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Research Article

Abrupt withdrawal after the chronic administration of propranolol has resulted in clinical syndromes that suggest adrenergic hypersensitivity. The effect of propranolol administration and withdrawal on beta-adrenergic receptors was studied in human lymphocyte membranes. Receptor density was quantitated by direct binding assays with the radioligand [125I]iodohydroxybenzylpindolol. Administration of propranolol (160 mg/d) for 8 d resulted in trough plasma levels of approximately 35 ng/ml. By day 5 of propranolol administration the density of beta-adrenergic receptors had increased 43 +/- 4% (P less than 0.01) above pretreatment levels. Abrupt withdrawal of propranolol was followed by the disappearance of propranolol from the plasma within 24 h. The density of beta-adrenergic receptors did not return to pretreatment level for several days. Physiologic supersensitivity of beta-adrenergic receptor-mediated responses was suggested by the appearance of significant increases in the orthostatic change in heart rate (P less than 0.05) and the orthostatic change in the heart rate-systolic blood pressure product (P less than 0.01) during the first 48 h after propranolol withdrawal. These data show that propranolol administration leads to an increase in the density of beta-adrenergic receptors in human tissue. The results are consistent with the hypothesis that some of the untoward effects observed after abrupt discontinuation of propranolol are caused by beta-receptor-mediated adrenergic hypersensitivity.

Elevation of β-Adrenergic Receptor Density in Human Lymphocytes after Propranolol Administration

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ABSTRACT Abrupt withdrawal after the chronic administration of propranolol has resulted in clinical syndromes that suggest adrenergic hypersensitivity. The effect of propranolol administration and withdrawal on β-adrenergic receptors was studied in human lymphocyte membranes. Receptor density was quantitated by direct binding assays with the radioligand [^{125}I]iodohydroxybenzylpindolol. Administration of propranolol (160 mg/d) for 8 d resulted in trough plasma levels of ~35 ng/ml. By day 5 of propranolol administration the density of β-adrenergic receptors had increased 43±4% ($P < 0.01$) above pretreatment levels. Abrupt withdrawal of propranolol was followed by the disappearance of propranolol from the plasma within 24 h. The density of β-adrenergic receptors did not return to pretreatment level for several days. Physiologic supersensitivity of β-adrenergic receptor-mediated responses was suggested by the appearance of significant increases in the orthostatic change in heart rate ($P < 0.05$) and the orthostatic change in the heart rate-systolic blood pressure product ($P < 0.01$) during the first 48 h after propranolol withdrawal. These data show that propranolol administration leads to an increase in the density of β-adrenergic receptors in human tissue. The results are consistent with the hypothesis that some of the untoward effects observed after abrupt discontinuation of propranolol are caused by β-receptor-mediated adrenergic hypersensitivity.

INTRODUCTION

β-Adrenergic receptor antagonists are widely used for the treatment of angina pectoris, atrial and ventricular arrhythmias, and hypertension. The most widely used drug of this class is propranolol, which produces therapeutic effects with relatively minimal toxicity even during chronic administration (1). However, recent reports have suggested that abrupt withdrawal of propranolol in patients with ischemic heart disease may precipitate increasingly severe and frequent anginal attacks, arrhythmias, myocardial infarction, and occasionally sudden death (2–7). This "propranolol withdrawal" phenomenon has been suggested to involve progression of underlying coronary artery disease (8), exceeding the level of angina-limited exercise activity (6), hyperaggregability of platelets (6, 9), increased plasma renin activity (6), alteration in the hemoglobin-oxygen dissociation curve (6), elevated levels of thyroid hormones (10), increased sympathetic tone (6), increased ventricular volume and wall stress (11), and rebound hypersensitivity of the β-adrenergic receptor to sympathetic stimuli (4, 12, 13).

Recently developed techniques for measuring the specific high affinity binding of radioligands to membrane preparations has made it possible to directly study β-adrenergic receptors in vitro (14). It has been observed that chronic changes in receptor occupancy lead to changes in receptor density. For example, incubation with agonists in vitro has been shown to lead to a decrease in the density of β-adrenergic receptors in frog erythrocytes (15), human astrocytoma cells (16, 17), and S-49 mouse lymphosarcoma cells (18). In vivo administration of agonists decreases β-adrenergic receptor density in frog erythrocytes (19) and in human polymorphonuclear leukocytes (20), and the administration of antidepressants, which block reuptake of catecholamines, also leads to a decrease in the density of β-adrenergic receptors in rat brain (21, 22). Conversely, chronic decreases in receptor occupation after denervation (23) or the administration of propranolol (22, 24) leads to increases in the density of β-adrenergic receptors in rat cerebral cortex and heart.

Smith and Parker (25) suggested that leukocytes,

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which possess β-adrenergic receptors, can be used to monitor effects on β-adrenergic receptors in human tissue. Direct identification of β-adrenergic receptors in human lymphocytes has been demonstrated by Williams et al. (26) using the radioligand [^{3}H]dihydroalprenolol. However, the comparatively low specific activity of [^{3}H]dihydroalprenolol necessitates the use of large amounts of blood, a disadvantage if repeated measurements are to be made on a given subject during a chronic study. [^{125}I]Iodohydroxybenzylpindolol (IHYP)[1] has also been used to identify and characterize β-adrenergic receptors in a variety of tissues (14). Since the specific activity of IHYP is more than 100 times greater than that of [^{3}H]dihydroalprenolol, IHYP should prove particularly useful in longitudinal studies requiring repeated sampling of tissue. In the current study, the application of direct IHYP binding techniques to the study of human lymphocyte β-adrenergic receptors is described. Propranolol administration led to a transitory increase in receptor density in lymphocytes. An increase of this type may be related to the clinical symptoms that have been observed after abrupt cessation of propranolol administration.

METHODS

Subjects and treatment. Blood was withdrawn by venipuncture from apparently healthy volunteers. The effects of propranolol were studied in 12 subjects (6 male, 6 female) ranging in age from 23 to 33 yr (mean, 27 yr). All subjects were drug free and provided a medical history and underwent a physical examination to exclude the presence of asthma, chronic pulmonary disease, diabetes, hypertension, cardiac disease of any kind, and signs or symptoms referable to the cardiopulmonary system. Propranolol (Inderal; Ayerst Laboratories, New York) was self-administered orally 160 mg/d for 8 d (40 mg every 6 h). Subjects were studied from 2 d before initiation of propranolol administration to 9 d after the last dose of propranolol. Informed consent was obtained.

Preparation of lymphocytes. Lymphocytes were isolated from heparinized blood according to the method of Böyum (27). Fresh heparinized whole blood (25 ml) was diluted with an equal volume of 0.9% NaCl. Aliquots (25 ml) of the mixture were carefully layered onto 10 ml of Isolymph (Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, N. Y.) using a variable speed peristaltic pump (Rainin Instrument Co., Inc., Woburn, Mass.). Tubes were centrifuged at 600 *g* for 40 min at 20°C. After careful removal of the plasma/platelet layer, the lymphocyte band (at least 90% small lymphocytes, <8% monocytes, <2% polymorphonuclear leukocytes) was harvested by vacuum aspiration and homogenized (Polytron homogenizer [Brinkmann Instruments, Inc., Westbury, N. Y.], speed 8 for 15 s). The homogenate was diluted in ice-cold glass distilled H_2O and centrifuged at 20,000 *g* for 10 min at 4°C. The supernate was discarded and the pellet resuspended in 40 ml of ice-cold glass distilled H_2O and allowed to stand on ice for 60 min to ensure adequate lysis. The preparation was centrifuged at 20,000 *g* for 10 min and the resulting pellet was resuspended in 2.5 ml of isotonic saline buffered with 20 mM Tris-HCl (pH 7.5). Samples were then frozen and stored at −80°C before being assayed. Storage for up to 8 wk had no effect on the density of receptors or on their pharmacological properties.

β-Adrenergic receptor binding assay. Hydroxybenzylpindolol was iodinated with ^{125}I, and IHYP was purified to theoretical specific activity (2.2 Ci/μmol) as described by Maguire et al. (28) and Harden et al. (29). IHYP and other drugs were prepared in 2.8 mM ascorbic acid that contained 10 μg/ml bovine serum albumin. Membranes were thawed and diluted with 2.5 ml of isotonic saline buffered with 20 mM Tris-HCl (pH 7.5). An aliquot (100 μl) of the membrane preparation containing 40–50 μg of protein was incubated with IHYP in a final volume of 250 μl which contained 0.09 M NaCl, 12 mM Tris-HCl (pH 7.5), 1 μg bovine serum albumin, 1 mM ascorbic acid, and 30 μM phentolamine. Binding assays were routinely carried out in new disposable polypropylene tubes (Walter Sarstedt, Inc., Princeton, N.J.). Samples were incubated for 30 min at 37°C to achieve equilibrium. Reactions were then stopped by adding 10 ml of 0.15 M NaCl in 10 mM Tris buffer (pH 7.5, 37°C) to each assay tube, and the samples were rapidly filtered through Gelman type AE glass fiber filters (Gelman Sciences, Inc., Ann Arbor, Mich.). Each filter was washed with an additional 10 ml of buffer and radioactivity was determined in a Searle gamma counter (Searle Radiographics Inc., Des Plaines, Ill.). Protein was determined by the method of Lowry et al. (30) with bovine serum albumin as a standard.

To determine the potency of drugs in inhibiting IHYP binding, various concentrations of each drug were incubated with IHYP (40,000–60,000 cpm; 41–62 pM) in the media described above. Specific binding of IHYP was defined as the amount of IHYP bound in the absence of competing ligand minus the amount bound in the presence of 10 μM (−)-isoproterenol. This concentration is 100 times the K_d of isoproterenol and with an observed Hill coefficient of 0.7 corresponds to occupancy of 95% of the receptors. To determine the density of binding sites, the amount of specifically bound IHYP was determined at nine concentrations of IHYP ranging from 10 to 227 pM (10,000–220,000 cpm). The data were analyzed by the method of Scatchard (31) to provide a value for the density of receptors and the K_d of IHYP. To examine the possibility that both β_1- and β_2-adrenergic receptors occur in lymphocytes the amount of specifically bound IHYP was determined at 14 concentrations of practolol in the presence of 100 μM guanosine 5′-triphosphate. The data were analyzed as described by Minneman et al. (32).

Plasma propranolol assay. The concentration of propranolol in plasma was determined by fluorescence using high-pressure liquid chromatography (HPLC [33]). Heparinized blood (5 ml) was centrifuged at 2,500 rpm for 15 min. The plasma layer was transferred by Pasteur pipette to a new polypropylene tube, capped, and stored frozen until the day of assay. Aliquots (1 ml) of standard (50 ng/ml of propranolol-HCl in 0.01 N HCl), controls (200 and 20 ng/ml propranolol-HCl in 3% bovine serum albumin), and patient samples were placed into individual conical screw-cap extraction tubes. A 500-μl aliquot of internal standard (500 ng/ml of 4-methylpropranolol in 0.01 N HCl) was added to each tube. NaOH (100 μl, 2 N) and ethyl acetate (3.0 ml; 'Baker Analyzed' reagent, J. T. Baker Chemical Co., Phillipsburg, N.J.) were added in sequence, the tubes were capped tightly and shaken briefly by hand. All tubes were subsequently mixed with a vortex mixer for 30 s and centrifuged at 2,500 rpm for 2 min. An aliquot (2.5 ml) of the upper layer was transferred to an evaporation tube, H_2SO_4 was added (100 μl, 0.1 N) and each tube was mixed with a vortex mixer for 60 s and centrifuged at 2,500 rpm for 1 min. After most of the top layer had been

[1] *Abbreviation used in this paper*: IHYP, 1 iodohydroxybenzylpindolol.

removed by aspiration, 40 μl of the bottom layer was injected onto a 30-cm × 3.9-mm μBondapak C18 reverse phase column (Waters Associates, Inc., Millford, Mass.). The mobile phase was 50% methanol in 0.01 M $NH_4H_2PO_4$. Fluorescence was measured with a model 204S fluorescence detector (Perkin-Elmer Corp., Norwalk, Conn.) employing an excitation wavelength of 293 nm and an emission wavelength of 350 nm. At a flow rate of 2 ml/min the retention times for propranolol and 4-methylpropranolol were 4.0 and 6.6 min, respectively. Plasma propranolol concentration was obtained by comparing the propranolol/internal standard peak height ratio to the standard peak height ratio. This assay procedure was linear from 5 ng/ml to at least 200 ng/ml. The smallest detectable concentration of propranolol was 3 ng/ml.

Blood pressure and heart rate. On each of 2 d before initiation of propranolol treatment and periodically during and for 5 d after propranolol treatment blood pressures and heart rates were measured. Measurements were obtained in the midmorning immediately before a dose of propranolol. Determinations were obtained after the subjects had been supine for five min and after they had assumed an upright posture for 2 min. Immediately after these measurements, 25–30 ml of venous blood was drawn for preparation of lymphocytes and determination of plasma propranolol concentration.

Statistical methods. Data were analyzed by the two-sided multiple comparison test described by Dunnett (34, 35) employing error estimates obtained from two-way analysis of variance. Unweighted linear regression analysis was performed by the method of least squares. All statistical procedures were carried out on a Wang 2200-T computer system (Wang Laboratories, Inc., Lowell, Mass.).

Materials. Hydroxybenzylpindolol was the generous gift of Dr. Daniel Hauser of Sandoz Pharmaceuticals, Basel, Switzerland. Na ^{125}I was purchased from New England Nuclear, Boston, Mass. Practolol and the *d*- and *l*-stereoisomers of propranolol were gifts of Ayerst Laboratories, New York. Phentolamine mesylate (Regitine) was obtained from CIBA Pharmaceutical Co., Summit, N. J. (*d*)-Isoproterenol-*d*-bitartrate was a gift from Winthrop Laboratories, Div. of Sterling Drugs, New York. 4-Methylpropranolol was the generous gift of Dr. P. Van den Broek of ICI, Pharmaceutical Div., Macceles Field, England. All other drugs and reagents were commercially available.

RESULTS

Effects of phentolamine on specific and nonspecific IHYP binding to lymphocyte membranes. Phentolamine, at concentrations of up to 100 μM, selectively reduces nonspecific binding of IHYP in regions of rat brain but has no effect on specific binding (32, 36). The effects of increasing concentrations of phentolamine (up to 300 μM) on total IHYP binding, IHYP binding in the presence of an arbitrary (10 μM) concentration of *d*-isoproterenol, and IHYP binding in the presence of the same concentration of *l*-isoproterenol was determined in lymphycyte membranes (Fig. 1). The difference between binding in the presence of *d*-isoproterenol and binding in the presence of *l*-isoproterenol represents specific binding to some but not all of the β-adrenergic receptors in the preparation. This difference does not represent binding to all the specific binding sites since *d*-isoproterenol at this concentration also inhibits some specific binding (see below). Although total binding was markedly reduced by phentolamine the specific binding sites measured in this manner were not affected by concentrations of phentolamine up to 30 μM.

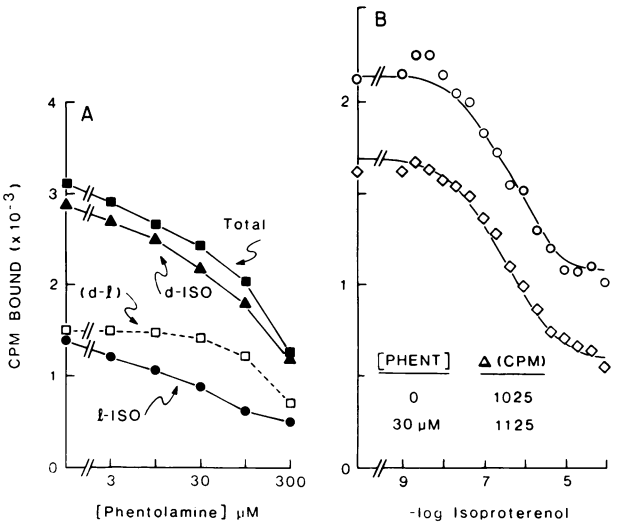

FIGURE 1 Effect of phentolamine on IHYP binding in human lymphocytes. (A) The effect of increasing concentrations of phentolamine on total (■) IHYP binding, IHYP binding in the presence of 10 μM *d*-isoproterenol (▲), IHYP binding in the presence of 10 μM *l*-isoproterenol (●), and the difference between binding in the presence of *d*- and *l*-isoproterenol (□) was determined. (B) The effect of increasing concentrations of *l*-isoproterenol was measured on IHYP binding in the absence (○) or the presence of 30 μM (◇) phentolamine. The inset shows the difference (in counts per minute) between total IHYP binding and IHYP binding in the presence of a maximally inhibitory concentration of *l*-isoproterenol. Assays were performed in the presence of 41–62 pM IHYP as described in the text and each point is the mean of triplicate determinations from two or three separate experiments.

To define total specific binding, dose-response curves for the inhibition of IHYP binding by *l*-isoproterenol were done in the absence and presence of 30 μM phentolamine. The difference between binding in the absence of *l*-isoproterenol and binding in the plateau region (isoproterenol concentrations > 5 μM) was identical in the presence or absence of phentolamine. However, the percentage of specific binding increased from 48% in the absence of phentolamine to 69% in the presence of 30 μM phentolamine. This decrease in nonspecific binding was not caused by a selective effect on α-adrenergic receptors since *d*- and *l*-propranolol and phentolamine were all approximately equipotent inhibitors of nonspecific binding. In addition, the concentration of phentolamine used was four orders of magnitude higher than is usually required to block α-adrenergic receptors (37, 38).

Characteristics of IHYP binding to lymphocyte

membranes. Specific binding of IHYP was rapid, reversible, and saturable, and Scatchard analysis (31) showed that IHYP bound to a single class of high affinity sites in lymphocyte membranes (Fig. 2). The calculated K_d value for IHYP binding to β-adrenergic receptors was 53±5 pM and the density of binding sites was 15.4±0.9 fmol IHYP bound/mg protein (n = 12).

Inhibition of IHYP binding by agonists and antagonists. Propranolol, isoproterenol, epinephrine, and norepinephrine inhibited IHYP binding to lymphocyte membranes (Fig. 3). The inhibition of IHYP binding showed stereospecificity in that *l*-propranolol and *l*-isoproterenol were two orders of magnitude more potent than their corresponding *d*-stereoisomers. The pharmacological specificity for the inhibition of IHYP binding, determined by the order of potency *l*-isoproterenol > *l*-epinephrine > *l*-norepinephrine, is that expected of a β_2-adrenergic receptor. The inhibition of specific IHYP binding to human lymphocyte membranes by the β_1-selective antagonist practolol resulted in linear Hofstee plots with a calculated IC_{50} for practolol of 39 μM. These data are consistent with the conclusion that human lymphocytes contain only a single subtype of β-adrenergic receptors. In a variety of mammalian tissues the K_d of practolol for β_1-adrenergic receptors ranges from 0.5 to 5.0 μM and for β_2-adrenergic receptors ranges from 20 to 90 μM (32). This also suggests that human lymphocytes contain only β_2-adrenergic receptors.

Effect of propranolol administration on plasma propranolol concentration. After the initiation of propranolol administration the plasma concentration of propranolol rose rapidly to steady-state level within 24 h (Fig. 4), which was maintained throughout the 8-d course of treatment. Propranolol rapidly disappeared from the plasma within 24 h after the last dose (Fig. 4).

Effect of propranolol administration on the K_d for IHYP binding. The apparent K_d for specific IHYP binding to lymphocyte membranes increased during treatment with propranolol (Fig. 5). There was a significant increase in the K_d for IHYP after 24 h of propranolol administration. This effect was maximal after 8 d of treatment at which time the K_d had risen threefold above control level. When drug administration was discontinued the K_d value returned to control level within 24 h.

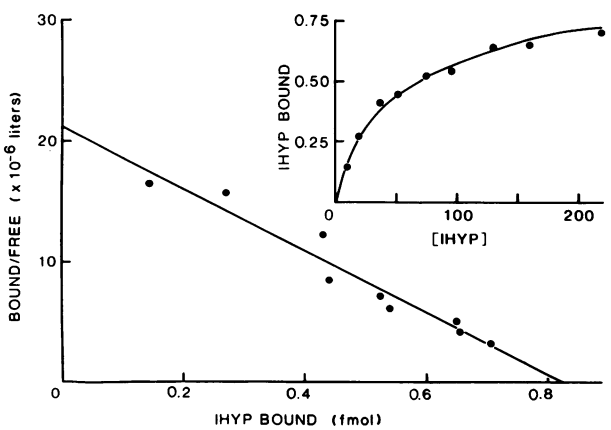

FIGURE 2 Binding of various concentrations of ^{125}I-IHYP to membranes from human lymphocytes. Assays were carried out under standard conditions as described in the text. The concentration of IHYP was varied between 10–227 pM. Data from a representative experiment is plotted by the method of Scatchard (31) to yield values for the K_d of ^{125}I-IHYP (39 pM) and the maximum number of specific binding sites (15.3 fmol/mg protein). The inset shows the amount of ^{125}I-IHYP bound (femtomoles) plotted as a junction of the concentration of ^{125}I-IHYP (picomolars).

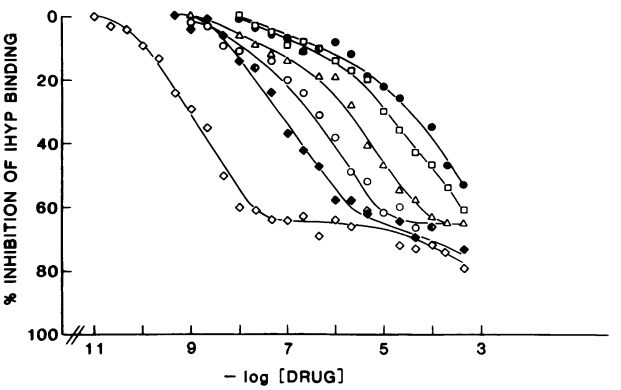

FIGURE 3 Binding of ^{125}I-IHYP to membrane fraction from human lymphocytes. Binding in the presence of 41–62 pM IHYP was carried out in the presence of various concentrations of *l*-propranolol (◇), *d*-propranolol (◆), *l*-isoproterenol (○), *d*-isoproterenol (●), *l*-epinephrine (△), or *l*-norepinephrine (□). The ordinate represents the percentage of inhibition of total ^{125}I-IHYP binding. Each point is the mean of triplicate determinations from two to four separate experiments.

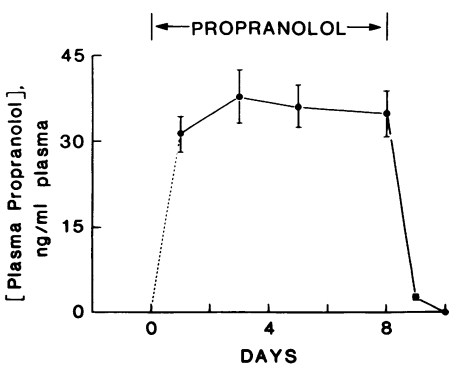

FIGURE 4 Effect of propranolol administration on plasma propranolol concentration. The amount of plasma propranolol (nanogram per milliliter of plasma) was determined by the fluorometric HPLC procedure described in the text. Each point is the mean±SEM of values from all 12 subjects.

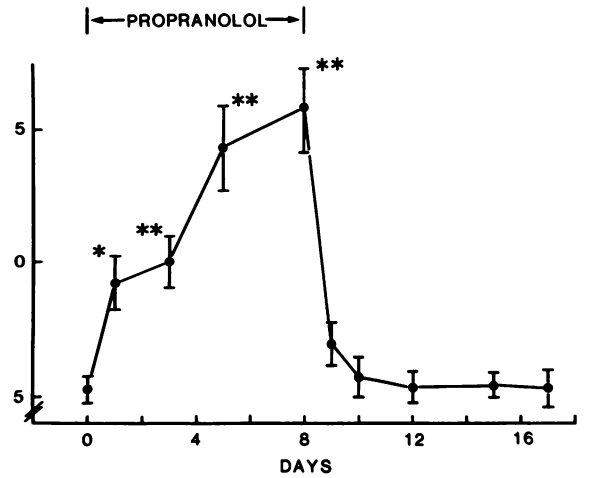

FIGURE 5 Effect of propranolol administration on the K_d for ^{125}I-IHYP binding to lymphocytes. The K_d for specific IHYP binding was determined in homogenates of human lymphocytes by the method of Scatchard (31) as described in the text. K_d values were determined at different times before, during, and after the termination of propranolol treatment. Each point is the mean±SEM of values from all 12 subjects. Assays were performed in duplicate. Values shown on day 0 are the mean of determinations from two separate pretreatment days. Statistical analysis was performed using the multiple comparisons method of Dunnett (34, 35) as described in the text (*$P < 0.05$; **$P < 0.01$).

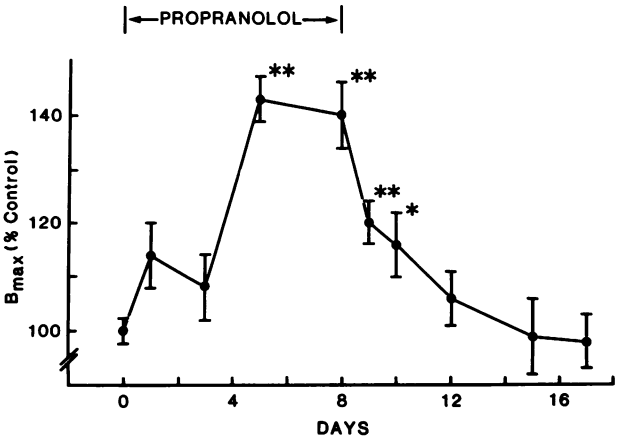

FIGURE 6 Effect of propranolol administration on the density of β-adrenergic receptors in human lymphocytes. The maximum amount of specifically bound ^{125}I-IHYP (femtomoles per milligram protein) was determined in homogenates of human lymphocytes by the method of Scatchard (31). Each point is the mean±SEM of values from all 12 subjects. Assays were performed in duplicate. Results are expressed as percentage of control binding where each subject served as his/her own control. The control value used for each subject was the mean of duplicate determinations from two separate pretreatment days. Statistical analysis was performed as described in Fig. 5 (*$P < 0.05$; **$P < 0.01$).

Effect of propranolol administration on the density of β-adrenergic receptors. The density of β-adrenergic receptors on lymphocyte membranes increased significantly during treatment with propranolol (Fig. 6). This effect was maximal after 5 d and persisted throughout continued propranolol administration. When propranolol administration was discontinued, the density of IHYP binding sites returned to the pretreatment level over the next several days. There was no significant alteration in the yield of membrane protein before, during, or after propranolol administration.

Physiological effects of chronic propranolol administration. Standing and supine heart rates decreased significantly during treatment with propranolol (Fig. 7). These decreases were maximal within 24 h and persisted for the 8 d of treatment. After the last dose of propranolol standing heart rate returned to the control level within 24 h. However, supine heart rate did not return to the pretreatment value until 4 d after discontinuation of propranolol. The orthostatic change in heart rate remained relatively unaffected during propranolol administration. However, after the last dose of propranolol there was a significant increase in the orthostatic change in heart rate that persisted for at least 2 d.

The heart rate-systolic blood pressure product (double product) has been used as an index of myocardial oxygen consumption (39). There were significant decreases in both standing and supine double products during treatment with propranolol (Fig. 8). This effect was also maximal within 24 h and persisted throughout continued propranolol administration. After the termination of propranolol administration the standing double product returned to the pretreatment level within 24 h. The supine double product returned to control level more slowly and was still significantly

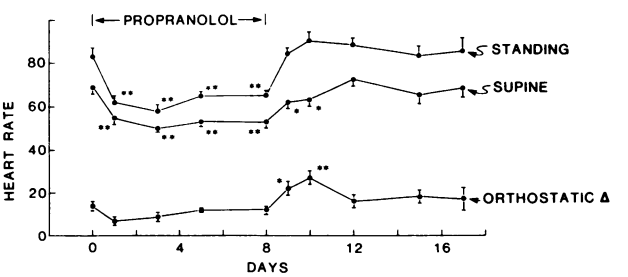

FIGURE 7 Effect of propranolol administration on heart rate. Standing and supine heart rates were determined before, during and after propranolol administration. Measurements were obtained in the midmorning immediately before a dose of propranolol. Determinations were obtained with the subject supine for 5 min and after the subjects had assumed upright posture for 2 min. The orthostatic change is the difference between standing and supine heart rates. Each point is the mean±SEM of values from all 12 subjects. Values shown on day 0 are the mean of determinations from two separate pretreatment days. The ordinate represents the heart rate measured in beats per minute. Statistical analysis was as described in Fig. 5 (*$P < 0.05$; **$P < 0.01$).

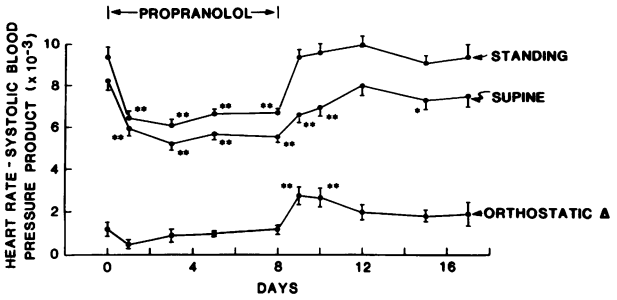

FIGURE 8 Effect of propranolol administration on the heart rate-systolic pressure double product. Measurements were obtained as described in Fig. 7. Each point is the mean±SEM of values from all 12 subjects. Statistical analysis was described in Fig. 5 (*$P < 0.05$; **$P < 0.01$).

decreased 48 h after the last dose of propranolol. The orthostatic change in the double product remained relatively unchanged during propranolol treatment. However, after the last dose of propranolol there was a significant increase in the orthostatic change in the double product that persisted for at least 2 d. Computer evaluation of individual subject responses demonstrated a linear correlation between the magnitude of change in β-adrenergic receptor density and the orthostatic change of both heart rate ($r = 0.63$; $P = 0.036$) and double product ($r = 0.67$; $P = 0.024$).

DISCUSSION

These studies show a significant elevation in the density of β-adrenergic receptors in lymphocytes from normal subjects taking propranolol. After 5 d of treatment receptor density had increased to an apparent steady-state level that was 40% above the pretreatment receptor density.

An unexpected finding of these studies was the increase in the apparent K_d for specific IHYP binding to the lymphocyte β-adrenergic receptors during propranolol treatment. This was probably caused by the presence of residual propranolol in the lymphocyte membranes that would compete with IHYP for binding to the β-adrenergic receptor and thereby generate the appearance of a decreased affinity of IHYP. The K_d values returned to control values in parallel with the disappearance of propranolol from the plasma (Fig. 4). It is possible that a true alteration in lymphocyte receptor affinity occurred. However, the observations that Scatchard analysis consistently demonstrated only a single high affinity binding site for IHYP throughout the study, and that the observed Hill coefficient of 1.0 for IHYP binding did not change during the study, argue convincingly against binding site heterogeneity. An elevation in the density of β-adrenergic receptors following chronic propranolol administration to rats (22, 24) has been observed without change in the K_d values for agonists or antagonists. The affect of chronic propranolol administration on the K_d of IHYP for β-adrenergic receptors in humans may be a reflection of the slower rate of propranolol disappearance in humans ($t_{1/2}$ = 3.4–6 h [40]) as compared with rats ($t_{1/2}$ = 1 h [41]). Furthermore, the time interval between the last dose of propranolol and the start of tissue isolation was 8–16 h in published studies with rats as compared to 4–6 h in the current studies.

The leukocyte preparation employed in this study contains at least 90% lymphocytes based on morphologic criteria. It has been suggested that cell identification using only simple morphologic criteria may overestimate the lymphocyte contribution to the leukocyte fraction obtained from the Ficoll-Hypaque procedure (42). It might be argued, therefore, that propranolol merely alters the relative contributions of different populations of leukocytes to the cell population that was studied. We believe several lines of evidence suggest this alternative to be highly unlikely. Previous investigators have demonstrated similar densities of β-adrenergic receptors on monocytes, lymphocytes purified on a nylon mesh column, and polymorphonuclear leukocytes (26). These three populations of leukocytes account for at least 99% of the cells obtained from the "lymphocyte band" of the Ficoll-Hypaque gradient. This would suggest that a change in the relative contribution of these leukocyte populations to our preparation would not cause any alteration in the observed density of receptors. However, it is still possible that propranolol administration can alter the relative contribution of subpopulations of lymphocytes (i.e., three classes of T cells, two known classes of B cells). However, since there was no overall change in the yield of total membrane protein before, during, or after propranolol treatment, any altered constituency of lymphocytes induced by β-adrenergic blockade would have been too small in magnitude to be quantifiable by morphologic or functional methods currently available to distinguish B cells from T cells. No practical methods yet exist for quantitating changes within subclasses of T or B cells.

The effectiveness of the propranolol regimen employed in this study was verified by decreases in heart rate and double product. To assess the β-adrenergic component of the physiological response to propranolol withdrawal recent studies have employed the method of bolus intravenous injections of isoproterenol (12, 13). This procedure was undesirable in the present study because repeated exposure to large concentrations of agonist could interfere with the natural history of the change in receptor density (14). Furthermore, maximal heart rate cannot be obtained with isoproterenol without serious risk of inducing cardiac arrhythmias. Unless maximal rate is obtained it is impossible to assess the pharmaco-dynamics of an effect. The observation that administration of propranolol to subjects

resting in the supine position does not alter ventricular dimensions and does not alter contractility, apart from the changes resulting from the slowing of heart rate (43), supports the view that there is relatively little basal sympathetic stimulation of the heart in supine, resting individuals (44). The cardiac acceleration produced on assuming an upright posture is mediated predominantly by an increase in β-adrenergic sympathetic stimulation (45). Therefore, orthostatic changes in heart rate were examined as an indirect index of β-adrenergic responsiveness. The heart rate-systolic blood pressure product (double product) has been used as an index of myocardial oxygen consumption (39, 46). Therefore, orthostatic changes in double product were also examined.

A significant elevation in the orthostatic change of the heart rate and the double product was observed 24 and 48 h after discontinuing propranolol administration. The increase in the orthostatic change of the double product suggests that β-adrenergic supersensitivity may be associated with an increase in the myocardial oxygen demand attributable to the act of standing. These results are consistent with the findings of Boudoulas et al. (12), who reported a significant rebound increase in heart rate and pulse pressure and a shortening of electromechanical systole produced by isoproterenol in normal volunteers between 24 and 48 h after abrupt propranolol withdrawal. Recent evidence reported by Nattel et al. (13) suggests that transient supersensitivity to β-adrenergic receptor mediated stimulation also occurs following abrupt propranolol withdrawal in patients with essential hypertension. Pedersen and coworkers (47) have reported that abrupt withdrawal of metoprolol or propranolol in hypertensive patients is associated with significant increases in both supine and standing heart rates between 24 and 48 h after the last dose, coinciding with the onset of subjective symptoms of rebound. The absence of pretreatment data precludes distinguishing between rebound and mere recovery in these patients. However, it is interesting to note that the orthostatic change in heart rate transiently increased more than threefold between 24 and 48 h after the last dose. It is unlikely that these experimental and clinical findings can be attributed merely to an increase in catecholamine levels during withdrawal since acute increases in plasma or urinary catecholamines have not been seen following abrupt withdrawal of either propranolol or metoprolol (13, 47–50).

Although the density of β-adrenergic receptors was increased by the 5th d of propranolol treatment, physiological expression of this change was prevented by the continued presence of propranolol. Within 24 h after the abrupt termination of propranolol administration only trace amounts of drug were detectable in the plasma, consistent with the observed half-life of propranolol previously shown to be 3.4–6 h after discontinuation of chronic administration (40). However, the decline of receptor density back to pretreatment level occurred over a considerably longer time. The disparity between the time-courses of these processes is consistent with the transient appearance of β-adrenergic supersensitivity. The significance of the 24–48 h period after abrupt propranolol withdrawal in humans has been repeatedly cited in clinical reports of the propranolol withdrawal rebound phenomenon (2–4, 7, 9, 11–13, 47).

The physiologic significance of the elevation in the density of β-adrenergic receptors rests in part on the studies cited above, which have directly demonstrated cardiac β-adrenergic supersensitivity in studies of peak myocardial responses to bolus isoproterenol. The orthostatic measures employed in this study are indirect and, hence, less sensitive since measurements were obtained after stable responses were achieved rather than at the peak response. In addition, the subjects were young and healthy and, therefore, potentially less susceptible to measurable physiologic supersensitivity than patients with angina or hypertension. Nevertheless, the time courses of the elevation in orthostatic change in heart rate and double product are consistent with previous studies and with the predictions based on the demonstrated changes in lymphocyte β-adrenergic receptor density and plasma propranolol concentration.

An unexpected finding of these studies was the dissociation between the time-courses for return to pretreatment values after withdrawal of propranolol for standing and supine measurements. The observed orthostatic changes are primarily caused by the depressed supine values rather than by elevated standing values. The mechanism accounting for the disparity between the recoveries of supine and standing measurements cannot be addressed in this study. Clearly a given stable response measure (e.g., supine heart rate) represents the summation of a variety of influences both parasympathetic as well as sympathetic, cardiac β-adrenergic as well as vascular β-adrenergic, etc. Understanding the mechanism behind a change in the absolute level of a single stable response measure would require knowledge of all of these factors. This does not, however, affect the validity of conclusions based only upon a change between two different stable response measures (e.g., supine vs. standing). The net effect of a change from supine to standing is a net increase in the level of sympathetic activity regardless of the absolute values of these two stable response measures. Peak heart rate response to bolus isoproterenol is a more purely cardiac β-adrenergic response, whereas an orthostatic change in stable heart rate is a more indirect response (i.e., not as purely cardiac or β-adrenergic) but nevertheless valid at least as an

index of the overall level of β-adrenergic activity. The correlation between the magnitude of change in β-adrenergic receptor density and orthostatic responses for individual subjects suggests a closer relationship between receptor density change and physiologic response. However, these correlations are limited by the sensitivity of the physiological measure and the unknown variability between subjects with regard to residual levels of propranolol in cardiovascular tissues. Furthermore, these correlations do not separate the influence of cardiac β-adrenergic responsiveness from the influence of other β-adrenergic-mediated functions regulating heart rate and double product such as renin release, thyroid hormone release, vascular responses, etc. Evidence suggests that our results with human lymphocyte β-adrenergic receptors may relate to other tissues as well. Our results are in agreement with Glaubiger and Lefkowitz (24) and Wolfe et al. (22) and suggest that the effect of chronic propranolol administration on β-adrenergic receptor density does not differ qualitatively between the heart (24), brain (22), and the lymphocyte. However, verification of the specific effects of chronic propranolol administration on the properties of β-adrenergic receptors in other tissues involved in cardiovascular regulation, and whether such changes might be related to a transient increase in the susceptibility to angina, must await the outcome of further investigation.

These studies clearly demonstrate the feasibility of using readily obtainable human lymphocytes to study chronic alterations in β-adrenergic receptor density in humans. Study of lymphocyte β-adrenergic receptors may aid the understanding of a variety of diseases including hypertension, asthma, and chronic obstructive pulmonary disease, as well as the long-term cellular consequences of pharmacologic intervention.

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