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Pharmacology

Efficacy of β_1-adrenergic receptors is lower than that of β_2-adrenergic receptors

(G protein/receptor–effector coupling/receptor subtypes/blocker selectivity)

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ABSTRACT **We investigated the relative activity at which fully occupied human β_1- and β_2-adrenergic receptors (β_1AR and β_2AR) activate the stimulatory G protein (G_s)/adenylyl cyclase (AC) system in isolated membranes. The receptors were cloned and coexpressed in permanent cell lines at β_1/β_2 ratios that varied from 1:2 to 3:1 and at total receptor abundance that ranged from 8 to 2200 fmol/mg of membrane protein. Cell lines expressing β_1AR or β_2AR alone were also obtained. Competitive inhibition of isoproterenol-stimulated AC activity by the β_2-selective antagonist ICI 118551 showed in all cases that maximal stimulation elicited by β_1AR was lower than when it was elicited by equivalent densities of β_2AR. This was especially noticeable at limiting concentrations of receptor, where the β_1AR-mediated effect was <10% of that mediated by β_2AR. At receptor concentrations > 1000 fmol/mg of protein, stimulation by β_2AR appeared to reach a maximum, while stimulation by β_1AR continued to increase, so that at 3200 fmol/mg, β_1AR-stimulated activity was 80% of β_2AR-stimulated activity. It is clear that the degree to which a given receptor system is able to activate the G_s/AC system depends not only on its abundance but also on an activity parameter determined by the nature of the receptor, which we refer to as receptor efficacy. For human βARs, this efficacy parameter is much lower for the β_1 subtype than for its β_2 counterpart. The more effective stimulation of AC through β_2AR than through β_1AR is an inherent property of the receptor and not the cell in which it is expressed.**

Tissues may have receptors for more than one drug or hormone, all of which elicit the same response but may do so to differing extents. One example is the adipose tissue of the rat. Cells from this tissue have receptors for corticotropin, epinephrine, secretin, and glucagon, all of which stimulate the same adenylyl cyclase (AC) in a nonadditive manner. A study of their individual effects showed them to vary by a factor of up to 2, so that full stimulation by glucagon was only 50% of that obtained with epinephrine (1, 2). Stimulations by corticotropin and secretin were intermediate. Given that the ligands are the natural hormones that coevolved with the receptors they interact with, explanations of the differing response intensities should be sought in the receptors and not in the ligands, which are likely to be the equivalent of "full agonists."

One possibility is that the lower of the responses is due to a low receptor abundance such that if the receptor number could be increased, the maximal response would be increased as well. Another possibility is that receptors, like ligands, may possess different intrinsic capacities to elicit a response. We report below experiments in which we compared the coupling—i.e., response-eliciting abilities—of the human β_1 and β_2 adrenergic receptors (β_1AR and β_2AR), and we show that, when compared to the β_2AR, the β_1AR is weaker in stimulating the G_s/AC system. We refer to this aspect of receptor function as receptor efficacy.

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METHODS

Applying procedures described previously (3–7), we cloned the complete coding sequences of the human β_1AR and β_2AR from a genomic DNA library, joined to their 5′ end the 41-bp alfalfa mosaic virus ribosomal recognition sequence of plasmid pAGA-2 (7, 8), and placed the constructs into pKNH (9), giving pK-hβ_1AR and pK-hβ_2AR, and pMAMneo (Stratagene), giving pMn-hβ_1AR and pMn-hβ_2AR. pKNH directs the constitutive transcription of inserts and pMAMneo directs dexamethasone-inducible transcription of inserts. The nucleotide sequences of the constructs were determined (10) and agreed with those reported for human β_1AR (11) and β_2AR (12).

Cell Lines Expressing β_1AR, β_2AR, and β_1AR Plus β_2AR. The expression vectors containing the βAR inserts were transfected alone and in combination into thymidine kinase-negative mouse fibroblasts (Ltk$^-$ cells) by the calcium phosphate/glycerol shock method (13). G418-resistant transformants were selected, seeded into 96-well plates, and tested *in situ* for isoproterenol-sensitive AC activity (14). Cells transfected with pK-hβ_1AR gave rise to Lβ1 cells, those transfected with pK-hβ_2AR gave rise to Lβ2 cells, and those transfected with either both pK-βAR plasmids or both pMn-βAR plasmids gave rise to Lβ1β2 cells. Cells identified by this method as having acquired isoproterenol responsiveness (primary transformants) were expanded and tested further. Several primary clones were obtained from each transfection, and some of these were subjected to clonal selection by limiting dilution to give clonal cell lines, Lβ1.x, Lβ2.x, and Lβ1β2.x, where x identifies individual cell clones.

Cell Growth, Membrane Preparation, and AC and Ligand Binding Assays. Cells were grown in minimal essential medium α plus 10% fetal bovine serum containing penicillin and streptomycin (6) supplemented with G418 (400 μg/ml; GIBCO). Cells were grown to close to confluence, split 1:5 into 150-mm dishes, and harvested 48–60 hr later. Dexamethasone (1 μM) was added 24 hr before harvest of cells expressing receptors under the control of pMAMneo's glucocorticoid-responsive mouse mammary tumor virus long terminal repeat.

Minor modifications (unpublished work) of methods described previously were used for preparation of membranes (5) and assay of AC activity (15–17) and [^{125}I]iodocyanopin-

Abbreviations: AC, adenylyl cyclase; AR, adrenergic receptor; ^{125}I-CYP, [^{125}I]iodocyanopindolol; PGE_1, prostaglandin E_1.

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Table 1. Scatchard analysis of ^{125}I-CYP binding and adrenergic stimulation of AC relative to that of prostaglandin E_1 (PGE_1) in membranes of L cells transfected with human β_1AR and/or β_2AR DNA in pKNH vector

Cell line	Receptor(s)	^{125}I-CYP binding* (mean ± SD) Exp	K_d, pM	B_{max}, fmol/mg	AC activity: Stimulation relative to PGE_1†	AC activity: PGE_1-stimulated pmol of cAMP per min per mg (*n*)
Lβ1.2	β_1		7.2 ± 0.2	3210 ± 30	1.6 ± 0.2	41 ± 13 (7)
Lβ1.3	β_1	1	8.1 ± 0.2	1910 ± 20	1.2 ± 0.1	26 ± 2 (2)
		2	9.2 ± 0.2	1980 ± 30		
Lβ1.4	β_1		7.6 ± 0.2	2370 ± 20	1.3	24 (1)
Lβ2.5	β_2		4.5 ± 0.2	720 ± 10	1.5	29 (1)
Lβ2.7	β_2	1	3.4 ± 0.2	3150 ± 40	1.9 ± 0.2	49 ± 12 (6)
		2	2.8 ± 0.1	ND		
Lβ2.20	β_2		4.1 ± 0.2	415 ± 10	1.6	14 (1)
Lβ1β2.1	$\beta_1+\beta_2$		6.2 ± 0.2	2590 ± 50	2.0 ± 0.3	35 ± 5 (5)

ND, not determined.
*Incubations were for 180 min at 32°C in 100 μl with 0.3–0.6 μg of membrane protein.
†Ratio of activity in the presence of (−)-isoproterenol (1–10 μM) divided by activity in the presence of PGE_1 (10 μg/ml). Incubations were for 20 min at 32°C in 50 μl with 2–5 μg of membrane protein. Values are means ± SD ($n > 2$) or half the range ($n = 2$) of *n* experiments.

dolol (^{125}I-CYP) binding (18). Membranes were frozen in aliquots and kept at −70°C until used. No significant loss of receptor binding or AC activity occurred over 9 months, the longest time tested.

RESULTS

We constructed, cloned by limiting dilution, and characterized 12 cell lines (Tables 1 and 2). Three express the human β_1AR (Lβ1 clones 2–4), 3 express the human β_2AR (Lβ2 clones 5, 7, and 20), and 6 express both the β_1AR and the β_2AR (Lβ1β2 clones 1, 8–11, and 14). Expression of the receptors is constitutive for all cell lines except Lβ1β2 clones 9–11, which were constructed by cotransfection of the β_1AR and β_2AR DNAs in pMAMneo and in which expression was induced by addition of dexamethasone 24 hr before harvest.

Scatchard analysis of ^{125}I-CYP binding to membranes of murine L cells expressing only β_1AR or only β_2AR showed that although the receptor abundance (fmol/mg of protein) varied from one clonal cell line to another, the lines had very similar affinities for ^{125}I-CYP that segregated into K_d values (mean ± SEM) of 8.0 ± 0.4 pM for cells expressing β_1AR and 3.7 ± 0.4 pM for those expressing β_2AR (Table 1). The binding data obtained with Lβ1β2.1 cell membranes were well fitted by a binding isotherm with a single K_d for ^{125}I-CYP of 6.2 ± 0.2 pM. Lβ1β2.1 cell membranes have about equal amounts of β_1AR and β_2AR (see below) and the binding data can be fitted equally well with a model of two independent binding sites with the K_d values derived from cell membranes expressing the individual receptors—i.e., 50% 8 pM plus 50% 3.7 pM (data not shown).

All βAR-transfected cell lines exhibited stimulation of AC by (−)-isoproterenol, which had been the functional screen used to identify them as receptor-positive. (−)-Norepinephrine and (−)-epinephrine also stimulated AC activity. In dose–response studies on membranes from cells that expressed either β_1AR or β_2AR alone, maximal stimulation of AC by the three agonists was the same (data not shown).

Table 2. Absolute and relative abundance of β_1AR and β_2AR and relative stimulation of AC by these receptors in membranes from stably transfected cell lines

Cell line	Vector	^{125}I-CYP binding* Added, pM	^{125}I-CYP binding* Bound, fmol/mg	Relative receptor abundance Probe	β_1/β_2 ratio‡	β_1,§ fmol/mg	AC activation† β_1, % of $\beta_1+\beta_2$ (*n*)	AC activation† $\beta_2+\beta_1$, isoproterenol/PGE_1 (*n*)	PGE_1-stimulated AC, pmol per min per mg (*n*)
Lβ1β2.1	pKNH	49	2134 ± 12	CGP	46:54	1000			
		52	2464 ± 13	ICI	54:46	1330	50 ± 7 (2)	2.0 ± 0.3 (5)	34 ± 6 (5)
Lβ1β2.8	pKNH	49	2214 ± 9	CGP	65:35	1440			
		52	2317 ± 14	ICI	74:26	1710	52 ± 4 (2)	1.9 ± 0.3 (4)	33 ± 8 (4)
Lβ1β2.14	pKNH	49	957 ± 8	CGP	63:37	600			
		49	1320 ± 16	ICI	70:30	920	47 ± 6 (2)	1.8 ± 0.2 (4)	31 ± 7 (4)
Lβ1β2.9	pMAMneo	49	126 ± 1	CGP	68:32	86			
		49	150 ± 2	ICI	75:25	113	38 ± 7 (2)	0.6 ± 0.1 (4)	30 ± 6 (4)
Lβ1β2.11	pMAMneo	49	50 ± 1	CGP	55:45	28			
		49	66 ± 2	ICI	62:38	41	29 ± 6 (2)	0.29 ± 0.05 (4)	26 ± 5 (4)
Lβ1β2.10	pMAMneo	49	8 ± 0.5	CGP	34:66	2.7			
			ND	ICI	ND	ND	7 ± 5 (2)	0.09 ± 0.03 (4)	48 ± 10 (4)

ND, not determined. Expression from pMAMneo was induced with dexamethasone.
*Incubations were for 180 min at 32°C in 100 μl with 0.5–0.6 μg (clones 1, 8, and 14), 2–2.5 μg (clone 9), 4–4.5 μg (clone 11), or 10–12 μg (clone 10) of membrane protein.
†Activities in the presence of (−)-isoproterenol (1–10 μM) or PGE_1 (10 μg/ml). Incubations were for 20 min at 32°C in 50 μl with 1.5–4.5 μg (Lβ1β2.1), 1.5–4.5 μg (Lβ1β2.8), 1.5–7.2 μg (Lβ1β2.14), 2.5–8.3 μg (Lβ1β2.9), 3.7–8.7 μg (Lβ1β2.11), or 13 μg (Lβ1β2.10) of membrane protein. Values are means ± SD; *n*, number of experiments.
‡Calculated from fitting curves for competitive inhibition of the binding of ^{125}I-CYP by CGP 20712A or ICI 118551 or curves for competitive inhibition of isoproterenol (1–10 μM)-stimulated AC by ICI 118551 with a model of two independent binding sites as shown in Fig. 2.
§^{125}I-CYP (fmol/mg) bound × fraction of binding calculated to be due to β_1AR.

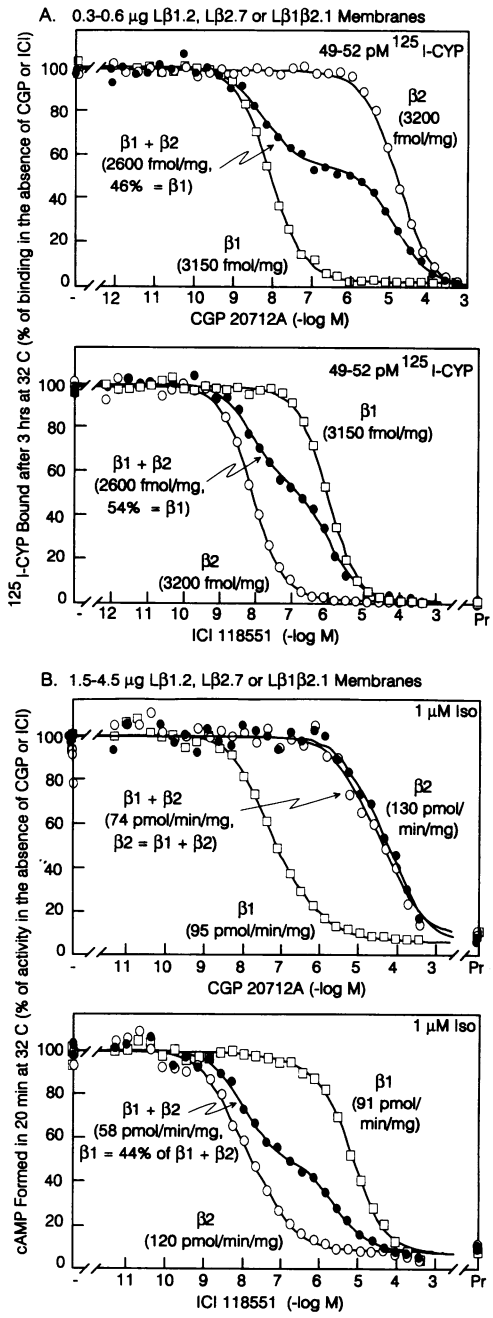

FIG. 1. Effect of the β_1-selective antagonist CGP 20712A (*Upper*) and the β_2-selective antagonist ICI 118551 (*Lower*) on the binding of ^{125}I-CYP (*A*) and stimulation of AC by 1 μM (−)-isoproterenol (*B*) in membranes from L cells expressing human β_1AR alone (□), human β_2AR alone (○), or both receptors together (●). Binding data represent total binding to membranes without subtraction of nonspecific binding. Pr, incubation mixtures contained 3 μM (−)-propranolol. Points are results from individual incubations except for ^{125}I-CYP binding and (−)-isoproterenol-stimulated activities in the absence of β_1 or β_2 blockers or in the presence of 3 μM (−)-propranolol (Pr), which are means of triplicates. Values below the β_1, β_2, and $\beta_1+\beta_2$ designations represent the 100% values of each of the curves; also given are the calculated amplitudes of β_1 or β_2 components in $\beta_1+\beta_2$ membranes (*A*) or activity behaving as β_1- or β_2-mediated in $\beta_1+\beta_2$ membranes (*B*). Lines represent computer generated fits of the data obtained with theoretical models for one (Lβ1 and Lβ2 cells) and two (Lβ1β2 cells) sites. Calculations were performed by an IBM PS/2 computer using nonlinear regression routines provided by the SIGMAPLOT 4.1 software (Jandel, Corte Madera, CA). One-site model: $y = f(x) = a + \{(b - a)[1/(1 + x/c)]\}$, where a is y at the bottom of the curve, b is y at the top of the curve, c is the midpoint of the displacement curve (IC_{50}), y is the y-axis value at x, and x is concentration of ligand along the x axis. Two-site model: $y = f(x) = a + \{(b - a)[e/(1 + x/c) + (1 - e)/(1 + x/d)]\}$; a, b, x, and y are as in the one-site model, e is the fraction of $b - a$ contributing to y with a midpoint value (IC_{50}) of c, and $1 - e$ is the fraction of $b - a$ contributing to y with a midpoint value (IC_{50}) of d.

However, there was considerable cell-to-cell difference in the stimulated activities that appeared to be dependent on the receptor subtype and receptor number, especially when related to activity stimulated by the resident L-cell PGE_1-responsive prostanoid receptor (Fig. 1*B*; Tables 1 and 2). Thus, the extent of AC stimulation by receptor at concentrations of isoproterenol giving maximal effects in membranes from three cell lines expressing the β_1AR at 1900–3200 fmol/mg ranged from 1.2- to 1.6-fold that obtained with PGE_1, while membranes from cells expressing the β_2AR at 2600–3200 fmol/mg had isoproterenol-stimulated activities that were 1.9- to 2.0-fold the PGE_1-stimulated activity. This suggested that β_1AR might be less efficacious than β_2AR in activating the G_s/AC system.

The lower activity of β_1AR is exemplified in Fig. 1. The effect of selectively blocking β_1AR on the stimulation of AC in membranes from cells coexpressing β_1AR and β_2AR was compared with the effect of selectively blocking β_2AR in the same membranes. The effect of β_1AR and β_2AR blockade in cell membranes with only β_1AR and only β_2AR was assessed as control. Competitive inhibition of ^{125}I-CYP binding by CGP 20712A and ICI 118551 to membranes of Lβ1.2 and Lβ2.7 cells (Fig. 1*A*) confirmed the respective β_1AR and β_2AR selectivity of these drugs (19) under our assay conditions. Quantitation of β_1AR and β_2AR in membranes of Lβ1β2.1 cells revealed that these cells coexpress β_1AR and β_2AR at a ratio of about 1:1 (displacement of ^{125}I-CYP by CGP 20712A gave a β_1/β_2 ratio of 0.85, and displacement of ^{125}I-CYP by ICI 118551 gave a β_1/β_2 ratio of 1.17; Fig. 1 and Table 2).

In contrast to the biphasic inhibition of ^{125}I-CYP binding, the dose–response curve for CGP 20712A-mediated inhibition of AC activity stimulated by 1 μM isoproterenol in membranes from Lβ1β2.1 cells was monophasic with an IC_{50} of 52 μM, which corresponds to the inhibition of the β_2AR component of the cell. This indicated that blockade of the β_1AR component (which in these membranes occurred with an IC_{50} of 5–10 nM) was without detriment to the overall stimulation of the G_s/AC system and that the β_2AR was driving the system to such an extent that an added β_1AR-mediated stimulation could not be expressed; i.e., that the two receptors were acting in a nonadditive manner and that the stimulation by β_2AR occluded the stimulation by the β_1AR.

Inhibition of isoproterenol stimulated AC activity by the β_2AR-selective blocker, ICI 118551, gave a different picture: the inhibition curve was biphasic, allowing clear distinction of the β_2AR and β_1AR components, with respective IC_{50} values of 12 nM and 3 μM. This indicated that, in contrast to subtraction of β_1AR action, which had been without effect, subtraction of β_2AR action resulted in a partial loss of stimulation by saturating isoproterenol and that, therefore, the intrinsic ability of β_1AR to activate the G_s/AC system was less than that of β_2AR. An overnight treatment of Lβ1β2.1 cells with pertussis toxin (0.1 μg/ml) prior to harvest for membrane preparation, which caused ADP-ribosylation of >95% of the cell's pertussis toxin substrates, left unaffected the biphasic inhibition of isoproterenol-stimulated AC activity by increasing concentrations of ICI 118551 (data not shown). This indicated that the lower stimulation by β_1AR observed after blockade of β_2AR was not due to β_1AR-mediated inhibition of AC activation by G_i.

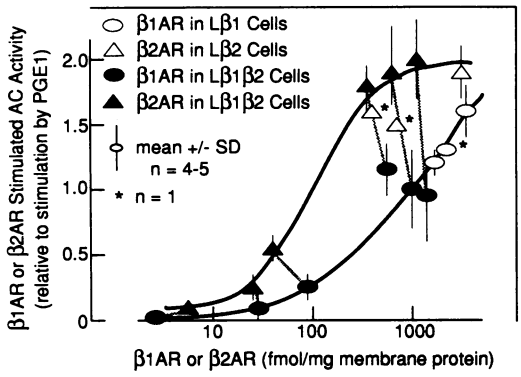

FIG. 2. Relationship between membrane receptor abundance and stimulation of the G_s/AC system for human β_1AR (ovals) and β_2AR (triangles). Data are derived from Tables 1 and 2. Open symbols, cells expressing only one receptor type; filled symbols, cells expressing both β_1AR and β_2AR. Gray lines connect data from the same cell line.

Table 2 presents results obtained with other Lβ1β2 cell lines, all expressing β_1AR and β_2AR at relative ratios ranging from 1:2 to 3:1, assessed by competitive inhibition of ^{125}I-CYP binding by CGP 20712A and ICI 118551, but differing significantly in the total number of βARs in the isolated membranes, ranging from 2300 fmol/mg (Lβ1β2.8) to 8 fmol/mg (Lβ1β2.10). In all but one case, the β_1AR component of AC stimulation was clearly uncovered after blocking the β_2AR component with ICI 118551 and ranged between 30% and 50% of the stimulation obtained with both receptors. For the lowest total βAR abundance (8 fmol/mg), stimulation of AC by β_1AR was marginal, 6–7% of β_1AR plus β_2AR, even though β_1AR was 30% of the total. This reflects the effect of absolute βAR level on AC stimulation, which reached a maximum at around 2-fold that obtained with the resident PGE_1-responsive receptor and decreased as total receptor levels fell below 500 fmol/mg (Fig. 2). In all cases, β_1AR was less efficacious than β_2AR. Nearly identical results were obtained when ICI 118551 was used to block the stimulation of AC by 1 or 10 μM isoproterenol. Both these concentrations of isoproterenol were supramaximal for AC stimulation in all membranes except those from Lβ1β2.10 cells, in which, presumably due to the very low receptor density, the dose–response curve for isoproterenol was right-shifted so that it required close to 10 μM to reach a maximum. EC_{50} values for isoproterenol-mediated stimulation in cells expressing transfected βARs have been shown to depend on receptor levels (20).

The β_1AR vs. β_2AR selectivities of CGP 20712A and ICI 118551 were determined from competitive shifts of dose–response curves for isoproterenol-mediated stimulation of the AC of Lβ1 and Lβ2 cells. Linear Schild plots with slopes of 0.9–1.0 were obtained (data not shown). The pK_B values derived for inhibition of isoproterenol action at β_1AR and β_2AR were 8.7 and 5.8, respectively, for CGP 20712A, and 7.2 and 9.5, respectively, for ICI 118551. Thus CGP 20712A has a β_1/β_2 selectivity ratio of 794:1 [antilog(8.7 − 5.8)] and ICI 118551 has a β_2/β_1 selectivity of 199 [antilog(9.5 − 7.2)]. Using the IC_{50} values obtained for competitive inhibition of ^{125}I-CYP binding and correcting for the 2-fold difference in affinity of the probe for β_2AR vs. β_1AR (Table 1), we calculated the β_1/β_2 selectivity of CGP 20712A to be 845:1 and the β_2/β_1 selectivity of ICI 118551 to be 228. These values are in excellent agreement with the values obtained from inhibition of stimulation of AC. Thus the method of assay does not affect the subtype selectivity of the blockers.

DISCUSSION

β_1AR and β_2AR are coexpressed and function in the human myocardium (21–25). The density of β_1AR is usually higher than that of β_2AR. Studies from several laboratories suggest that β_1AR may be "weaker" than β_2AR or even be ineffective in activating the G_s/AC system. For example, Waelbroeck *et al.* (26) found virtually no stimulation of AC through β_1AR but only through β_2AR. An analytical study by Kaumann and Lemoine (19) on isolated ventricular tissues from patients with mitral valve lesion showed an asymmetry between a lower abundance of β_2AR relative to β_1AR and a greater stimulation of AC by β_2AR than by β_1AR in cardiac membrane particles. Thus, while about 70% of the total βAR population was β_1AR, competitive blockade of both epinephrine- and norepinephrine-induced stimulation of AC showed a β_1AR component (stimulation resistant to β_2AR blocker) of 25–36%, but a β_2AR component (stimulation resistant to β_1AR blocker) of 63–89% (19). Relating β_1AR and β_2AR densities to the corresponding β_1AR and β_2AR stimulation of AC yielded a ratio of $\geq$6:1 for activation of AC by β_2AR relative to β_1AR (19, 25). Golf and Hansson (33) and Bristow *et al.* (27) extended these findings to membranes of nonfailing human heart, by also reporting more β_1AR than β_2AR in membranes but a much greater stimulation of AC through β_2AR than β_1AR. There are, however, at least two problems with a definitive interpretation of experiments of this sort: (*i*) data from human heart AC show a large patient-to-patient variability (24, 28, 29), suggesting that a factor(s) other than an intrinsic property of the βARs themselves contribute to the estimation of the efficiency with which they transduce a signal, and (*ii*) it is not possible to know the cellular origin of the membranes whose βARs are assessed biochemically by ligand binding or AC activation. Although it is assumed that most membranes are from myocardial cells, it cannot be ruled out that a significant contribution of βARs comes from other cells (endothelial, neuronal, etc.) in the particular tissue fragments used for the studies.

Green *et al.* (30) eluded the uncertainty about the cellular origin of membrane used by evaluating the properties of the cloned human β_1AR and β_2AR in two clonal CHW-1102 fibroblast lines, one expressing the β_1AR and the other expressing the β_2AR at nearly identical levels. Like receptor levels, isoproterenol-stimulated AC activities were very similar for the two cell lines. The interaction of these receptors with isoproterenol was studied in terms of binding in the absence and presence of GTP and in terms of the EC_{50} with which isoproterenol stimulated AC activity. K_i values, assessed by displacement of ^{125}I-CYP in the presence of GTP, and low-affinity K_L constants, computed from the biphasic displacement of ^{125}I-CYP by isoproterenol in the absence of GTP, differed <2-fold in comparisons of β_1AR with β_2AR or K_i with K_L. On the other hand, the high-affinity K_H constants, computed from the biphasic displacement curves obtained in the absence of GTP, differed depending on the comparison made: K_H for β_1AR was 5-fold that for β_2AR, and the K_H/K_L ratio for β_1AR was 12 whereas that for β_2AR was 140. The higher K_H/K_L ratio was positively correlated to a 4-fold lower EC_{50} for AC stimulation in β_2AR cells than β_1AR cells. Since K_H values are determined by the association of the receptors with the G_s protein of the cell (31), these experiments indicate that subtype-specific factors other than the inherent affinity for the ligand (assessed as K_i or K_L) influence the efficiency with which the signal is transduced. The results of Green *et al.* (30) agree with the general concept that βAR coupling is subtype-selective. They did not, however, show a difference between the two receptor subtypes other than the affinity of the agonist for what is thought to be the G_s/βAR complex and provided no clues as to possible differences in the coupling efficiency suggested by the studies with human myocardium.

Our studies show that the extent to which a receptor may activate an AC system (i.e., its efficacy) may depend on the nature of the receptor. Unlike Green *et al.* (30), by coexpressing β_1AR and β_2AR we were able to compare the ability

of the two receptor subtypes to stimulate AC in an identical biochemical background. As is shown in Fig. 2 and Table 2, in all of the cells tested, and over a total receptor abundance ranging from 8 to 2500 fmol/mg of protein, β_1AR stimulation of AC was always partial and nonadditive to that elicited by the β_2AR. Increases in β_2AR density resulted in increasing isoproterenol-stimulated AC activities (in comparison to stimulation by PGE_1), and this leveled off at about twice the stimulation elicited by PGE_1. Thus, the response system saturates and further increases in receptor density no longer lead to further increases in activity. The same applies to β_1AR, with the difference that at matching receptor levels in the membranes the ability of the β_1AR to stimulate the G_s/AC system is less than that of the β_2AR. Although at high levels of β_1AR expression, AC stimulation approached that of saturating levels of β_2AR, it is not clear from our studies whether β_1AR will ever be able to activate as many G_s molecules per unit time as β_2AR.

Our experiments may be relevant to the function of β_1AR and β_2AR in human heart. Working with single isolated human ventricular myocytes, Del Monte *et al.* (32) have recently confirmed *in situ* both the coexistence of β_1AR and β_2AR in a single cell and that the contribution of the β_2AR predominates over that of the more abundant β_1AR. We conclude that β_2AR stimulates AC with greater efficacy than β_1AR and that efficacy is an inherent property of G-protein-coupled receptors.

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1. Birnbaumer, L. & Rodbell, M. (1969) *J. Biol. Chem.* **244,** 3477–3482.
2. Rodbell, M., Birnbaumer, L. & Pohl, S. L. (1970) *J. Biol. Chem.* **245,** 718–722.
3. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
4. Liao, C.-F., Themmen, A. P. N., Joho, R., Barberis, C., Birnbaumer, M. & Birnbaumer, L. (1989) *J. Biol. Chem.* **264,** 7328–7337.
5. Levy, F. O., Gudermann, T., Perez-Reyes, E., Birnbaumer, M., Kaumann, A. J. & Birnbaumer, L. (1992) *J. Biol. Chem.* **267,** 7553–7562.
6. Gudermann, T., Birnbaumer, M. & Birnbaumer, L. (1992) *J. Biol. Chem.* **267,** 4479–4488.
7. Sanford, J., Codina, J. & Birnbaumer, L. (1991) *J. Biol. Chem.* **266,** 9570–9579.
8. Gudermann, T., Nichols, C., Levy, F. O., Birnbaumer, M. & Birnbaumer, L. (1992) *Mol. Endocrinol.* **6,** 272–278.
9. Nukada, T., Mishina, M. & Numa, S. (1987) *FEBS Lett.* **211,** 5–9.
10. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
11. Frielle, T., Collins, S., Daniel, K. W., Caron, M. G., Lefkowitz, R. J. & Kobilka, B. K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7920–7924.
12. Emorine, L. J., Marullo, S., Delavier-Klutchko, C., Kaveri, S. V., Durieu-Trautmann, O. & Strosberg, A. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6995–6999.
13. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52,** 456–467.
14. Birnbaumer, M., Hinrichs, V. & Themen, A. P. N. (1990) *Mol. Endocrinol.* **4,** 245–254.
15. Birnbaumer, L., Yang, P. C., Hunzicker-Dunn, M., Bockaert, J. & Duran, J. M. (1976) *Endocrinology* **99,** 163–184.
16. Salomon, Y., Londos, C. & Rodbell, M. (1974) *Anal. Biochem.* **58,** 541–548.
17. Bockaert, J., Hunzicker-Dunn, M. & Birnbaumer, L. (1976) *J. Biol. Chem.* **251,** 2653–2663.
18. Abramowitz, J., Iyengar, R. & Birnbaumer, L. (1982) *Endocrinology* **110,** 336–346.
19. Kaumann, A. J. & Lemoine, H. (1987) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **335,** 403–411.
20. George, S. T., Berrios, M., Hadcock, J. R., Wang, H.-y. & Malbon, C. C. (1988) *Biochem. Biophys. Res. Commun.* **150,** 665–672.
21. Brodde, O. E., Karad, K., Zerkowski, H. R., Rohm, N. & Reidemeister, J. C. (1983) *Circ. Res.* **53,** 752–758.
22. Heitz, A., Schwartz, J. & Velly, J. (1983) *Br. J. Pharmacol.* **80,** 711–717.
23. Stiles, G. L., Taylor, S. A. & Lefkowitz, R. J. (1983) *Life Sci.* **33,** 467–473.
24. Gille, E., Lemoine, H., Ehle, B. & Kaumann, A. J. (1985) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **331,** 60–70.
25. Kaumann, A. J. (1987) *Biomed. Biochim. Acta* **46,** S411–S416.
26. Waelbroeck, M., Taton, G., Delhaye, M., Chatelain, P., Camus, J. C., Pochet, R., Leclerc, J. L., De Smet, J. M., Robberecht, P. & Christophe, J. (1983) *Mol. Pharmacol.* **24,** 174–182.
27. Bristow, M. R., Hershberger, R. E., Port, J. D., Minobe, W. & Rasmussen, R. (1988) *Mol. Pharmacol.* **35,** 295–303.
28. Lemoine, H., Schönell, H. & Kaumann, A. J. (1988) *Br. J. Pharmacol.* **95,** 55–66.
29. Kaumann, A. J., Hall, J. A., Murray, K. J., Wells, F. C. & Brown, M. J. (1989) *Eur. Heart J.* **10,** Suppl. B, 29–37.
30. Green, S. A., Holt, B. D. & Ligget, S. B. (1992) *Mol. Pharmacol.* **41,** 889–893.
31. Cerione, R. A., Codina, J., Benovic, J. L., Lefkowitz, R. J., Birnbaumer, L. & Caron, M. G. (1984) *Biochemistry* **23,** 4519–4525.
32. Del Monte, F., Kaumann, A. J., Poole-Wilson, P. A., Wynne, D. G., Pepper, J. & Harding, S. E. (1993) *Circulation* **88,** 854–863.
33. Golf, S. & Hansson, V. (1986) *Cardiovasc. Res.* **20,** 637–644.