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β_1- and β_2-Adrenergic Receptor-Mediated Adenylate Cyclase Stimulation in Nonfailing and Failing Human Ventricular Myocardium

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SUMMARY

Prenalterol (β_1-agonist), denopamine (β_1-agonist), and zinterol (β_2-agonist) were partial agonists of adenylate cyclase (AC) stimulation in human ventricular myocardium obtained from nonfailing chambers whose β_1/β_2 receptor subtype ratio was approximately 80/20. At a concentration less than its low affinity (β_2) K_i, betaxolol, a highly selective β_1-antagonist, inhibited isoproterenol (nonselective agonist), denopamine, and prenalterol stimulation of AC, indicating that isoproterenol, denopamine, and prenalterol are all capable of stimulating AC through β_1-receptor activation. At a concentration less than its low affinity (β_1) K_i, ICI 118,551, a highly selective β_2-agonist, inhibited both isoproterenol and zinterol stimulation of AC, indicating that isoproterenol and zinterol stimulate AC through β_2-receptors. Zinterol stimulation of AC was mediated entirely by β_2-receptors, inasmuch as 10^{-7} M betaxolol had no effect on the zinterol dose-response curve and ICI 118,551 produced a degree of blockade ($K_B = 5.2 \pm 1.6 \times 10^{-9}$ M), consistent with the β_2-receptor K_i of the latter ($2.0 \pm .4 \times 10^{-9}$ M, p, not significant). In nonfailing myocardium, analysis of β_1 versus β_2 stimulation by the nonselective agonist isoproterenol revealed that the numerically small (19% of the total) β_2 fraction accounted for the majority of the total adenylate cyclase stimulation. In failing ventricular chambers with a β_1/β_2 receptor subtype ratio reduced from 82/19 (nonfailing) to 64/36 ($p < 0.001$) and a β_1-receptor density reduced by 61% ($p < 0.001$), maximal denopamine stimulation was reduced by 49% ($p < 0.001$). Moreover, in preparations from failing heart, the component of denopamine stimulation that was inhibited by 10^{-7} M betaxolol (β_1 component) was reduced by 77% ($p < 0.05$). Finally, in preparations derived from failing ventricular myocardium, β_2-receptor density was not significantly decreased, but zinterol stimulation of AC was reduced by 32% ($p < 0.05$). We conclude that heart failure results in subsensitivity to both selective β_1 and β_2 stimulation of adenylate cyclase, with β_1 subsensitivity due to selective β_1 receptor down-regulation and β_2 subsensitivity due to partial uncoupling of β_2 receptors from subsequent events in the β_2-adrenergic pathway.

β-Adrenergic receptors are coupled to adenylate cyclase in mammalian myocardial cell surface membranes (1). The β-receptor subtype that mediates adenylate cyclase stimulation in the ventricular myocardium of mammalian laboratory animals is the β_1-receptor (2), which is thought to be linked to muscle contraction through cAMP-mediated activation of protein kinase A and subsequent phosphorylation reactions that lead to an increase in calcium influx (1).

Human myocardium, in contrast to the hearts of small laboratory animals, contains a relatively high proportion of β_2-adrenergic receptors (3–7). Estimation of the β_2/total receptor ratio has ranged from 50% in right atrial tissue (5) to 14% in nonfailing human left ventricle (3). Moreover, in human heart β_2-receptors appear to be coupled to adenylate cyclase stimulation (8–11) and muscle contraction (6, 7).

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The β_2-adrenergic receptor was the first subtype to be shown to be coupled to adenylate cyclase in human myocardium (8). In that initial study, no such coupling could be demonstrated for β_1-receptors, and the authors speculated that in the human heart β_1-receptors were linked to muscle contraction by a non-cAMP mechanism (8). However, more recent reports in human tissue indicate that both β_1- and β_2-receptors are coupled to adenylate cyclase in atrial (9) and ventricular myocardium (10, 11), with ventricular β_1-receptors apparently less efficiency coupled (10, 11). Therefore, there are conflicting reports on the coupling of β_1- versus β_2-receptors to adenylate cyclase in human heart.

We have recently reported that β_1-receptors selectively down-regulate in heart failure and that this selective down-regulation is transmitted to a loss of β_1-mediated inotropic stimulation (7). Because the majority of tissue samples used in previous studies of β-receptor coupling to adenylate cyclase were taken

ABBREVIATIONS: NS, not significant; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; Gpp(NH)p, guanosine 5'-(β, γ-imido)triphosphate; ICYP, [^{125}I]iodocyanopindolol.

from subjects with cardiac dysfunction (8–10), the apparent "inefficiency" of coupling of β_1-receptors to adenylate cyclase could have been due to selective subsensitivity of the β_1 pathway related to β_1-receptor loss.

In the current investigation, we examine the behavior of adenylate cyclase stimulation in response to β_1-, β_2-and nonselective β-agonist stimulation in preparations derived from nonfailing and failing human ventricular myocardium and compare these data with radioligand measurements of β_1- and β_2-receptor density. The results indicate that in nonfailing ventricular myocardium β_1-receptors are indeed much less efficiently coupled to adenylate cyclase than are β_2-receptors. Moreover, heart failure causes a loss in both β_1- and β_2-mediated adenylate cyclase responsiveness, via β_1-receptor downregulation and β_2-receptor uncoupling.

Materials and Methods

Tissue procurement. Nonfailing human ventricular myocardium was obtained from the left ventricles of 20 brain-dead kidney organ donors, age 8–58 years, for whom no suitable cardiac recipient was available. These donors had been maintained on respirators for 1–3 days and they had not been given β-adrenergic agonists for inotropic support. None of these subjects had any history of heart disease, and all had normal left ventricular function by echocardiography, performed as part of the organ donation screening process (12). Additional nonfailing left ventricles were taken from seven heart-lung transplant recipients with isolated right ventricular failure due to primary pulmonary hypertension, age 23–40. The mean age ± standard error of the 27 subjects from whom a nonfailing left ventricle was obtained was 29 ± 2, with 54% being male. For donors, written consent was obtained from a family member for organ donation for research purposes, whereas, for heart-lung recipients, informed written consent was given by the patient.

Failing human ventricular myocardial tissue was obtained at the time of cardiac transplantation from the left ventricles of 28 subjects with end-stage biventricular failure from idiopathic dilated cardiomyopathy. All had left ventricular ejection fractions of <0.20 (average, 16 ± 0.02) and no subject had received a β-agonist within 5 days of undergoing transplantation. The mean age of these 26 subjects was 35.4 ± 2.9 years (p, NS versus nonfailing controls) and 86% were male ($p < 0.05$ versus nonfailing controls). Both nonfailing and failing hearts were rapidly removed, placed in ice-cold oxygenated Tyrode's solution, and transported to the laboratory as previously described.

Adenylate cyclase. Adenylate cyclase activity was assayed in crude human ventricular myocardial membranes by previously described techniques (13, 14). Briefly, a 2-g aliquot of left or right ventricular myocardium was removed from the explanted heart of cardiac transplant recipients or prospective donors, placed in ice-cold oxygenated Tyrode's solution, weighed, and homogenized in ice-cold 250 mM sucrose, 5 mM Tris, 1 mM EGTA buffer, pH 7.5. Washed membranes were frozen in this same buffer and stored at −70° for periods of 2 days to 4 weeks until use.

Two adenylate cyclase assay conditions were employed. The standard condition consisted of 75–250 μg of membrane protein in 100 mM Tris buffer, pH 7.30 at 30°, containing 0.1 mM Mg ATP, 0.5 mM $MgCl_2$, 10 μM GTP, 1 mM cAMP, [^{3}H]cAMP, 10 mM phosphocreatine, and 14.5 μg of creatine kinase. For experiments in which effects of β-adrenergic antagonists were assessed or required, the assay mixture was preincubated with betaxolol (β_1-antagonist) or ICI 118,551 (β_2-antagonist) for 15 min at 30°. For experiments in which the activity of selective β_1 agonists was assessed, the assay conditions were identical to the above with the exceptions of 10^{-5} M Gpp(NH)p substituting for GTP and the addition of 10^{-6} M forskolin and 10^{-7} M ICI 118, 551 (β_1 assay condition).

After a 5-min warm-up period, measurement of adenylate cyclase activity was begun by adding 1.0–2.5 μCi of [α-^{32}P]ATP (New England Nuclear, Boston, MA). After incubation at 30° for 20 min, the reaction was stopped by the addition of 1% sodium dodecyl sulfate. Formed [^{32}P]cAMP was then isolated by column chromatography. The methods yielded reagent blanks that were <10% of basal activity in all cases, and recovery of [^{3}H]cAMP was 60–90%.

Adenylate cyclase dose-response curve ED_{50}, maximum, and minimum were determined by computer modeling of a four-parameter logistic equation (7). Dissociation constants (K_B values) for compounds inhibiting cyclase dose-response curves were determined from the equation (15):

$$K_B = \frac{\text{antagonist}}{(\text{dose ratio} - 1)}$$

Creatine kinase. Creatine kinase activity was measured by a spectrophotometric technique as previously described (13), with activity expressed as IU/g wet weight.

β-Receptor radiolabeling. β-Adrenergic receptor density was assessed by ICYP binding, as previously described (7, 14). Crude membranes were prepared from a 5-g aliquot of left ventricular free wall by contractile protein extraction and washing of a 50,000 × g pellet, as previously described (7, 13, 14). The binding parameters B_{max} and K_D were determined by nonlinear least squares methodology, as previously described (16). Additionally, the proportion of β_1- versus β_2-receptors was assessed by betaxolol- (nonfailing and failing hearts) and ICI 118,551- (nonfailing hearts) ICYP (Amersham, Arlington Heights, IL) competition curves, with the proportion of β_1- and β_2-receptors and their K_I values determined by computer modeling using the MLAB program (7). β_1- and β_2-receptor densities were determined by multiplying their respective fractions times the total β-receptor density measured by ICYP saturation curves. In competition curve measurements, the concentration of ICYP was 50 pM for ICYP K_D values <20 pM and 100 pM if the ICYP K_D from saturation curves was >20 pM; the receptor concentration was 2–5 pM. The average ICYP concentration in competition curve experiments was 6.0 ± 0.5 times the ICYP K_D. Additional equilibrium assay conditions for ICYP binding were as described previously (7, 14).

Statistical analysis. The method of analyzing radioligand-unlabeled ligand competition curves has been previously described (7), as has the analysis of radioligand saturation curves (7). Differences between the groups were assessed by Student's t test, with a $p < 0.05$ in a two-tailed distribution being statistically significant.

Source of compounds and regents. ICYP was purchased from Amersham. [^{32}P]ATP was purchased from New England Nuclear (Boston, MA). Betaxolol was a gift from Synthelabs (L.E.R.S.), Paris, France; ICI 118,551 was provided by ICI (Cheshire, England); propranolol was a gift from Ayerst Laboratories (New York, NY). Zinterol was obtained from Bristol Myers (Evansville, IN); denopamine (TA 064) was a gift from Marion Laboratories (Kansas City, MO), and prenalterol was obtained from AB Hassle (Molndal, Sweden). All chemicals were purchased from standard commercial suppliers.

Results

Characterization of β-adrenergic receptors in nonfailing and failing ventricles. In Table 1 are given β-receptor binding and creatine kinase data for the 27 nonfailing left ventricles. These ventricles yielded an ICYP B_{max} (total β-adrenergic receptor density) of 86.1 fmol/mg, with a K_D of 11.6 pM. Fig. 1A is a representative experiment of the β_1-selective antagonist betaxolol (7, 17) and the β_2-selective antagonist ICI 118,551 (18) inhibiting ICYP binding; both selective ligands resulted in Hill slope values consistently <1.0. Mean values ± standard errors of computer modeling of curves from all 27 preparations are shown in Table 1. With betaxolol, 23 of 27 preparations gave a best fit when modeled for two sites, with

TABLE 1

β-Receptor binding and creatine kinase data in 27 nonfailing and 28 failing left ventricles

Values are mean ± standard error.

Group	ICYP B_{max}	ICYP K_D	ICYP-Unlabeled ligand competition curves								Creatine Kinase
			Betaxolol				ICI 118,551				
			K_H (β_1)	K_L (β_2)	β_1	β_2	K_H (β_2)	K_L (β_1)	β_1	β_2	
	fmol/mg	pM	nM	nM	%		nM	nM	%		IU/g
Nonfailing	86.1 ± 3.9	11.6 ± 1.4	6.88 ± 0.90	258 ± 58	81.4 ± 1.9	18.4 ± 2.0	1.78 ± .30	247 ± 32	83.4 ± 2.2	14.7 ± 2.0	1098 ± 69
Failing	47.2 ± 2.5	11.7 ± 1.7	5.49 ± 0.80	307 ± 61	63.9 ± 2.9	35.6 ± 2.1					947 ± 48
p Value	<0.001	NS	NS	NS	<0.001	<0.001					NS

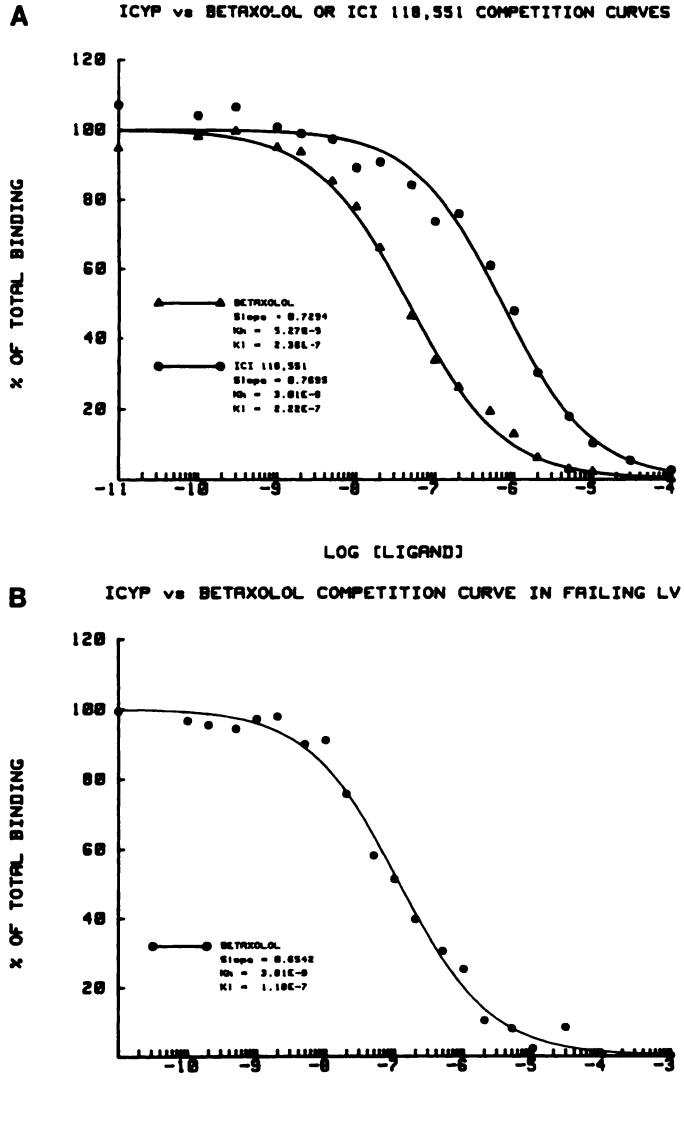

Fig. 1. A, Representative experiment, betaxolol (△) and ICI 118,551 (●) competition for ICYP binding in membrane preparations derived from a nonfailing left ventricle. Betaxolol gave significant fits for a high (K_H) and a low (K_L) affinity site with respective proportions of 78.8 and 22.4%. ICI 118,551 gave significant fits for a high and a low affinity site with respective proportions of 19.8 and 80.5%. ICYP concentration was 50 pM. B, Representative ICYP-betaxolol competition curve in membranes derived from a failing left ventricle. The best fit was for two sites with respective K_H and K_L percentages of 63.1 and 36.1%. ICYP concentration was 50 pM.

the remaining four preparations modeling for a one-site fit. With ICI 118,551, 25 of 27 preparations modeled best as two sites, with two preparation modeling as a one-site fit. Betaxolol was 38-fold selective (K_L + K_H) for β_1-receptors, whereas ICI 118,551 was 139-fold selective for β_2-receptors.

The effect of heart failure on total β, β_1-, and β_2-adrenergic receptor density in preparations derived from 28 failing left ventricles removed from subjects with idiopathic dilated cardiomyopathy and end-stage heart failure is show in Table 1. Total β-receptor density was decreased by 45% ($p < 0.001$), whereas β_1-receptor density calculated from the data shown in Table 1 was decreased by 56.3% (69.8 ± 3.4 fmol/mg in nonfailing versus 30.5 ± 2.2 in failing heart, $p < 0.001$). On the other hand, β_2-receptor density was not changed in failing heart (16.5 ± 1.2 fmol/mg versus 16.2 ± 2.0 in nonfailing heart; p, NS). The dissociation constant for ICYP was not affected by heart failure nor were the high and low affinity dissociation constants of betaxolol; betaxolol was 53-fold selective for β_1-receptors. Creatine kinase activity did not differ significantly in the two groups.

A representative betaxolol-ICYP competition curve performed in a preparation derived from a failing ventricle is shown in Fig. 1B. In the membrane preparations derived from failing heart, the curve slopes were more shallow (0.655 ± 0.022 versus 0.743 ± 0.018, $p < 0.01$), the β_2 proportion was higher (Table 1), and the β_1 proportion was lower (Table 1) than these respective parameters obtained in nonfailing heart.

Stimulation of adenylate cyclase by nonselective or selective β-agonists in preparations derived from nonfailing myocardium. Fig. 2 gives the stimulation of adenylate cyclase by isoproterenol (nonselective β-agonist), zinterol [selective β_2-agonist (19)], and prenalterol [selective β_1-agonist (20)] in preparations derived from nonfailing myocardium. As can be seen in Fig. 2, zinterol is a "partial agonist" for adenylate cyclase stimulation, inasmuch as it produced a stimulation that was 33 ± 1.9% of that obtained by isoproterenol in the 14 nonfailing preparations examined. The selective β_1-antagonist betaxolol at 10^{-7} M did not block the effects of zinterol (Fig. 3) but did antagonize slightly the effects of isoproterenol (Fig. 4). In contrast, the effects of zinterol were fully antagonized by the selective β_2-antagonist ICI 118,551 (Fig. 3); the K_B for zinterol-ICI-118,551 was similar to and not statistically different from the high affinity K_I (K_H) for ICI 118,551 derived from ICYP-ICI 118,551 competition curves (Table 2). The average K_B for the inhibition of isoproterenol by betaxolol was 6.4 ± 0.9 × 10^{-8} M (Table 2), which is significantly less than the low affinity K_I (1.7 ± 0.3 × 10^{-7} M; $p < 0.05$) and significantly greater than the high affinity K_I (8.1 ± 2.0 × 10^{-9} M, $p < 0.05$)

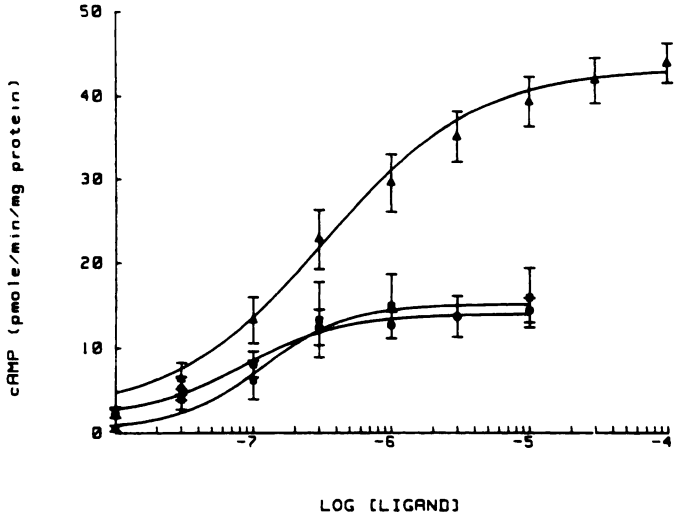

Fig. 2. Isoproterenol (▲) (n = 10), zinterol (●) (n = 10), and prenalterol (■) (n = 5) stimulation of adenylate cyclase, mean ± standard error. Prenalterol was assayed under conditions different from zinterol and isoproterenol (see Materials and Methods).

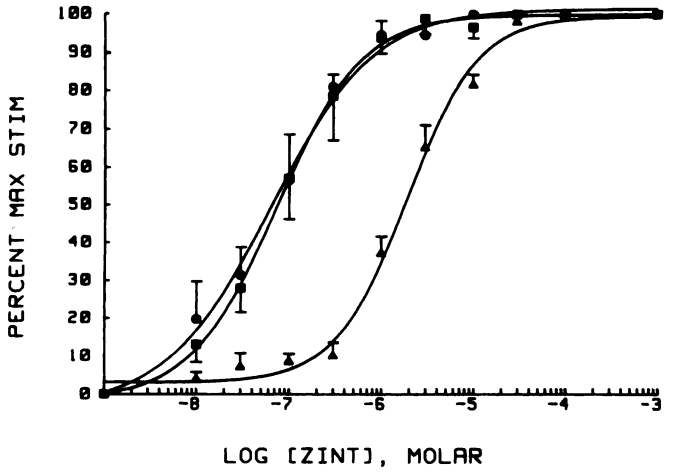

Fig. 3. Effect of 10^{-7} M betaxolol (■) or 10^{-7} M ICI 118,551 (▲) on the zinterol (*ZINT*, ●) dose response for adenylate cyclase stimulation, percent of maximum response ± standard error (preparations from five nonfailing human left ventricles).

obtained from computer modeling of ICYP-betaxolol competition curves.

In Fig. 2 it is shown that the selective β_1-agonist prenalterol (20) stimulates adenylate cyclase activity, whereas in Figs. 4 and 5A it can be seen that, compared with its antagonism of isoproterenol, betaxolol produces a greater degree of antagonism of prenalterol. Prenalterol produced a maximum net stimulation that was 13.5 ± 2.7% of the maximum effect of isoproterenol under the same assay conditions (in Fig. 2 the isoproterenol dose-response data were obtained under assay conditions different from the prenalterol data). The K_B obtained for betaxolol blockade of prenalterol was 2.1 ± 0.6 × 10^{-8} M, which is significantly lower than the K_B obtained for betaxolol-isoproterenol but significantly greater than the high affinity betaxolol K_H from radioligand binding data.

In order to achieve consistent adenylate cyclase stimulation, as shown in Figs. 2 and 5A, it was necessary to modify the assay conditions to maximize the effects of the weak β_1 partial agonist prenalterol (β_1 assay conditions). Prenalterol did not produce measurable stimulation under the assay conditions employed for isoproterenol and zinterol but did produce consistent stimulation when assayed in the presence of 10^{-6} M forskolin and the nonhydrolyzable guanine nucleotide GppNHp, with 10^{-7} M ICI 118,551 in the assay medium to block β_2 stimulation.

Similar results were achieved with the selective partial β_1-agonist denopamine (7) (Fig. 5B), when, in four experiments, the denopamine response was blocked by betaxolol (mean K_B = 2.5 ± 1.4 × 10^{-8} M) to a degree similar to blockade of prenalterol. However, with the β_1 assay condition denopamine produced a greater degree of stimulation in preparations derived from nonfailing myocardium than did prenalterol (denopamine, 1.42 ± 0.04; prenalterol, 1.17 ± 0.04 × basal activity; $p < 0.05$).

The selective β_2-antagonist ICI 118,551 shifted zinterol dose-response curves to the right in a parallel fashion, as shown in Fig. 3. In contrast, the effect of ICI 118,551 on isoproterenol stimulation of adenylate cyclase was to produce a nonparallel shift caused by a marked decrease in slope (Fig. 4). As a result, the isoproterenol ED_{50} as an index of overall degree of curve shift approaches what would be predicted by the ICI 118,551 β_2 K_I (Table 2). However, at lower concentrations the degree of curve shift is less than would be expected if isoproterenol were acting solely on β_2-receptors.

Additional experiments under standard assay conditions indicated that dobutamine and norepinephrine, each of which has been reported to have selective β_1 properties in cardiac tissue (9, 21), produced adenylate cyclase stimulation by a predominately β_2 mechanism, with only slight or no shifts in the dose-response curve by a β_1-blocking dose (10^{-7} M) of betaxolol. The K_B for norepinephrine-betaxolol (six experi-

TABLE 2

Comparison of betaxolol and ICI 118,551 K_B values (from antagonism of isoproterenol, prenalterol, or zinterol stimulation of adenylate cyclase) with high affinity K_I(K_H) values (from ICYP competition curves)

Data are mean ± standard error.

Agonist	Cyclase stimulation		ICYP competition curves (K_H and K_L)	
	Antagonist (n)	K_B	K_H	K_L
		nM		
Isoproterenol	Betaxolol, 10^{-7} M (n = 8)	64.3 ± 8.9[a,b,c]	8.3 ± 2.3	179 ± 41
Prenalterol	Betaxolol, 10^{-7} M (n = 5)	21.4 ± 5.7[b,d]	4.2 ± 0.9	144 ± 13
Isoproterenol	ICI 118,551, 10^{-7} M (n = 8)	7.5 ± 2.2[b]	2.1 ± 0.5	275 ± 57
Zinterol	ICI 118,551, 10^{-7} M (n = 8)	4.8 ± 1.4[b]	2.1 ± 0.5	275 ± 57

[a] $p < 0.001$ versus K_H for same antagonist in same preparations.
[b] $p < 0.05$ versus K_L for same antagonist in same preparations.
[c] $p < 0.05$ versus prenalterol-betaxolol K_B.
[d] $p < 0.05$ versus K_H for same antagonist in same preparations.

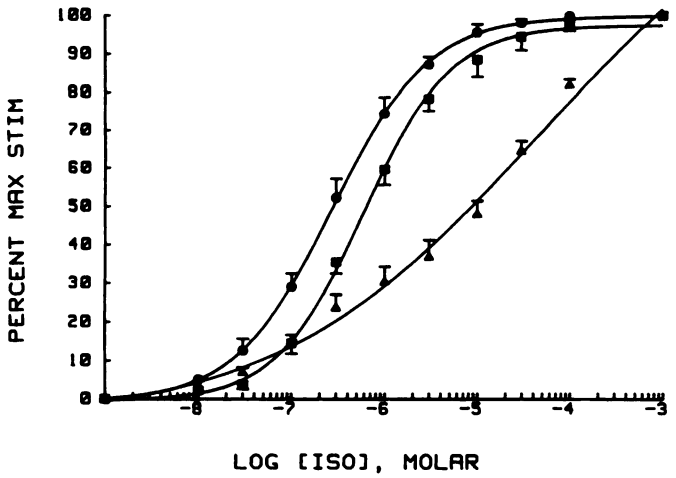

Fig. 4. Effect of 10^{-7} M betaxolol (■) or 10^{-7} M ICI 118,551 (▲) on the isoproterenol (*ISO*, ●) dose response for adenylate cyclase stimulation, percent of maximum response ± standard error (preparations from nine nonfailing human left ventricles).

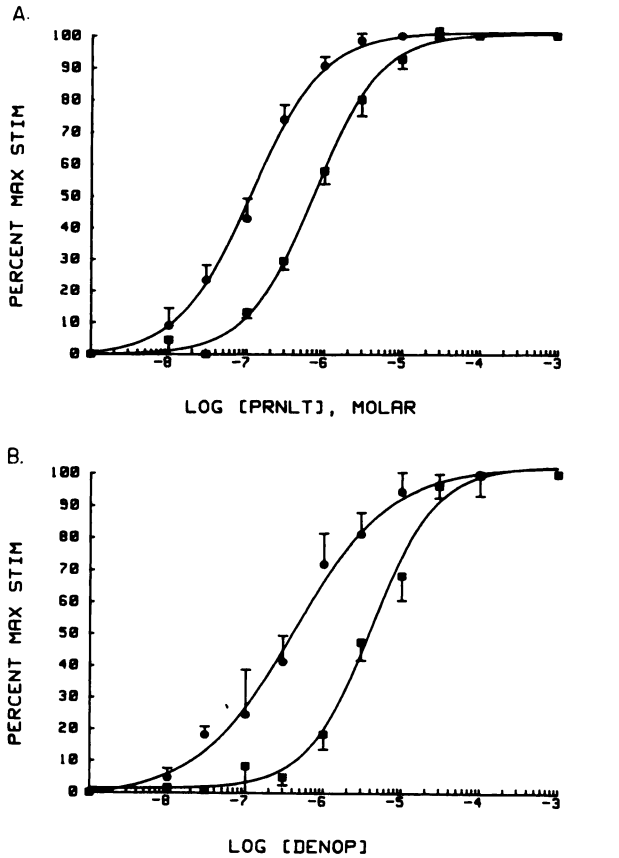

Fig. 5. A, effect of 10^{-7} M betaxolol (■) on the prenalterol (*PRNLT*, ●) dose response for adenylate cyclase stimulation, percent of maximum response ± standard error (preparations from five nonfailing human left ventricles). ED_{50} of the prenalterol control curve is 1.1×10^{-7} M. B, Effect of 10^{-7} M betaxolol (■) on the denopamine (*DENOP*, ●) dose response for adenylate cyclase stimulation, percent of maximum response ± standard error (preparations from four nonfailing human left ventricles). ED_{50} of the denopamine control curve is 4.1×10^{-7} M.

ments) was $7.7 \pm 1.9 \times 10^{-8}$ M (p, NS versus isoproterenol-betaxolol) and in three experiments betaxolol did not significantly shift the dobutamine dose-response curve.

Based on a betaxolol K_H value of 6.88 nM (Table 1), 10^{-7} M of the β_1-selective compound would occupy >90% of the β_1-receptor sites. Because the zinterol dose-response curve was not shifted by 10^{-7} M betaxolol, it can be concluded that zinterol is acting purely through β_2-receptors and that the K_B calculated for ICI 118,551 and zinterol is the β_2 K_B for ICI 118,551 (Table 2). Because the ICI 118,551 K_B calculated from antagonism of isoproterenol curves approached the value obtained for zinterol-ICI 118,551 (respective K_B values of 7.5 versus 4.8 nM; Table 2), it can be determined that the minority β_2 fraction was producing the majority of the adenylate cyclase stimulation in response to the nonselective β-agonist isoproterenol in particulate fractions derived from nonfailing ventricular myocardium. In the eight preparations in which ICI 118,551 antagonism of isoproterenol was conducted, the relative β_1/β_2 fraction averaged 80/19, based on mean values of ICYP-betaxolol and ICYP-ICI 118,551 competition curves.

Effect of heart failure on selective and nonselective β-agonist stimulation of adenylate cyclase. Based on the data presented in above, assay conditions can be employed whereby selective stimulation of adenylate cyclase by either β_1- or β_2-receptor activation can be accentuated and measured. For β_1-receptor stimulation, the best assay condition is with denopamine as the agonist in the presence of forskolin and GppNHp to enhance agonist efficacy and ICI 118,551 to selectively block β_2 responses. Under these β_1 assay conditions, maximal stimulation by 10^{-5} M denopamine is approximately 1.4–1.5 times greater than basal activity, and the K_B for betaxolol blockade approached (3 times higher) the high affinity β_1 K_H for betaxolol, measured by radioligand binding. Shown in Table 3 are values for denopamine net stimulation (denopamine stimulation minus basal activity) of adenylate cyclase under β_1 assay conditions in 18 preparations derived from nonfailing and 17 preparations derived from failing left ventricular myocardium. As can be seen in Table 3, in preparations derived from failing heart, denopamine produced only 51% of the stimulation produced by denopamine in nonfailing heart. That is, there was a 49% reduction in denopamine-stimulated adenylate cyclase activity in failing preparations, which had a reduction in β_1-receptor density of 61%.

Even with the β_1 assay condition, the selective β_1-agonist denopamine produces some β_2 stimulation, as shown by the denopamine-betaxolol K_B being 3 times higher than the betaxolol K_H. In order to measure selective β_1 stimulation by denopamine, it is necessary to determine what component of stimulation is blockable by a selective β_1-blocking dose of betaxolol, as shown in Table 3. In Table 3 it can be seen that, at a denopamine concentration of 10^{-6} M, which is approximately the EC_{50} (Fig. 5B), the component of denopamine stimulation of adenylate cyclase that is blocked by betaxolol is reduced from 26.5 pmol/min/mg cAMP in seven nonfailing preparations to 6.0 ± 3.1 pmol/min/mg cAMP in eight failing preparations ($p < 0.05$). That is, selective β_1 stimulation of adenylate cyclase was reduced by 77% in preparations derived from failing ventricles. In these same failing left ventricles, β_1-receptor density was reduced by 61%, from 82.1 ± 11.5 fmol/mg in nonfailing heart to 32.0 ± 5.5 in failing myocardium ($p < 0.001$).

Zinterol is a selective activator of β_2-receptor-mediated adenylate cyclase in both nonfailing and failing preparations under standard assay conditions employing GTP as the guanine nucleotide. Consequently, the maximal zinterol response in stand-

TABLE 3

β_1-Agonist (denopamine) stimulation of adenylate cyclase (AC) and β_1- and β_2-receptor density in preparations derived from nonfailing and failing human heart

Group	Receptor densities			AC stimulation by denopamine	
	β_1	β_2	β Total	Net, 10^{-5} M[a]	Net β_1, 10^{-5} M[b]
	fmol/mg			pmol of cAMP/min/mg	
Nonfailing (n = 18)	71.9 ± 4.8	16.8 ± 2.2	87.3 ± 5.1	53.5 ± 4.8	26.5 ± 6.8
Failing (n = 17)	28.4 ± 7.6[c]	15.7 ± 1.8	44.3 ± 2.5[c]	27.5 ± 4.5[d]	6.0 ± 3.1[d]

[a] Net stimulation, denopamine stimulation minus basal activity.
[b] Net stimulation by denopamine minus net denopamine stimulation in the presence of 10^{-7} M betaxolol, from seven nonfailing and eight failing ventricles.
[c] $p < 0.001$.
[d] $p < 0.05$.

ard curve conditions may be used as a probe for β_2-mediated cyclase stimulation. In Fig. 6 are shown full dose-response curves for zinterol stimulation of adenylate cyclase in preparations derived from nonfailing and failing human left ventricular myocardium. Significantly less adenylate cyclase stimulation occurs in failing heart ($p < 0.05$ for the entire curves) compared with data in nonfailing heart. However, when the ED_{50} values for each curve (normalized to the maximum of each curve) were compared, there was no significant difference ($8.14 \pm 2.45 \times 10^{-8}$ M in nonfailing versus $1.18 \pm 0.37 \times 10^{-7}$ M in failing, p, NS). In Table 4 are given data for zinterol activation of adenylate cyclase in preparations derived from 14 nonfailing and 17 failing left ventricles. Despite the fact that β_2-receptors were not decreased in density in failing heart (Tables 1, 3, and 4), zinterol produced less stimulation of adenylate cyclase than it did in nonfailing preparations, with a 32% reduction of maximal β_2-stimulated activity. In Fig. 6 and Table 4, five of the nonfailing and six of the failing ventricles were also used to measure selective β_1 stimulation (Table 3).

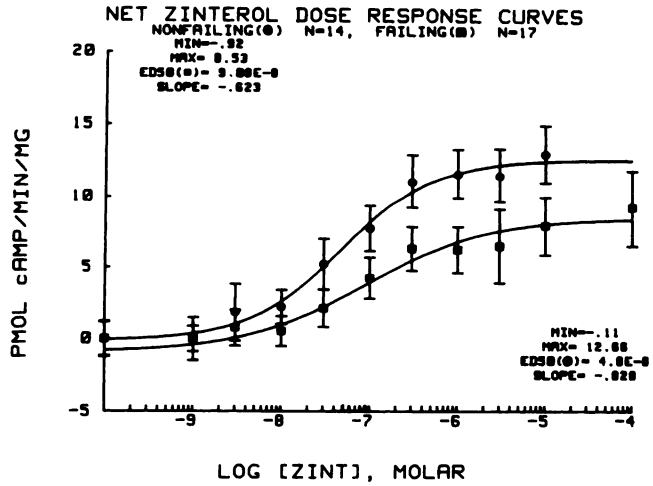

Fig. 6. Dose response for adenylate cyclase stimulation of the selective β_2-agonist zinterol (*ZINT*) in preparations derived form nonfailing (●, n = 14) and failing (■, n = 17) human left ventricles.

Stimulation of adenylate cyclase by the nonselective β-agonist isoproterenol in preparations derived from nonfailing and failing ventricles is shown in Fig. 7 and Table 4. Isoproterenol full dose-response curves are significantly blunted in preparations derived from failing heart, as shown in Fig. 7. Again, there is no difference in ED_{50} ($4.98 \pm 1.67 \times 10^{-7}$ M in nonfailing versus $7.80 \pm 2.08 \times 10^{-7}$ M in failing, p, NS). In preparations that had a reduction in total β-receptor density of 42%, isoproterenol stimulation of adenylate cyclase was reduced by 29% (Table 4). Stimulation of adenylate cyclase by fluoride or histamine (Table 4) and total creatine kinase activity (data not shown) were not different in failing versus nonfailing hearts.

Discussion

Previous investigations have documented that human myocardium has significant numbers of β_2-adrenergic receptors (3–7) and that β_2-receptors are coupled to a positive inotropic response (6, 7, 10, 22, 23). Surprisingly, in the initial report of the coupling of β_1- and β_2-receptors to adenylate cyclase, it appeared that only the β_2-receptor stimulated formation of cAMP (8). However, in this previous investigation only a single ventricular preparation that was obtained from a dysfunctional human heart was examined, and heart failure may decrease the number of β_1-receptors (7) and/or reduce receptor cyclase coupling.

Although subsequent studies (10, 11) in small numbers of samples were able to demonstrate coupling of human ventricular myocardial β_1-receptors to adenylate cyclase, β_2-receptors appeared to be more efficiently coupled. Again, however, specimens from subjects who likely had varying degrees of cardiac dysfunction were used in one of these studies (10) and, there-

TABLE 4

β_2-Agonist (zinterol) and nonselective β-agonist (isoproterenol) stimulation of adenylate cyclase (AC) and β_1 and β_2 receptor density in nonfailing and failing human heart

Iso, isoproterenol maximum from dose-response curves; Zint, zinterol maximum from dose-response curves; Hist, response to 10^{-4} M histamine; NaF, response to 10^{-2} M NaF.

Group	Receptor densities			Net AC Stimulation				Agonist/NaF	
	β_1	β_2	β total	Iso	Zint	Hist	NaF	Iso	Zint
	fmol/mg			pmol of cAMP/min/mg					
Nonfailing (n = 14)	67.5 ± 4.6	15.9 ± 3.0	84.6 ± 4.7	41.7 ± 2.5	13.9 ± 1.2	5.0 ± 0.8	49.1 ± 5.2	0.92 ± 0.07	0.32 ± 0.04
Failing (n = 17)	32.6 ± 3.0[a]	15.8 ± 1.5	48.7 ± 3.4[a]	29.6 ± 3.5[b]	9.4 ± 0.9[b]	4.3 ± 0.4	44.7 ± 5.5	0.72 ± 0.07[b]	0.23 ± 0.03[b]

[a] $p < 0.001$.
[b] $p < 0.05$.

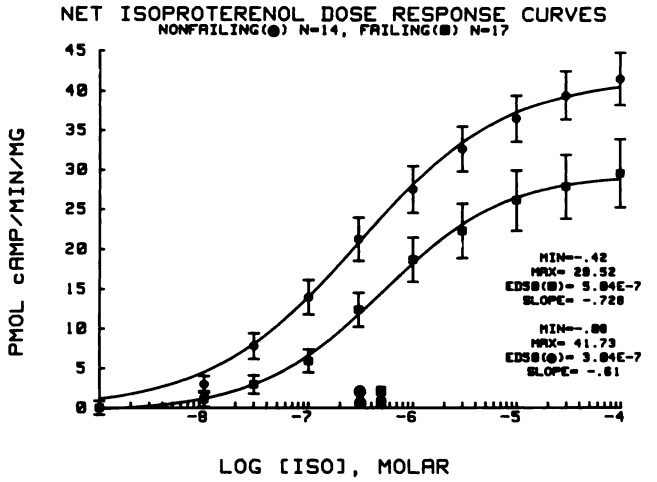

Fig. 7. Dose response for adenylate cyclase stimulation of the nonselective β-agonist isoproterenol (*ISO*) in preparations derived from nonfailing (●, $n = 14$) and failing (■, $n = 17$) left ventricles.

fore, this investigation is also potentially hampered by being conducted in a starting material that did not contain a normal population of β_1-receptors.

In the current investigation, we demonstrate that nonfailing human ventricular myocardium contains predominately β_1-receptors, in agreement with previous studies (3–7). Despite the relatively small proportion of receptors of the β_2 subtype in nonfailing ventricular myocardium, the majority of adenylate cyclase stimulation in particulate fractions was mediated by this receptor. That is, in preparations derived from nonfailing human ventricular myocardium, the β_2 subtype that constituted 19% of the total β-receptors mediated the majority of the adenylate cyclase stimulation in response to the nonselective full agonist isoproterenol.

β_1-Receptors were weakly but definitely coupled to adenylate cyclase in nonfailing myocardium, as shown by betaxolol inhibition of cyclase stimulation produced by the selective β_1-agonists prenalterol and denopamine. Additionally, 10^{-7} M betaxolol, a concentration of this selective β_1-antagonist that would occupy >90% of β_1- but <5% of β_2-receptors, produced a significant rightward shift in the isoproterenol-cyclase dose-response curve. Because 10^{-7} M betaxolol had no effect on cyclase stimulation produced by the selective β_2-agonist zinterol, this rightward shift in the isoproterenol-cyclase dose-response curve was due to blockade of β_1 stimulation.

Although the selective β_1-agonists denopamine and prenalterol stimulated cyclase through a β_1 mechanism, it was necessary to assay these weak partial agonists in the presence of Gpp(NH)p and forskolin, to take advantage of the synergistic effect of the latter on hormone-coupled cyclase stimulation (14) and the ability of the former to enhance efficacy (24). Also, a selective β_2-blocking concentration of ICI 118,551 had to be added to achieve measurable selective β_1-agonist stimulation by these agents. Using these assay conditions, it was possible to demonstrate selective β_1 stimulation of adenylate cyclase by both prenalterol and denopamine, inasmuch as for both agonists betaxolol produced a degree of blockade that approached its β_1 K_H, as measured by radioligand binding. Interestingly, two β-agonists that have been previously identified as having selectivity for β_1-adrenergic receptors, dobutamine (21) and norepinephrine (2, 9), were not selective β_1-agonists in our adenylate cyclase system, inasmuch as stimulation of adenylate cyclase by either agent appeared to be no more selective than for isoproterenol.

Our results on relative coupling efficiency are in general agreement with the results of a recent investigation (10) that showed differential coupling of β_2- versus β_1-receptors in small numbers (two or three) of samples removed from human left ventricle at the time of valve replacement. These authors also showed that β_2-receptors are in the minority (26–29% of the total) but produced the majority of adenylate cyclase stimulation. Our own observations extend this discrepancy in coupling to the nonfailing human left ventricle.

There are several potential explanations for the difference between our results and those of an earlier investigation (8) that did not detect β_1-cyclase coupling in human ventricular myocardium. The first, and probably most important, is that our preparations were derived from nonfailing human myocardium that contained 80% β_1-receptors. Also, our methods are designed to minimize loss of β_1-receptors in storage, which is negligible up to several months when the tissue and membranes are processed as described (7). Finally, our experiments with selective antagonists were performed under equilibrium conditions.

When the degree of receptor down-regulation in preparations derived from failing heart was compared with the degree of loss of maximum adenylate cyclase activity in response to nonselective and selective β-receptor subtype activation, an interesting observation emerged. In preparations derived from failing heart, the degree of β_1-receptor down-regulation (61%) was in reasonable agreement with the 49% reduction in denopamine stimulation and the 77% reduction in denopamine stimulation blocked by betaxolol (β_1 stimulation). However, selective β_2 stimulation of adenylate cyclase by zinterol was reduced despite the fact that β_2-receptor density was unchanged. That is, in preparations derived from failing heart, β_2-receptor density was preserved in failing ventricle, in agreement with previous studies (7), whereas maximal β_2 stimulation of adenylate cyclase was reduced by 32% ($p < 0.05$). These data indicate that β_2-receptors were partially uncoupled from subsequent biochemical events in the adenylate cyclase pathway.

Investigations of "homologous" β-adrenergic subsensitivity in model systems have indicated that uncoupling may precede receptor down-regulation. For example, when cultured heart cells are exposed to a high concentration of β-agonist, uncoupling appears to occur first (25, 26), followed by receptor loss (25). Because β_1-receptors have much higher affinity for norepinephrine than do β_2-receptors (2, 27), these observations suggest that exposure to increased quantities of norepinephrine in the failing human heart (28, 29) may have led to "low affinity" uncoupling of β_2-receptors and "high-affinity" down-regulation.

In preparations derived from failing heart, the response to isoproterenol was reduced by 29% ($p < 0.05$), which was somewhat less than the 42% reduction in the total number of β-receptors. The reason for this difference between reduction in total β-receptor density and reduction in isoproterenol maximum stimulation of adenylate cyclase in failing heart is apparently because the tighter coupled β_2-receptor is disproportionately represented in the response to isoproterenol.

The adenylate cyclase response to a maximum dose of histamine (10^{-4} M) was not different in preparations taken from

nonfailing versus failing left ventricles, indicating that H_2 receptor-mediated responses were intact, similar to previously reported observations (24). Similarly, the response to fluoride was not different in preparations taken from nonfailing and failing heart, indicating that G_s-C function is intact in failing heart, consistent with previous reports (13, 14, 30). Finally, there was no difference in creatine kinase activity in failing versus nonfailing hearts, indicating that the amount of viable myocardium is not reduced in failing left ventricles with idiopathic dilated cardiomyopathy (13, 30).

Despite the finding that the β_2-receptor is markedly more efficiently coupled to adenylate cyclase stimulation in particulate fractions of failing human ventricle, this differential β_2/β_1 coupling does not appear to be transmitted to stimulation of muscle contraction. That is, previous work reported by us (7) and others (10) in isolated human myocardial tissue indicates that selective β-receptor subtype activation produces positive inotropic effects in proportion to the number of β_1- or β_2-receptors present in the tissue, which creates a "discrepancy" (10) between cyclase activation and muscle contraction stimulation. These observations are consistent with the hypothesis that cAMP is the second messenger for the catecholamine-mediated positive inotropic response but that specific pools of cAMP may be utilized for contraction versus other biochemical events. In human ventricular myocardium, discrepancies between the amount of adenylate cyclase stimulation produced and the amount of positive inotropic response generated have been previously described (15). Alternatively, responses in membrane preparations may differ from responses in intact cells, as has been emphasized by others (31).

In a previous study (7), the muscle contraction responses to zinterol were decreased by 34% in preparations derived from failing heart, which was a statistically insignificant difference. In this previous study the small, nonsignificant decrease in zinterol response in failing preparations was in contrast to a marked reduction in denopamine stimulation, which was by 90% (7). The results of the current study are, therefore, in agreement with these previously determined measurements for muscle contraction, with β_2 responses mildly decreased in failing ventricular myocardium despite preservation of β_2-receptor density and β_1 responsiveness markedly reduced.

Perhaps the most important question raised by our findings concerns the basis for the marked difference in the efficiency of coupling of β_1- versus β_2-receptors. There are two general possibilities, 1) the difference is an *in vitro* phenomenon that has been created in the preparation of the particulate fraction used to assay cyclase activity or 2) the difference is truly biologic, reflecting fundamental differences in the way β_1- and β_2-adrenergic receptors interface with subsequent components of the adenylate cyclase pathway. Against the former possibility is the fact that our particulate fraction is prepared quite gently and avoids the use of steps that uncouple β-receptors. Support for the latter possibility, that β_1 and β_2-receptors are truly coupled differently to adenylate cyclase derives from the observation that β_1- and β_2-receptors may behave differently when exposed to down-regulating influences (7, 32–34). This suggests the possibility of differential regulation of β_1- and β_2-receptors, which could presumably be accomplished through differences in components of the respective receptor-cyclase complexes. Further studies will be required to elucidate the cause(s) of the remarkable difference in biochemical coupling of β_1- and β_2-receptors in particulate fractions of nonfailing human ventricular myocardium.

In summary, in nonfailing human ventricular myocardium, β_2-adrenergic receptors are coupled markedly more efficiently to adenylate cyclase than are β_1- receptors. Heart failure produces a loss in the amount of adenylate cyclase stimulation that can be mediated by each subtype. For β_1-receptors, this is due to down-regulation and, for β_2-receptors, the loss in activity is due to partial uncoupling. In the human heart β_1- and β_2-receptors exhibit at least two fundamental differences; the β_1-receptor selectively down-regulates in heart failure, whereas the β_2-receptor is markedly more efficiently coupled to adenylate cyclase in nonfailing heart and then uncouples in response to heart failure.

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