methods.

Methods

Receptor binding studies at beta-adrenoceptors present in rat salivary glands and at beta-adrenoceptors present in rat reticulocytes were carried out as described earlier[1,11]. A hydrophilic antagonist was used as a radiolabelled tracer for beta-adrenoceptors (^{3}H-CGP 12177; spec. act. 35–45 Ci mmol^{-1}; Amersham-Buchler; Braunschweig, F.R.G.). The receptor occupancy was calculated[1,2] from the inhibition of radioligand binding by an antagonist present in the plasma samples, and is shown relative to the maximum occupancy possible.

Studies in healthy, male volunteers ($N = 24$; 22–26 years of age) were carried out after written and informed consent in four different groups ($N = 6$ for each group). The protocol was identical to that used in a previous study with propranolol[1]. At different time intervals (see figures) after double-blind drug administration (7 am; 200 ml of water, overnight fasting), blood was sampled into heparinized syringes and plasma was separated by centrifugation and stored frozen until use. Thereafter, the volunteers exercised for 4 minutes on a bicycle ergometer at 75% of maximum work capacity (horizontal position with the head elevated by 15°). Heart rate was read from an ECG every minute. Only the data from minute 3 are shown here. Data from minutes 1, 2 and 4 gave similar results.

STATISTICS

Mean ± SEM values are shown for the heart rates. From receptor binding studies mean data only are shown, since the SEM did usually not exceed 5%. Drugs were derived from the sources described earlier[11,15]. Racemates of propranolol (Dociton 80 tablets), atenolol (Tenormin 50 tablets) and bisoprolol (Concor 10 tablets) were administered enclosed in a wafer.

Results

As can be seen from Table 1, propranolol shows a small subtype$_2$-selectivity at beta-adrenoceptors, atenolol a 35-fold and bisoprolol a 75-fold beta$_1$ selectivity in the *in vitro* systems employed. Penbutolol, carteolol and bupranolol can be termed rather non-selective, whereas metoprolol and betaxolol clearly belong to the class of beta$_1$-selective antagonists; the drug ICI 118,551 still under investigation is the most selective ligand with a pronounced 300-fold selectivity for beta$_2$ versus beta$_1$-adrenoceptors.

Table 1 Beta$_1$/beta$_2$-adrenoceptor subtype selectivity ratios of different beta-adrenoceptor blocking drugs. Given are the ratios of K_i-values derived from receptor binding studies at rat salivary and rat reticulocyte membranes[1,2]

Non-selective:	Beta$_1$ selective:	Beta$_2$ selective:
Carteolol 1/1·2	Metoprolol 20/1	Propranolol 1/1·8
Bupranolol 1/1·1	Atenolol 35/1	ICI 118,551 1/300
Penbutolol 1/1	Betaxolol 35/1	
^{3}H-CGP 12177 1/1	Bisoprolol 75/1	

Exercise heart rates in the placebo group showed only minor variations and thus rule out relevant diurnal bias of the antagonist effects (not shown). After 240 mg of propranolol (Fig. 1, top), the reduction of exercise heart rate declined in parallel with beta-adrenoceptor occupancy in agreement with our earlier studies[1]. After 200 mg of atenolol (Fig. 2, top) a reduction of exercise tachycardia was apparent until 36 h after administration. Beta$_2$-adrenoceptor occupancy *in vitro* by the antagonist present in the plasma samples was detectable only within the first 12 hours, and thus cannot explain the inhibition of tachycardia. *In vitro* beta$_1$-adrenoceptor occupancy, however, showed a good relation to the *in vivo* effects. A subtype$_1$ occupancy was observed until 36 hours after atenolol intake. At the maximum effect of atenolol (3 h after administration), the relation between beta$_2$- and beta$_1$-adrenoceptor occupancy was 25%/80%. From the data shown, it can be estimated, that single daily doses of 50 to 100 mg of atenolol should be sufficient to inhibit exercise tachycardia for a 24 hours period.

100 mg of bisoprolol inhibited exercise tachycardia for 60 hours after administration (Fig. 3, top). 24 hours after drug administration no more beta$_2$-adrenoceptor occupancy was detected from the plasma samples (Fig. 3, bottom). Beta$_1$-

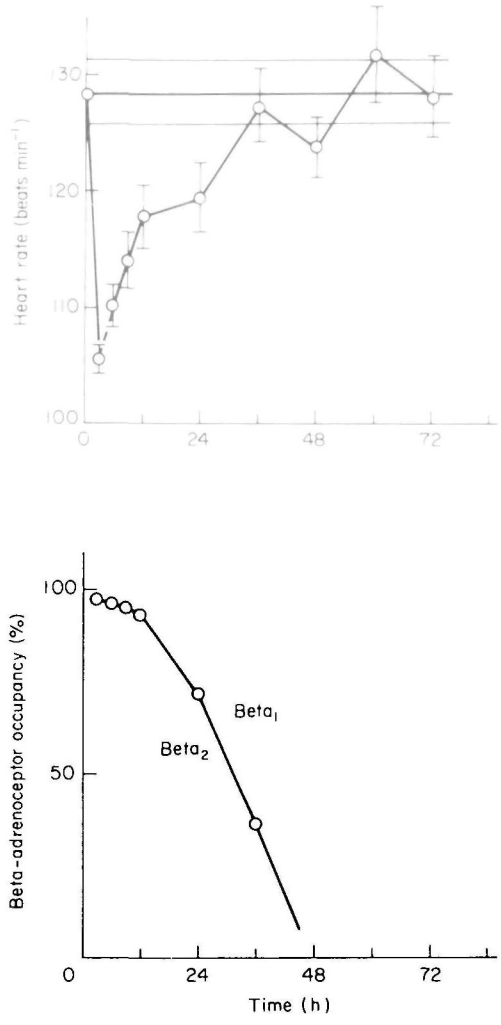

Figure 1 Reduction of exercise tachycardia (top; mean ± SEM; $N = 6$) and *in vitro* beta-adrenoceptor occupancy (bottom; $beta_1 = beta_2$) after a single oral dose of propranolol (240 mg).

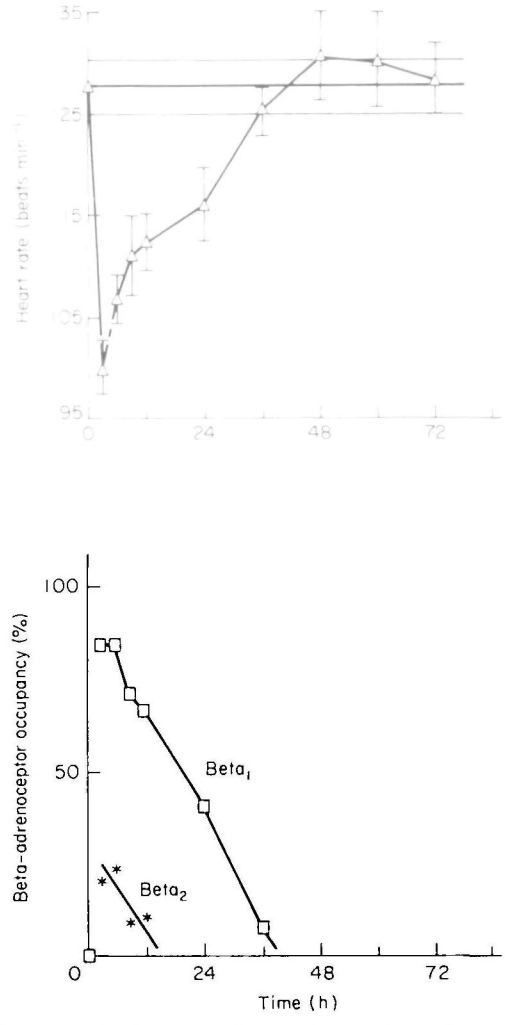

Figure 2 Reduction of exercise tachycardia (top; mean ± SEM; $N = 6$) and *in vitro* beta-adrenoceptor occupancy (bottom; $beta_1 > beta_2$) after a single oral dose of atenolol (200 mg).

adrenoceptor occupancy, however, paralleled the effects on exercise tachycardia with respect to the relative duration and extent. At a $beta_2$-adrenoceptor occupancy of 25% comparable to the maximum observed with atenolol (time point 12 hours), 95% of $beta_1$-adrenoceptors were occupied during the bisoprolol regimen. Thus, the higher $beta_1$-selectivity ratio of bisoprolol versus atenolol (see Table 1) can also be read from these *in vitro/ex vivo* data. From the effect data shown in Fig. 3, one can estimate, that a single daily dose of 5 to 10 mg of bisoprolol should be sufficient to antagonize the sympathetic drive monitored in the present study from the exercise procedure without a relevant occupancy of $beta_2$-adrenoceptors.

Discussion

In the present study, we have extended our comparison of beta blockers in man and in receptor binding studies *in vitro*[1,3–5] to the use of $beta_1$-selective antagonists. As expected from simulations with *in vitro* data of atenolol and data taken from literature[18], we are able to predict the relative reduction of exercise tachycardia (RET) also for this $beta_1$-selective antagonist by the use of

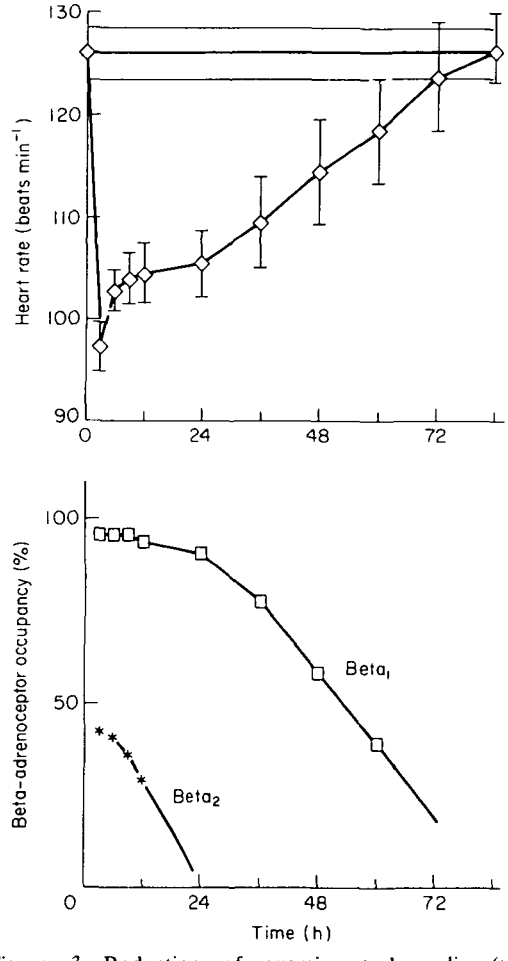

Figure 3 Reduction of exercise tachycardia (top; mean ± SEM; $N = 6$) and *in vitro* beta-adrenoceptor occupancy (bottom; $beta_1 > beta_2$) after a single oral dose of bisoprolol (100 mg).

a selective assay of beta-adrenoceptor occupancy *in vitro*. More interestingly, one can even relate exercise tachycardia mainly to a $beta_1$ effect, if there is disappearing $beta_2$-blockade after 24 h whereas a submaxial reduction of exercise heart rate still persists (Fig. 3).

The question of reliable estimates of subtype selectivity measurements with the receptor binding assay shown here (Table 1) can be solved by a comparison with data from independent experimental approaches. The selectivity ratios of the drugs stated in Table 1 have been validated by functional studies and receptor binding studies in a wide range of tissues from different species with different response measurements and numerous selective agonists and antagonists[10,12,13,19–27]. Furthermore, the advantageous selectivity of bisoprolol versus atenolol also prevails in the *ex vivo/in vitro* part of the study: at a comparable $beta_2$-adrenoceptor occupancy, the $beta_1$ occupancy of bisoprolol present in the plasma samples is indeed higher than that of atenolol.

Finally, the prediction from the *in vitro* studies of the effective doses of atenolol or bisoprolol is confirmed by the findings in therapeutic studies. For atenolol daily doses of 50 mg to 100 mg are approved doses for cardiovascular therapy. From our studies, this recommendation can be fully supported as described in the 'Results' and Fig. 2. For bisoprolol 5 to 20 mg were efficient in therapy of angina pectoris[16] and hypertension[17] with the major response at doses between 5 and 10 mg. These doses were also approved for general use in the Federal Republic of Germany. From our studies *in vitro* (Fig. 3 bottom) one can read, that the antagonist concentration within the 24 hours interval between 36 h and 60 h is sufficient to occupy $beta_1$-adrenoceptors from 75% to 30%. The half-life of bisoprolol in plasma was described as about 10 hours[14,15]. Thus, at 36 hours after 100 mg of bisoprolol, i.e. after 3·6 elimination half-lives, less than 1/10 of drug is still in the body. Doses below 10 mg should therefore be sufficient for beta blockade without a significant blockade of $beta_2$-adrenoceptors. Taking a small accumulation by daily intake of the drug into consideration, one can predict that 5 mg to 10 mg should suffice during beta-blocker therapy using bisoprolol. It is worthwhile to mention that at the time interval after administration of the 100 mg that reflects this low dose, an *in vitro* $beta_2$ blockade was not apparent (Fig. 3 bottom).

We conclude, that the approach presented, allows for a rational comparison of various drugs from one class. Such a comparison and consequences therefrom, may be essential in view of the constantly extending market of antihypertensive drugs. We have recently presented a parallel comparison of angiotensin converting enzyme inhibitors on the basis of a classic pharmacologic technique[28]: Schild-plots in man[29–31]. We feel, that the wide spectrum of methods used in basic pharmacology can serve for therapeutic purposes much more than imagined at present.

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References

[1] Wellstein A, Palm D, Belz GG, Pitschner HF. Receptor binding of propranolol is the missing link between plasma concentration kinetics and effect time course in man. Eur J Clin Pharmacol 1985; 29: 131–47.

[2] Wellstein A, Palm D, Wiemer G, Schäfer-Korting M, Mutschler E. Simple and reliable radioreceptor assay for β-adrenoceptor antagonists and active metabolites in native human plasma. Eur J Clin Pharmacol 1984; 27: 545–53, 1984.

[3] Wellstein A, Palm D. Penbutolol: In vitro β-adrenoceptor interaction and the time course of plasma concentrations explain it's prolonged duration of action in man. Eur J Clin Pharmacol 1985; 29: 293–300.

[4] Wellstein A, Palm D, Matthews JH, Belz GG. In vitro receptor occupancy allows to establish equieffective doses of β-blockers with different pharmacodynamic profile in man. Investigations with propranolol and bufuralol. Meth Find Exptl Clin Pharmacol 1985; 7: 645–651.

[5] Wellstein A, Küppers H, Pitschner HF, Palm D. Transdermal delivery of bupranolol: pharmacodynamics and beta-adrenoceptor occupancy. Eur J Clin Pharmacol 1986; 31: 419–22.

[6] Sriwatanakul K, Nahorski SR. Disposition and activity of β-adrenoceptor antagonists in the rat using an ex vivo receptor binding assay. Eur J Pharmacol 1980; 66: 169–78.

[7] DeLeve LD, Endrenyi L, Leenen FHH. Plasma concentration–response relationships of two formulations of propranolol. J Clin Pharmacol 1985; 25: 182–86.

[8] McDevitt DG, Shand DG. Plasma concentrations and the time-course of β-blockade due to propranolol. Clin Pharmacol Ther 1975; 18: 708–13.

[9] Shanks RG, Carruthers SG, Kelly JG, McDevitt DG. Correlation of reduction of exercise heart rate with blood levels of atenolol after oral and intravenous administration. Postgraduate Med J 1977; (Suppl 3): 70–3.

[10] Schliep HJ, Harting J. β_1-selectivity of bisoprolol, a new β-adrenoceptor antagonist, in anesthetized dogs and guinea pigs. J Cardiovasc Pharmacol 1984; 6: 1156–1160.

[11] Wellstein A, Palm D, Belz GG. Affinity and selectivity of β-adrenoceptor antagonists in vitro. J Cardiovasc Pharmacol 1986; 8 (Suppl. 11): S36–40.

[12] Wang XL, Brinkmann M, Brodde OE. Selective labelling of β_1-adrenoceptors in rabbit lung membranes by (-)^{3}H-bisoprolol. Eur J Pharmacol 1985; 114: 157–65.

[13] Kaumann AJ, Lemoine H. Direct labelling of myocardial β_1-adrenoceptors. Comparison of binding affinity of ^{3}H-(-)-bisoprolol with it's blocking potency. Naunyn-Schmiedeberg's Arch Pharmacol 1985; 331: 27–39.

[14] Leopold G, Pabst J, Ungethüm W, Bühring KU. Basic pharmacokinetics of bisoprolol, a new highly beta$_1$-selective adrenoceptor antagonist. J Clin Pharmacol 1986; 26: 616–21.

[15] Wellstein A, Palm D, Belz GG, Leopold G, Bühring KU, Pabst J. Concentration kinetics of propranolol, bisoprolol and atenolol in humans assessed with chemical detection and a subtype selective β-adrenoceptor assay. J Cardiovasc Pharmacol 1986; 8 (Suppl 11): S41–5.

[16] Wagner, G. Summary of short- and long-term studies with bisoprolol in coronary heart disease (CHD). J Cardiovasc Pharmacol 1986; 8 (Suppl 11): S160–6.

[17] Weiner L, Frithz G. Antihypertensive effects of bisoprolol during once daily administration in patients with essential hypertension. Eur J Clin Pharmacol 1986; 29: 517–21.

[18] Wellstein A, Palm D, Belz GG, Pitschner HF. Receptor binding characteristics and pharmacokinetic properties as a tool for the prediction of clinical effects of β-blockers. Arzneimittelforsch/Drug Res 1985; 35: 2–6.

[19] Gille E, Lemoine H, Ehle B, Kaumann AJ. The affinity of (-)-propranolol for β_1- and β_2-adrenoceptors of human heart. Differential antagonism of the positive inotropic effects and adenylate cyclase stimulation by (-)-noradrenaline and (-)-adrenaline. Naunyn-Schmiedeberg's Arch Pharmacol 1985; 331: 60–70.

[20] Kaumann AJ, Lemoine H. β_2-adrenoceptor-mediated positive inotropic effect of adrenaline in human ventricular myocardium. Quantitative discrepancies with binding and adenylate cyclase stimulation. Naunyn-Schmiedeberg's Arch Pharmacol 1987; 335: 403–11.

[21] Lemoine H, Ehle B, Kaumann AJ. Direct labelling of β_2-adrenoceptors. Comparison of binding potency of ^{3}H-ICI 118,551 and blocking potency of ICI 118,551. Naunyn-Schmiedeberg's Arch Pharmacol 1985; 331: 40–51.

[22] O'Donnell SR, Wanstall JC. Evidence that ICI 118,551 is a potent, highly beta$_2$-selective adrenoceptor antagonist and can be used to characterize beta-adrenoceptor populations in tissues. Life Sci 1980; 27: 671–7.

[23] Robberecht P, Delhaye M, Taton G *et al.* The human heart β-adrenergic receptors. I. Heterogeneity of the binding sites: Presence of 50% β_1- and 50% β_2-adrenergic receptors. Mol Pharmacol 1983; 24: 169–73.

[24] Schliep HJ, Schulze E, Harting J, Haeusler G. Antagonistic effects of bisoprolol on several β-adrenoceptor-mediated actions in anaesthetized cats. Eur J Pharmacol 1986; 123: 253–61.

[25] Vago T, Bevilacqua M, Dagani R *et al.* Comparison of rat and human left ventricular beta-adrenergic receptors: subtype heterogeneity delineated by direct radioligand binding. Biochem Biophys Res Comm 1984; 121: 346–54.

[26] Zerkowski HR, Ikenzo K, Rohm N, Reidemeister JC, Brodde OE. Human myocardial β-adrenoceptors: demonstration of both β_1- and β_2-adrenoceptor mediating contractile responses of β-agonists on the isolated right atrium. Naunyn-Schmiedeberg's Arch Pharmacol 1986; 332: 142–7.
[27] Ask JA, Stene-Larsen G, Helle KB, Resch F. Functional β_1- and β_2-adrenoceptors in the human myocardium. Acta Physiol Scand 1985; 123: 81–8.
[28] Arunlakshana O, Schild HO. Some quantitative uses of drug antagonists. Br J Pharmacol 1959; 14: 48–58.
[29] Belz GG, Essig J, Wellstein A. Hemodynamic responses to angiotensin I in normal volunteers and the antagonism by the angiotensin-converting enzyme inhibitor cilazapril. J Cardiovasc Pharmacol 1987; 9: 219–24.
[30] Wellstein A, Essig J, Belz GG. Inhibition of angiotensin-I response by cilazapril and it's time course in normal volunteers. Clin Pharmacol Ther. 1987; 41: 639–44.
[31] Wellstein A, Essig J, Belz GG. A new method for estimating the potency of angiotensin converting enzyme inhibitors in man. Br J Clin Pharmacol 1987; 24: 397–99.