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Presence of Both *Beta*$_1$- and *Beta*$_2$-Adrenergic Receptors in a Single Cell Type

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SUMMARY

Many tissues, including lung, heart, and brain, have been shown to contain *beta*$_1$ and *beta*$_2$-adrenergic receptor subtypes. This raises the question of whether receptor subtype heterogeneity corresponds to cell type heterogeneity within the tissue, or whether *beta*$_1$- and *beta*$_2$-receptors can coexist in the same cell. We have shown, by both radioligand binding studies and adenylate cyclase experiments, that these two receptor subtypes coexist in C_6 cloned glioma cells and in three derived subclones. Competition experiments in binding and adenylate cyclase assays were conducted using membranes of C_6 glioma cells and of three derived subclones. When [^{3}H]dihydroalprenolol was used as the radioactive ligand, graphic and computer analysis of the competition binding curves obtained with *beta*$_1$- or *beta*$_2$-specific drugs always indicated a heterogeneity of *beta*-adrenergic receptors. For C_6 glioma cell membranes, computer analysis indicated the presence of 80–90% *beta*$_1$-receptors and 10–20% *beta*$_2$-receptors. The same results were obtained with the three subclones. Analysis of the curves for the inhibition of isoproterenol-stimulated adenylate cyclase by practolol, a *beta*$_1$-selective antagonist, showed the presence of two components. The heterogeneity of these practolol inhibition curves indicated that both types of *beta*-adrenergic receptors are coupled to the cyclase. Analysis of the dose-response curves of adenylate cyclase activation obtained with specific *beta*$_2$-agonists also showed a heterogeneity of the response. This finding suggested that occupation of the *beta*$_2$-receptors by a *beta*$_2$-agonist was responsible for most of the cyclase activation and also that occupation of *beta*$_1$-receptors by such an agonist can lead to stimulation of the enzyme but with a less efficient coupling. In conclusion, both *beta*$_1$- and *beta*$_2$-adrenergic receptors coupled to adenylate cyclase can coexist on a single cell.

INTRODUCTION

Lands *et al.* (1) were the first to describe, from physiological studies, two subtypes of *beta*-adrenergic receptors. *Beta*-adrenergic responses are classified as *beta*$_1$ if epinephrine and norepinephrine are equipotent. Such responses, for instance, have been observed in heart (2), adipose tissue (3), and cerebral cortex (4). *Beta*$_2$-adrenergic responses are characterized by a higher potency of epinephrine than of norepinephrine, and have been reported in liver (5), lung (6), muscle (1), and frog erythrocytes (7).

It is now possible to determine the relative proportions of *beta*$_1$- and *beta*$_2$-receptors in a given tissue (8). This can be done by graphic (4, 9) or computer analysis (6, 10) of the data reflecting competition for binding between a labeled nonselective *beta*-adrenergic antagonist and *beta*-adrenergic ligands with selective affinity for one receptor subtype.

The presence of *beta*$_1$ and *beta*$_2$-receptor subtypes in a tissue could be interpreted in one of two ways: either each receptor subtype is present on a different cell population within the tissue, or a single cell type contains both receptors. As far as we know, this important question has not yet been answered. Furchgott and Wakade (11) have shown in guinea pig tracheal smooth muscle that both *beta*$_1$- and *beta*$_2$-adrenergic receptors are implicated in the contractile response. However, these studies did not demonstrate that both receptors are on the same smooth muscle cell type.

We recently showed that primary cultures of glial cells, 96% of which were astrocytes, contained both *beta*$_1$- and *beta*$_2$-adrenergic receptors (12). Although this might indicate that both of these receptors are located on a single cell, we cannot rule out the possibility that two astrocyte cell populations are present in these cultures. We therefore examined this problem in homogeneous populations

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of glial cells, the C_6 glioma cells and three derived subclones.

The following analyses were used to detect the possible presence of both *beta*$_1$- and *beta*$_2$ subtypes in these cell lines: (a) the usual method, comprising both graphic and computer analysis of the biphasic curves for displacement of [^{3}H]DHA[3] by three selective *beta*$_1$-adrenergic antagonists (practolol, atenolol, and metoprolol), one *beta*$_1$-selective agonist (norepinephrine), and one *beta*$_2$-selective agonist (procaterol); (b) a new method, which consists of analyzing the curves obtained for practolol inhibition of the isoproterenol-stimulated adenylate cyclase; and (c) analysis of biphasic adenylate cyclase activation by beta$_2$-selective agonists.

MATERIALS AND METHODS

Cloned C_6 glioma cells were taken from glial tumors induced by repeated injections of *N*-nitrosomethylurea into Wistar rats (13, 14).

Subclones. A cell suspension at a theoretical final density of one cell per 200 μl was seeded in microwells (microtest Falcon F 3040). Microwells in which only one cell was observed were selected, and three of the subclones obtained were studied.

Adenylate cyclase assays and [^{3}H]DHA binding. Culture of C_6 glioma cells, particulate fraction preparations, and [^{3}H]DHA binding and adenylate cyclase activity measurements were performed as previously described (15). [^{3}H]DHA binding and adenylate cyclase activities were determined under strictly identical conditions. Briefly stated, particulate fractions of C_6 glioma cells (20–25 μg of protein) were incubated at 30° in a total volume of 50μl containing 100 mM Tris-HCl (pH 8), 5 mM $MgSO_4$, 1 mM cyclic AMP, 0.2 mM ATP, creatine kinase (0.2 mg/ml), 20 mM phosphocreatine, and 1 mM EDTA. For adenylate cyclase assays, tracer amounts of [α-^{32}P]-ATP and cyclic [^{3}H]AMP were added together 8 min after the beginning of incubation and the reaction was stopped 5 min later. For binding measurements, [^{3}H]DHA was added at the beginning of incubation. The incubation was terminated 10 min later by the addition of 1 ml of cold (4°) 50 mM Tris-HCl (pH 8) containing 20 mM $MgCl_2$, and the samples were filtered through GF/C Whatman filters. The filters were then washed and the bound radioactivity was determined by scintillation counting. Specific binding was defined as the difference between the amount of [^{3}H]DHA bound in the absence (total binding) and in the presence (nonspecific binding) of 10 μM unlabeled alprenolol.

Analysis of the results. The data were analyzed by a nonlinear least-squares curve-fitting procedure using a "Minuit" routine for function analysis described by James and Roos (16). The formulae used for mathematical analysis by computer were as follows:

a. For binding experiments:

$$Y = N_1 \frac{F}{F + K_D(1 + I/K_{D_1})} + N_2 \frac{F}{F + K_D(1 + I/K_{D_2})} \quad (1)$$

in which Y is the specifically bound [^{3}H]DHA and F is the free concentration of [^{3}H]DHA. Under our experimental conditions, because of the low receptor concentration, the free concentration can be considered equal to the total concentration. At 8 nM [^{3}H]DHA, in the presence of 30 μg of membrane in 50 μl, less than 3% of ligand is bound to the receptors. K_D is the dissociation constant of [^{3}H]DHA for the two receptor subtypes (experimentally determined), I is the concentration of the competing ligand, N_1 and N_2 are the respective concentrations of *beta*$_1$ and *beta*$_2$-adrenergic receptor subtypes, and K_{D_1} and K_{D_2} are the respective dissociation constants of the competing ligand for these two receptor subtypes.

b. For inhibition of the isoproterenol-stimulated adenylate cyclase by antagonists:

$$Y = N_1 \frac{F}{F + K_{A_{app}}(1 + I/K_{I_1})} + N_2 \frac{F}{F + K_{A_{app}}(1 + I/K_{I_2})} \quad (2)$$

in which Y is the adenylate cyclase minus basal activity, F is the isoproterenol concentration (10^{-7} M), $K_{A_{app}}$ the apparent activation constant of isoproterenol for adenylate cyclase, I is the concentration of the antagonist, N_1 and N_2 are the respective maximal activities of *beta*$_1$- and *beta*$_2$-adrenergic-sensitive adenylate cyclases, and K_{I_1} and K_{I_2} are the respective adenylate cyclase inhibitory constants of the antagonist.

c. For adenylate cyclase dose-activation curves:

$$Y = N_1 \frac{F}{F + K_{A_{app\,1}}} + N_2 \frac{F}{F + K_{A_{app\,2}}} \quad (3)$$

in which Y is the adenylate cyclase minus basal activity, F is the free agonist concentration, N_1 and N_2, are the respective maximal activities of *beta*$_1$ and *beta*$_2$-adrenergic-sensitive adenylate cyclases, and $K_{A_{app\,1}}$ and $K_{A_{app\,2}}$ are the respective apparent activation constants of the agonist for *beta*$_1$- and *beta*$_2$-adrenergic-sensitive adenylate cyclases.

We assumed that the system contained only two *beta*-adrenergic receptor subtypes and that drug-receptor interactions followed simple mass-action kinetics. Minneman *et al.* (6) gave good arguments in favor of both of these assumptions. Our earlier demonstration that, in C_6 glioma cell membranes, both [^{3}H]DHA binding and isoproterenol stimulation followed simple mass-action kinetics (17) was essential for the present investigation.

Using the "extra sum of squares" principle as applied by de Lean *et al.* (18) the goodness of the fit was evaluated between a model having only one receptor subtype and a model having the two *beta*$_1$ and *beta*$_2$-receptor subtypes. Fisher's coefficients (F) and probability error levels (p) are given from computer analysis of nontransformed data.

Graphic analyses were shown to give only a better visualization of the heterogeneity of the *beta*-adrenergic receptors, all parameter values arising from computer analysis.

Chemicals. [^{3}H]DHA was purchased from New England Nuclear Corporation (Boston, Mass.); (−)-Isoproterenol and (−)-norepinephrine were obtained from

[3] The abbreviation used is: [^{3}H]DHA, (−)-[^{3}H]dihydroalprenolol.

Sigma Chemical Company (St. Louis, Mo.). (−)-Alprenolol and metoprolol were kindly donated by Ciba Geigy (Reuil-Malmaison, France). Procaterol (OPC 2009) was obtained from Otsuka (Tokushima, Japan). Zinterol was kindly donated by Mead Johnson (Evanston, Ind.). Atenolol and practolol were generous gifts from ICI Pharma (Enghein-les-Bains, France), and salbutamol was obtained from Allen and Hanburys (Ware, England).

Except where noted all drugs were racemic mixtures.

RESULTS

Binding experiments were conducted using a radioactive nonselective *beta*-adrenergic drug, [^{3}H]DHA. *Beta*-adrenergic-sensitive adenylate cyclase was stimulated by isoproterenol, a nonselective *beta*-adrenergic agonist. Figure 1 shows experiments illustrating the inhibition of [^{3}H]DHA binding and isoproterenol-stimulated adenylate cyclase by either alprenolol, a nonselective drug, or practolol, a *beta*$_1$-selective antagonist.

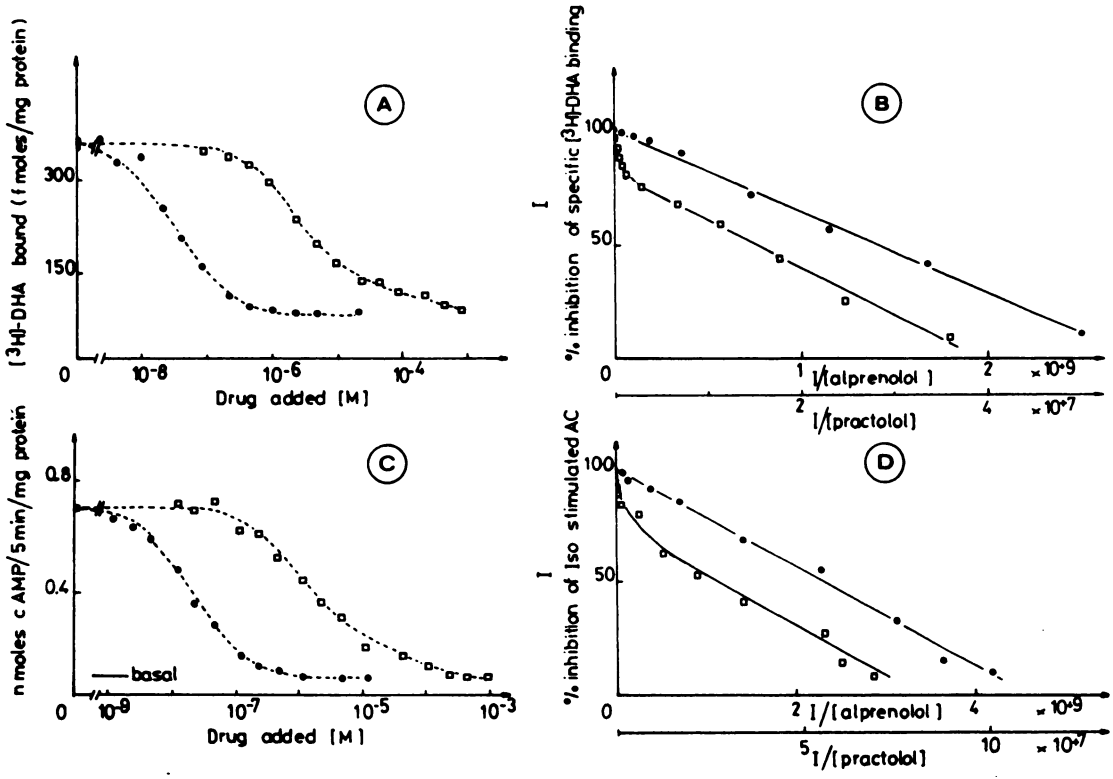

FIG. 1. *Inhibition of [^{3}H]DHA binding and isoproterenol-stimulated adenylate cyclase by alprenolol and practolol in C_6 glioma cells*

C_6 glioma cell membranes were prepared, and binding and adenylate cyclase assays ere performed as previously described (15).

A and B. Binding experiments.

A. Total [^{3}H]DHA bound to C_6 glioma cell membranes in the presence of different concentrations of alprenolol (●) or practolol (□). The [^{3}H]DHA concentration was 9.6 nM (K_D = 3 nM). Each *point* is the mean of duplicate determinations. *Dotted lines* are drawn from computer analysis of the experimental data. The best fit was obtained for (a) alprenolol, with only one class of binding sites having a K_D of 8.1×10^{-9} M (modeling the data for two classes of binding sites did not significantly improved the goodness of fit); and (b) for practolol with two classes of binding sites: 85% *beta*$_1$-receptors with a K_D of 6.1×10^{-7} M and 15% *beta*$_2$-receptors with a K_D of 10^{-4} M ($F = 45$; $p < 0.001$ as compared with a model for a single class of binding sites).

B. Hofstee plots for the inhibition of specific [^{3}H]DHA binding by alprenolol (●) and practolol (□). Experimental data are taken from A. The ordinate represents the percentage inhibition of specific [^{3}H]DHA binding (I). The abscissa represents the ratio of I over the concentrations of competing drugs.

C and D. Adenylate cyclase experiments. The activity of isoproterenol-stimulated adenylate cyclase (10^{-7} M isoproterenol) was determined in the presence of different concentrations of alprenolol (●) and practolol (□). In the same experiment, the $K_{A_{app}}$ for isoproterenol (concentration giving half-maximal stimulation) was 3.5×10^{-8} M.

C. Direct dose-inhibition curves. Basal activity was 0.08 nmole of cyclic AMP/5 min/mg of protein. Each point is the mean of two experimental determinations. *Dotted lines* are drawn from computer analysis of these experimental data. The best fit was obtained (a) for alprenolol with a single class of sites having a K_I of 3.9×10^{-9} M (modeling the data for two classes of *beta* adrenergic-sensitive adenylate cyclases did not significantly improve the goodness of fit) and (b) for practolol with two classes of *beta*-adrenergic-sensitive adenylate cyclases, 75% of which was *beta*$_1$-sensitive ($K_I = 2.2 \times 10^{-7}$ M) and 25% *beta*$_2$-sensitive ($K_I = 1.3 \times 10^{-5}$ M) ($F = 13$; $p < 0.01$ as compared with a model for a single class of *beta*-adrenergic-sensitive adenylate cyclase.

D. Hofstee plot for the inhibition of isoproterenol-stimulated adenylate cyclase by alprenolol (●) and practolol (□). Experimental data are taken from C. The ordinate represents the percentage of inhibition of isoproterenol-stimulated adenylate cyclase activity (I) and the abscissa represents the ratio of I over the concentrations of the competing drugs. Two other experiments gave similar results.

A and C. For computer analysis, we assumed, like other authors (6), that the system contained only two *beta*-adrenergic subtypes and that drug-receptor interaction followed simple mass-action kinetics. The formula used for computerization is given under Materials and Methods.

The curves for both the displacement of [^{3}H]DHA binding and for the inhibition of isoproterenol-stimulated adenylate cyclase by alprenolol were fitted by a computer model involving a single class of sites (Fig. 1A and C) (K_D of alprenolol for binding sites = 8.1×10^{-9} M; K_I for adenylate cyclase inhibition = 3.9×10^{-9} M). The Hofstee plot of these experimental data resulted in straight lines (Fig. 1B and D). The curve for [^{3}H]DHA displacement by practolol, on the contrary, was best fitted by a two-site model ($F = 45$; $p < 0.001$ when compared with a one-site model) (Fig. 1A). Computer analysis indicated that 85% of sites had a high-affinity for practolol ($K_D = 6.1 \times 10^{-7}$ M) and that the affinity of the remaining 15% was lower ($K_D = 10^{-4}$ M). Since practolol is characterized as a $beta_1$ selective ligand we assume that the high-affinity sites correspond to the $beta_1$-receptor subtype and that the low-affinity sites correspond to the $beta_2$-receptor subtype. The Hofstee plot of the above-mentioned practolol displacement curves was biphasic and indicated a greater proportion of $beta_1$ than $beta_2$-adrenergic receptor subtype (Fig. 1B).

The dose-inhibition curve for isoproterenol-stimulated adenylate cyclase obtained with practolol was also biphasic (Fig. 1C). Computer analysis of this experiment indicated 75%, $beta_1$-sensitive adenylate cyclase ($K_I = 2.2 \times 10^{-7}$ M) and 25% $beta_2$-sensitive adenylate cyclase ($K_I = 1.3 \times 10^{-5}$ M). The Hofstee plot of these data gave a biphasic curve which confirmed the presence of both $beta_1$ and $beta_2$-sensitive adenylate cyclases (Fig. 1D).

Binding experiments were conducted with two other $beta_1$-selective antagonists, atenolol and metoprolol; a $beta_1$-selective agonist, norepinephrine; and a $beta_2$-selective agonist, procaterol (8). The Hofstee plots drawn from the inhibition curves were always biphasic (Fig. 2) the four direct displacement curves were best fitted by a computer model involving two classes of sites. The proportions of $beta_1$-adrenergic receptor subtypes detected by the two $beta_1$-selective adrenergic antagonists and with norepinephrine were very similar to those determined with practolol: 88%, 87%, and 86% for atenolol, metoprolol, and norepinephrine, respectively. The affinities of these compounds, respectively were, 3.1×10^{-7} M, 8.3×10^{-8} M, and 9.4×10^{-7} M for the $beta_1$-adrenergic receptors and 7.0×10^{-5} M, 3.4×10^{-6} M, and 4.5×10^{-5} M for the $beta_2$-subtype.

The presence of $beta_1$- and $beta_2$-adrenergic receptors

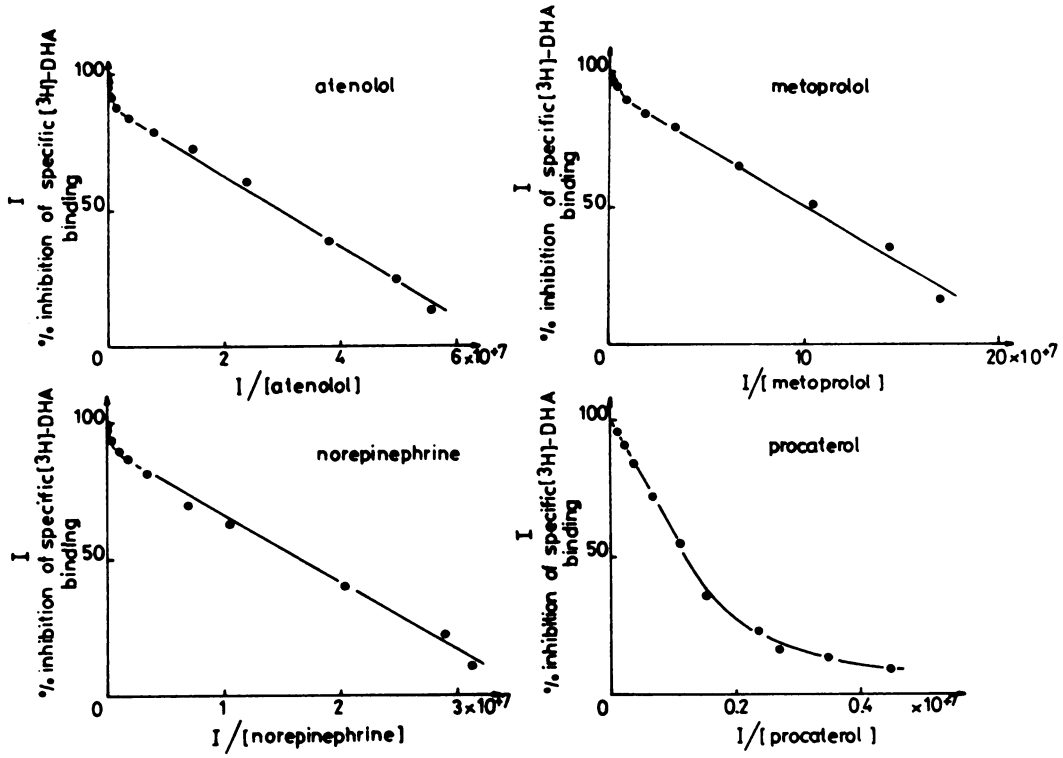

FIG. 2. *Hofstee plots for the inhibition of specific [^{3}H]DHA binding by two $beta_1$-selective antagonists, atenolol and metoprolol; one $beta_1$-selective agonist, norepinephrine; and one $beta_2$-selective agonist, procaterol*

Computer analysis of direct [^{3}H]DHA/competing drug displacement curves indicated the following percentages and affinities for $beta_1$- and $beta_2$-receptors:

	$Beta_1$		$Beta_2$	
	%	K_D	%	K_D
		M		M
Atenolol	88	3.1×10^{-7}	12	7.0×10^{-5}
Metoprolol	87	8.3×10^{-8}	13	3.4×10^{-6}
Norepinephrine	86	9.4×10^{-7}	14	4.5×10^{-5}
Procaterol	91	1.7×10^{-5}	9	3.8×10^{-7}

was confirmed by using the *beta*$_2$-selective agonist procaterol. Computer analysis of the direct [^{3}H]DHA/procaterol displacement curve indicated 91% of sites with a low affinity for this drug (1.7×10^{-5} M). Similar percentages of *beta*$_1$- and *beta*$_2$-receptors were also found with another *beta*$_2$-selective agonist, zinterol (data not shown).

We have studied three subclones of the C_6 glioma cells. The percentages of *beta*$_1$- and *beta*$_2$-adrenergic receptors were determined by a computer analysis of the curves representing binding or adenylate cyclase competition experiments. In all of these clones, both the competition curve for [^{3}H]DHA binding by alprenolol and the curve reflecting isoproterenol-stimulated adenylate cyclase inhibition by alprenolol were monophasic. The affinity values of this nonselective antagonist for *beta*-adrenergic receptors were very similar to these subclones and parental cells (Table 1). Furthermore, all of these cell lines contained comparable proportions of *beta*$_1$- and *beta*$_2$-adrenergic receptor subtypes. The respective affinities of these receptors for practolol were not significantly different from the affinities of C_6 parental cells (Table 1).

Drugs such as procaterol, zinterol, and salbutamol are considered *beta*$_2$-selective agonists (19, 20). Figure 3 shows that they partially activated the adenylate cyclase of C_6 glioma cells. Maximal stimulations were 59, 46, and 40% of total isoproterenol stimulation for zinterol, salbutamol, and procaterol, respectively (mean value for salbutamol, 45 ± 2%; n = 3). Similar activation by salbutamol was obtained in the three subclones (36, 43, and 37% for C_{6_1}, C_{6_2}, and C_{6_3}, respectively). Although the isoproterenol dose-response curve was fitted with a single adenylate cyclase component, the zinterol, salbutamol,. and procaterol dose-response curves were best fitted with two adenylate cyclase components. For the three drugs, the proportions of component having the lower $K_{A_{app}}$ were 75, 71, and 46% for salbutamol, zinterol, and procaterol, respectively (mean value for salbutamol 66 ± 8%; n = 3). The apparent activation constants ($K_{A_{app}}$) of salbutamol, zinterol, and procaterol for the two components of adenylate cyclase are given in the legend to Fig. 3.

The [^{3}H]DHA/salbutamol displacement curve was monophasic ($K_D = 1.3 \times 10^{-5}$ M; Fig. 3B), indicating that this compound had no selective affinity for either the *beta*$_1$- or *beta*$_2$-receptor subtypes in this system as in some others (9, 19).

DISCUSSION

To detect whether both *beta*$_1$- and *beta*$_2$-adrenergic receptor subtypes are present in a homogeneous C_6 cell population we performed competition experiments with selective and nonselective *beta*-adrenergic ligands in binding and adenylate cyclase assays.

When alprenolol, a nonselective *beta*-adrenergic ligand was used, graphic or computer analysis of either [^{3}H]DHA/alprenolol competition curves or of isoproterenol-stimulated adenylate cyclase/alprenolol inhibition curves showed that all *beta*-adrenergic receptors of the C_6 glioma cell membranes had the same affinity for this ligand (Fig. 1A and B).

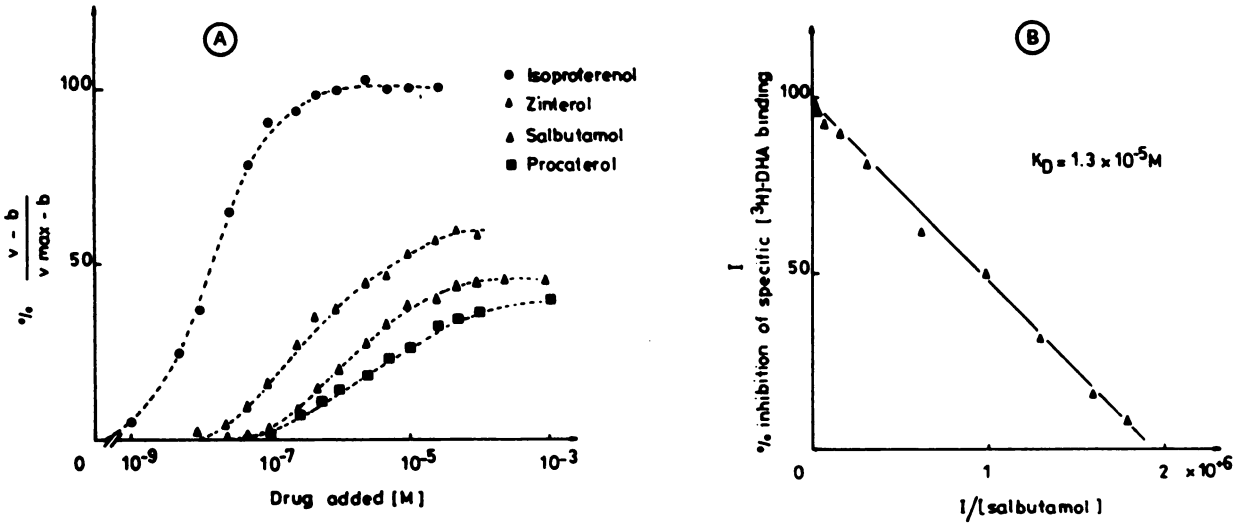

FIG. 3. *Adenylate cyclase stimulation by a nonselective beta-adrenergic agonist and by three beta*$_2$*-selective agonists in C_6 glioma cell membranes (A) and Hofstee plot for the inhibition of specific [^{3}H]DHA binding by salbutamol (B)*

A. The ordinate represents the percentage of drug-stimulated adenylate cyclase to maximal isoproterenol-stimulated activity. Basal (*b*) and maximal isoproterenol-stimulated activities (V_{max}) were 0.1 and 1.5 nmoles of cyclic AMP/5 min/mg of protein. Each *point* represents the mean of duplicate determinations. *Dotted lines* were drawn from computer analysis of these experimental data. The best fit was obtained for isoproterenol (●) with only one class of sites with an apparent activation constant ($K_{A_{app}}$) of 1.5×10^{-8} M. Modeling the data for two classes of *beta*-adrenergic-sensitive adenylate cyclase did not significantly improved the goodness of fit. For the other agonists, the best fits were obtained with two classes of sites: zinterol (▲), 71% ($K_{A_{app}} = 1.6 \times 10^{-7}$ M) and 29% ($K_{A_{app}} = 5.7 \times 10^{-6}$ M) ($F = 8$; $p < 0.01$ as compared with a model for a single class of *beta*-adrenergic-sensitive adenylate cyclase); salbutamol (△), 75% ($K_{A_{app}} = 7.9 \times 10^{-7}$ M) and 25% ($K_{A_{app}} = 1.1 \times 10^{-5}$ M)($F = 10$; $p < 0.01$); and procaterol (■), 46% ($K_{A_{app}} = 4.3 \times 10^{-7}$ M) and 54% ($K_{A_{app}} = 1.8 \times 10^{-5}$ M) ($F = 15$; $p < 0.001$). The formula for computer analysis is given under Materials and Methods.

B. The [^{3}H]DHA concentration was 8.4 nM and the specifically bound [^{3}H]DHA was equal to 220 fmoles/mg of protein. The K_D calculated for salbutamol was 1.3×10^{-5} M.

When *beta*$_1$- or *beta*$_2$-selective ligands were used the situation was different. We performed binding competition experiments with three *beta*$_1$-selective antagonists, practolol, atenolol, and metoprolol; one *beta*$_1$-selective agonist, norepinephrine; and one *beta*$_2$-selective agonist, procaterol. For all of these ligands, both graphic and computer analyses of the competition curves indicated the existence of two populations of sites. The percentages of low- and high-affinity sites were almost the same for all of the *beta*$_1$-selective drugs, i.e., 80–90% high-affinity sites and 10–20% low-affinity sites (Figs. 1 and 2). Comparison of respective affinities of practolol for these sites with those reported in the literature for this ligand for *beta*$_1$- and *beta*$_2$-adrenergic receptors (4, 6) suggests that the high-affinity sites correspond to the *beta*$_1$-receptor subtype and that the low-affinity sites correspond to the *beta*$_2$-receptor. With the *beta*$_2$-selective agonist procaterol, binding experiments also showed the presence of 91% *beta*$_1$- and 9% *beta*$_2$-receptors (Fig. 2).

Although in our system norepinephrine had a higher affinity for *beta*$_1$- than *beta*$_2$ receptors ($K_D = 9.4 \times 10^{-7}$ M and 4.5×10^{-5} M, respectively; Fig. 2), epinephrine and isoproterenol did not show any selectivity ($K_D = 2 \times 10^{-6}$ M and 2×10^{-7} M, respectively; data not shown). Thus, as first classified by Lands *et al.* (1), the order of potency for *beta*$_1$-adrenergic receptors was isoproterenol > norepinephrine ≃ epinephrine, and for *beta*$_2$-adrenergic receptors it was isoproterenol > epinephrine > norepinephrine.

Analysis of the inhibition of isoproterenol-sensitive adenylate cyclase by practolol confirmed the presence of two types of *beta*-adrenergic receptors in C_6 glioma cells and showed that both are coupled to the adenylate cyclase (Fig. 1C and D). The nonlinearity of the coupling between *beta*-adrenergic receptors and adenylate cyclase in these cells (15) might explain why the proportions of *beta*$_1$- and *beta*$_2$-adrenergic receptor subtypes on the one hand and the proportions of *beta*$_1$- and *beta*$_2$-adrenergic-sensitive adenylate cyclases on the other are not strictly identical (Table 1). Drugs such as zinterol, salbutamol, and procaterol are considered *beta*$_2$-selective agonists and have been reported to activate adenylate cyclase only in tissues containing mainly *beta*$_2$-receptors (19, 20). When these drugs are used to activate the adenylate cyclase in C_6 glioma cell membranes, two components are detected in the dose-activation curves (Fig. 3A). The heterogeneity of these adenylate cyclase stimulations is interesting since it suggests that not only the occupation of *beta*$_2$-adrenergic receptors but also that of *beta*$_1$-adrenergic receptors by these agonists led to a stimulation of the enzyme.

In the experiments with zinterol and procaterol, the relationships between the occupation of *beta*$_1$- and *beta*$_2$-adrenergic receptor subtypes and the corresponding adenylate cyclase activations are complex for two reasons: (a) these *beta*$_2$-selective agonists have different affinities for the *beta*$_1$- and *beta*$_2$-adrenergic receptors (ref. 6 and Fig. 2), and (b) the relationship between the occupation of these receptors and the adenylate cyclase activation is nonlinear (15).

In the case of salbutamol the situation is simpler, since it has the same affinity for both receptor subtypes ($K_D = 1.3 \times 10^{-5}$ M; Fig. 3B and refs. 9 and 19), but the dose-activation curve for adenylate cyclase by this agonist was heterogeneous (Fig. 3A). This curve was best fitted with two components ($F = 10$; $p < 0.01$ when compared with one component) having different $K_{A_{app}}$ (see Materials and Methods). We assumed that the component having a $K_{A_{app}}$ of 7.9×10^{-7} M (75% of the total salbutamol stimulation) (Fig. 3A) and the component having a $K_{A_{app}}$ of 1.1

TABLE 1

Relative concentrations of beta$_1$*- and beta*$_2$*-adrenergic receptors and their affinities for alprenolol and practolol in C_6 glioma cells and three subclones*

The percentages of *beta*$_1$- and *beta*$_2$-adrenergic receptors and their affinities were determined from binding curves for the competition between [^{3}H]DHA and alprenolol or practolol, and from curves reflecting inhibition of isoproterenol-stimulated adenylate cyclase by alprenolol or practolol (Fig. 1). Values were obtained from computer analysis of these results. Each value is the mean ± standard error of the mean of four separate experiments for C_6 glioma cells and of three separate experiments for the subclones. Subclones were used between passages 6 and 9. The values of 100% receptor subtypes of *beta*-adrenergic-sensitive adenylate cyclase obtained with alprenolol indicated that this ligand binds to all *beta*$_1$- and *beta*$_2$-receptors without selectivity.

Binding	C_6 parental clone ($n = 4$)		Subclones					
	% Receptor subtypes	K_D (10^{-7} M)	$C_6$1 ($n = 3$)		$C_6$2 ($n = 3$)		$C_6$3 ($n = 3$)	
			%	K_D (10^{-7} M)	%	K_D (10^{-7} M)	%	K_D (10^{-7} M)
Alprenolol	100	0.089 ± 0.025	100	0.095 ± 0.006	100	0.090 ± 0.005	100	0.076 ± 0.02
Practolol								
Beta$_1$	81.2 ± 0.5	6.2 ± 0.8	87.3 ± 1.4	3.9 ± 0.5	81.7 ± 1.8	7.2 ± 0.8	75.0 ± 5.1	5.4 ± 0.6
Beta$_2$	18.8 ± 0.5	930 ± 190	12.7 ± 1.4	440 ± 130	18.3 ± 1.8	610 ± 260	25.0 ± 5.1	1020 ± 600
Adenylate cyclase	% *Beta*-adrenergic-sensitive adenylate cyclase	K_I (10^{-7} M)	%	K_I (10^{-7} M)	%	K_I (10^{-7} M)	%	K_i (10^{-7} M)
Alprenolol	100	0.099 ± 0.013	100	0.065 ± 0.009	100	0.051 ± 0.007	100	0.070 ± 0.017
Practolol								
Beta$_1$	71.7 ± 7	4.2 ± 0.1	77.0 ± 6.4	2.9 ± 0.5	80.0 ± 2.9	3.7 ± 1.1	63.4 ± 4.9	3.2 ± 0.4
Beta$_2$	28.3 ± 7	340 ± 200	23.0 ± 6.4	190 ± 30	20.0 ± 2.9	210 ± 50	36.6 ± 4.9	270 ± 150

$\times 10^{-5}$ M (25% of the total salbutamol stimulation; Fig. 3A) were due to the interaction of salbutamol with $beta_2$- and $beta_1$-adrenergic receptors, respectively. Thus (a) occupation by salbutamol of all $beta_1$-adrenergic receptors [81.2 ± 0.5% (n = 4) of total receptors (Table 1)] leads to 34 ± 8% (n = 3) of total salbutamol stimulation, whereas occupation of all $beta_2$-adrenergic receptors [18.8 ± 0.5% (n = 4) of total receptors] by this agonist leads to 66 ± 8% (n = 3) of the stimulation. (b) The $K_D/K_{A_{app}}$ ratio for $beta_2$-adrenergic stimulation is 1.3×10^{-5} M/7.9×10^{-7} M = 16.4, whereas this ratio is close to 1 (1.3×10^{-5} M/1.1×10^{-5} M) for the $beta_1$-adrenergic stimulation. A high value of this ratio indicates high efficiency of coupling between the receptor and the adenylate cyclase (21).

These two observations suggest that, when a $beta_2$-selective agonist such as salbutamol occupied a $beta_2$-adrenergic receptor, the coupling with the adenylate cyclase was more efficient than the coupling obtained when this $beta_2$-selective agonist occupied a $beta_1$-adrenergic receptor. The reverse hypothesis might be true: occupation of a $beta_1$-adrenergic receptor by $beta_1$-selective agonist would give a more efficient coupling than occupation of a $beta_2$-adrenergic receptor by this agonist.

However, an attempt to demonstrate such a "reverse hypothesis" with norepinephrine did not give clear results. This was probably due to the fact that our system contained about 85% $beta_1$-adrenergic receptors which are, according to our hypothesis, well coupled, and therefore will give more than 95% of the maximal stimulation which can be obtained.

Thus we can distinguish different types of selectivity of ligands. Some have different affinities for receptor subtypes; others, such as salbutamol, have equal affinities for the two receptor subtypes but their apparent selectivity derives from differing agonist coupling efficacy.

In conclusion, the most important result of this study is the detection of both $beta_1$- and $beta_2$-adrenergic receptors in a C_6 glioma cell line and in three subclones derived from a single cell. The fact that both $beta_1$- and $beta_2$-adrenergic receptors are present in all four homogeneous cell populations indicates that these two receptor subtypes can coexist in a single cell. The biochemical and physiological consequences of these findings will now have to be considered in both astrocytes and other mammalian cell populations.

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REFERENCES

1. Lands, A. M., A. Arnold, J. P. McAuliff, F. P. Luduena, and I. G. Brown. Differentiation of receptor systems activated by sympathomimetic amines. *Nature (Lond.)* **214**:597–598 (1967).
2. Minneman, K. P., and P. B. Molinoff. Differential distribution of β_1 and β_2 adrenergic receptors in cat and guinea-pig heart. *J. Pharmacol. Exp. Ther.* **212**:503–508 (1980).
3. Murad, F. Beta blockade of epinephrine induced cyclic AMP formation in heart, liver, fat and trachea. Biochim. Biophys. Acta **304**:181–187 (1973).
4. Nahorski, S. R. Heterogeneity of cerebral β adrenoceptor binding sites in various vertebrate species. *Eur. J. Pharmcol.* **51**:199–209 (1978).
5. Mayer, S. E. Effects of adrenergic agonists and antagonists on adenylate cyclase activity of dog heart and liver. *J. Pharmacol. Exp. Ther.* **181**:116–125 (1972).
6. Minneman, K. P., L. R. Hegstrand, and P. B. Molinoff. Simultaneous determination of β_1 and β_2 adrenergic receptors in tissues containing both receptor subtypes. *Mol. Pharmacol.* **16**:34–46 (1979).
7. Mukherjee, C., M. G. Caron, M. Coverstone, and R. J. Lefkowitz. Identification of adenylate cyclase coupled β adrenergic receptors in frog erythrocytes with (−)-[^{3}H]alprenolol. *J. Biol. Chem.* **250**:4869–4876, (1975).
8. Minneman, K. P., and P. B. Molinoff. Classification and quantitation of β adrenergic receptor subtypes. *Biochem. Pharmacol.* **24**:1317–1322 (1980).
9. Rugg, E. L., D. B. Barnett, and S. R. Nahorski. Coexistence of β_1 and β_2 adrenoceptors in mammalian lung: evidence from direct binding studies. *Mol. Pharmacol.* **14**:996–1005 (1978).
10. Hancock, A. A., A. L. De Lean, and R. J. Lefkowitz. Quantitative resolution of β adrenergic receptor subtypes by selective ligand binding: application of a computerized model fitting technique. *Mol. Pharmacol.* **16**:1–9 (1979).
11. Furchgott, R. F., and T. D. Wakade. Evidence for both β_1 and β_2 receptors in guinea-pig tracheal smooth muscle and variation of the β_1: β_2 ratio in different animals, in *Abstracts of the Sixth Int. Congr. Pharmacol., Helsinki*, 622 (1975).
12. Ebersolt, C., M. Perez, G. Vassent, and J. Bockaert. Characteristics of the β_1 and β_2 adrenergic sensitive adenylate cyclases in glial cell primary cultures and their comparison with β_2 adrenergic sensitive adenylate cyclase of meningeal cells. Brain Res. **213**:151–161 (1981).
13. Benda, P., K. Someda, J. Messer, and W. H. Sweet. Morphological and immunochemical studies on rat glial tumors and clonal strains propagated in culture. *J. Neurosurg.* **34**:310–315 (1971).
14. Benda, P., J. Lightbody, G. Sato, L. Levine, and W. H. Sweet. Differentiated rat glial cell strain in tissue culture. *Science (Wash. D. C.)* **161**:370–372 (1968).
15. Lucas, M., and J. Bockaert. Use of (−)-[^{3}H]dihydroalprenolol to study *beta*-adrenergic receptor adenylate cyclase coupling in C_6 glioma cells: role of 5′-guanylylimidodiphosphate. *Mol. Pharmacol.* **13**:314–329 (1977).
16. James, F., and M. Roos. Minuit: a system for function minimization and analysis of the parameter errors and correlations. *Computer Physics Commun.* **10**:343–367 (1975).
17. Homburger, V., M. Lucas, J. Barabe, B. Cantau, J. Penit, and J. Bockaert. Further evidence that desensitization of β adrenergic sensitive adenylate cyclase proceeds in two steps. *J. Biol. Chem.* **255**:10436–10444 (1980).
18. De Lean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase coupled-β-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
19. Minneman, K. P., L. R. Hegstrand, and P. B. Molinoff. The pharmacological specificity of β_1 and β_2 adrenergic receptors in rat heart and lung *in vitro*. *Mol. Pharmacol.* **16**:21–33 (1979).
20. Minneman, K. P., G. A. Weiland, and P. B. Molinoff. A comparison of the β adrenergic receptor of the turkey erythrocyte with mammalian β_1 and β_2 receptors. *Mol. Pharmacol.* **17**:1–7 (1980).
21. Maguire, M. E., E. M. Ross, and A. G. Gilman. β Adrenergic receptor: ligand binding properties and interaction with adenyl cyclase. *Adv. Cyclic Nucleotide Res.* **8**:1–81 (1977).

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