β-Adrenergic Receptor–G Protein–Adenylate Cyclase Complex in Experimental Canine Congestive Heart Failure Produced by Rapid Ventricular Pacing

Kevin P. Marzo, Martin J. Frey, John R. Wilson, Bruce T. Liang, David R. Manning, Vita Lanoce, and Perry B. Molinoff

Changes in the β-adrenergic receptor–G protein–adenylate cyclase complex were investigated in an experimental canine model of low-output heart failure produced by chronic rapid ventricular pacing. The contractile response occurring after exposure to the β-adrenergic agonist dobutamine, measured as peak left ventricular +dP/dt, was decreased after 3 weeks of pacing. To further characterize the diminished functional responsiveness to β-adrenergic receptor stimulation, β-adrenergic receptor–adenylate cyclase coupling was investigated using membranes prepared from both control and paced animals. The density of β-adrenergic receptors was decreased by 40% with a selective downregulation of the β1-subtype. The affinity of the receptor for the antagonist radioligand [125I]iodocyanopindolol remained unchanged. A defect in coupling was suggested by a decreased ability of isoproterenol, fluoride, and forskolin to stimulate adenylate cyclase in membranes prepared from failing hearts. Determination of the levels of Gααi (the α-subunit of Gα) by immunoblotting and pertussis toxin labeling revealed modest increases of −30%. Furthermore, Mn2+ and purified Gαi failed to stimulate adenylate cyclase in membranes prepared from failing hearts, indicating an impairment in the catalytic moiety of adenylate cyclase itself or in the ability of adenylate cyclase to couple to Gαi. In contrast, complementation assay did not reveal differences in the functional activity of Gααi (the α-subunit of Gαi). Taken together, these data demonstrate a selective decrease in the β1-subtype of adrenergic receptors and an increase in a 40-kd Gαi-like protein in the failing heart. Similar changes have been described in human idiopathic dilated cardiomyopathy. In addition to these changes, we identified a possible defect at the level of the catalytic subunit of adenylate cyclase. (Circulation Research 1991;69:1546–1556)

Cardiac responsiveness to β-adrenergic stimulation is impaired in experimental animals and in humans with congestive heart failure.1–3 The primary signaling system in the heart that mediates the inotropic effects of catecholamines is the β-adrenergic receptor complex. This transmembrane complex consists of three distinct proteins: the β-adrenergic receptor, which binds the stimulatory hormone; the catalytic moiety of adenylate cyclase, which synthesizes cAMP from ATP; and the stimulatory guanine nucleotide–binding regulatory protein (Gαi), which couples the β-adrenergic receptor to its effector.

The decreased response of β-adrenergic stimulation in the failing human heart results in part from downregulation of β-adrenergic receptors. Bristow et al.1 described a selective decrease in the density of β-receptors in failing human heart, as well as a significant impairment in the development of contractile force in response to the β-agonists isoproterenol and dobutamine.4,5 Additional abnormalities distal to the receptor may also be responsible for reduced cAMP production during β-adrenergic stimulation.6 For example, an increase in the level and/or function of the inhibitory G protein Gi, which antag-
onizes the action of Gβ, has been observed in humans and in animal models of heart failure.7,8 Studies of the β-adrenergic complex in experimental models of heart failure have revealed divergent results compared with the changes seen in idiopathic heart failure in humans. Studies by Vatner et al9 and Longabaugh et al11 in a canine model of heart failure due to left ventricular (LV) pressure overload revealed an increased density of β-receptors associated with a decrease in Gβ. In a guinea pig model of heart failure, Karliner et al10 also showed an increase in the density of β-receptors. In contrast, a canine model of right heart failure revealed a decrease in the density of β-receptors restricted to the right ventricle.11 Furthermore, studies of the cardiomyopathic Syrian hamster revealed a functionally abnormal Gβ in the presence of unchanged β-receptor density.3 The diverse abnormalities in signal transduction in various animal models may arise from the different pathogenic mechanisms for the various forms of heart failure. Although these models remain useful for studying signal transduction, the availability of an animal model with changes in the β-adrenergic complex similar to those found in human idiopathic cardiomyopathy remains desirable.

The purpose of the present study was to characterize the β-adrenergic receptor–adenylate cyclase system in an experimental canine model of heart failure produced by chronic rapid ventricular pacing. Results of several studies from this laboratory and elsewhere have shown that rapid ventricular pacing of dog hearts at 240–260 beats/min results in low-output heart failure characterized by biventricular pump dysfunction and dilation, as well as hemodynamic changes similar to those observed in human biventricular dysfunction.12

Materials and Methods

Animals

Twenty-two adult mongrel dogs weighing 18–25 kg were studied during the course of the present investigation. An initial set of experiments was performed on 14 (six paced and eight sham-operated) dogs. The dogs were preanesthetized with atropine (0.5 mg) and morphine sulfate (10 mg) and were anesthetized with sodium pentobarbital (10 mg/kg) and morphine sulfate (8 mg/kg). Through a left lateral thoracotomy, epicardial pacing leads were sutured onto the LV apex. The leads were tunneled to the back and connected to a multiprogrammable pulse generator (Medtronic Inc., Minneapolis, Minn.) implanted subcutaneously. After recovery from anesthesia, the dogs were returned to a chronic care facility. Two days later, the pacemaker was programmed to 260 beats/min in six dogs; eight dogs remained nonpaced and served as controls. At 4 weeks, blood was obtained at rest to measure plasma levels of norepinephrine and epinephrine. After the fourth week of pacing, dogs were anesthetized with morphine sulfate (4–6 mg/kg) and chloralose (75 mg/kg) and intubated. A continuous infusion of chloralose was initiated at 10 mg/kg/hr. A Swan-Ganz catheter was inserted into the external jugular vein and positioned in the pulmonary artery. Five minutes later, the following hemodynamic measurements were recorded: right atrial pressure, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and arterial pressure. Cardiac output was measured in triplicate by thermodilution. Dogs were then killed with an overdose of sodium pentobarbital (50 mg/kg).

A separate set of studies was performed on four different dogs to assess the cardiac responses to β-adrenergic stimulation with dobutamine. The study was designed such that each dog would serve as its own control. Pacemaker implantation was performed as described above. To avoid early postoperative effects on adrenergic responsiveness, the dogs were given 7 days to recover, at which time a baseline hemodynamic assessment was performed. Dogs were anesthetized with sodium pentobarbital and valium, intubated, and maintained on a ventilator (Harvard Apparatus, South Natick, Mass.) with anesthesia maintained using intravenous morphine given as a 2–4 mg bolus every 20 minutes. Arterial pH was maintained between 7.35 and 7.45 by altering respiratory rate. A Swan-Ganz catheter was inserted through an external jugular vein and a 5F micromanometer-tipped catheter (Millar Instruments, Houston, Tex.) was inserted via a femoral artery and placed in the LV. A side-arm sheath (Cordis) in the right femoral artery was used to monitor arterial pressure. LV pressure was calibrated against a mercury manometer and against the arterial pressure measurements. Hemodynamic measurements were obtained at paced heart rates of 150 beats/min by reprogramming the permanent pacemaker. After placement of catheters, baseline hemodynamic measurements were taken and then averaged from two consecutive sets of data separated by at least 10 minutes and differing by <10%. Hemodynamic measurements and peak LV +dp/dt (by continuous electronic differentiation) were recorded both on paper and on FM magnetic tape for subsequent data analysis. Baseline hemodynamic data were obtained during continuous infusion of 5% dextrose in water (D5W) using a Harvard pump at a rate of 1.9 ml/min. Dobutamine diluted in D5W was then infused at 5-minute intervals according to the following schedule: 2.5, 5.0, 7.5, 10.0, and 15.0 μg/kg/min. Hemodynamic measurements were made during the fifth minute of each infusion period. At the termination of the study, pacing was discontinued, femoral arteries were repaired, and the dogs were allowed to recover from anesthesia and were returned to a chronic-care facility. Two days later, rapid ventricular pacing was initiated by programming pacemakers to 260 beats/min. After 3 weeks, the same protocol used in obtaining hemodynamic measurements and LV +dp/dt responses to dobutamine infusion was repeated. Rapid ventricular pacing was resumed 2 days...
into recovery. One week later, at the end of the fourth week of rapid pacing, the dogs were killed with an intravenous overdose of pentobarbital (50 mg/kg), and LV tissue was obtained. Four noninstrumented dogs were killed simultaneously, and LV tissue was removed to serve as control samples. The tissue samples obtained from the eight dogs of this latter set of studies (four paced and four control) were used only for studies of pertussis toxin–catalyzed ribosylation and of adenylate cyclase stimulation by MnCl₂.

Membrane Preparation

After the dogs were killed, the hearts were immediately excised, and the LV free wall was removed and placed in buffer containing the following components at 4°C: 50 mM Tris-HCl (pH 7.4), 1 mM MgCl₂, 1 mM EDTA, 20 μg/ml leupeptin, and 32 μg/ml lima bean trypsin inhibitor. Cardiac tissue was minced and homogenized with a Polytron tissue disruptor (Brinkmann Instruments, Inc., Westbury, N.Y.) for 10 seconds. The homogenate was filtered through sterile cheesecloth and centrifuged at 1,000g for 5 minutes. The supernatant was recentrifuged at 18,000g for 15 minutes, and the pellet was resuspended in buffer. The homogenization and centrifugation were repeated twice. The pellet was resuspended in a protein concentration of 3 mg/ml and stored at −70°C until assayed. Adenylate cyclase activity was stable (±9%) over 3 months of storage; receptor binding was stable (±6%) over 6 months of storage.

β-Adrenergic Receptor Binding

The density of β-adrenergic receptors was measured with [125I]iodocyanopindolol ([125I]ICYP) as previously described. Briefly, membranes were incubated at 37°C for 1 hour with increasing concentrations of [125I]ICYP (3–150 pM). Specific binding was defined as binding inhibited by 100 μM isoproterenol, and binding parameters were determined by linear least-squares fitting by the method of Scatchard.

β-Adrenergic Receptor Subtype Analysis

The relative proportion of β₁- and β₂-adrenergic receptors was determined by inhibition of the binding of 55 pM [125I]ICYP by 18 concentrations of the β₁-selective antagonist ICI 89,406. To determine the density of β₁- and β₂-adrenergic receptors in a membrane preparation, the relative percentage of each subtype was determined from a four-parameter logistic equation using the program NEWFITTIMES on the National Institutes of Health PROPHET system. The result was multiplied by the Bₘₐₓ value derived from Scatchard analysis of a simultaneously performed saturation isotherm.

Binding of Dihydropyridine

The density of binding sites for dihydropyridine was determined using [3H]PN-200-110 (Amersham Corp., Arlington Heights, Ill.) as previously described. Crude membranes were incubated at 37°C for 30 minutes with increasing concentrations of [3H]PN-200-110. Specific binding was defined as binding inhibited by 10 μM nifedipine, and binding parameters were determined by the method of Scatchard.

Adenylate Cyclase Assay

Adenylate cyclase activity was determined in crude cardiac membranes (50–100 μg) by measuring the conversion of [α-32P]ATP to cyclic [α-32P]AMP as described previously. Basal activity in the presence of 10 μM GTP was used to assess the net basal activity of both G₁ and G₄ on adenylate cyclase activity in control and failing membranes before the addition of specific agonists.

Labeling of Gᵢ₄ by ADP-Ribosylation With Pertussis Toxin

Pertussis toxin–mediated [32P]ADP-ribosylation was performed on membranes prepared from LV tissue immediately after excision of four paced hearts and four control canine hearts. Tissue was placed in buffer containing the following components at 4°C: 50 mM Tris-HCl (pH 7.4), 1 mM MgCl₂, 1 mM EGTA, 1 mM EDTA, 20 μg/ml leupeptin, and 32 μg/ml lima bean trypsin inhibitor. Tissue was homogenized with a Dounce homogenizer, filtered through cheesecloth, and spun for 15 minutes at 30,000g in a Sorvall centrifuge, and the supernatant was discarded. The pellet was resuspended in the same buffer, homogenized, and refiltered through cheesecloth. [32P]ADP-ribosylation of G proteins was carried out at 37°C for 40 minutes on membrane homogenates as previously described. The assay mixture contained: 0.01 mM NAD⁺, 2 μCi [32P]NAD⁺, 2.5 mM ATP, 2 mM GTP, 10 mM isoniazid, 10 mM thymidine, 5 mM MgCl₂, 20 μg/ml pertussis toxin, 10 mM phosphocreatine, 0.84 IU creatine phosphokinase, and 100–200 μg tissue in a total volume of 50 μl. The reaction was stopped by the addition of 2% sodium dodecyl sulfate in sample buffer followed by boiling for 2 minutes and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Equal amounts of protein were placed in each lane. Analysis of the protein bands after ADP-ribosylation was performed on 11% polyacrylamide gels. Dry gels were exposed to Kodak film with enhancing screens for 2–3 days at −70°C. The autoradiograms from the dried gels were scanned on a densitometer, and the labeled 40-kd band representing the Gᵢ₄ (the α-subunit of Gᵢ₄) was cut out and weighed. The weights obtained represent the relative levels of Gᵢ₄.

Reconstitution of Gᵢ₄ in Cyc⁻ Membranes

Reconstitution of adenylate cyclase in membranes prepared from cyc⁻ mouse S49 lymphoma cells was performed according to the methods of Sternweis et al., as previously described. Heart membranes (2.5 mg/ml) were solubilized in buffer containing 10 mM Tris, 10 mM MgCl₂, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.2% Lubrol for 60 minutes at room temperature. After centrifugation at 20,000g, the supernatant was heated at 30°C for 10 minutes; the extract was then...
Enzyme-Linked Immunotransfer

Rabbit peptide-directed antibodies against a defined region of G\textsubscript{\alpha} were raised as previously described (antiserum 1398).\textsuperscript{24} The antibodies were not specific and could recognize G\textsubscript{\alpha1}, G\textsubscript{\alpha2}, G\textsubscript{\alpha3}, and G\textsubscript{\alpha}G\textsubscript{\alpha}. The antiserum against G\textsubscript{\alpha} (antiserum 5357) was generated by immunization with G\textsubscript{\alpha} purified from bovine brain. Antiserum 5357 does contain some reactivity toward G\textsubscript{\alpha} and G\textsubscript{\alpha}, but it is negligible at the concentration used. In addition, the antiserum that recognizes G\textsubscript{\alpha} does not produce overlapping signals in the 35-kd region in our laboratory’s experience with various cell tissues. Immunoblotting was carried out as previously described.\textsuperscript{23} Briefly, after transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels, cardiac membranes were suspended in 20 mM Tris-HCl and 500 mM NaCl (TBS), then with 3% gelatin in TBS for 30 minutes. Membranes were incubated with a 1:100 dilution of antiserum in 1% gelatin in TBS for 2 hours, washed twice in TBS containing 0.05% Tween 20, then incubated with a 1:2,000 dilution of goat anti-rabbit immunoglobulin G conjugated to horseradish peroxidase for 1–3 hours. Membranes were washed twice in TBS/0.05% Tween 20 and placed in TBS containing 0.5 mg/ml of 4-chloro-1-naphthol and 0.015% H\textsubscript{2}O\textsubscript{2}. Color development was allowed to occur for 10–20 minutes and was terminated by washing membranes in distilled water. The intensity of the developed color was a linear function of the amount of membrane protein subjected to these procedures.

Stimulation With Purified G\textsubscript{\alpha}

Purification of G\textsubscript{\alpha} from rabbit liver was accomplished as described by Sternweis et al.\textsuperscript{22} Purified GTP-\gamma-S–activated G\textsubscript{\alpha} was prepared by incubation of G\textsubscript{\alpha} (40 \mu g/ml) for 60 minutes in a solution containing 2 \mu M GTP-\gamma-S, 30 mM MgCl\textsubscript{2}, 0.1 mM EDTA, 0.1 mM dithiothreitol, 25 mM NaCl, and 0.1% Lubrol PX. Activated G\textsubscript{\alpha} was added to membranes by dilution into the reaction mixture for assay of adenylate cyclase as previously described.\textsuperscript{24}

Plasma Catecholamines and Protein Assays

Plasma catecholamine levels were measured after high-pressure liquid chromatography as described by Goldstein et al.\textsuperscript{25} Protein concentration was measured by the method of Lowry et al\textsuperscript{26} using bovine serum albumin as a standard.

Statistical Analysis

All values are expressed as mean±SEM. Statistical evaluation was performed by Student’s t test. Serial changes within each group were analyzed by repeated-measures analysis of variance. A probability value of p<0.05 was considered statistically significant.

Results

Baseline Hemodynamic Studies

After 4 weeks of ventricular pacing at 260 beats/min, all dogs had hemodynamic evidence of biventricular heart failure. Right atrial pressure was increased (14±1 mm Hg in dogs with heart failure versus 3±1 mm Hg in control dogs) as were pulmonary capillary wedge pressure (24±2 versus 2±1 mm Hg) and LV end-diastolic pressure (19±3 versus 7±1 mm Hg, Table 1). The cardiac output in paced dogs was markedly reduced (1.5±0.1 l/min in dogs with heart failure versus 3.2±0.5 l/min in control dogs) (all p<0.01). LV +dP/dt was obtained on four dogs in the baseline state and after 3 weeks of pacing. Resting peak LV +dP/dt decreased from 2,122±185 to 1,322±103 mm Hg/sec (p<0.01) (Table 1).

Effect of Dobutamine Infusion on +dP/dt

The effect of dobutamine infusion on peak LV +dP/dt was assessed. To control for changes in heart rate responses, hemodynamics were obtained at fixed heart rates of 150 beats/min. The significantly impaired contractile response was reflected by the percent increase in peak LV +dP/dt in the failing dogs compared with the baseline state (Table 1, Figure 1).

| Table 1. Response to Dobutamine at Baseline and After 3 Weeks of Pacing in Dogs |
|-----------------------------------|------------------|------------------|------------------|------------------|------------------|
| Hemodynamic value                | Dobutamine (\mu g/kg/min) | Dobutamine (\mu g/kg/min) | Dobutamine (\mu g/kg/min) | Dobutamine (\mu g/kg/min) | Dobutamine (\mu g/kg/min) |
| Peak LV dP/dt (mm Hg/sec)        | 0                | 2.5              | 5                | 7.5              | 10               | 15               |
| Baseline                         | 2,122±185        | 2,625±143        | 3,617±137        | 3,870±220        | 4,247±360        | 4,100±305        |
| Heart failure                    | 1,322±103        | 1,522±95         | 1,874±128        | 1,870±275        | 1,925±196        | 2,017±161        |
| Mean arterial pressure (mm Hg)   | 99±6             | 107±15           | 110±10           | 109±5            | 110±3            | 102±5            |
| LV end-diastolic pressure (mm Hg)| 7±1              | 8±2              | 7±1              | 9±2              | 8±2              | 9±3              |
| Heart failure                    | 19±3             | 17±2             | 16±1             | 17±3             | 14±2             | 16±2             |

Values are paced and represent mean±SEM. LV, left ventricular; Baseline, values in dogs before chronic rapid pacing; Heart failure, values in paced hearts with heart failure.
FIGURE 1. Graph showing effect of dobutamine on percent increase in peak left ventricular +dP/dt. CHF, congestive heart failure. The percent increase in peak left ventricular +dP/dt during dobutamine infusion in four dogs was measured before and after 3 weeks of rapid ventricular pacing. Values were obtained at heart rates of 150 beats/min and represent mean±SEM (n=4 dogs).

Plasma Catecholamines

Plasma norepinephrine and epinephrine were higher (p<0.01) in heart failure dogs (1,287±210 and 423±83 pg/ml) as compared with control dogs (88±10 and 65±10 pg/ml).

Density of β-Adrenergic Receptors

Saturation analysis of binding of [125I]ICYP demonstrated a 42% decrease (p<0.05) in the density of β-adrenergic receptors in membranes prepared from the chronically paced heart failure dogs compared with the normal membranes (Table 2). The affinity for [125I]ICYP was not significantly different between the two groups.

Identification and Quantification of β₁ and β₂ Adrenergic Populations

ICI 89,406, a selective β₁-competitive antagonist, was used to determine the relative percentage of β₁- and β₂-adrenergic receptors. A representative set of ICI 89,406–ICYP competition curves is given in Figure 2. Table 2 gives the summary of the density of β₁- and β₂-receptors for the competition curves in all the paced and control LV membranes, and demonstrates the decrease in β₁-receptor density and unchanged density of β₂-receptors in paced dogs compared with control dogs. The ratio of β₁/β₂ changed from approximately 70:30 in control dogs to 54:46 in paced dogs. These results suggest a selective downregulation of β₁-receptors in chronically paced failing dogs.

Binding of Dihydropyridine

The density of binding sites for dihydropyridines has been shown in human idiopathic dilated cardiomyopathy not to change significantly compared with normal values and thus was used as a reference marker in the present study. The density of binding sites from paced hearts (128±15 fmol/mg protein) was not significantly different from that of control hearts (113±12 fmol/mg protein) (p=NS), suggesting that the alterations in the density of β-receptors were not related to alterations in the membrane preparation.

G Proteins

The guanine nucleotide–binding regulatory proteins were evaluated with several techniques. Gᵢ, the inhibi-
Adenylate cyclase activity was measured in membranes from control and failing hearts to assess coupling between the β-adrenergic receptor and adenylate cyclase (Table 3). Basal adenylate cyclase (with GTP) was reduced by 27% in failing LV tissue (p<0.05) when compared with control tissue. Failing ventricles had a 60% reduction in net maximal isoproterenol-stimulated adenylate cyclase activity compared with control ventricles. Fluoride is a selective activator of Gs, and forskolin potentiates the activation of the catalytic moiety of adenylate cyclase by Gs, respectively.29-32 Sodium fluoride (10 mM)– and forskolin (30 μM)–stimulated adenylate cyclase activities (without GTP) were reduced 40% and 49%, respectively, in failing myocardium compared with control myocardium. To further investigate a potential defect in the catalytic subunit, Mn2+ (10 mM)–stimulated adenylate cyclase activity in membranes prepared from normal and failing hearts was studied. Mn2+–stimulated adenylate cyclase activity was significantly less than that observed in the normal myocardium (201.5±7.8 pmol cAMP/mg/min in heart failure dogs versus 257.7±27.2 pmol cAMP/mg/min in control dogs, n=4, p=0.01).

The functional integrity of adenylate cyclase was additionally assessed by measuring activities elicited with purified GTP-γ-S–activated Gs. A marked enhancement of activities for membranes from both control and failing hearts was observed (Figure 6). Enhancement in both cases was nearly maximal at 0.8 μg/ml Gs. At all concentrations of GTP-γ-S–activated Gs, activities of adenylate cyclase in membranes from control hearts were greater than those in membranes from failing hearts, strengthening the argument that an abnormality exists in the catalytic

1551

Figure 3. Enzyme-linked immunotransfer blotting of the α-subunit of Gβ (ai) and the β-subunit of the G protein (B). Crude membranes (30 μg of protein) were incubated with a 1:1,000 dilution of antisera as described in “Materials and Methods” in six control (C) and six heart failure (H) dogs. A single gel of four control and four heart failure dogs is shown.
adenylate cyclase in this model of experimental heart failure.

In a separate set of experiments, the possibility that alterations in adenylate cyclase activity resulted from the presence of a nonspecific inhibitor of adenylate cyclase was investigated. Membrane from failing hearts was incubated in a ratio of 1:1 with control membrane. Forskolin (30 μM)—stimulated adenylate cyclase activity in the 1:1 mixture was not statistically different from the sum of the forskolin-stimulated activities of control and failed LV membranes when determined separately (p=NS).

Discussion

A canine model of low-output heart failure induced by chronic rapid ventricular pacing has been used for more than 20 years. Results of prior studies have shown that this model produces a preparation that is similar both hemodynamically and neurohormonally to human dilated cardiomyopathy. Typical manifestations of heart failure in this model include ascites, pulmonary edema, reduced cardiac output, as well as elevated right atrial, pulmonary capillary wedge, and LV end-diastolic pressures. The levels of plasma renin, angiotensin II, aldosterone, vasopressin, atrial natriuretic factor, norepinephrine, and epinephrine are increased as in the failing human heart. Biventricular cardiac dilation and dysfunction and markedly impaired LV contractile function can be detected echocardiographically after 2 weeks of rapid pacing. However, biochemical abnormalities associated with or underlying the hemodynamic derangements seen in this model of heart failure are not understood. Specifically, it is not clear whether a potential defect in the β-adrenergic receptor-effector coupling resides at the level of the β-receptor or the level distal to the receptor or both. The present results show that the canine pacing model of heart failure is associated with diminished β-adrenergic responsiveness, a decreased density of β-adrenergic receptors, and post-receptor defects in signal transduction, including changes in G proteins and a possible defect in adenylate cyclase.

The failing heart shows a diminished responsiveness to β-adrenergic stimulation. This is in part the result of β-receptor downregulation. Fowler et al and Bristow et al demonstrated in failing human hearts a selective decrease in the β₁-subpopulation of receptors as well as a diminished contractile response to β-agonists. Although unchanged in density, stimulatory responses mediated by β₂-adrenergic receptors are also impaired, suggesting an uncoupling from distal effector mechanisms. Postreceptor defects in signal transduction have also been observed in the failing myocardium. The coupling mechanisms distal to the surface-membrane β-receptor include the catalytic moiety of adenylate cyclase and guanine nucleotide–binding regulatory proteins called G proteins. These proteins are from a family of membrane-bound homologous GTP-binding proteins sharing a common heterotrimeric αβγ-subunit structure that transduces stimulatory (G₁) or inhibitory (G₃) signals to adenylate cyclase. In myocardium from patients with idiopathic cardiomyopathy, Feldman et al observed a 36% increase in the α-subunit of G₁, whereas G₃ remained unchanged, suggesting that postreceptor inhibitory signals contributed to diminished adrenergic responsiveness.

In the canine model of low-output heart failure, the physiological responsiveness to stimulation with
dobutamine is markedly impaired. This assessment was made using the contractile response to dobutamine as determined by peak LV +dP/dt. In humans, infusion of dobutamine, a nonselective β-agonist with mild α-agonist properties, has been shown to be a useful pharmacological probe of in vivo β-adrenergic receptor sensitivity. The advantage of a nonselective β-agonist is that it may provide a better approximation of the full β-adrenergic response of the myocardium that depends on both β₁- and β₂-receptors for maximal inotropic response. Heart rate was controlled in the present study by pacing. Although ventricular dimensions were not assessed directly, the responses of arterial pressure and end-diastolic pressure to dobutamine were similar in each state, suggesting that the changes in loading conditions were not a principal determinant of impaired responsiveness. To minimize the cardiovascular effects of anesthesia, the dogs were studied with agents associated with minimal cardiac effects.

To further study the mechanisms underlying the decreased β-adrenergic–mediated functional responsiveness, the ability of isoproterenol to stimulate adenylate cyclase was examined in membranes prepared from both control and failing hearts. Maximal isoproterenol-stimulated adenylate cyclase activity in the failing heart was ~40% of that in the controls. Potential mechanisms underlying the decreased β-adrenergic–mediated contractile response in intact dogs and the diminished β-adrenergic–mediated activation of adenylate cyclase in membranes include 1) downregulation of the β-receptor, 2) decreased levels of G₉, 3) increased levels of G₁, and 4) defects in the adenylate cyclase itself.

The density of β-adrenergic receptors was reduced by 40% in failing canine LV compared with control LV, whereas the affinity for the antagonist [¹²⁵I]ICYP was unchanged. Subtype analysis revealed a selective decrease in the density of β₁-receptors with no change in the density of β₂-receptors. The reduction in density was unlikely to be due to nonspecific changes in the membrane, as neither the density of binding sites for dihydropyridine nor levels of G₉ showed differences between control and experimental dogs. The selective downregulation of β₁-receptors is reminiscent of changes described in the failing human heart. The reduced density of β-receptors in the membranes from failing dogs was associated with a proportional loss in maximal isoproterenol-stimulated adenylate cyclase activity.

G proteins were investigated with several techniques. The assessment of G₁ by immunochemical quantification using antisera and pertussis toxin–catalyzed ADP-ribosylation labeling revealed modest increases of 34% and 28%, respectively, in failing canine hearts compared with control hearts. Since the pertussis toxin–mediated labeling of the G₁-like proteins provides information on the G protein available for ADP-ribosylation, it has been suggested that the toxin labeling may represent a functional property of the G protein. Nevertheless, the increase in the level of G₁ by immunoblotting and enhanced pertussis toxin labeling of the protein suggest an increased amount and/or

![Figure 5](image.png) Graph showing reconstitution of left ventricular membranes with S49 cyc- membranes. Cardiac membranes (2.5 mg/ml) were solubilized in 0.2% Lubrol detergent. After centrifugation, 25, 50, and 100 μg solubilized membranes and 60 μg cyc- membranes were incubated for 20 minutes at 30°C with 10 mM NaF. Adenylate cyclase activity was determined as described in “Materials and Methods” (n=6 control dogs and n=6 heart failure dogs).

**Table 3. Adenylate Cyclase Activity in Control and Failing Canine Hearts**

<table>
<thead>
<tr>
<th>Group</th>
<th>Basal (with GTP)</th>
<th>Net Iso</th>
<th>Net NaF</th>
<th>Net forskolin</th>
<th>Net Mn²⁺</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>7.3±0.5</td>
<td>144±18</td>
<td>61.4±9.8</td>
<td>158±21.5</td>
<td>257±27</td>
</tr>
<tr>
<td>CHF</td>
<td>5.3±0.6*</td>
<td>58±10*</td>
<td>36.7±5.2*</td>
<td>81.6±8.0*</td>
<td>201±7.8*</td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=6, except n=4 for Mn²⁺); all assays were performed in triplicate. Iso, Isoproterenol; Control, membranes from nonpaced dogs; CHF, membranes from paced dogs with congestive heart failure.

*p<0.05 vs. Control.
Figure 6. Graph showing activities of adenylate cyclase elicited by GTP-γ-S-activated Gs (GTP S Gs). Cardiac membranes (30 μg) from six control and six heart failure dogs were incubated with GTP-γ-S-activated Gs purified from rabbit liver (40 μg/ml). Adenylate cyclase was determined as described in "Materials and Methods." Each point represents the mean±SEM of specific activity of adenylate cyclase determined in triplicate (*p<0.01).

Function of the Gs in membranes of animals with heart failure. At least three isofoms of Gs have been shown to exist, with the predominant Gsα in dogs being Gsα-1, and that in humans being Gsα-3.42 Shifts in expression of isofoms of Gsα may occur in heart failure. Although Gsα was shown to increase in the failing paced canine heart, isofom shifts could not be detected with the use of a nonspecific antibody nor after pertussis toxin labeling, which measures only the total ribosylatable substrate.

The functional activity of Gs was assessed by an in vitro complementation assay using membranes prepared from cys-549 lymphoma cells that are genetically deficient in Gsα. The equivalent abilities of extracts from failing and normal hearts to reconstitute fluoride-stimulated activities suggest that the levels of Gs are similar in the two types of membranes. Immunochemical quantification of Gs was not performed. The possibility remains, however, that in the present study, differential extraction of Gs by cholate or the influence of extrated factors other than Gsα may have restored activity and obliterated differences in Gs activity.

A defect beyond the β-receptor was evidenced in the measurement of adenylate cyclase activity in failing and control dogs in the presence of various pharmacological agents. Basal adenylate cyclase activity as well as activity in the presence of fluoride and forskolin (without GTP) were significantly reduced in failing dogs. Since both fluoride and forskolin require Gs for maximal adenylate cyclase stimulation, this would imply that levels or function of Gs are decreased, that there is an increase in Gsα or, perhaps more distally, that there is a defect in the catalytic moiety of adenylate cyclase itself. Although results of reconstitution suggest normal Gsα bioactivity, a defect in Gs may in fact still be present. There is evidence that both fluoride and forskolin stimulate adenylate cyclase by a mechanism that is independent of dissociation of αβγ-subunits of Gs.4,4 The defect in the failing canine heart may be manifest only when Gs is in a heterotrimeric form and not dissociated as occurs during reconstitution. Elevated levels of Gsα may also contribute to the enhanced inhibition of Gs and thus of adenylate cyclase.

An abnormality in the catalytic moiety of adenylate cyclase may also contribute to impaired production of cAMP. Evaluation of the catalytic subunit is difficult in crude membranes where contamination with G proteins invariably occurs. In the pressure overload-induced model of canine heart failure, a postreceptor defect at the level of the catalytic unit independent of changes in Gs has been shown to occur.44 The impaired stimulation by Mn-i2 is consistent with a defect in the catalytic unit in this model as well. The markedly reduced stimulation of adenylate cyclase activity by forskolin in the failing heart may represent the combined effect of decreased Gs and catalytic activity or may be secondary to a decreased level of the catalytic unit or an impairment in the ability of the catalytic unit to interact with Gs. The functional integrity of the catalyst was further assessed by measuring enzyme activity elicited with purified, GTP-γ-S-activated Gsα. The results suggest that the level of stimulation of adenylate cyclase activity was significantly reduced in the failing myocardium and strengthen the argument that the activity of the catalytic subunit is abnormal and independent of changes in G proteins.3

The alterations in the regulation of cardiac adenylate cyclase in the pacing model of congestive heart failure differ from those described in the well-studied genetic animal model of cardiac failure, the myopathic Syrian hamster. Feldman et al3 and Kessler et al45 have shown evidence that decreased bioactivity of the Gs may contribute to the contractile abnormalities seen in this model at an early preneocrotic, nondilated stage. The Gs and catalytic unit do not appear abnormal. In our canine model, despite obtaining tissue at a late, dilated stage of the disease process when nonspecific responses to heart failure may be evident, we were unable to show evidence of altered Gsα bioactivity. In contrast to the changes found in the Syrian hamster model, we demonstrated an impaired Mn2+ -stimulated adenylate cyclase activity, suggesting a defective catalytic subunit as well as an increase in the Gsα. The mechanism of these acquired defects remains unclear.

Changes in the β-adrenergic complex of the canine model of low-output heart failure have multiple similarities to the changes observed in human idiopathic cardiomyopathy including 1) a diminished responsiveness to stimulation with β-agonists as assessed by the contractile response to dobutamine in intact animals and by a decreased level of isoproterenol-stimulated adenylate cyclase, 2) a selective decrease in β1-adrenergic receptors with a proportional loss of maximal isoproterenol-stimulated adenylate cyclase activity, and 3) postreceptor alterations in-
cluding increases in G, and unchanged bioactivity of G as measured by in vitro complementation assays. In contrast to canine heart failure, however, the chronically paced canine heart manifests a decreased activity of adenylate cyclase, suggesting a defect in the structure and/or function of the catalytic unit.

In conclusion, this model provides a useful system with which to investigate changes in the β-receptor–adenylate cyclase system using a preparation that is rapidly produced, stable, and free of the pharmacological effects of heart failure therapies that can alter components of the complex and are inherent in studies with human tissue. Further studies on the structure and function of the defective cardiac adenylate cyclase are warranted. This initial investigation cannot ascertain whether the change in G proteins or postreceptor alterations was an acquired defect or a nonspecific response to heart failure. In an attempt to elucidate mechanisms by which postreceptor changes occur, studies should be directed at differential tissue expression and developmental regulation of G protein subunits in the failing ventricle at various stages of heart failure development.

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K P Marzo, M J Frey, J R Wilson, B T Liang, D R Manning, V Lanoce and P B Molinoff

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