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The Human Heart *Beta*-Adrenergic Receptors

II. Coupling of $Beta_2$-Adrenergic Receptors with the Adenylate Cyclase System

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SUMMARY

The *beta*-adrenergic stimulation of adenylate cyclase in membranes from human auricles, ventricles, and fetal heart was compared with the binding properties of *beta*-adrenergic receptors in human auricles. In terms of adenylate cyclase activation, three full agonists (isoproterenol, epinephrine, and norepinephrine), four partial agonists (procaterol, salbutamol, fenoterol, and zinterol), and four antagonists (propranolol, metoprolol, atenolol, and practolol) were tested. The *beta*-adrenergic activation of adenylate cyclase in membranes from rat heart (with a majority of $beta_1$-adrenergic receptors), rat erythrocytes, and rat reticulocytes (with a homogeneous population of $beta_2$-adrenergic receptors) served as reference. The reactivity of human heart adenylate cyclase, estimated by the K_{act} or K_i values of 11 *beta*-adrenergic agents, indicated that the activation of this enzyme occurred through receptors of the $beta_2$-subtype only. Receptors of the $beta_1$-subtype (50% of the total population) were not coupled to the enzyme.

INTRODUCTION

In the preceding paper (1), binding data established the relative proportions of $beta_1$- and $beta_2$-adrenergic receptor subtypes in crude membrane preparations from human auricles. Equal proportions of both types of receptors were observed, in contrast to the majority of $beta_1$-receptors identified in heart preparations from other animal species (2).

An increase in tissue cyclic AMP levels, due to the activation of membrane adenylate cyclase, is generally considered as the major intracellular signal of *beta*-adrenergic agonists (3). This has been documented in tissues endowed with a majority of $beta_1$-adrenergic receptors [such as rat heart (4)], a majority of $beta_2$-adrenergic receptors [such as rat lung (4)], and in cells possessing only $beta_2$-adrenergic receptors [such as rat reticulocytes (5, 6)].

The response of a tissue with both *beta*-adrenergic subtypes is unpredictable. As shown by Pike *et al.* (7), the intrinsic activity of a given agonist may, indeed, differ from one tissue to the other, and probably also from one cell type to the other: the intensity of the adenylate cyclase response to *beta*-adrenergic agonists depends not only on the number of receptors but also, and probably mainly, on the efficacy of coupling between the occupied receptor and the subunits of the adenylate cyclase system. Therefore, in membranes prepared from a heterogeneous tissue, a small proportion of receptors efficiently coupled to the adenylate cyclase system might mask the influence of a large number of poorly coupled receptors. Furthermore, the role of cyclic AMP as second messenger for *beta*-adrenergic agonists in heart is still controversial (8, 9). Finally, Erdos *et al.* (10) presented recent evidence that *beta*-adrenergic agonists could modulate the transport of magnesium independently of the cyclic AMP pathway in mutant cells.

It was therefore of interest to compare the pattern of *beta*-adrenergic adenylate cyclase activation with the characteristics of *beta*-adrenergic receptors in the human auricular membranes studied in the preceding paper (1). In the present report, the adenylate cyclase system in membranes from human auricles is shown to exhibit a typical $beta_2$-adrenergic response, notwithstanding the existence of 50% $beta_1$-adrenergic receptors in the same membranes. A similar activation pattern of adenylate cyclase was found in membranes from human ventricles and fetal heart.

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MATERIALS AND METHODS

Human heart membranes. The human heart specimens consisted of 12 right auricles identical with those described previously (1), one fetal heart obtained during a medical abortion at 17 weeks of age, and muscle fragments from three left ventricles. Two of the last specimens were obtained during the resection of a postinfarction ventricular aneurysm; the third specimen was from a 35-year-old patient with cerebral death from head trauma who was a donor for kidney transplantation. All tissue specimens were frozen as soon as possible in liquid nitrogen and stored at −80° until use. A particulate fraction was prepared as previously described (1) and used immediately to assay adenylate cyclase activity.

Rat heart membranes. Ventricles were obtained from male Wistar albino rats weighing 200–250 g. Animals were exsanguinated by decapitation, and ventricles were removed and rinsed at room temperature in 0.15 M NaCl and immediately frozen in liquid nitrogen. The procedure for preparing a particulate fraction was identical with that described for human heart specimens.

Membranes from rat erythrocytes and rat reticulocytes. Erythrocyte membranes were prepared from normal Wistar albino rats (200–250 g) and reticulocyte membranes from similar rats treated with phenylhydrazine according to the method of Dickinson *et al.* (5). The methodology of Dickinson *et al.* (5) was also followed for red cell membrane preparation with only two modifications: (a) blood was collected by aortic puncture under ether anesthesia with a heparinized syringe, and (b) the solution used for the lysis of red blood cells was made of 5 mM Tris-HCl/0.5 mM EDTA (pH 7.8). The membranes were rapidly frozen in liquid nitrogen and stored at −80° until use.

Adenylate cyclase assay. Adenylate cyclase activity was determined with minor modifications of the procedure of Salomon *et al.* (11). Membrane protein (50–100 μg for human heart, 100–150 μg for rat heart, 50–60 μg for rat reticulocytes, and 200–300 μg for rat erythrocytes) was incubated in a total volume of 60 μl containing 0.5 mM [α-^{32}P]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, pyruvate kinase (30 μg/ml), 10 μM GTP, and 30 mM Tris-HCl at a final pH of 7.5. The reaction was initiated by addition of the membranes and was terminated after an 8-min incubation at 37° by adding 0.5 ml of a 0.5% sodium dodecyl sulfate solution containing 0.5 mM ATP, 0.5 mM cyclic AMP, and [8-^{3}H]cyclic AMP (20,000 cpm) (for determination of cyclic nucleotide recovery). When red blood cell membranes were used, the samples were then boiled for 3 min to facilitate the separation procedure. Cyclic AMP was separated from ATP by two successive chromatographies on Dowex-50-WX 8 and neutral alumina. Under all conditions tested, cyclic AMP production was linear for at least 8 min.

Determination of protein. Protein concentration was determined according to the method of Lowry *et al.* (12), using bovine serum albumin as a standard.

Drugs and chemicals. [8-^{3}H]Cyclic AMP (24 Ci/mmole) was obtained from the Radiochemical Centre (Amersham, Bucks, England), and [α-^{32}P]ATP (25 Ci/mmole) was from New England Nuclear Corporation (Boston, Mass.). (±-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.). (−)-Propranolol, practolol, and atenolol were from ICI Ltd. (Alderly Park, England), salbutamol was from Glaxo Group Research (Ware, England), fenoterol was from Boehringher (Ingelheim, Federal Republic of Germany), procaterol was from Otsuka (Tokushima, Japan), zinterol was from Mead Johnson (Evansville, Ind.), and metoprolol was from Ciba-Geigy Corporation (Basel, Switzerland). Other drugs and reagents were commercially available.

Determination of K_{act} and K_i values for adenylate cyclase activity. K_{act} values were determined as the drug concentration achieving 50% of maximal enzyme stimulation. K_i values for the inhibition of isoproternol-stimulated adenylate cyclase by various antagonists were determined by two approaches: (a) by Schild's plot analysis of activation curves by isoproterenol, in the presence of 3- to 5-fold concentrations of antagonist (13); and (b) by correcting IC_{50} values by the equation of Cheng and Prusoff (14), after incubation in the presence of a submaximal dose of isoproterenol and increasing concentrations of antagonist.

RESULTS

Isoproterenol-, epinephrine-, and norepinephrine-stimulated adenylate cyclase activity in membranes from human auricles and in three reference systems. The effects of isoprotrenol and of two natural catecholamines on adenylate cyclase activity in membranes from human auricles were compared with those observed in membranes from rat heart (taken as a reference system with a predominance of *beta*$_1$-receptors), rat reticulocytes (taken as a reference system with tightly coupled homogeneous *beta*$_2$-receptors), and rat erythrocytes (taken as a reference system with poorly coupled homogeneous *beta*$_2$-receptors). The results are illustrated in Fig. 1; K_{act} values for adenylate cyclase are reported in Table 1.

Adenylate cyclase activation in membranes from human auricles, rat reticulocytes, and rat erythrocytes displayed a pattern compatible with activation by *beta*$_2$-adrenergic receptors, based on the relative agonist potencies: isoproterenol > epinephrine > norepinephrine. In contrast, the response of rat heart membranes suggested the presence of *beta*$_1$-adrenergic receptors that mediated adenylate cyclase activation, as the relative potency of the agonists was isoproterenol > norepinephrine = epinephrine. The K_{act} values for the three full agonists were almost identical in membranes from human auricles and rat erythrocytes, and 3- to 5-fold lower in membranes from rat reticulocytes.

Stimulatory effects of the four partial agonists procaterol, salbutamol, fenoterol, and zinterol on adenylate cyclase stimulation in membranes from human auricles and in three systems of reference. Dose-effect curves of adenylate cyclase activation are illustrated in Fig. 2; K_{act} values for adenylate cyclase activation are reported in Table 1, and the efficacy of the partial agonists (as compared with that of isoproterenol) is shown in Table 2.

The relative potencies of the partial agonists, as shown by K_{act} values, were similar in membranes from human auricles, rat erythrocytes, and rat reticulocytes (zinterol being 4- to 8-fold *more* potent than isoproterenol), in contrast to rat heart membranes, where zinterol was 2.6-fold *less* potent than isoproterenol (Table 1).

The relative efficacies (intrinsic activities) of partial agonists were similar in membranes from human auricles, rat erythrocytes, and rat reticulocytes, decreasing in the order procaterol ⩾ fenoterol > zinterol > salbutamol. In contrast, procaterol was markedly less active than fenoterol in rat ventricle membranes and had the same intrinsic activity as salbutamol (Table 2).

All of the drugs tested were 2- to 8-fold more potent, and the partial agonists were more efficient in membranes from rat reticulocytes than in membranes from rat erythrocytes and human auricles.

Inhibitory effects of the four beta-adrenergic antagonists L *-propranolol, metoprolol, atenolol, and practolol on isoproterenol-stimulated adenylate cyclase in mem-*

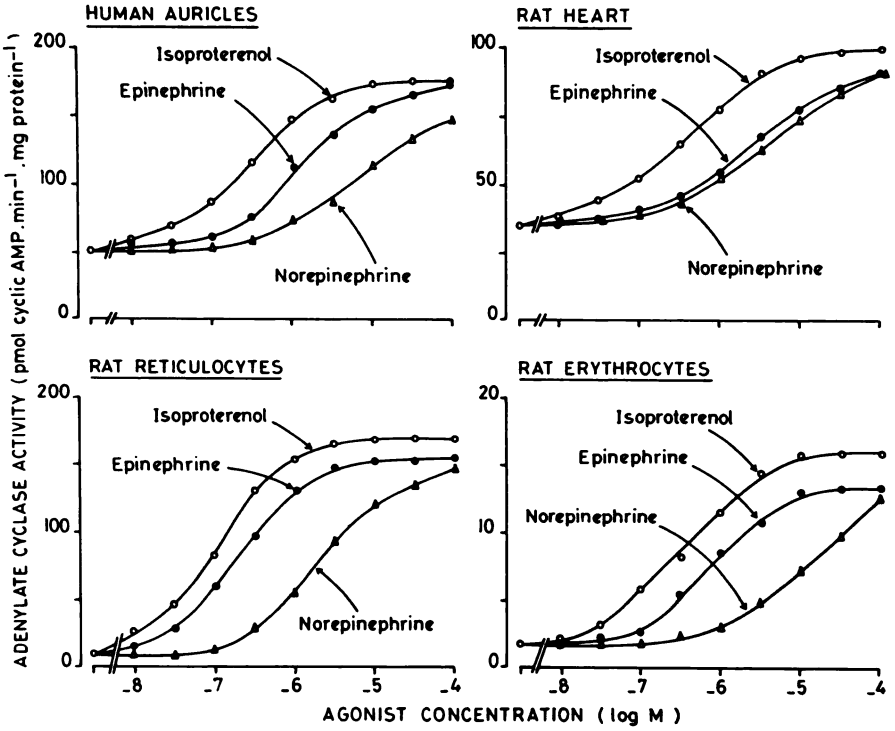

FIG. 1. *Dose-effect curves of adenylate cyclase activation of human auricular membranes (upper left), rat heart membranes (upper right), rat reticulocyte membranes (lower left), and rat erythrocyte membranes (lower right) in the presence of isoproterenol (○), epinephrine (●), and norepinephrine (△)*

The results were obtained as described under Materials and Methods and are the means of three experiments performed in duplicate.

branes from human auricles, rat reticulocytes, and rat heart. The relative efficacies and/or potencies of the agonists in human auricular membranes indicated that adenylate cyclase was activated mainly through *beta*$_2$-adrenergic receptors. This hypothesis was further tested by comparing the ability of selective and nonselective antagonists to inhibit isoproterenol-stimulated adenylate cyclase activity in membranes from human auricles, rat reticulocytes, and rat heart.

The K_i values were measured by two approaches:

1. Dose-effect curves of isoproterenol were established in the presence of various concentrations of each antagonist. In each experiment, isoproterenol activation curves remained parallel and reached the same maximal activity with the four antagonists tested. Schild's plots derived from these curves were compatible with competitive inhibition. The calculated K_i values in membranes from human auricles and rat reticulocytes are shown in Figs. 3 and 4; the calculated K_i values for rat heart membranes are shown in Table 3.

2. The effects of increasing concentrations of antagonist were tested on membranes from human auricles and rat reticulocytes in the presence of a fixed submaximal concentration (1 μM) of isoproterenol. These experiments allowed us to calculate K_i values according to the method of Cheng and Prusoff (14) and the Hill coefficient for selective antagonists, as a test for the heterogeneity of receptors involved in adenylate cyclase inhibition (Fig. 5). The Hill coefficients (mentioned in Fig. 5) were not different from 1 with the four antagonists tested, sug-

TABLE 1

Effect of full and partial beta-adrenergic agonists on adenylate cyclase activation in particulate fractions from human auricles, rat ventricles, rat reticulocytes, rat erythrocytes, human ventricles, and human 17-week-old fetal heart

The results were derived from those presented in Figs. 1, 2, 6, and 7 and are the means of three experiments performed in duplicate (except for fetal heart, one specimen).

Drug	K_{act} of adenylate cyclase					
	Human auricles	Rat ventricles	Rat reticulocytes	Rat erythrocytes	Human ventricles	Human fetus
	μM					
(±)-Isoproterenol	0.30	0.35	0.12	0.40	0.40	0.30
(−)-Epinephrine	1.00	2.50	0.25	1.00	1.50	1.00
(−)-Norepinephrine	10.00	2.80	2.00	10.00	10.00	12.00
Procaterol	0.20	0.30	0.10	0.30	0.20	0.10
Salbutamol	0.80	1.00	0.10	0.80	0.80	1.00
Fenoterol	0.95	0.80	0.10	0.75	0.60	0.40
Zinterol	0.08	0.95	0.02	0.05	0.08	0.03

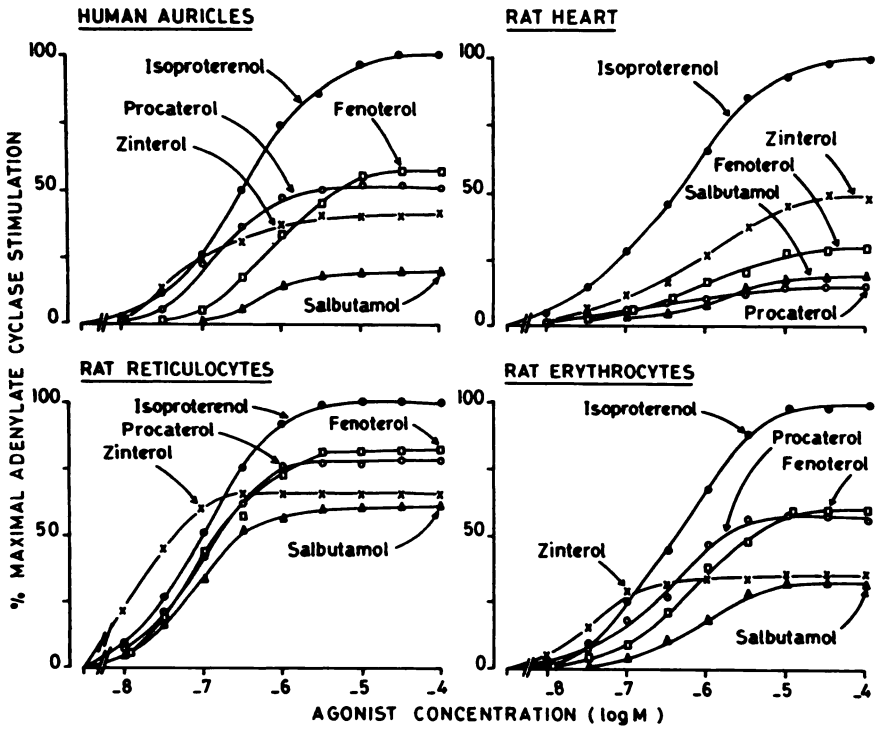

FIG. 2. *Dose-effect curves of adenylate cyclase activation of human auricular membranes (upper left), rat heart membranes (upper right), rat reticulocyte membranes (lower left), and rat erythrocyte membranes (lower right) in the presence of isoproterenol (●), procaterol (○), fenoterol (□), salbutamol (△), and zinterol (×). The results, expressed as percentage of maximal adenylate cyclase activation achieved with 100 μM isoproterenol, are the means of three experiments performed in duplicate.*

gesting that isoproterenol activation of adenylate cyclase involved a single homogeneous class of receptors in both preparations.

The K_i values for the three tissues studied are compared in Table 3 with those found in the literature (4). Membranes from human auricles and rat reticulocytes showed similar K_i values for a nonselective antagonist [(−)-propranolol] and for three selective antagonists (metoprolol, atenolol, and practolol), these K_i values being comparable to those observed in rat lung membranes (a heterogeneous tissue with a majority of $beta_2$-adrenergic receptors) but different from those found for rat cardiac membranes (a heterogeneous tissue with a majority of $beta_1$-adrenergic receptors).

Effects of three full beta-adrenergic agonists and five partial agonists on adenylate cyclase activation in membranes from human ventricle and human fetal heart. Dose-effect curves of adenylate cyclase activation in the presence of isoproterenol, norepinephrine, epinephrine, procaterol, fenoterol, salbutamol, and zinterol are illustrated in Fig. 6 for membranes from human heart ventricle and in Fig. 7 for membranes from a single human fetal heart. The K_{act} values and the relative efficacies of the partial agonists are collected in Tables 1 and 2.

These results were similar to those observed for human auricles, the order of potency of isoproterenol and the natural catecholamines being again isoproterenol > epinephrine > norepinephrine, and for the partial agonists zinterol > procaterol ≥ isoproterenol > fenoterol > salbutamol. The efficacies of the partial agonists as compared with that of isoproterenol were fenoterol ≥ procaterol > salbutamol > zinterol.

It must also be noted that the absolute value of adenylate cyclase activity was comparable in membranes from auricles and ventricles but was much higher in membranes from the single specimen of fetal heart.

TABLE 2

Effect of partial agonists on adenylate cyclase activity in particulate fractions from human auricles, rat ventricles, rat reticulocytes, rat erythrocytes, human ventricles, and human 17-week-old fetal heart

The results were derived from Figs. 2, 6, and 7 and are the means of three experiments performed in duplicate (except for human 17-week-old fetal heart). Efficacy refers to the V_{max} relative to isoproterenol for the stimulation of adenylate cyclase by the drug in the tissue examined.

Drug	Efficacy (isoproterenol = 1.00)					
	Human auricles	Rat ventricles	Rat reticulocytes	Rat erythrocytes	Human ventricles	Human fetus
(±)-Isoproterenol	1.00	1.00	1.00	1.00	1.00	1.00
Procaterol	0.50	0.15	0.78	0.57	0.49	0.52
Salbutamol	0.20	0.18	0.60	0.32	0.23	0.22
Fenoterol	0.56	0.28	0.80	0.60	0.48	0.54
Zinterol	0.40	0.50	0.60	0.37	0.33	0.30

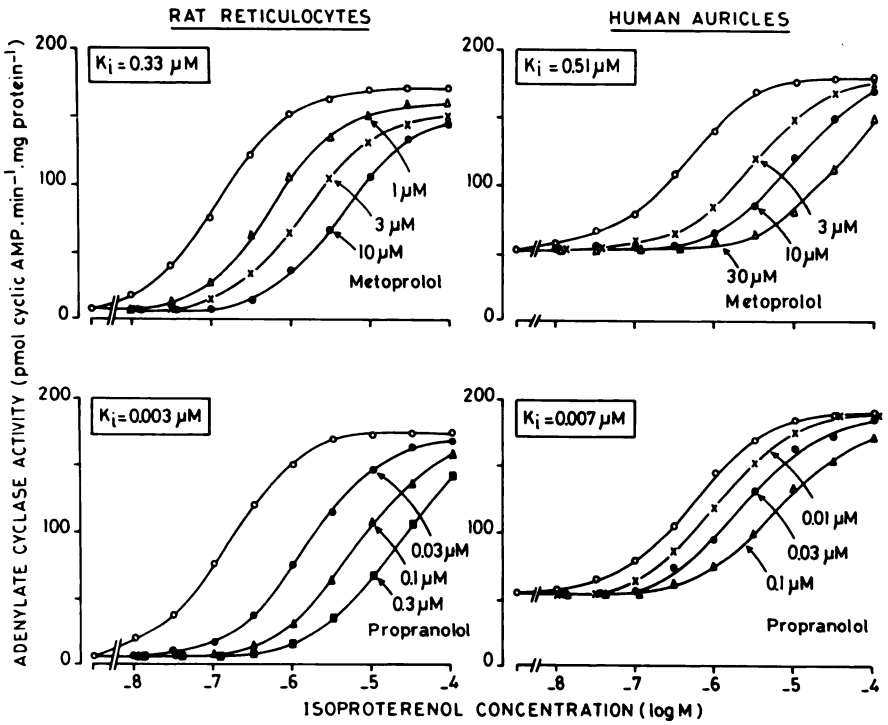

FIG. 3. *Dose-effect curves of isoproterenol-stimulated adenylate cyclase from rat reticulocyte membranes (upper and lower left) and human auricular membranes (upper and lower right) in the presence of various concentrations of metoprolol (upper panels) and* L *-propranolol (lower panels)*

The metoprolol concentrations tested were 0 μM (○), 1 μM (△), 3 μM (×), 10 μM (●), and 30 μM (▲). The L-propranolol concentrations tested were 0 μM (○), 0.01 μM (×), 0.03 μM (●), 0.1 μM (▲), and 0.3 μM (■). The results are the means of three experiments performed in duplicate. The K_i values were calculated according to ref. 13.

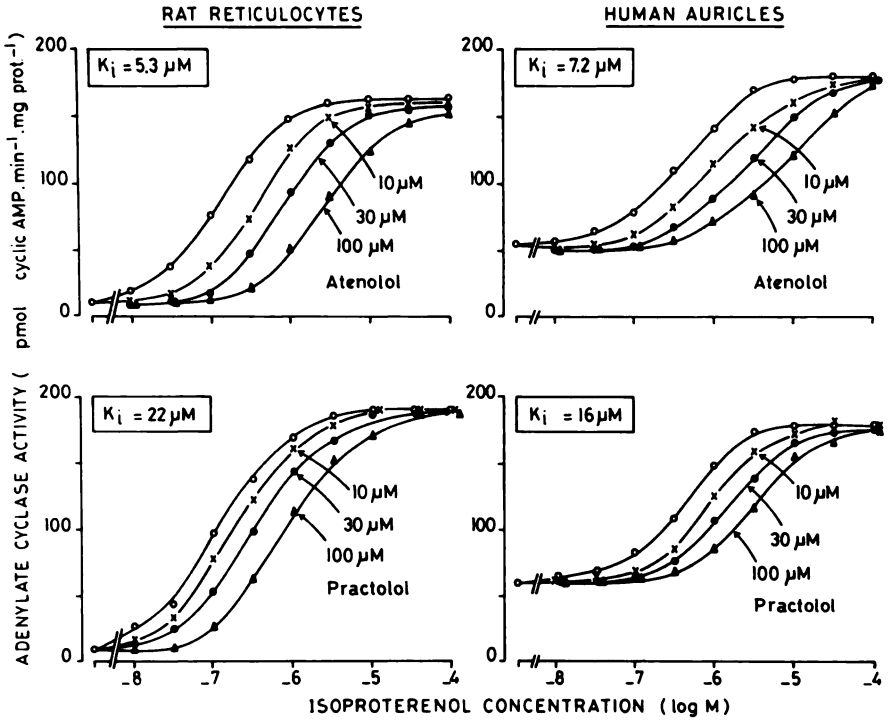

FIG. 4. *Dose-effect curves of isoproterenol-stimulated adenylate cyclase from rat reticulocyte membranes (upper and lower left) and human auricular membranes (upper and lower right) in the presence of various concentrations of atenolol (upper panels) and practolol (lower panels)*

The atenolol and practolol concentrations tested were 0 μM (○), 10 μM (x), 30 μM (●), and 100 μM (▲). The results are the means of three experiments performed in duplicate. The K_i values were calculated according to ref. 13.

TABLE 3

Effect of beta-adrenergic antagonists on adenylate cyclase activity in particulate fractions from human auricles, rat reticulocytes, and rat heart

The K_i values for human auricles and rat reticulocytes are the means of values presented in Figs. 3, 4, and 5 and are compared with the values obtained for rat heart and those described for rat lung in ref. 4.

Drug	K_i of adenylate cyclase activity			
	Human auricles	Rat reticulocytes	Rat heart	Rat lung (4)
		μM		
(−)-Propanolol	0.0055	0.0030	0.0048	0.0014
Metoprolol	0.43	0.38	0.27	1.0– 1.2
Atenolol	5.90	5.10	0.90	1.5– 2.0
Practolol	16.30	22.0	3.2	8.0–11.0

Because of the limited amount of tissue obtained, the effects of *beta*-adrenergic antagonists could not be established in preparations from adult ventricles and from the single fetal heart specimen.

DISCUSSION

The present results demonstrated that the adenylate cyclase in human heart preparations was activated through a single population of *beta*-adrenergic receptors with $beta_2$ specificity. Three arguments support this conclusion.

The effects of isoproterenol, norepinephrine, and epinephrine were typical of a $beta_2$-adrenergic receptor-mediated response: epinephrine was 8- to 12-fold more potent than norepinephrine, as in membranes from rat reticulocytes and rat erythrocytes, used as $beta_2$ reference systems.

The effects of the four partial agonists procaterol, fenoterol, salbutamol, and zinterol were also in line with the proposed conclusion.

Procaterol and zinterol are considered (15) as selective molecules displaying a higher affinity for $beta_2$- than for $beta_1$-adrenergic binding sites, and both are reported to be potent agonists for $beta_2$-receptors and poor antagonists on $beta_1$-receptors [on the basis of adenylate cyclase activation studies (4)]. This was indeed the case for procaterol in our reference systems including rat heart membranes [where the modest activating effect of procaterol on adenylate cyclase could be attributed to a 20% proportion of $beta_2$-adrenergic receptors (2)]. For zinterol, the efficient stimulation of adenylate cyclase in rat heart membranes, however, gave a K_{act} value more compatible with an interaction with $beta_1$-adrenergic receptors.

Fenoterol and salbutamol are reported (15) to be nonselective molecules (on the basis of binding studies), but to activate adenylate cyclase essentially through $beta_2$-receptors. We confirmed this finding for fenoterol, but salbutamol was a poor agonist in membranes from rat reticulocytes, erythrocytes, and heart.

The K_{act} values of adenylate cyclase activation and the relative efficacies of procaterol, fenoterol, and zinterol indicate that adenylate cyclase was essentially stimulated by $beta_2$-adrenergic receptors in human heart membranes. Indeed, the pattern of adenylate cyclase activation was identical with that observed in rat erythrocyte and reticulocyte membranes. Rat reticulocyte membranes were more sensitive and more responsive to *beta*-adrenergic agonists than were rat erythrocytes and human heart membranes.

Membranes from rat erythrocytes and rat reticulocytes contain a homogeneous population of $beta_2$-adrenergic receptors (5). The receptor density was lower in erythrocytes than in reticulocytes (5, 6, 16, 17), and adenylate cyclase activation in erythrocytes was drastically reduced (6, 17) owing to the inability of *beta*-adrenergic agonists to promote an efficient coupling of the occupied receptor with the guanine nucleotide regulatory protein in mature cells (17). This deficient coupling might be responsible for lower K_{act} values for all agonists and the lower intrinsic activities of partial agonists in erythrocytes as compared with reticulocytes.

Variations of potency (K_{act}) and efficacy (intrinsic activity) among *in vitro* systems have been demonstrated not only in closely related systems, such as the membranes from reticulocytes and erythrocytes (possessing a homogeneous population of $beta_2$-adrenergic receptors), but also in cells endowed with a mixed population of

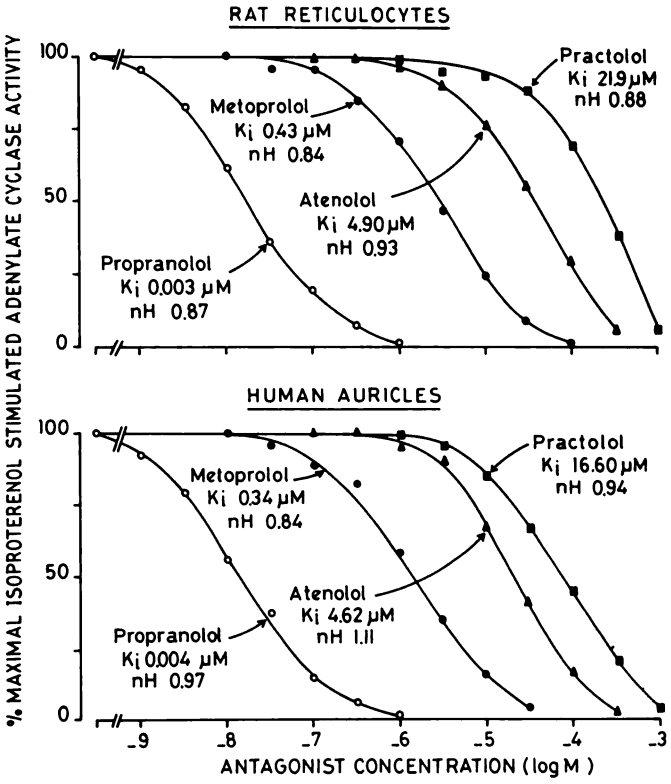

FIG. 5. *Dose-effect curves of inhibition of 1 μM isoproterenol-stimulated adenylate cyclase from rat reticulocyte (upper panel) and human auricular membranes (lower panel) in the presence of increasing concentrations of* L*-propranolol (○), metoprolol (●), atenolol (▲), and practolol (■)*

The results, expressed as percentage of adenylate cyclase activity observed in the presence of 1 μM isoprotrenol, are the means of two experiments performed in duplicate. On the same preparations, a complete dose-effect curve of isoproterenol-stimulated adenylate cyclase was performed in order to establish the K_{act} of the agonist and to calculate the K_i for antagonists according to ref. 14. The Hill coefficients (n_H) and K_i values for antagonists are also shown.

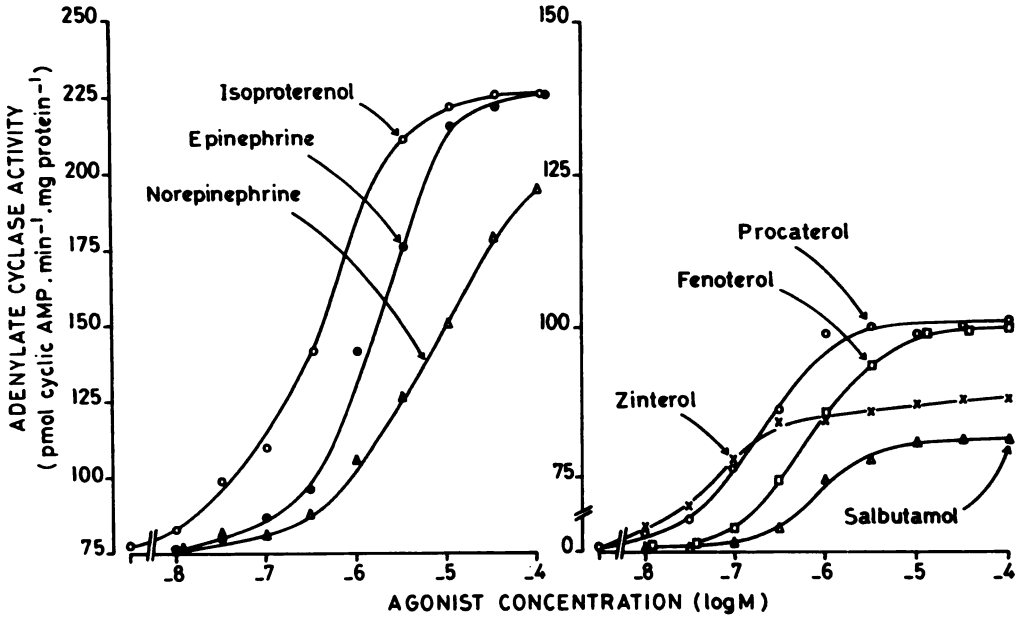

FIG. 6. *Dose-effect curves of adenylate cyclase activation by beta-adrenergic agonists in human heart ventricular membranes*
Left, the effects of isoproterenol (○), epinephrine (●), and norepinephrine (△). Right, the effects of procaterol (○), fenterol (□), salbutamol (△), and zinterol (×). The results are the means of three experiments performed in duplicate.

beta$_1$- and *beta*$_2$-adrenergic receptors. For instance, C_6 glioma cells possess *beta*$_1$- and *beta*$_2$-adrenergic receptors that are coupled differently with adenylate cyclase (18). Pike *et al.* (7) have shown that components distal to the receptors may be responsible for the intrinsic activity of *beta*-adrenergic agonists.

The effects of the three *beta*$_1$-selective adrenergic antagonists practolol, metoprolol, and atenolol suggest that only *beta*$_2$-adrenergic receptors were involved in isoproterenol-stimulated adenylate cyclase from human auricles. The inhibition was competitive and complete with the three antagonists tested. A similar Hill coefficient of 1 for inhibition curves in membranes from human auricles and rat reticulocytes indicates that the antagonists interacted with a single class of receptors. The K_i values found in both systems were those described for pure *beta*$_2$-adrenergic systems. The affinity of metoprolol was higher, and those of atenolol and practolol were lower, in our *beta*$_2$-adrenergic receptor preparations than those found by Minneman *et al.* (15) but identical with those found by Dickinson *et al.* (5) on membranes from rat reticulocytes and rat erythrocytes.

The potencies (K_{act} or K_i) of selective and nonselective agonists and antagonists in human auricles are compared in Fig. 8 with (a) their dissociation constants (K_D) on *beta*$_1$- and *beta*$_2$-adrenergic receptors as cited in ref. 4, and (b) with K_{act} values in rat heart and rat erythrocyte membranes and with K_i values in rat heart and rat

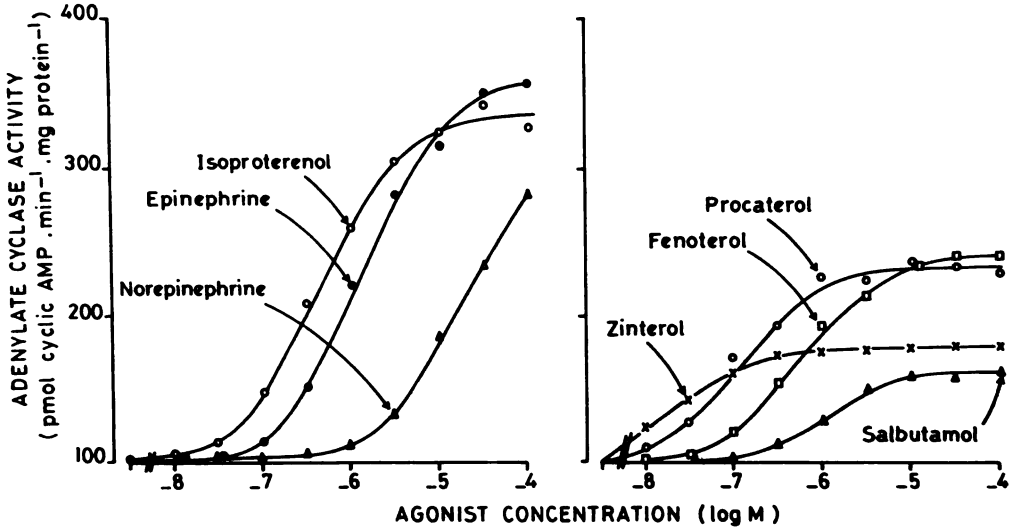

FIG. 7. *Dose-effect curves of adenylate cyclase activation by beta-adrenergic agonists in heart membranes from a human 17-week-old fetus*
Left, the effect of isoproterenol (○), epinephrine (●), and norepinephrine (△). Right, the effect of procaterol (○), fenoterol (□), salbutamol (△), and zinterol (×).

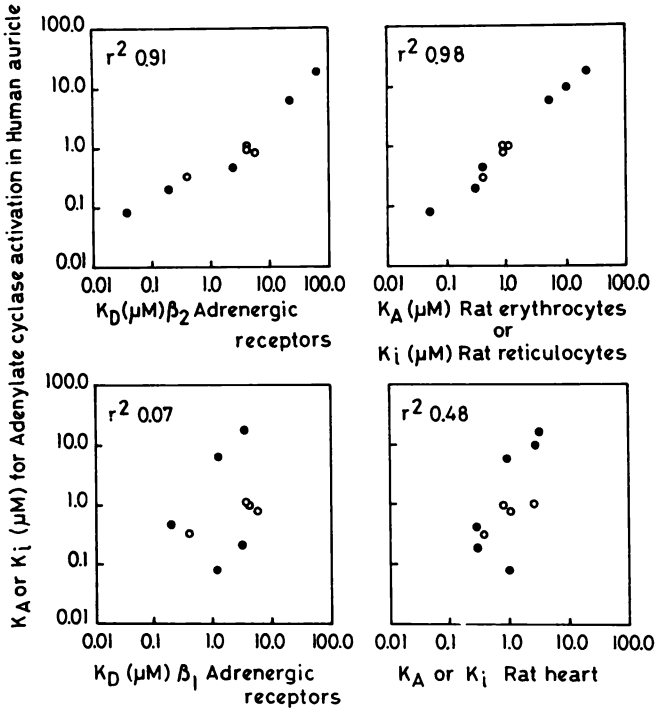

FIG. 8. *Activation constants (K_A) or inhibition constants (K_i) of selective (●) and nonselective (○) drugs are compared with their dissociation constants (K_D) for $beta_2$- (top left) or $beta_1$- (bottom left) adrenergic receptors (values from ref. 4), with the activation (rat erythrocytes) or inhibition (rat reticulocytes) constants in pure $beta_2$-adrenergic systems (top right), and with the activation or inhibition constants found in a predominantly $beta_1$-adrenergic system (rat heart) (bottom right)*

reticulocyte membranes. A good correlation coefficient was found between adenylate cyclase activation in human auricles and binding to *$beta_2$*-adrenergic receptors ($r^2 = 0.91$) or adenylate cyclase activation in the pure *$beta_2$*-adrenergic systems of rat reticulocytes and erythrocytes ($r^2 = 0.98$). No such correlation could be found between adenylate cyclase activity in human auricles and rat heart (a predominantly *$beta_1$*-adrenergic system: $r^2 = 0.48$) or binding to *$beta_1$*-adrenergic receptors ($r^2 = 0.07$) (Fig. 8).

In the preceding paper (1), we established by direct binding studies the presence of an equal proportion cf *$beta_1$*- and *$beta_2$*-adrenergic receptors in membranes from human auricles. A direct comparison of these data and the present results is warranted, as the methodology in both studies was almost identical (the only difference being a 20-min incubation period for binding studies and a more limited 8-min period for adenylate cyclase assays). Two conclusions emerge: (a) the *$beta_1$*-adrenergic receptors identified by binding studies were not coupled or were very poorly coupled to adenylate cyclase as compared with *$beta_2$*-adrenergic receptors, and (b) the K_D values for *$beta_2$*-receptor occupancy (1) were higher than K_{act} values for adenylate cyclase activation and K_i values for inhibition, with the single exception of procaterol, which displayed the same values for K_D and K_{act}. This suggests the existence of an amplification mechanism between receptor occupancy and adenylate cyclase activation, such as "spare" receptors, a process already proposed to play a role in heart (19).

If we assume that membranes from both human heart auricles (1) and ventricles contain *$beta_1$*- as well as *$beta_2$*-adrenergic receptors and that, at variance with heart preparations from all other animal species studied thus far, *$beta_2$*-adrenergic receptors were coupled only to adenylate cyclase, three questions arise concerning the human heart: (a) What is the second messenger of *$beta_1$*-adrenergic receptors? (b) What is the evidence that cyclic AMP is involved in the physiological role of catecholamines? (c) What are the respective functional roles of *$beta_1$*- and *$beta_2$*-adrenergic receptors?

As discussed above, the coupling between *$beta_1$*- and *$beta_2$*-adrenergic receptors and adenylate cyclase activation may differ markedly from one tissue to another (6, 17) and even within cells where *$beta_1$*- and *$beta_2$*-adrenergic receptors coexist (18). It is possible, therefore, that *$beta_1$*-adrenergic receptors were so poorly coupled to adenylate cyclase in membranes from human heart as compared with *$beta_2$*-adrenergic receptors that only the *$beta_2$*-adrenergic component of the response could be detected.

Alternatively, *beta*-adrenergic receptors could exert metabolic effects in the absence of cyclic AMP production. In S49 lymphoma cells, the inhibition of magnesium transport by *beta*-adrenergic agonists, acting through *beta*-adrenergic receptors, is independent of adenylate cyclase activation (10, 20–22). A similar role for *$beta_1$*-adrenergic receptors is conceivable in human heart.

The relationship between *beta*-adrenergic receptors, cyclic AMP accumulation, and mechanical properties of the heart is still a matter of debate. Cyclic AMP might well be a second messenger for *beta*-adrenergic agents on cardiac activity (reviewed in refs. 8 and 9), but a dose-effect relationship between cyclic AMP and the cardiac actions of catecholamines is not unequivocally established. For instance, isoproterenol derivatives with a high molecular weight display a potent inotropic action but do not increase cyclic AMP levels in cardiac tissue (23). Unfortunately, most of these studies did not discriminate the *beta*-adrenergic receptor subpopulations, and no data other than those in the present study are available on human heart.

Catecholamines exert multiple effect on heart, including positive inotropic and chronotropic effects, increased rate of tension development, alterations in calcium and potassium movements, and stimulated glycogenolysis and lipolysis. At least one of these effects, the chronotropic effect, could be mediated partially by *$beta_2$*-adrenergic receptors in human atrium [see discussion of the preceding paper (1)]. Considering the rapid development of selective *beta*-adrenergic blockers for therapeutic purpose, our results emphasize the need to study carefully the role of *$beta_2$*-adrenergic receptors in human atrium and ventricle.

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