β-Adrenoceptor and Adenylate Cyclase Function in the Infarct Model of Rat Heart Failure (43398)

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Abstract. **In order to determine the possible etiology for diminished inotropic responsiveness to catecholamines in the infarction model of chronic congestive heart failure in rats, we studied β-adrenoceptor number and site-specific stimulated adenylate cyclase activity in noninfarcted left ventricular tissue of rats at 3 months after ligation of the left coronary artery. Rats were divided into sham, small infarct, and large infarct groups according to infarct size. The large infarct groups showed increased right ventricle to body weight ratio (0.93 ± 0.07 mg/g for the large infarcts vs 0.52 ± 0.02 and 0.54 ± 0.02 mg/g for the shams and small infarcts, respectively). β-Adrenoceptor number among the groups was similar (shams, 27 ± 1 fmol/mg; small infarcts, 26 ± 1 fmol/mg; and large infarcts, 29 ± 1 fmol/mg), as was K_d (20 ± 1 pmol, 18 ± 2 pmol, and 18 ± 2 pmol, respectively). Site-specific stimulation of adenylate cyclase using isoproterenol, Gpp(NH)p, forskolin, and $MnCl_2$ revealed no significant differences among the groups. We conclude that this system is not responsible for the altered inotropic responsiveness to catecholamines seen in this model.** [P.S.E.B.M. 1992, Vol 200]

Chronic congestive heart failure (CHF) may be produced in rats by ligation of the left coronary artery. This model demonstrates hemodynamic abnormalities and contractile dysfunction proportional to the size of the infarct (1). Myomorphometric studies reveal ventricular remodeling with both concentric and eccentric hypertrophy of the remaining myocardium that do not completely compensate for the loss of myocytes in large infarctions (2, 3). While loss of functioning myocardium certainly contributes to the hemodynamic abnormalities described in this model (1), impaired responsiveness to catecholamines in the noninfarcted papillary muscle also exists (4). This could be related to alterations in the β-receptor (BAR)-adenylate cyclase (AC) system in the cell membrane of the hypertrophied myocytes. Changes in this system are evident in a number of animal models of CHF and seem to depend on the model and species being investigated. The increased sympathetic tone that accompanies congestive failure may play a major role in these chronic compensatory changes (5). We postulated that diminished inotropic responsiveness to catecholamines of noninfarcted myocardium in rats may be related to alterations in the BAR-AC system, as observed in other species (6–9). Accordingly, we performed biochemical studies in order to further elucidate the mechanisms contributing to this impaired catecholamine sensitivity in the chronic infarction model of CHF.

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Methods

Surgery. Male Sprague-Dawley rats weighing 200–275 g (Charles River Breeders, Wilmington, MA) were anesthetized with ether, intubated endotracheally, and placed on a small animal respirator (Harvard Instruments, Cambridge, MA). A left parasternal incision was made under sterile conditions, the pericardium was opened, and the left coronary artery was ligated approximately 2 mm from its origin. In sham animals, the suture was not tied. The chest wall was closed in layers and pneumothorax aspirated prior to extubation. Animals were housed in the University of Rochester

vivarium in separate cages and given standard laboratory chow and water *ad libitum*.

This study was approved by the University of Rochester Committee on Animal Resources, and conformed to the guiding principles approved by the Council of the American Physiological Society and the National Institutes of Health Guide on the Humane Care and Use of Laboratory Animals.

Sacrifice. Three months after surgery, surviving animals were weighed and sacrificed by decapitation. The heart was rapidly removed and placed in ice-cold, oxygenated 50 m*M* Tris HCl (pH 7.7). The right ventricle was dissected free and an incision was made through the septum to open the left ventricular chamber for the infarct-sizing procedure. In order to preserve tissue for biochemical studies, a rapid technique for deriving an accurate estimate of infarct size needed to be developed. Infarct sizing was accomplished by transilluminating the cooled and opened left ventricle between two transparent plates and directly tracing left ventricular endocardial and translucent scar areas, taking care to avoid stretching of the myocardium. The average time for this process was approximately 3 min. Planimetry was later performed to give infarct size as a percentage of total left ventricular endocardial surface area. The scar and septum were then dissected from the left ventricular free wall and each component was blotted dry and weighed. The noninfarcted left ventricular free wall was then stored at −70°C for biochemical analysis.

Membrane Preparation. Crude membrane preparations were used for receptor binding and adenylate cyclase assays. LV tissue was minced and suspended in ice-cold 50 m*M* Tris HCl (pH 7.7) then homogenized by Polytron (Brinkman Instruments, Luzern, Switzerland) for 8-sec bursts three times at speed setting 8. The suspension was then centrifuged for 15 min at 500*g* (4°C). The supernatant was spun at 90,000*g* for 15 min at 4°C and resulting pellets were stored at −70°C until additional studies were performed. Because limited amounts of noninfarcted LV remained, it was not always possible to use the same rat left ventricular free wall for both assays.

Radioligand Studies. β-Receptor number was determined by [^{125}I]iodocyanopindolol (sp act 2200 mCi/mmol [New England Nuclear, Boston, MA]) saturation binding using 12 dilutions of radioligand and 100-μg crude membrane filtered through 50-μm mesh with and without isoproterenol (10^{-4}) to determine specific binding. Membranes were incubated at 37°C for 1 hr and harvested by rapid vacuum filtration (Brandel, Gaithersburg, MD) through Whatman GF/B filters using 5 ml of iced reaction buffer and two 5-ml washes. Gamma scintillation with an efficiency of 60% was used to assess bound radioactivity. Specific binding was greater than 85%. Experiments were performed in duplicate.

Adenylate Cyclase Activity. Maximal stimulation of adenylate cyclase was assessed by generation and then competitive protein assay for quantification of cAMP (10). Briefly, 60 μg of fresh membranes were suspended in 50 m*M* Tris HCl (pH 7.7) with 10 strokes in a ground glass homogenizer and filtered through 50-μm nylon mesh. Prepared membranes were then incubated with 5 m*M* phosphocreatine, 15 units of creatine phosphokinase, 2 m*M* $MgCl_2$, 0.5 m*M* 1-isobutyl-1-methoxyxanthine, and 0.4 m*M* EGTA, with 0.5 μmol of ATP added for 10 min at 37°C in the presence or absence of stimulator. The solvent for stimulators was distilled and deionized water, except for forskolin, which required 50% dimethylsulfoxide to dissolve completely. Concentrations of stimulators to elicit maximal production of cAMP were determined in pilot studies. They were as follows: 1-isoproterenol, 100 m*M* with 0.1 m*M* GTP; Gpp(NH)p, 100 m*M*; forskolin, 100 μM; and $MnCl_2$, 40 m*M*. The reaction was terminated by boiling for 3 min. Centrifugation at 1000*g* for 20 min at 4°C removed denatured protein and supernatant was used to determine amount of cAMP generated. Competition with [^{3}H]cAMP, sp act 3350 mCi/mmol (New England Nuclear, Boston, MA), was performed using 3′5′cAMP-dependent protein kinase in 0.2% bovine serum albumin for a 90-min incubation period at 4°C. Activated charcoal in 0.2% bovine serum albumin was then added to remove free ligand and separated by centrifugation at 1000*g* for 20 min. The supernatant was removed and added to 5 ml of Ecoscint A (Dupont, Wilmington, DE) for beta scintillation counting. A standard curve was used to translate these results into amount of cAMP generated. Experiments were performed in triplicate.

Protein Determination. Protein concentrations were determined by the Lowry method using bovine serum albumin as the standard (11).

Statistical Analysis. Data are expressed as mean ± SE. Maximum number of β-receptor binding sites (B_{max}) and dissociation constant (K_d) were determined by linear regression analysis using the Ligand program (12). Nonparametric statistical tests were used because group data showed some variation from random distribution about the mean. Intergroup comparisons were made using the Kruskal-Wallis statistic with the Mann-Whitney rank sum test and Bonferroni correction for pairwise comparisons. The level of significance was determined by a P-value < 0.05. Biological variability in the ability of the tissues to produce cAMP made a calculation of the power of our sample size to detect a significant change important for determining no effect. Using an arbitrary change of 25% in adenylate cyclase activity as evidence of an infarction effect, our study had a power of 75%.

Results

Surgery was performed on 176 animals during a 4-month period. Postoperative mortality in the infarction groups at 48 hr was 42%. In the remaining 102 animals, there were eight deaths over the 3-month period, with seven of these in the large infarct group. Animals in which a suture was tied that had no evidence of infarction were not included in the sham group. Table I shows gross characteristics of the infarct groups. Left coronary ligation produced a wide range of infarct sizes, from 8% to 46% of total LV. Only one rat developed a nontransmural infarction and was not included in the analysis. Infarct size is expressed as a percentage of total left ventricular area. The division point between infarct groups is 25%, and this is used to distinguish compensated small infarctions from decompensated large infarctions, with increased right ventricular weight suggesting more severe left ventricular dysfunction. Mean infarct size for the small (n = 14) and large (n = 15) groups was 17 ± 1% and 35 ± 1%, respectively.

Table I. Sacrifice Characteristics of Infarct Groups[a]

Group	Sham (n = 17)	Small (n = 14)	Large (n = 15)
Infarct size (%LV)	No scar	17 ± 1[b]	35 ± 1
Body wt (g)	584 ± 12	601 ± 16	579 ± 22
LV wt (g)	0.87 ± 0.01	0.79 ± 0.05[c]	0.62 ± 0.05[d]
RV/body wt (mg/g)	0.52 ± 0.02	0.54 ± 0.02	0.93 ± 0.07[d]
Heart wt/body wt (mg/g)	2.4 ± 0.03	2.5 ± 0.10	2.9 ± 0.14[d]

[a] Abbreviations used in this table: LV, left ventricle; RV, right ventricle.
[b] Values are expressed as mean ± SE.
[c] Differs significantly from sham group at $P < 0.001$.
[d] Differs significantly from sham and small infarct groups at $P < 0.001$.

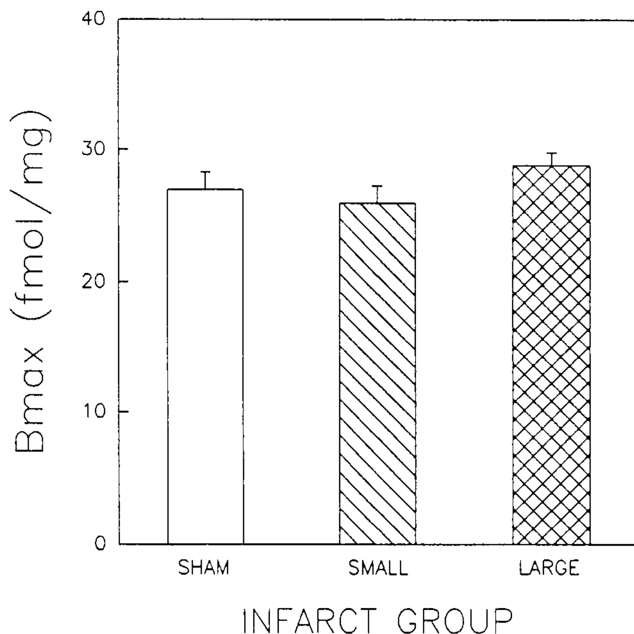

Figure 1. Effect of infarct size on B_{max} of left ventricular crude membrane fractions by [^{125}I]iodocyanopindolol. Bars denote SE. The differences among the groups were not statistically significant.

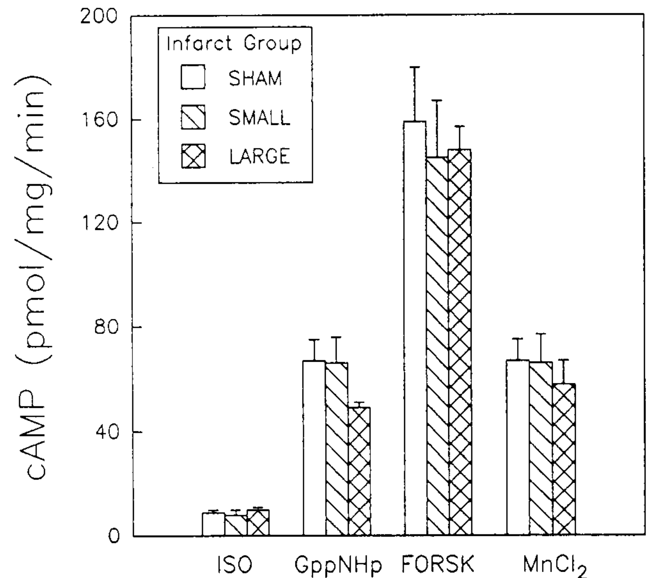

Figure 2. Changes from basal adenylate cyclase activity in response to isoproterenol (ISO), Gpp(NH)p, forskolin (FORSK), and $MnCl_2$ in left ventricular crude membrane fractions from the infarct groups. Bars denote SE. There were no significant differences among the groups.

Body weights did not differ significantly. While left ventricular weight was inversely proportional to scar size, both heart and right ventricular to body weight ratios were increased for the large infarct group ($P <$ 0.001), indirectly supporting the existence of right ventricular hypertrophy seen in this model by myomorphometric techniques (2).

As shown in Figure 1, β-receptor number did not change in the infarction groups as compared with sham-operated controls. Receptor binding of the antagonist was both saturable and reversible. Mean B_{max} were (fmol/mg protein): shams, 27 ± 1; small infarcts, 26 ± 1; and large infarcts, 29 ± 1. K_d for the respective groups were (pmol) 20 ± 1, 18 ± 2, and 18 ± 2 (P = NS), indicating no change in the affinity of the receptor for antagonist.

Figure 2 shows stimulated AC activity expressed as absolute change in production of cAMP from basal. The values (pmol/mg/min) for isoproterenol were 9 ± 1, 8 ± 2, and 10 ± 1 pmol/mg/min; for Gpp(NH)p 67 ± 8, 66 ± 10, and 49 ± 2; for forskolin 159 ± 21, 145 ± 22, and 148 ± 9; and for $MnCl_2$ 67 ± 8, 66 ± 11, and 58 ± 9, for sham (n = 11), small infarct (n = 9), and large infarct (n = 9) groups, respectively. The trend toward a decrease in cAMP production in the large infarct group for stimulators beyond the level of the BAR is not significant (all P = NS). Isoproterenol and forskolin stimulation are not altered by infarction, suggesting intact G protein function. The trend in the large

infarct group Gpp(NH)p data may represent an alteration in G protein structure caused by increased time of tissue manipulation required for dissection of larger scars at the time of sacrifice.

Discussion

Site-specific stimulation is used to look for potential abnormalities that may exist along the pathway from the β-receptor to adenylate cyclase when diminished sensitivity to catecholamines is demonstrated. Gpp(NH)p is a nonhydrolyzable analog of GTP and irreversibly activates $G_s\alpha$. Adenylate cyclase is the principal site for the effect of both Mn^{2+} and forskolin, with the latter also interacting with the G protein (13). Because site-specific stimulation of adenylate cyclase among the groups was equivalent, we cannot invoke an abnormality in the BAR-AC system as the cause for the diminished responsiveness to β-agonists shown in previous studies. Congestive heart failure is accompanied by a number of well-described biochemical abnormalities in the BAR-AC system that vary with each model. In the dog model of pressure overload left ventricular hypertrophy and congestive heart failure, BAR number increases with a loss of high affinity sites and a decrease in functional $G_s\alpha$ (5, 7). The Syrian hamster model of cardiomyopathy shows no change in BAR number with the loss of $G_s\alpha$ function that accompanies the development of heart failure, with no change in immunologically detectable $G_s\alpha$ or its mRNA (14). In the spontaneously hypertensive rat, some investigators have shown that there is indirect evidence supporting no significant change in the BAR-AC system despite altered responsiveness to catecholamines (15), whereas others have demonstrated diminished numbers of β-receptors (16). A recent report described loss of inotropic responsiveness to β-agonists in aortic-constricted rats at 3 weeks, with no change in β-receptor number but a decrease in tissue cAMP levels (17).

In the rat model of CHF due to chronic myocardial infarction, our laboratory (4) has also found changes similar to those described in the spontaneously hypertensive rat (15). While diminished inotropic response to catecholamines exists, there is no change in lusitropy, which argues against a cAMP-mediated effect on the contractile proteins. This suggests that the BAR-AC system may not be directly related to the changes we observed. Because alterations in other models are known to exist, we needed to ascertain whether the infarct model had any significant alterations in known transmembrane signaling pathways for β-agonists. The complexity of the β-receptor-adenylate cyclase system is becoming increasingly apparent in light of the recent discoveries of novel effector pathways for the G proteins (18, 19). Our data do not support a significant change, leading one to hypothesize that the altered catecholamine response after infarction may be due to cellular mechanisms beyond cAMP production or changes within the contractile proteins. A change from V1 to V3 myosin has been described (20) and this may lead to inhibition of ATPase activity by the cAMP produced during β-agonist stimulation (21). An alteration in cAMP-dependent phosphorylation of troponin I might lead to a change in calcium sensitivity of the contractile apparatus (21). These changes, as well as others, may be responsible for the alteration in sensitivity to β-agonists that occurs with congestive failure in this model.

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