Journal of Cardiovascular Pharmacology
8(**Suppl. 11**): S29–S35 © 1986 Raven Press, New York

Bisoprolol (EMD 33512), a Highly Selective β_1-Adrenoceptor Antagonist: In Vitro and In Vivo Studies

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Summary: The properties of the newly developed selective β_1-adrenoceptor antagonist bisoprolol (EMD 33152) were investigated by in vitro and in vivo studies. Binding studies with (−)-[^{125}I] iodocyanopindolol (ICYP) revealed that the affinity of (±)-bisoprolol to β_1-adrenoceptors was ~100 times higher than to β_2-adrenoceptors. This high β_1-adrenoceptor selectivity of bisoprolol could be confirmed in binding studies with the tritiated compound (−)[^{3}H] bisoprolol, which labelled in rabbit lung membranes — a tissue known to contain 80% β_1- and 20% β_2-adrenoceptors — exclusively β_1-adrenoceptors. In physiological studies, (±)-bisoprolol was found to be devoid of any intrinsic sympathomimetic activity (ISA), since it had no positive chronotropic effects on spontaneously beating right atria of reserpinized rats. On isolated electrically driven human right atria, (±)-bisoprolol was ~30 times more potent in antagonizing the β_1-adrenoceptor–mediated positive inotropic effect of noradrenaline (pA_2-value, 8.42) than the β_2-adrenoceptor–mediated positive inotropic effect of procaterol (pA_2-value, 6.99). To test the β_1-adrenoceptor selectivity in vivo, the effects of bisoprolol administration (1 × 10 mg/day for 9 days) on lymphocyte β_2-adrenoceptor density [assessed by (−)-ICYP binding] in healthy volunteers were compared with those of the nonselective β-adrenoceptor antagonists propranolol (4 × 40 mg/day for 9 days) without ISA, and pindolol (2 × 5 mg/day for 9 days) with ISA. Lymphocyte β_2-adrenoceptor density was not affected by bisoprolol, neither during treatment nor after withdrawal, in contrast to the effects of propranolol, which increased β_2-adrenoceptor density by ~35%, and pindolol, which decreased β_2-adrenoceptor density by ~50%. On the other hand, in healthy volunteers bisoprolol (2.5 mg i.v. 30 min before the experiments) completely prevented the intrarenal β_1-adrenoceptor–mediated increase in plasma renin activity following isoprenaline infusion, indicating that bisoprolol is also in vivo a potent β_1-adrenoceptor antagonist. These results demonstrate in vitro and in vivo that bisoprolol is a selective β_1-adrenoceptor antagonist without ISA, possessing low affinity to β_2-adrenoceptors. Bisoprolol is, therefore, a suitable tool to study β-adrenoceptor subtypes in the human being. **Key Words:** Bisoprolol (EMD 33512) — β_1-Adrenoceptors — β_2–Adrenoceptors — β-Adrenoceptor subtypes in human heart — Plasma renin activity.

Bisoprolol (EMD 33512; Fig. 1) is a newly developed β-adrenoceptor antagonist possessing a high affinity for β_1-adrenoceptors (1). In this study, the properties of bisoprolol were investigated in several in vitro and in vivo models.

IN VITRO STUDIES

Affinity of (±)-bisoprolol to β_1- and β_2-adrenoceptors

To determine the affinity of (±)-bisoprolol to β_1- and β_2-adrenoceptors, inhibition of binding of the nonselective β-adrenoceptor radioligand (−)-[^{125}I]-iodocyanopindolol (ICYP), which labels β_1- and β_2-adrenoceptors with the same affinity (2) to rabbit lung membranes (in the presence of 50 n*M* ICI 118,551 = homogeneous population of β_1-adrenoceptors) and to rat lung membranes (in the presence of 7.5 μM atenolol = homogeneous population of β_2-adrenoceptors) by (±)-bisoprolol was investigated. (±)-Bisoprolol inhibited binding of ICYP to both tissues with steep monophasic displacement curves and pseudo Hill coefficients (n_H) not significantly different from 1.0 (3). From these displacement curves, a β_1/β_2-ratio for (±)-bisoprolol of ~100 was found (Table 1).

A similar β_1-selectivity of (±)-bisoprolol was ob-

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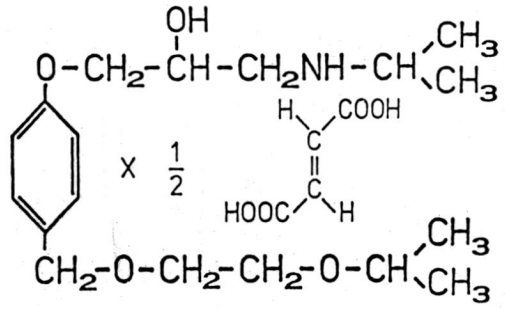

Bisoprolol hemifumarate (EMD 33 512)

FIG. 1. Structural formula of bisoprolol.

tained in physiological experiments. Recently, it has been shown that, on the isolated electrically driven human right atrium, both β_1- and β_2-adrenoceptors are involved in the positive inotropic action of β-agonists (4,5). On this preparation, noradrenaline (10^{-9}–10^{-5} M) produced its positive inotropic effect predominantly through β_1-adrenoceptor stimulation (4) (Fig. 2). (±)-Bisoprolol (10^{-9}–10^{-5} M) led to a concentration-dependent shift to the right of the dose–response curve for the positive inotropic effect of noradrenaline (Fig. 2). The threshold concentration for (±)-bisoprolol was between 10^{-9} and 10^{-8} M. The slope of the Schild plots for the antagonistic effect of (±)-bisoprolol versus noradrenaline was not significantly different from 1.0 (0.89 ± 0.12, n = 3), suggesting interaction with a homogeneous population of β-adrenoceptors. From the Schild plot, a pA_2-value for (±)-bisoprolol of 8.42 was calculated (Table 1). Similar pA_2-values, ranging from 8.65 to 8.87, have been reported recently for the antagonistic effect of (−)-bisoprolol on the positive inotropic effects of noradrenaline on isolated electrically driven muscle strips derived from three different regions of the kitten heart (6). On the other hand, 10–50 times higher concentrations of (±)-bisoprolol (threshold concentration, 10^{-7} M) were needed to induce a shift to the right of the dose–response curve for the positive inotropic effect of the β_2-agonist procaterol (Fig. 3), which produces its positive inotropic effect on the isolated electrically driven human right atrium, mainly through β_2-adrenoceptor stimulation (5). The slope of the Schild plots for antagonism of (±)-bisoprolol against procaterol-induced positive inotropic effects was again not significantly different from 1.0 (0.93 ± 0.08; n = 3); the pA_2-value for (±)-bisoprolol amounted to 6.99 (Table 1). Thus, on human myocardial β_1- and β_2-adrenoceptors, the β_1/β_2-selectivity ratio for bisoprolol was ~30.

(−)[^{3}H] Bisoprolol labels selectively β_1-adrenoceptors in rabbit lung membranes

To further characterize the β_1-adrenoceptor selectivity of bisoprolol, the binding properties of (−)[^{3}H] bisoprolol to rabbit lung membranes — a tissue known to contain ~80% β_1- and 20% β_2-adrenoceptors (7,8) — were investigated (3). Binding of (−)[^{3}H] bisoprolol to rabbit lung membranes was saturable at 25°C, of high affinity, rapid, and readily reversible. K_d-values obtained from equilibrium studies (4.7 ± 0.6 nM; n = 4) were in good agreement with those determined kinetically (K_d = 9.37 nM). Binding of (−)[^{3}H] biso-

TABLE 1. *Affinity of bisoprolol to β_1- and β_2-adrenoceptors determined in vitro in three different models*

Model	β_1-Adrenoceptors	β_2-Adrenoceptors
Inhibition of (−)-ICYP binding to membranes derived from rabbit lung (β_1) and rat lung (β_2) by (±)-bisoprolol	K_T-value (nM)	K_T-value (nM)
	26.9 ± 4.4 (5)	2,726 ± 233 (5)
Antagonism of (±)-bisoprolol against noradrenaline (β_1)- and procaterol (β_2)-induced positive inotropic effects on the isolated electrically driven human right atrium	K_B-value (nM)	K_B-value (nM)
	3.98 ± 0.11 (3)	103 ± 31 (3)
Binding of (−)[^{3}H] bisoprolol to rabbit lung membranes	K_d-value (nM)	—
	4.7 ± 0.6 (4)[a] 9.37[b]	

[a] Assessed by equilibrium studies.
[b] Assessed kinetically.

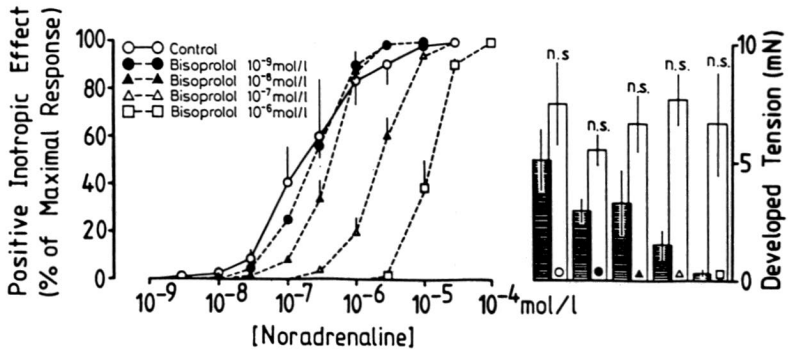

FIG. 2. Influence of bisoprolol on the positive inotropic effect of noradrenaline on the isolated muscle strip of human right atrial appendage. **Ordinate left:** positive inotropic effect in % of maximal response; **right:** basal (▤) and maximal developed tension (□) in the absence and presence of bisoprolol (in m*N*). **Abscissa:** molar concentrations of noradrenaline. Means ±SEM of three experiments are given. ○ = control, $pD_2 = 6.80 \pm 0.21$; ● = bisoprolol 10^{-9} mol/l, $pD_2 = 6.61 \pm 0.06$; ▲ = bisoprolol 10^{-8} mol/l, $pD_2 = 6.36 \pm 0.09$; △ = bisoprolol 10^{-7} mol/l, $pD_2 = 5.65 \pm 0.09$; □ = bisoprolol 10^{-6} mol/l, $pD_2 = 4.92 \pm 0.08$. n.s., not significantly different from the value obtained in the absence of bisoprolol.

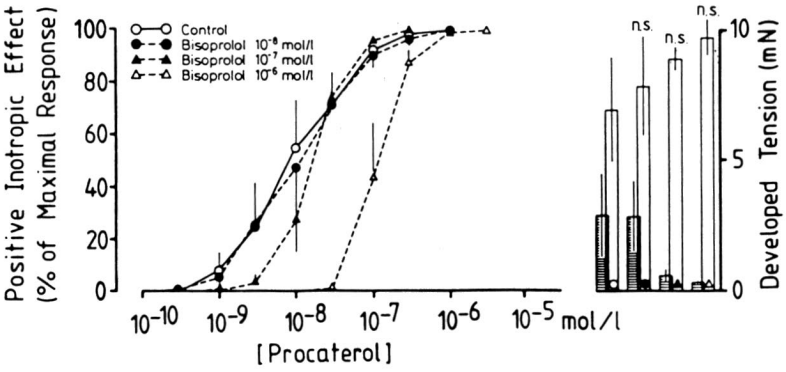

FIG. 3. Influence of bisoprolol on the positive inotropic effect of procaterol on the isolated muscle strip of human right atrial appendage. **Ordinate left:** positive inotropic effect in % of maximal response; **right:** basal (▤) and maximal developed tension (□) in the absence and presence of bisoprolol (in m*N*). **Abscissa:** molar concentrations of procaterol. Means ±SEM of three experiments are given. ○ = control, $pD_2 = 8.02 \pm 0.32$; ● = bisoprolol 10^{-8} mol/l, $pD_2 = 7.96 \pm 0.27$; ▲ = bisoprolol 10^{-7} mol/l, $pD_2 = 7.79 \pm 0.04$; △ = bisoprolol 10^{-6} mol/l, $pD_2 = 6.97 \pm 0.13$. n.s., not significantly different from the value obtained in the absence of bisoprolol.

TABLE 2. *K_I-values (nM) and pseudo Hill coefficients (n_H) for inhibition of (−)[^{3}H] bisoprolol binding by β-adrenergic antagonists and agonists in rabbit lung membranes*

Antagonists	K_I (nM)	n_H
(−)-Propranolol	1.69 ± 0.22	0.97 ± 0.04
(−)-Alprenolol	2.91 ± 0.27	1.04 ± 0.03
(±)-Bisoprolol	8.51 ± 0.77	0.99 ± 0.10
Metoprolol	61.97 ± 7.33	0.95 ± 0.05
ICI 118,551	169.0 ± 21.1	1.08 ± 0.07
(+)-Alprenolol	253.5 ± 27.7	0.99 ± 0.06
Practolol	281.7 ± 24.4	0.96 ± 0.09

	In the absence of Gpp(NH)p			In the presence of Gpp(NH)p	
Agonists	K_{IH} (nM)	K_{IL} (nM)	n_H	K_I (nM)	n_H
(−)-Isoprenaline	59 ± 4.4	667 ± 39	0.64 ± 0.07	477 ± 52	0.98 ± 0.05
(−)-Noradrenaline	161 ± 22	3931 ± 277	0.62 ± 0.09	3124 ± 288	1.00 ± 0.09
(−)-Adrenaline	277 ± 38	5250 ± 511	0.58 ± 0.06	4832 ± 379	0.96 ± 0.11

Rabbit lung membranes were incubated with (−)[^{3}H] bisoprolol (10–15 nM) and 15 concentrations of the competing agents, and specific binding was determined. IC_{50}-values for inhibition of binding by β-adrenoceptor antagonists were calculated from the resulting displacement curves and converted into K_I-values, according to the equation of Cheng and Prusoff (29). Inhibition of binding by β-adrenoceptor agonists was assessed in the presence or absence of 10 μM Gpp(NH)p; the resulting displacement curves were analyzed by nonlinear regression analysis, as described by Engel et al. (2). K_{IH} and K_{IL} refer to dissociation constants for high- and low-affinity states, respectively. Each value is the mean ± SEM of four experiments.

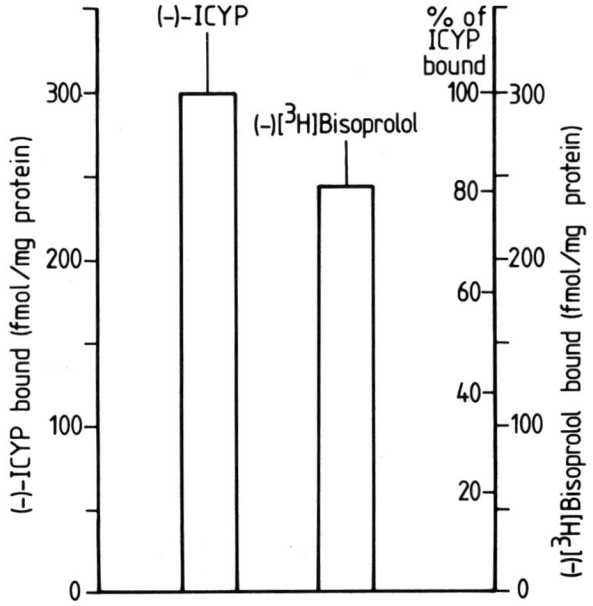

FIG. 4. Maximal number of (−)-ICYP (β_1- and β_2-adrenoceptors) and (−)[^{3}H]-bisoprolol (β_1-adrenoceptors) binding sites in rabbit lung membranes. **Ordinate left:** Total β-adrenoceptor number, assessed by Scatchard-analysis (28) of (−)-ICYP binding, in fmol (−)-ICYP specifically bound/mg protein; **right:** β_1-adrenoceptor number, assessed by Scatchard-analysis (28) of (−)[^{3}H]-bisoprolol binding, in fmol (−)[^{3}H]-bisoprolol specifically bound/mg protein. Means ±SEM of four experiments are given.

prolol to rabbit lung membranes was stereospecific, as indicated by the 100 times greater potency of (−)-alprenolol for inhibiting binding compared with its (+)-isomer (Table 2). Nonselective as well as subtype-selective β-adrenoceptor antagonists inhibited (−)[^{3}H] bisoprolol binding compared with monophasic displacement curves and pseudo Hill coefficients of 1.0, indicating that in rabbit lung membranes (−)[^{3}H] bisoprolol labels a homogeneous class of β-adrenoceptors (Table 2). Agonists inhibited binding with an order of potency: (−)-isoprenaline > (−)-noradrenaline ≥ (−)-adrenaline (Table 2), which is a typical one for β_1-adrenoceptors. These results therefore indicate that (−)[^{3}H] bisoprolol labels in rabbit lung membranes exclusively β_1-adrenoceptors. This assumption is supported by the fact that the maximal number of (−)[^{3}H] bisoprolol binding sites in rabbit lung membranes (244 ± 31 fmol bound/mg protein; n = 4) was only 80% of the number of sites labeled by the non-selective β-adrenoceptor radioligand (−)-ICYP (299 ± 36 fmol/mg protein, n = 4, Fig. 4). Thus, the amount of β_1-adrenoceptors in rabbit lung membranes determined directly by (−)[^{3}H] bisoprolol binding (80%) was identical with that previously assessed indirectly by analyzing the inhibition of binding of the nonselective β-adrenoceptor radioligands (−)[^{3}H] dihydroalprenolol (7) or ICYP (8) by β_1- and β_2-selective agents. (−)[^{3}H] Bisoprolol therefore is a suitable ligand to determine directly β_1-adrenoceptors in tissues containing both β-adrenoceptor subtypes.

Bisoprolol is devoid of any intrinsic sympathomimetic activity (ISA)

The spontaneously beating right atrium of rats pretreated with reserpine (10 mg/kg i.p., 24 h before experiments) is a suitable model to determine the ISA of β-adrenoceptor antagonists (9,10). On this preparation, the maximum increase in heart rate evoked by pindolol amounted to 39% of that induced by saturating concentrations of the full agonist isoprenaline (Fig. 5). Accordingly, the ISA of pindolol is 0.39 (10). (±)-Bisoprolol, however, showed no positive chronotropic activity, but caused negative chronotropic effects in concentrations $> 0.1\ \mu M$ (Fig. 5).

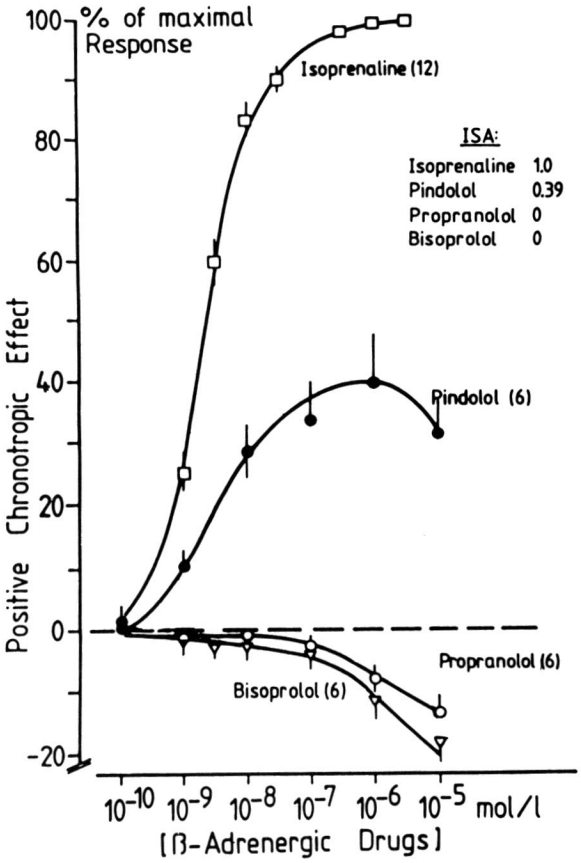

FIG. 5. Positive chronotropic effects of β-adrenoceptor antagonists on spontaneously beating right atria from reserpinized rats. **Ordinate:** Positive chronotropic effect in % of maximal response (i.e., increase in heart rate evoked by 10^{-6} mol/l isoprenaline = +132.5 ± 8.2 beats/min = 100%). Basal rates of beating (beats/min) amounted to 208.3 ± 5.6 (isoprenaline); 198.8 ± 6.5 (pindolol); 220.5 ± 3.9 (propranolol); 234.3 ± 8.0 (bisoprolol). **Abscissa:** molar concentrations of the β-adrenergic drugs. Means ±SEM are given. Number of experiments in parentheses.

IN VIVO STUDIES

Comparison of the effects of propranolol, pindolol, and bisoprolol on lymphocyte β_2-adrenoceptor density in healthy volunteers

Circulating lymphocytes containing a homogeneous population of β_2-adrenoceptors (11–13), excitatorily coupled to the adenylate cyclase (14,15), are a model frequently used to study drug-induced alterations of β-adrenoceptors in humans (16). In this model, the effects of propranolol (nonselective β-adrenoceptor antagonist without ISA) and pindolol (nonselective β-adrenoceptor antagonist with strong ISA) were compared with those of bisoprolol. Administration of propranolol (4 × 40 mg/day for 9 days) to healthy volunteers caused significant increases in lymphocyte β_2-adrenoceptor density, which, after 2 days, amounted to ~30–35% (Fig. 6A). A similar increase in lymphocyte β_2-adrenoceptor density in healthy volunteers following propranolol treatment has also been observed by Aarons et al. (17) and Wood et al. (18). After abrupt withdrawal of propranolol, β_2-adrenoceptor density declined slowly, still being significantly elevated for 3–5 days, although 24 h after withdrawal no propranolol was detectable in plasma. Such a "supersensitivity" of β-adrenoceptors following the withdrawal of propranolol has been suggested to be the cause of the "propranolol-withdrawal phenomenon" (19). Administration of pindolol (2 × 5 mg/day) on the contrary, caused significant decreases in lymphocyte β_2-adrenoceptor density (Fig. 6B), in accordance with data recently reported by Molinoff and Aarons (20) and Giudicelli et al. (21). After withdrawal of pindolol, β_2-adrenoceptor density recovered slowly, still being significantly reduced after 4 days, although no pindolol was detectable in plasma after 36 h. It is very likely that under these conditions, rapid cessation of drug treatment, should not induce rebound effects. In fact, pindolol does not show any symptoms of adrenergic hypersensitivity after rapid withdrawal (22,23).

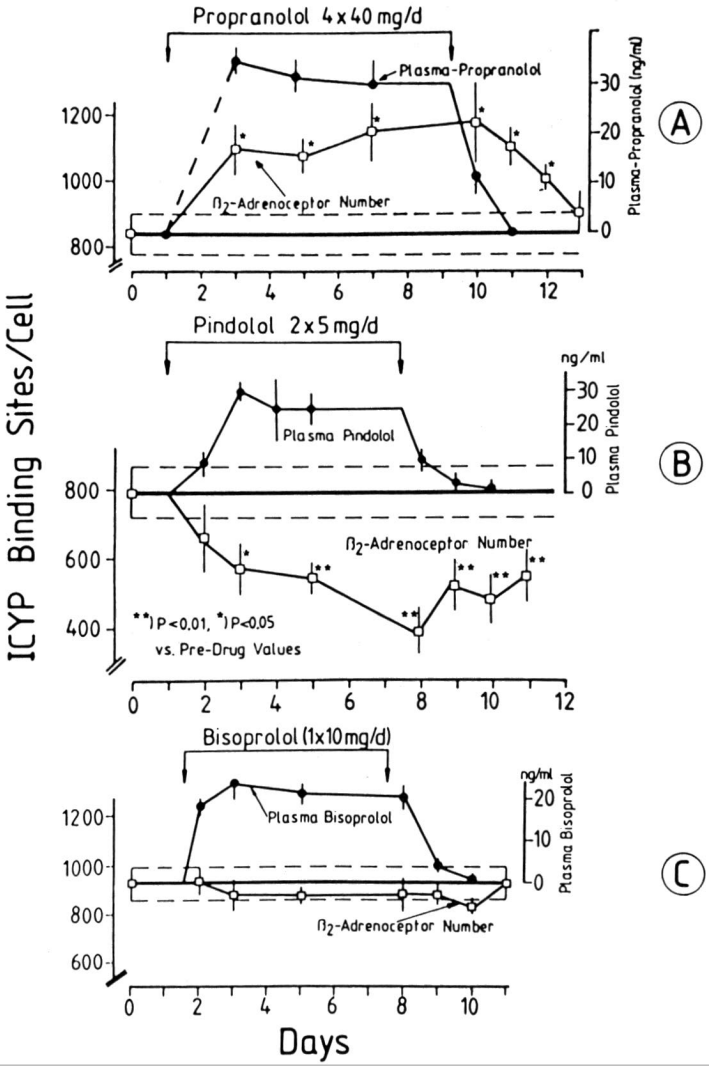

FIG. 6. Effects of propranolol (4 × 40 mg/day) (**A**), pindolol (2 × 5 mg/day), (**B**), and bisoprolol (1 × 10 mg/day) (**C**) on β_2-adrenoceptor density in lymphocytes of healthy volunteers. **Ordinates:** β_2-adrenoceptor density in lymphocytes, assessed by Scatchard-analysis (28) of ICYP binding, in ICYP binding sites/cell. **Abscissa:** Days of study. Means ±SEM of six (**A**), eight (**B**), and twelve (**C**) experiments, respectively, are given. Horizontal lines and broken lines represent means ±SEM of predrug levels.

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