MOLECULAR PHARMACOLOGY, 24:169–173

The Human Heart *Beta*-Adrenergic Receptors

I. Heterogeneity of the Binding Sites: Presence of 50% $Beta_1$- and 50% $Beta_2$-Adrenergic Receptors

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Received January 24, 1983; Accepted April 11, 1983

SUMMARY

Beta-adrenergic receptors were characterized in a particulate fraction of human auricles obtained from patients operated upon for coronary insufficiency or valvular disease. [^{125}I] Hydroxybenzylpindolol binding was evaluated in terms of kinetics; K_D and B_{max} values; and inhibition of binding in the presence of 10 μM GTP and of increasing concentrations of four nonselective agonists giving a Hill coefficient of 1 (isoproterenol, salbutamol, fenoterol, and epinephrine), of two nonselective antagonists giving a Hill coefficient of 1 (pindolol and propranolol), and of a series of selective drugs giving a Hill coefficient of 0.60–0.72 that included three $beta_1$-selective antagonists (practolol, metoprolol, and atenolol) and two $beta_2$- selective agonists (procaterol and zinterol). K_D values for all drugs were compatible with the coexistence in membranes from human auricles of $beta_1$- and $beta_2$-adrenergic receptors, the relative proportions of receptors of each subclass being approximately the same.

INTRODUCTION

Lands *et al.* (1, 2) have suggested that the pharmacological response to *beta*-adrenergic agonists can be related to occupancy of two types of receptors: $beta_1$-adrenergic receptors displaying an equal affinity for the endogenous catecholamines epinephrine and norepinephrine, and $beta_2$-adrenergic receptors with a significantly higher affinity for epinephrine than for norepinephrine. These studies were later confirmed and extended, using a whole range of selective agonists and antagonists, and with *in vitro* techniques allowing a direct identification of membrane receptors (the relevant binding sites for catecholamines). Rugg *et al.* (3) and Minneman *et al.* (4, 5) have recently analyzed, in tissue homogenates and membranes, displacement curves of a nonselective radiolabeled ligand by selective molecules using a computerized graphic program determining the relative proportion of receptors of each subtype. With this approach, the pharmacological specificity of $beta_1$- and $beta_2$-adrenergic receptors was shown to be the same in several mammalian tissues and cells, and the interpretation of the experimental data did not require the existence of other subtype(s) of *beta*-adrenergic receptors (5).

It is now considered that cardiac tissue usually contains a majority of $beta_1$-adrenergic receptors [100% in cat and guinea pig left ventricle (6), 83% in rat heart (4)], whereas rat lungs (4) possess a majority of $beta_2$-adrenergic receptors (85%). In selected areas of rat brain, the relative concentration of $beta_1$- and $beta_2$-receptors varies greatly: there are 77%, 82%, 15%, 81%, and 71% $beta_1$-adrenergic receptors in caudate nucleus, cortex, cerebellum, hippocampus, and diencephalon, respectively (4). Using a similar methodological approach, Dickinson *et al.* (7) demonstrated the existence of a homogenous population of $beta_2$-adrenergic receptors in membranes from rat erythrocytes and reticulocytes.

The purpose of the present work was to characterize the nature of *beta*-adrenergic receptors in human cardiac tissue by *in vitro* binding techniques, and to establish the relative proportion of receptors of the $beta_1$- and $beta_2$-subtypes.

MATERIALS AND METHODS

The research program was approved by the Ethics Committee of the Medical School of the Université Libre de Bruxelles.

This work was supported by Grant 3317 from I.R.S.I.A. (Belgium), by Grant 3.4504.81 from the Fonds de la Recherche Scientifique Médicale (Belgium), and by a grant from the Ministère de la Politique Scientifique (Belgium).

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0026-895X/83/050169-05$02.00/0

Human heart specimens. Twelve human right auricles were obtained surgically during the establishment of extracorporeal circulation in patients operated upon for coronary insufficiency or valvular disease. Most of the patients with coronary disease had been maintained by classical treatment for angina pectoris, including the use of nonselective or $beta_1$-selective adrenergic blocker(s). The dosage of adrenergic blockers was reduced by one-half the day before the operation and replaced by 10 mg of propranolol at the time of the operation.

The auricles were immediately frozen in liquid nitrogen, in the operating room, and were stored at −80° until use.

Preparation of a particulate fraction from human heart auricles. Thawed tissue (0.5 g) was homogenized first with an Ultraturax (Janke and Kunkel KG, Staufer i. Bresgau, Federal Republic of Germany) for 10 sec at 2° and then by five up-and-down movements of a glass-Teflon homogenizer in 5 ml of 10 mM Tris-HCl buffer (pH 7.5) containing 2 mM dithioerythritol and 0.25 M sucrose. After filtration through two layers of medical gauze, the homogenate was centrifuged for 10 min at 1,500 × *g*. The pellet was resuspended in a volume of the homogenization buffer, allowing a final protein concentration of 3 mg/ml [as determined by the method of Lowry *et al.* (8), using bovine serum albumin as standard], and used immediately to assay *beta*-adrenergic receptors.

The fraction prepared with this buffer yielded basically a low-speed pellet that differed from the high-speed (20,000 × *g* for 10 min) sedimentation fraction frequently used in other studies, and contained the highest concentration of plasma membranes as indicated by marker enzymes (9, 10).

Assay of beta-adrenergic receptors. [^{125}I]HYP[4] (30–40 pM), obtained from New England Nuclear Corporation (Dreieich, Federal Republic of Germany) and with a specific activity of 2.2 Ci/μmole, was incubated in the presence of 50–100 μg of membrane protein in 0.24 ml of 30 mM Tris-HCl buffer (pH 7.5) enriched with 0.5 mM ATP, 5 mM $MgCl_2$, 0.5 mM ethylene glycol bis (β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 1 mM cyclic AMP, 0.5 mM theophylline, 10 mM phospho(enol)pyruvate, and pyruvate kinase (30 μg/ml). This medium was chosen to allow a direct comparison of binding data with the adenylate cyclase activity documented in the accompanying paper (11). GTP was added at a final 10 μM concentration in all experiments (except those described in Fig. 2) to simplify the analysis of competition curves with agonists (see Results). Heart membranes were incubated at 37° for 20 min, except in experiments described in Fig. 1. Binding was stopped by the addition of 3 ml of 0.15 M NaCl in 20 mM tris-HCl buffer (pH 7.5) at room temperature and immediate filtration through glass-fiber GF/C filters (Whatman, Maidstone, England). Each filter was washed three times with 3 ml of buffer, and the radioactivity was determined in an Autogamma Counter (Packard).

Specific binding of [^{125}I]HYP was defined as the amount of tracer bound in the absence of competing ligand minus the amount bound in the presence of 1 μM pindolol. All assays were carried out in duplicate. Total tracer binding never exceeded 15% of the radioactivity offered, and specific binding represented 70% of total binding, on an average. In the concentration range tested, specific binding was proportional to the protein concentration.

Hofstee plots (i.e., percentage of inhibition of [^{125}I]HYP binding versus percentage of inhibition of [^{125}I]HYP binding over the concentration of competing drug) were established with two competing drugs, the $beta_1$-selective antagonist practolol and the $beta_2$-selective agonist procaterol. These plots were nonlinear and were examined by computer-aided iterative graphic analysis as described by Minneman *et al.* (4). the K_D values for the inhibition of specific [^{125}I]HYP binding with various drugs were calculated according to the method of Cheng and Prusoff (12).

It is of interest that, when rat cardiac membranes were prepared and tested like human cardiac membranes, more than 80% of the *beta*-adrenergic receptors were of the $beta_1$ subtype (data not shown), in agreement with Minneman *et al.* (4).

[4] The abbreviation used is: [^{125}I]HYP, iodinated hydroxybenzylpindolol.

Drugs and chemicals. (±)-Isoproterenol, (−)-epinephrine, (−)-norepinephrine, phospho(enol)pyruvate, pyruvate kinase, cyclic AMP, GTP, and ATP (sodium salt, Grade I, obtained by phosphorylation of adenosine) were purchased from Sigma Chemical Company (St., Louis, Mo.). Pindolol was obtained from Sandoz Ltd. (Basle, Switzerland); (−)-propranolol, practolol, and atenolol from ICI Ltd. (Alderly Park, England); salbutamol from Glaxo Group Research (Ware, England); fenoterol from Boehringer (Ingelheim, Federal Republic of Germany); procaterol from Otsuka (Tokushima, Japan); zinterol from Mead Johnson (Evansville, Ind.); and metoprolol from Ciba-Geigy Corporation (Basel, Switzerland). Other drugs and reagents were commercially available.

RESULTS

Association kinetics and Scatchard plots of [^{125}I]HYP binding to membranes from human auricles. At 37° and in a buffer system identical with that used for adenylate cyclase assays, the specific binding of [^{125}I]HYP was rapid, reaching equilibrium after 20 min (Fig. 1, left). A Scatchard analysis (13) of data collected at that time showed that the tracer bound to a single class of receptors (Fig. 1, right). A program taking into account the concentration of free active stereoisomer for Scatchard plot analysis was kindly made available to us by Dr. P. B. Molinoff (University of Pennsylvania, Philadelphia, Pa.). The calculated K_D value for [^{125}I]HYP binding was 6.3 ± 0.5 × 10^{-11} M, and the density of binding sites was 51.1 ± 7.0 fmoles/mg of protein (means of three experiments).

Inhibition of [^{125}I]HYP binding by isoproterenol. (±)-Isoproterenol inhibited tracer binding in a dose-dependent fashion: displacement of the tracer was obvious at 10^{-8} M and was complete at 10^{-4} M in the absence of GTP (Fig. 2). Under these conditions, the Hill coefficient was 0.6, suggesting the presence of negative cooperativity due to the coexistence of high- and low-affinity states or receptors for the agonist (14). In the presence of 10 μM GTP, the inhibition curve was shifted to the right (Fig. 2) and the Hill coefficient became close to 1.0 (0.94 ± 0.08, mean of three experiments), indicating that isoproterenol recognized a single class of receptors under these conditions (4). Therefore, in order to simplify the analysis of competition curves with agonists, GTP was added systematically in the following experiments.

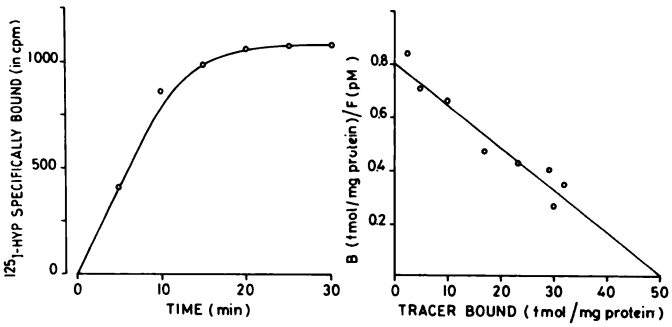

FIG. 1. *General characteristics of [^{125}I]HYP binding in a particulate fraction from human auricles*

Left. The time course of binding of the tracer in one experiment representative of two others. Membrane protein (100 μg) was incubated under standard conditions in the presence of [^{125}I]HYP (30,000 cpm). Right. A saturation curve of [^{125}I]HYP binding plotted according to Schatchard. The curve was the mean of three experiments performed in triplicate on membranes from individual auricles.

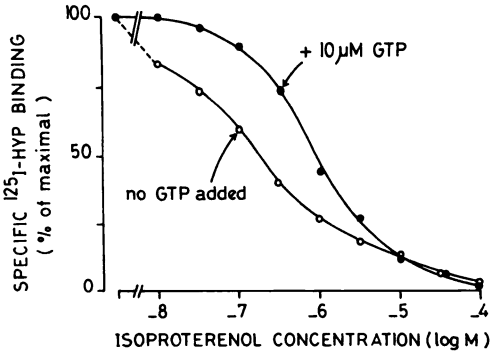

FIG. 2. *Effects of 10 μM GTP on dose-effect curves of inhibition of [^{125}I]HYP binding by isoproterenol*

Membrane protein (80 μg) from human auricles was incubated at 37° for 20 min in the absence of (○) or presence (●) of 10 μM GTP in the medium as described under Materials and Methods, and in the presence of increasing concentrations of isoproterenol. The results are expressed as percentage of [^{125}I]HYP specifically bound and represent the mean of two experiments, performed in duplicate, on membranes from individual auricles.

Inhibition of [^{125}I]HYP binding by nonselective and selective beta-adrenergic agonists and antagonists. *Beta*-adrenergic receptors were further characterized in human auricles by testing the ability of several drugs to inhibit tracer binding. According to the classification of Minneman *et al.* (5), the ligands tested were either nonselective (i.e., having the same affinity for *beta*$_1$-adrenergic and *beta*$_2$-adrenergic receptors) or selective (i.e., preferring one subtype of *beta*-adrenergic receptors). Figure 3 illustrates competition curves obtained with nonselective antagonists (left) and with nonselective agonists (right). The corresponding K_D values in Table 1 were compared with the values found in ref. 5. The nonselective character of these six drugs was confirmed by a Hill coefficient not different from 1.

Competition curves with selective antagonists (Fig. 4, left), and selective agonists (Fig. 4, right) gave Hill coefficients lower than 1.0 (0.60–0.72), suggesting the presence of more than one class of *beta*-adrenergic receptors in membranes from human auricles. Assuming the presence of two classes of *beta*-adrenergic receptors, the competition curves with the *beta*$_1$-selective antagonist practolol and the *beta*$_2$-selective agonist procaterol were converted to a Hofstee representation and subjected to computer-aided iterative graphic analysis (4). The results

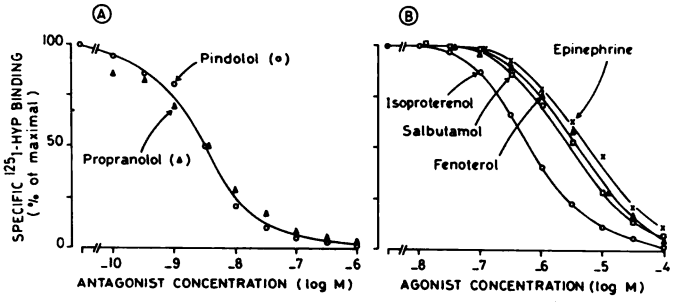

FIG. 3. *Inhibitory effects of two nonselective beta-adrenergic antagonists and four nonselective beta-adrenergic agonists on the binding of [^{125}I]HYP to membranes from human auricles.*

Binding was studied at 37° after a 20-min incubation period in the complete medium (with 10 μM GTP) as described under Materials and Methods, in the presence of increasing concentrations of two antagonists (A: ○, pindolol; △, (−)-propanolol) and four agonists (B: ○, isoproterenol; □, salbutamol; △, fenoterol; ×, epinephrine). The results are expressed as percentage of [^{125}I]HYP specifically bound and represent the mean of three experiments, performed in duplicate, on membranes from individual auricles.

TABLE 1

Corrected EC_{50} values of nonselective and selective drugs for beta-adrenergic receptors in a human auricle particulate fraction as compared with K_D values in reference tissues (5)

The EC_{50} values were corrected according to Cheng and Prusoff (12) to allow a direct comparison with the K_D values of ref. 5, obtained under similar experimental conditions. The correction factor varied from 0.50 to 0.65 at the tracer concentrations used in these experiments.

Drug	Human auricles		Reference tissues[a]	
	μM		μM	
Nonselective				
Isoproterenol	0.80 ± 0.11[b]		0.22–0.56	
Epinephrine	5.00 ± 0.42[b]		3.20–4.20	
Salbutamol	3.00 ± 0.40[b]		2.90–8.70	
Fenoterol	3.50 ± 0.15[b]		2.00–5.20	
	Beta$_1$	*Beta*$_2$	*Beta*$_1$	*Beta*$_2$
Beta$_1$-selective				
Practolol	3.0 ± 0.2[c]	60.0 ± 10.0[c]	1.6 –5.0	29.0–91.0
Metoprolol	0.06[d]	3.0[d]	0.05–0.3	1.2– 3.5
Atenolol	0.8[d]	20.0[d]	0.53–1.7	14.0–30.0
Beta$_2$-selective				
Procaterol	17.0 ± 2.5[e]	0.23 ± 0.04[e]	2.1–5.4	0.10–0.27
Zinterol	3.0[d]	0.10[d]	0.9–1.5	0.02–0.06

[a] Data from Minneman *et al.* (5) mentioning extreme values found when comparing tissues containing homogeneous or heterogeneous populations of *beta*-adrenergic receptors.

[b] Mean ± standard error of the mean calculated from Fig. 3B.

[c] Mean ± standard error of the mean calculated from Fig. 5.

[d] Values calculated from Fig. 4A and B assuming that all curves were compatible with the existence of 50% "*beta*$_1$"-adrenergic receptors and 50% "*beta*$_2$"-adrenergic receptors. The K_D values mentioned were calculated from EC_{25} and EC_{75}.

[e] Mean ± standard error of the mean calculated from Fig. 6.

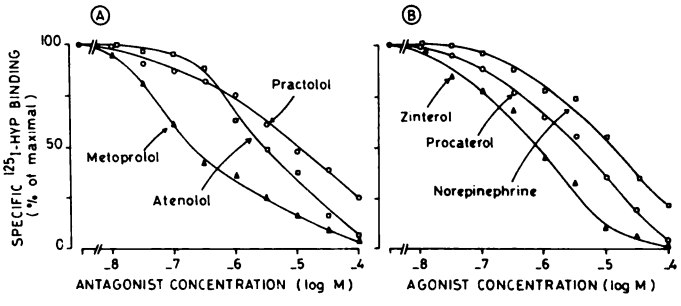

FIG. 4. *Inhibitory effects of three selective beta$_1$-adrenergic antagonists, two selective beta$_2$-adrenergic agonists, and one selective beta$_1$-adrenergic agonist on the binding of [^{125}I]HYP to membranes from human auricles*

Binding was studied at 37° after a 20-min incubation period in the complete medium (with 10 μM GTP) as described under Materials and Methods, in the presence of increasing concentrations of three *beta*$_1$-selective antagonists (A: ○, practolol; △, metoprolol; □, atenolol), two *beta*$_2$-selective agonists (B: ○, procaterol; △, zinterol), and one *beta*$_1$-selective agonist (B: □, norepinephrine).

The results are expressed as percentage of [^{125}I]HYP specifically bound and represent the mean of three experiments, performed in duplicate, on membranes from individual auricles.

indicated that *beta*-adrenergic receptors represented one-half of the total number of *beta*-adrenergic receptors: 48.7 ± 5.4% using practolol on membranes from three auricles (Fig. 5) and 50.7 ± 4.6% using procaterol on membranes from three other auricles (Fig. 6).

The Hofstee plots obtained using the other selective ligands (experiments presented in Fig. 4) were in good agreement with this conclusion. Because of the limited number of experimental points in these experiments, the two "K_D values," calculated as indicated in the legend to Table 1, should be considered as only good approximations of the true K_D values. Our data in Table 1, when compared with those obtained by Minneman *et al.* (5) under similar experimental conditions, suggest that the two components of *beta*-adrenergic binding sites present in human auricles were identical with the $beta_1$- and $beta_2$-adrenergic receptors described in other mammalian tissues.

DISCUSSION

Physiological and pharmacological data suggest that *beta*-adrenergic receptors can be classified into subtypes that may coexist in the same organ (1, 2, 15). Recent *in vitro* binding studies have allowed a quantitative analysis of the number and relative proportion of receptors of each *beta*-adrenergic receptor subtype (3–7, 16), thus confirming the pharmacological data. It is also now evident that distinct subclasses of *beta*-adrenergic receptors can coexist in a single cell type (17). Only two subclasses of *beta*-adrenergic receptors, the $beta_1$ and $beta_2$ types, have been distinguished thus far by their affinity, as determined by *in vitro* binding studies, for selective agonists and antagonists in a number of mammalian tissues (5). However, these two subtypes may be distinct from the *beta*-adrenergic receptors present in turkey (18), chick, and frog erythrocytes (19).

The main results of the present study on membranes from human auricles indicated that (a) $beta_1$- and $beta_2$-adrenergic receptors coexist and have recognition patterns for adrenergic compounds similar to those observed in other mammalian tissues, and (b) the number of $beta_1$- and $beta_2$-adrenergic receptors is approximately equal.

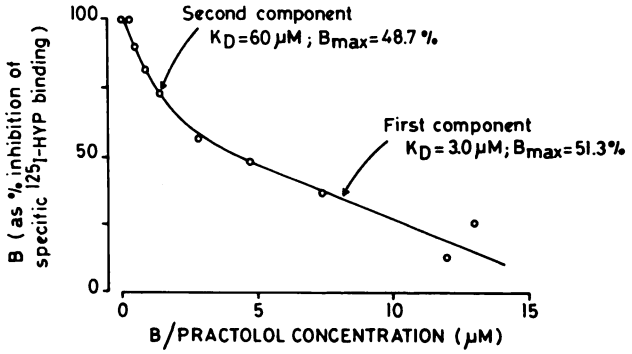

FIG. 5. *Hofstee plot of the inhibition of specific [^{125}I]HYP binding by the $beta_1$-adrenergic antagonist practolol in membranes from human auricles*

Additional experiments, using a larger number of practolol concentrations, were performed for Hofstee analysis of the competition curves. The amount of competing drug bound (*B*, expressed as percentage of inhibition of specific [^{125}I]HYP binding) was plotted on the ordinate. The same value, divided by the concentration of competing drug (micromolar) was plotted on the abscissa. The data represent the mean of three experiments, performed in duplicate, on membranes from individual auricles.

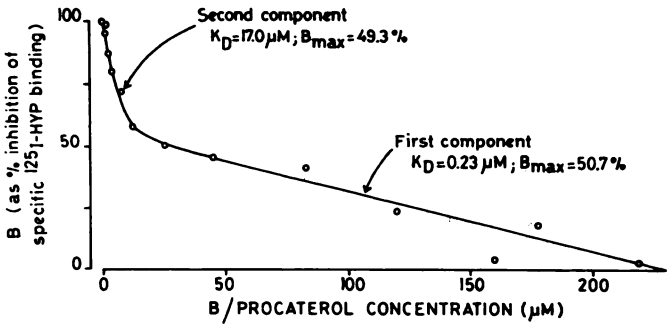

FIG. 6. *Hofstee representation of the inhibition of specific [^{125}I] HYP binding by the $beta_2$-adrenergic agonist procaterol in membranes from human auricles.*

Results are presented as in Fig. 5 and also represent the mean of three additional experiments, performed in duplicate, on membranes from individual auricles.

The heterogeneity of *beta*-adrenergic receptors in human auricular preparations was established by considering that all agonists and antagonists reported to display some selectivity for a given receptor subtype displayed a Hill coefficient significantly lower than 1 despite the presence of 10 μM GTP, whereas nonselective ligands reported to recognize all *beta*-adrenergic receptors equally well displayed a Hill coefficient of 1. The present identification of the two *beta*-receptor populations as $beta_1$- and $beta_2$-adrenergic receptors was based on the close relationship between K_D values in human auricles and K_D values reported for other systems (Table 1 and ref. 5). Procaterol appeared to be more selective in our study than in the study of Minneman *et al.* (5), where it was called OPC 2009: the preferential affinity of this partial agonist was only 20-fold higher for $beta_2$- than for $beta_1$-adrenergic receptors in ref. 5, as opposed to the specificity factor of 80 in the present study and 100 in the report by Dickinson *et al.* (7) on subpopulations of rat lung receptors.

According to binding studies, cardiac tissues are often thought to possess mostly $beta_1$-adrenergic receptors; e.g., rat heart is reported to contain only 17% $beta_2$-adrenergic receptors (4), the ventricles of cat and guinea pig might even be devoid of $beta_2$-adrenergic receptors (6), and atria in these two species contain no more than 20% $beta_2$-adrenergic receptors (6). In contrast, our results on human auricles showed a much higher proportion of $beta_2$-adrenergic receptors (50%).

At this point, a methodological point should be discussed. The auricles were obtained from patients undergoing surgery for coronary insufficiency or valvular disease. No patient suffered severe hypertension or cardiac failure, but most of them were under drug therapy, including nonselective or $beta_1$-selective adrenergic blockers; theoretically, this might have affected the number of heart receptors. Indeed, chronic treatment with a nonselective *beta*-adrenergic blocker is known to increase the number of *beta*-adrenergic receptors in human lymphocytes (20). Furthermore, Minneman *et al.* (21) have shown that increasing norepinephrine (a $beta_1$-preferring agonist) in rat brain leads to a selective decrease in $beta_1$-

adrenergic receptors, the opposite situation being observed after the destruction of adrenergic neurons. To the best of our knowledge, there is no information available on the effects of *beta*$_1$-selective antagonists. There is no reason to believe that their chronic administration might induce a selective elevation of *beta*$_2$-adrenergic receptors, and, were such drugs selectively increasing the number of *beta*$_1$-adrenergic receptors, an underestimation of the relative proportions of *beta*$_2$-adrenergic receptors would follow. In practice, no obvious differences were recorded in the proportions of *beta*$_1$- and *beta*$_2$-adrenergic receptors in the auricles from three nontreated patients, one patient receiving a selective *beta*-blocker and two patients receiving a nonselective *beta*-blocker.

The relationship between the distribution and functional roles of *beta*-adrenergic receptor subtypes in mammalian heart is not completely understood. Hedberg *et al.* (6) reported an apparent homogeneous population of *beta*$_1$-adrenergic receptors in the left ventricle of cat and guinea pig, whereas the atria of both species contained both *beta*$_1$- and *beta*$_2$-adrenergic receptors in a ratio of approximately 3:1. These results are in agreement with physiological data reported by Carlsson *et al.* (22) for cat heart, suggesting that both types of adrenergic receptors are responsible for the chronotropic control of the sinoatrial mode. In contrast, data from O'Donnell and Wanstall (23) on guinea pig heart suggest that only *beta*$_1$-adrenergic receptors are involved in the chronotropic response, although *beta*$_2$-adrenergic receptors are also present. In human heart, the respective roles of *beta*$_1$- and *beta*$_2$-adrenergic receptors obviously must be documented further. The present results might explain why, at variance with isoproterenol, selective *beta*$_2$-adrenergic bronchodilators affect rather selectively the pulse rate without influencing blood pressure (24–26) and why mepindolol, a *beta*-antagonist, is as active on heart rate as on pulmonary capillary pressure and peripheral resistance (two effects mediated by *beta*$_2$-adrenergic receptors) but is less active on stroke volume and contractility in the right ventricle in man (27). When considering that *beta*$_2$-receptors are more sensitive to epinephrine (considered a hormonal mediator) than to norepinephrine (considered a neurotransmittor), it is tempting to suggest that the control of human cardiac function might be of particular importance (28, 29).

ACKNOWLEDGMENT

We thank Dr. P. B. Molinoff for pertinent advice during the performance of the experiments and for access to the computer programs for analyzing Scatchard plots and Hofstee plots.

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