Dysfunction of the $\beta$- and $\alpha$-Adrenergic Systems in a Model of Congestive Heart Failure

The Pacing-Overdrive Dog

Angelino Calderone, Michel Bouvier, Kai Li, Carl Juneau, Jacques de Champlain, and Jean-Lucien Rouleau

The functional integrity of the $\beta$- and $\alpha$-adrenergic stimulatory pathways in a rapid ventricular pacing model of congestive heart failure in dogs was investigated; normal dogs served as controls. Total $\beta$-adrenergic receptor density was 35% lower ($p<0.01$) in the pacing-overdrive dogs, and the $\beta$-adrenergic receptor–mediated stimulation of adenylyl cyclase ($V_{\text{max}}$) was found to be 68% and 72% lower ($p<0.01$) in the left and right ventricles of the paced dogs. In addition, the basal adenylyl cyclase activity was found to be 56% and 68% lower ($p<0.01$) in the left and right ventricles of the failing heart. Similarly, the $V_{\text{max}}$ of 5'-guanyllylimidodiphosphate (Gpp(NH)p) and forskolin stimulation of adenylyl cyclase activity was significantly lower, 70% and 55%, respectively ($p<0.01$), in both ventricles of the paced dogs. However, although the concentration yielding half-maximal velocity for $\beta$-agonist and Gpp(NH)p stimulation of adenylyl cyclase was similar in both groups, that for forskolin stimulation of the enzyme was significantly increased ($p<0.01$). Pertussis toxin–mediated ADP-ribosylation of membranes from control and failing hearts revealed a significant decrease in the inhibitory guanine nucleotide binding protein content (48±9%, $p<0.01$) in the hearts of the paced dogs. Moreover, although the pertussis toxin treatment increased the basal and the forskolin-stimulated adenylyl cyclase activity in both normal and failing heart membranes, the adenylyl cyclase activity remained significantly depressed in the failing heart after pertussis toxin treatment ($p<0.01$). Consistent with the depressed adenylyl cyclase activity, mechanical studies on isolated papillary muscles and trabeculae revealed a decrease in baseline total tension (from 7.0±0.7 to 3.8±0.4 g/mm², $p<0.01$) and $dT/dt$ (from 26±8 to 13±1 g/mm²/sec, $p<0.01$) in the pacing-overdrive model. Tension generation and $dT/dt$ observed in the paced dogs in response to increasing concentrations of forskolin demonstrated a rightward shift in the dose–response curve and a decrease in maximal forskolin stimulation ($p<0.01$). Similarly, maximal tension and $dT/dt$ in the presence of isoproterenol was significantly lower than in the normal dogs ($p<0.01$). The decrease in $\beta$-adrenergic responsiveness was accompanied by a decrease and rightward shift in $\alpha_1$-adrenergic responsiveness (increase in tension was 1.1±0.1 g/mm² in paced dogs versus 2.1±0.1 g/mm² in controls, $p<0.01$). This decrease in $\alpha_1$-adrenergic responsiveness occurred despite no change in receptor density, suggesting that the abnormality resides beyond the receptor. We suggest that a decrease in adenylyl cyclase reactivity contributes to the blunted $\beta$-adrenergic response to catecholamine stimulation in the pacing-overdrive model of heart failure. These abnormalities to the $\beta$-adrenergic signal transduction pathway were accompanied by a decrease in activity of the membrane-bound enzyme Na⁺,K⁺-ATPase ($p<0.01$). These defects may be a reflection of more widespread dysfunction of sarcolemmal-bound enzymes and may play a role in the decrease in contractility and the development of heart failure in this model. (Circulation Research 1991;69:332–343)
A decrease in inotropic response to \( \beta \)-adrenergic stimulation has been documented in human and experimental end-stage heart failure.\(^1\)\(^-\)\(^4\) However, the mechanism suggested to explain this phenomenon differs according to the model being studied. In end-stage human heart failure, \( \beta \)-adrenergic receptor downregulation has been suggested as playing a major role.\(^1\) In an experimental model of heart failure induced by pressure overload, an increase in the total \( \beta \)-adrenergic receptor population has been associated with a selective decrease in high-affinity receptors,\(^3\) thus suggesting a decrease in the coupling of the receptor with the stimulatory guanine nucleotide–binding protein, \( G_s \). Recent reports\(^5\)\(-\)\(^9\) have suggested that changes in the stimulatory and inhibitory binding proteins, \( G_s \) and \( G_i \), respectively, contribute to the diminished cAMP response. Indeed, a decrease in myocardial \( G_s \) content has been shown in a model of left ventricular hypertrophy and in lymphocytes of patients with end-stage heart failure.\(^6\)\(^-\)\(^7\) Furthermore, an increase in \( G_s \) content has been reported in patients with idiopathic cardiomyopathy.\(^8\) Finally, adenylate cyclase activity has been found to be variable, with some studies demonstrating a decrease in basal activity\(^9\) and others demonstrating a normal basal and stimulated adenylate cyclase activity.\(^9\) Thus, the cause of the decreased \( \beta \)-agonist responsiveness of the failing myocardium appears to be multifactorial and variable according to the model being studied.

What happens to myocardial \( \alpha \)-adrenergic responsiveness in heart failure has been less well documented. In all but one study, in which \( \alpha \)-adrenergic receptor density was found to be increased,\(^10\) receptor density was normal.\(^11\)\(^-\)\(^13\) When receptor-mediated inositol 1,4,5-trisphosphate (IP\(_3\)) studies were done,\(^12\) the generation of IP\(_3\) was also found to be normal. Nevertheless, one recent study in cardiomyopathic hamsters suggests an increase in \( \alpha \)-adrenergic–mediated effects on \( [Ca^{2+}]_c \) and contractility. These effects were found to be mediated by a peritissus toxin–sensitive G protein or proteins and to occur despite normal \( \alpha \)-adrenergic receptor density.\(^13\) However, because an increase in cardiac sympathetic tone and enhanced tissue adrenergic sensitivity are thought to be important factors in the pathogenesis of the hereditary cardiomyopathy itself,\(^14\) it may be difficult and inappropriate to extrapolate these results to other species or models of heart failure.

In the present study, an animal model of low-output heart failure induced by rapid ventricular pacing, a model likened to heart failure due to chronic tachycardia in humans, was examined. This model is characterized by a decrease in cardiac output\(^15\)\(^,\)\(^16\) and an increase in ventricular filling pressures.\(^17\) There is marked ventricular dilatation but no hypertrophy, and light microscopy studies reveal few histological changes. As in most models of heart failure, plasma catecholamines are elevated and myocardial catecholamines are depleted.\(^15\)\(^-\)\(^17\) In our study, the contributions of the \( \beta \)-adrenergic receptor, \( G_s \), and adenylate cyclase to the diminished myocardial contractility of this model of heart failure were investigated. Furthermore, the negative influence of \( G_i \) on the \( \beta \)-adrenergic stimulatory pathway was studied. Finally, an evaluation was made of \( \alpha \)-adrenergic receptor density and responsiveness.

### Materials and Methods

**Materials**

Chemical reagents used in the radioligand binding and cAMP studies were obtained from Sigma Chemical Co., St. Louis, and Fisher Scientific Co., Ottawa, Canada, unless otherwise indicated. Chemical reagents used for the sodium dodecyl sulfate–polyacrylamide gel were obtained from Bio-Rad Laboratories, Mississauga, Canada.

**Methods**

Thirty-six mongrel dogs weighing between 25 and 30 kg were paced until overt evidence of heart failure occurred (the pacing-overdrive model); another 36 similar dogs served as controls (normal). Although these control dogs did not have sham operations, they were kept alongside the paced dogs and were housed, fed, and cared for in a similar fashion. The pacemakers were introduced into the pacing-overdrive dogs under general anesthesia that was induced with thiopental (25 mg/kg) and maintained with halothane while the dogs were being mechanically ventilated. The right internal jugular vein was isolated and distally ligated through a right paramedian cervical incision under sterile conditions. A bipolar pacemaker electrode was then inserted through a small incision in the vein, and the tip was positioned and secured in the right ventricular apex under fluoroscopic guidance. The pacemaker (multiprogrammable pulse generator, Medtronic Inc., Minneapolis, Minn.) was inserted in a cervical subcutaneous pocket, and the rate was set at 30 stimuli/min. After recovery from surgery (3 days), the rate was set to 250 stimuli/min, and the capture rate was assessed daily by electrical recording and heart rate measurements. The pacing was continuously maintained until the development of overt heart failure occurred. The symptoms included ascites, fatigue, shortness of breath, and a lack of appetite. An average weight gain of 10% had occurred in the paced dogs before death.

Control and pacing-overdrive dogs were anesthetized with thiopental (25 mg/kg) and ventilated mechanically. After a paramedical incision, the heart was removed and immediately placed in an oxygenated Krebs-Henseleit solution at 4°C. The left and right ventricles were opened, and either a papillary muscle and/or a trabecula was removed for the mechanical studies. The remaining tissue was used for biochemical analysis of the integrity of the \( \beta \)-adrenergic pathway, \( \alpha \)-adrenergic receptor density, and Na\(^+\),K\(^+\)-ATPase activity.
At the time of death, a 1-ml blood sample was taken and mixed with 20 μl of 0.25 M EGTA and 0.2 M glutathione (pH 7) for plasma catecholamine measurements.

**Preparation of Myocardial Membranes**

The tissues were isolated from the left and right ventricular free walls and minced in 30 ml buffer A, which contained 0.25 M sucrose, 5 mM Tris-HCl, 1 mM MgCl₂, 1 mM EDTA, and 10 μM phenylmethylsulfonyl fluoride (PMSF, pH 7.4) at 4°C. The samples were then homogenized with a polytron (three bursts of 5–7 seconds at maximum speed, Brinkmann T, Brinkmann Instruments, Inc., Westbury, N.Y.). The homogenate was filtered through three layers of cheesecloth and centrifuged at 1,000g for 10 minutes at 4°C. The supernatant was removed and centrifuged at 45,500g at 4°C for 25 minutes. The supernatant was discarded, and the pellet was resuspended in 30 ml buffer B, which contained 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM EDTA, and 10 μM PMSF, with five strokes in a 40-ml dounce-type puffer and centrifuged at 45,500g at 4°C for 25 minutes. This step was repeated twice, and the pellet was resuspended in an appropriate volume of buffer B to obtain a protein content of 1 mg/ml. Recovery of membrane protein was 3.5% and 4% for the pacing-overdrive dogs and control dogs, respectively. Protein content was determined by the method of Lowry et al.¹⁸

α₁- and β-Adrenergic Receptor Binding Studies

The radioligand [7-methoxy-³H]prazosin (82 Ci/mmol) and (-)[³H]dihydroalprenolol ([³H]DHA, 31.3 Ci/mmol), both obtained from New England Nuclear Corp., Boston, were used to measure α₁-adrenergic¹⁹,²⁰ and β-adrenergic²¹,²² receptor density, respectively. Duplicate aliquots of membrane preparations (90–110 μg/100 μl) were incubated for 20 minutes at 30°C with varying concentrations of [7-methoxy-³H]prazosin (0.025–1.0 nM) or [³H]DHA (0.5–15 nM) in buffer B to give a total volume of 150 μl. Nonspecific binding was determined in the presence of the β-antagonist alprenolol (10 μM) or the α-antagonist phenolamine (50 μM) (CIBA-GEIGY Corp., Summit, N.J.). The incubations were terminated by a rapid dilution of the assay mixture with 1 ml cold buffer B, followed by a rapid vacuum filtration through Whatman GF/C glass fiber filters (Whatman Inc., Clifton, N.J.). The filters were rapidly washed four times using 3-ml aliquots of cold buffer B. After the filters were dried, the radioactivity was counted in 5 ml scintillation fluid (liquifluor PPO-POPOP toluene concentrate, New England Nuclear). Specific binding to the α₁-adrenergic receptor population was defined as the difference between the total amount of radioactivity bound in the presence of [7-methoxy-³H]prazosin alone and the nonspecific binding in the presence of [7-methoxy-³H]prazosin and 50 μM phenolamine; 60–70% specific binding was routinely obtained. Similarly, the total β-adrenergic receptor population was defined as the difference between the total amount of radioactivity bound in the presence of [³H]DHA alone and the nonspecific binding in the presence of [³H]DHA and 10 μM unlabeled (−)-alprenolol; 70–80% specific binding was routinely obtained. Receptor density and the equilibrium dissociation constant Kᵦ for [7-methoxy-³H]prazosin and [³H]DHA binding to membrane preparations were assessed by a Scatchard analysis. Four additional control and four additional paced dogs had β-adrenergic receptor studies done with [¹²⁵I]iodocyanopindolol ([¹²⁵I]CYP). Binding of β-adrenergic receptors with [¹²⁵I]CYP was measured by incubating 10 μl membrane preparation (1 mg/ml) with eight concentrations of radioligand (10–300 pmol) at 25°C for 2 hours in a total volume of 250 μl. Nonspecific binding was determined in the presence of 20 μM (−)-alprenolol. The specific binding routinely obtained was 80%. Termination of binding and washing of filters were done as described above.

**Measurement of Adenylate Cyclase Activity in Membrane Preparations**

The membrane preparations used for receptor analysis were also used to study adenylate cyclase activity. Aliquots of 100–125 μg protein/100 μl were used to assess cAMP production as previously described²³ with the following modifications. Aliquots were incubated at 37°C for 5 minutes in a total volume of 200 μl containing 25 mM Tris-HCl, 5 mM MgCl₂, 0.5 mM EDTA, 5 μM PMSF (pH 7.5), 1 mM ATP, 0.1 mg/ml creatine kinase, and 2 mM creatine phosphate in the presence or absence of the phosphodiesterase inhibitor theophylline (1.4 mM). Dose-response curves for the stimulation of adenylate cyclase activity via the G, binding protein were obtained with the nonhydrolyzable GTP analogue 5'-guanylylimidodiphosphate (GppNHp) dissolved in buffer B. Dose-response curves of the direct stimulation of the adenylate cyclase were obtained with forskolin dissolved at a concentration of 100 mM in 100% ethanol. Catecholamine stimulation was done in the presence of 1 mM ascorbic acid, 5.0×10⁻³ M GTP, and 0.7 mM theophylline. The reactions were terminated by placing the tubes in a boiling water bath for 2 minutes. The tubes were then centrifuged at 6,500g for 2 minutes in a microcentrifuge (model 5413, Eppendorf Inc., Fremont, Calif.). The supernatant was removed and frozen at −80°C for later estimation of the cAMP content. cAMP levels were determined by a radioligand binding assay, which involves the competition between unlabeled cAMP and a fixed quantity of tritium-labeled cAMP for binding to a protein that has a high specificity and affinity for cAMP (Amersham, Oakville, Canada).²⁴

[¹²⁵I]ADP-Ribosylation of the Inhibitory Guanine Nucleotide Binding Protein Gi

Membrane preparations (1.25 mg protein/ml) were stirred at 4°C for 60 minutes in the presence of 0.7% lubrol PX. The mixture was then centrifuged, and the supernatant (detergent extract) was used for ADP-
ribosylation. ADP-ribosylation was performed in a 50-μl solution containing 25 mM Tris-HCl, 2.5 mM MgCl₂, 0.5 mM EDTA, 5 μM PMSF (pH 7.4), 5 mM thymidine, 1 mM ADP-ribose, 1 mM NAD, 0.1 mM GTP, 0.5 mM ATP, 0.1 mg/ml creatine kinase, 2 mM creatine phosphate, 5 mM dithiothreitol, 50 μM NAD ([³²P]NAD, 10,000 cpm/pmol) (all from New England Nuclear), 2.5 μg activated pertussis toxin (preactivated with 50 mM dithiothreitol for 20 minutes at 30°C), 0.25% lubrol PX, and 17.0 μl detergent extract. The reaction was initiated by the addition of [³²P]NAD and allowed to proceed for 60 minutes at 30°C. The reaction was terminated by the addition of 50 μl of sample buffer (0.25 mM Tris-HCl at pH 6.5, 8% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and 0.5% bromophenol blue). Samples were then loaded on a 12.5% sodium dodecyl sulfate–polyacrylamide gel. The gel was then washed in the presence of 30% ethanol, 10% acetic acid, and ionic exchange resin, dried, and then subjected to autoradiography at −80°C for 24–72 hours. The extent of [³²P]NAD labeling of the 40,000-Da band was determined with a densitometric analyzer (Pharmacia LKB Biotechnology, Piscataway, N.J.).

The pertussis toxin–catalyzed ribosylation of the pacing-overdrive membranes was done simultaneously with membranes derived from a control dog and was prepared in an identical fashion. The levels of the ADP-ribosylated G protein were then calculated as a percentage of the control in each experiment.

Adenylate Cyclase Activity in Pertussis Toxin–Treated Membranes

Membrane preparations were ADP-ribosylated as described above, except the lubrol extraction step was omitted. After the ribosylation period of 60 minutes, [³²P]ATP (1–2×10⁶ cpm/assay, New England Nuclear) was added, and basal and 10⁻⁴ M forskolin–stimulated adenylate cyclase activity was assayed for 30 minutes at 30°C. The [³²P]cAMP formed was separated as previously described by Salomon.²⁶ Control samples were treated in an identical fashion but with no toxin.

Na⁺,K⁺-ATPase Activity in Membrane Preparations

Freeze-thawed membrane preparations (1.0–1.25 mg protein/ml) were incubated in a medium containing (mM) histidine 50, NaCl 120, KCl 20, MgCl₂ 3, NaN₃ 5, EGTA 2, and ATP 5 (pH 7.4). Na⁺,K⁺-ATPase activity was evaluated as the difference between the activity in the absence and in the presence of 1 mM ouabain (Sigma). The reaction was performed at 37°C for 60 minutes, and the inorganic phosphate released from ATP was measured by the method of Fiske and Subbarow.²⁶

In Vitro Myocardial Mechanical Studies

While the heart was immersed in an oxygenated Krebs-Henseleit solution at 4°C, the left and right ventricles were opened, and either left or right ventricular trabeculae or papillary muscle located in the basal portion of the free wall was dissected and mounted in an isolated bath. Only muscles free from the ventricular wall for at least 4 mm with a cross-sectional area of <1.2 mm² were used for this study. In previous studies²⁷ we have shown that there was no difference in total tension (T) or dT/dt between left and right ventricular muscles and that muscles from both ventricles responded similarly to β-adrenergic stimulation. Twenty-nine muscles (15 from the right and 14 from the left ventricle) were obtained from normal dogs, and 31 muscles (18 from the right and 13 from the left ventricle) were obtained from the pacing-overdrive dogs. Two muscles were obtained from one control dog and from each of two pacing-overdrive dogs. The base of the muscle was held by a stainless-steel clamp, and the other end was tied to a prototype lever with an electromagnetic feedback system identical to that described by Brutsaert et al.²⁸ The muscles were bathed in Krebs-Henseleit solution containing (mM) NaCl 118, KCl 3.5, MgSO₄ 2.43, CaCl₂ 1.25, KH₂PO₄ 1.2, NaHCO₃ 24.9, and dextrose 5 and bubbled with 95% O₂–5% CO₂ at a pH of 7.4 and a temperature of 29°C. The muscles were stimulated through platinum field electrodes with a stimulator (model S-88, Grass Instrument Co., Quincy, Mass.) at 6 stimuli/min until the stimulation was 10% above the threshold level. In all the experiments, the preload on the muscle was adjusted so that the initial muscle length was at maximum length. The muscle was then stabilized at maximum length for 3 hours, and an isometric contraction was recorded at 100 mm/sec on a recorder (Brush 200, Gould, Cleveland, Ohio).

Protocol A. A baseline isometric contraction was recorded for each of five muscles from the control dogs and 10 from the pacing-overdrive dogs. Ethanol (0.5% [concentration in maximal forskolin dose]) was then added to the bath to assess the effect on T and dT/dt. Fifteen minutes later, a second isometric contraction was recorded. The bath was washed, and a third isometric contraction was recorded. There were no differences between the three contractions. Forskolin was then added to the bath in increasing concentrations (from 0.025 to 5 μM), with isometric contractions being recorded after the muscle had been allowed to stabilize for at least 30 minutes at each concentration.

Protocol B. Once the baseline isometric contractions were recorded for the muscles from 15 control and 12 pacing-overdrive dogs, isoprotenerol was added in increasing concentrations (from 0.01 to 20 μM). The isometric contractions were recorded at peak effect of each concentration.

Protocol C. Once the baseline contractions were recorded for the muscles from eight control and nine pacing-overdrive dogs, phenylephrine, in the presence of 5 μM propranolol, was added in increasing concentrations (from 0.1 pM to 0.1 mM). An isometric contraction was recorded at each concentration. Pilot studies had demonstrated that prazosin could completely inhibit the inotropic response of phenylephrine,
indicating that the positive inotropic effect of phenylephrine was mediated via α1-adrenergic receptors.

**Plasma Catecholamine Measurements**

Plasma catecholamines were measured by the radioreceptor assay of Peuler and Johnson,29 with a few minor modifications, including addition of 2N perchloric acid (1:9) to deproteinize the plasma and a doubling of the buffer capacity of the reactive mixture.

**Analysis of Data**

Determinations of the apparent concentration of the specific agent at which a given enzyme yields one half of its maximum velocity (K<sub>a</sub>) and the maximal rate of conversion of ATP to cAMP (V<sub>max</sub>) for adenylate cyclase activation by the different agents were made with a linear regression analysis of the double reciprocal of 1/(V<sub>0</sub>−V<sub>i</sub>) versus 1/S, where V<sub>i</sub> is the adenylate cyclase activity in the absence of the agent and V is the adenylate cyclase activity in the presence of the agent at concentration S.30 Double reciprocal plots have a slope equal to K<sub>a</sub>V<sub>max</sub> and an intercept of 1/V<sub>max</sub>. With respect to forskolin, the solubility problems encountered with this agent did not allow us to reach a plateau at the highest dose tested (10<sup>−4</sup> M); therefore, maximal response was assumed to occur at 0.1 mM, as previously described.9,30

**Statistics**

Values are expressed as mean±SEM. The effects of forskolin, GppNHP, and isoproterenol at each concentration were compared to baseline by a two-way analysis of variance, followed by a Dunnett’s test. The control and pacing-overdrive model dose–response curves were compared by a one-way analysis of variance. Student’s unpaired t test was used to determine the statistical difference of the parameters between normal and pacing-overdrive dogs found in Tables 1–6 and in Figure 3.

**Results**

Evidence of heart failure developed after 4–7 weeks of pacing. At autopsy, all of the pacing-overdrive dogs had ascites, pleural effusions, and marked cardiac dilatation (Table 1). However, despite obvious ventricular dilatation, there was no cardiac hypertrophy, as evidenced by the similar heart weight/body weight ratio between control and heart failure dogs (Table 1). Furthermore, plasma norepinephrine was found to be elevated in the dogs with heart failure (Table 1).

**Radioligand Binding Studies**

Specific binding of [3H]DHA to canine cardiac membrane preparations is shown in Figure 1. The β-adrenergic receptor density was decreased by 35% (p<0.001) in the myocardium from the left and right ventricles of the pacing-overdrive dogs (Table 2). No difference in the equilibrium dissociation constant (K<sub>d</sub>) for [3H]DHA binding was observed between the two groups (Table 2). Qualitatively, similar results were observed with the β-adrenergic antagonist [125I]CYP. B<sub>max</sub> for pacing-overdrive dogs was significantly decreased as compared with controls (220±26 versus 396±70 fmol/mg protein, respectively). α<sub>1</sub>-Adrenergic density was unchanged in myocardium from the ventricles of pacing-overdrive dogs (Table 3). Also, no difference in K<sub>d</sub> for [3H]prazosin binding was observed between the two groups (Table 3).

**Basal Adenylate Cyclase Activity**

Basal adenylate cyclase activity, as measured by the cAMP accumulation (see “Materials and Methods”), was decreased in both the left (56%) and right (68%) ventricles of the pacing-overdrive dogs (Table 2). When the phosphodiesterase inhibitor theophylline was added to the bath, net cAMP increased in both groups but remained significantly lower in the cardiac membrane preparations obtained from the pacing-overdrive dogs (Table 2).
TABLE 2. Basal Adenylate Cyclase Activity and β-Adrenergic Receptor Studies Done on Canine Cardiac Membrane Preparations

<table>
<thead>
<tr>
<th></th>
<th>Basal adenylate cyclase activity (pmol cAMP/mg protein/min)</th>
<th>Basal cyclase activity in the presence of 1.4 mM theophylline (pmol cAMP/mg protein/min)</th>
<th>β-Adrenergic receptor density (fmol/mg protein)</th>
<th>( K_d ) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>12</td>
<td>12</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>LV</td>
<td>50±3</td>
<td>215±14</td>
<td>123±3</td>
<td>7.2±0.3</td>
</tr>
<tr>
<td>RV</td>
<td>51±3</td>
<td>215±17</td>
<td>121±7</td>
<td>6.9±0.3</td>
</tr>
<tr>
<td>Paced dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>LV</td>
<td>22±3*</td>
<td>77±10*</td>
<td>80±10*</td>
<td>7.5±0.5</td>
</tr>
<tr>
<td>RV</td>
<td>18±2*</td>
<td>64±6*</td>
<td>80±11*</td>
<td>6.9±0.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. \( n \), Number of dogs in each group; LV, left ventricle; RV, right ventricle. *\( p<0.01 \) vs. corresponding value in normal dogs.

Catecholamine Stimulation of Adenylate Cyclase Activity

Increasing concentrations of norepinephrine or isoproterenol increased adenylate cyclase activity in the membrane preparations of both control and paced dogs (Figure 2). There was no significant difference in the \( K_{act} \) of these agents between the two groups (Table 4). However, the \( V_{max} \) of norepinephrine- and isoproterenol-stimulated adenylate cyclase activity was found to be decreased by 70% in the membranes of the pacing-overdrive dogs (Table 5).

Stimulation of Adenylate Cyclase Activity by GppNHp

The \( G_s \)-mediated stimulation of adenylate cyclase was assessed using GppNHp, a nonhydrolyzable GTP analogue. Increasing concentrations of GppNHp stimulated an increase in cAMP production in both groups. There was no significant difference in \( K_{act} \).
TABLE 4. Concentration Yielding Half-Maximal Velocity for cAMP Generation in Canine Membrane Preparations

<table>
<thead>
<tr>
<th></th>
<th>Forskolin</th>
<th>GppNHp</th>
<th>Norepinephrine</th>
<th>Isoproterenol</th>
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<tbody>
<tr>
<td></td>
<td>K_{cat} (μM)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>LV</td>
<td>2.5±0.3</td>
<td>1.5±0.3</td>
<td>5.0±0.3</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>RV</td>
<td>2.6±0.3</td>
<td>1.6±0.3</td>
<td>5.3±0.5</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>Paced dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>LV</td>
<td>4.0±0.6*</td>
<td>1.1±0.2</td>
<td>5.5±0.5</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>RV</td>
<td>4.6±0.6*</td>
<td>1.2±0.2</td>
<td>6.3±0.6</td>
<td>4.0±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM. K_{cat} concentration of the specific agent at which a given enzyme yields one half of its maximum velocity; GppNHp, nonhydrolyzable GTP analogue 5'-guanylimidodiphosphate; n, number of dogs in each group; LV, left ventricle; RV, right ventricle.

*p<0.05 vs. corresponding value for normal dogs.

between the dose–response curves of the two groups (Figure 2, Table 4); however, a 68% and 73% decrease in maximal GppNHp-promoted cAMP formation was found in the left and right ventricles, respectively, of the paced dogs (Table 5).

Forskolin-Stimulated Adenylate Cyclase Activity

The forskolin dose–response curves of adenylate cyclase stimulation for both the right and left ventricles of the pacing-overdrive dogs were shifted to the right such that K_{cat} was significantly increased (Table 4, Figure 2). V_{max} for forskolin stimulation of adenylate cyclase activity was also reduced in the pacing-overdrive dogs (Table 5). Interestingly, the percent decrease in V_{max} stimulation with forskolin was similar to the reduction in basal adenylate cyclase activity.

Pertussis Toxin ADP-Ribosylation and Adenylate Cyclase Activity in Membrane Preparations

The level of G_{i}, as quantitated by pertussis toxin-promoted ADP-ribosylation with [32P]NAD was found to be 48±9% lower in the heart failure membranes as compared with control membranes (Figure 3). Nevertheless, despite these quantitative differences in G_{i} membrane content, pertussis toxin treatment of cardiac membrane preparations resulted in a similar increase in adenylate cyclase activity in both groups. The difference in basal and forskolin-stimulated adenylate cyclase activity between the control and pacing-overdrive dogs persisted after the pertussis toxin treatment (Figure 3).

Na^+,K^+-ATPase Activity

Na^+,K^+-ATPase activity was found to be 22% lower (p<0.05) in the ventricles of the pacing-overdrive dog (8.8 μmol P/mg protein/hr, n=11) as compared with normal (11.2 μmol P/mg protein/hr, n=11).

Mechanical Characteristics of the Isolated Papillary Muscle

There was no difference in muscle length (control, 6.0±1.0 mm; heart failure, 5.8±1.0 mm) or cross-sectional area (control, 0.80±0.3 mm²; heart failure, 0.76±0.3 mm²) between the two groups. Muscles from the pacing-overdrive dogs produced a significantly lower total tension (3.7±0.4 versus 7.1±0.6 g/mm², p<0.01) and had a decrease in dT/dt (13.1±1.2 versus 26.8±2.9 g/mm²/sec, p<0.01). Increasing concentrations of forskolin failed to normalize these values (Table 6). Furthermore, as in the membrane preparations, the forskolin dose–response curve was shifted to the right in the muscles of the pacing-overdrive dogs (Figure 4). Increasing concentrations of isoproterenol also failed to normalize tension and dT/dt (Table 6), but there was no shift in the dose–response curve to isoproterenol (Figure 4). Increasing concentrations of phenylephrine (in the presence of 5 mM propranolol) caused less of an increase in tension in the muscles of the pacing-overdrive dogs (Figure 5). Also, as was the case with forskolin, the dose–response curve to phenylephrine was shifted to the right (Figure 5).

Discussion

Various abnormalities in the β-adrenergic stimulating pathway of adenylate cyclase were observed in the myocardium of the pacing-overdrive dog model of heart failure. Indeed, we have shown here that this

TABLE 5. Maximum cAMP Generation in Canine Membrane Preparations

<table>
<thead>
<tr>
<th></th>
<th>Forskolin</th>
<th>GppNHp</th>
<th>Norepinephrine</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V_{max} (pmol cAMP/mg protein/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>LV</td>
<td>2,004±233</td>
<td>389±57</td>
<td>271±21</td>
<td>312±37</td>
</tr>
<tr>
<td>RV</td>
<td>1,911±247</td>
<td>403±56</td>
<td>225±20</td>
<td>256±34</td>
</tr>
<tr>
<td>Paced dogs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>10</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>LV</td>
<td>939±174*</td>
<td>174±25*</td>
<td>88±17*</td>
<td>115±20*</td>
</tr>
<tr>
<td>RV</td>
<td>854±184*</td>
<td>116±15*</td>
<td>51±10*</td>
<td>60±10*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. V_{max}, maximal rate of conversion of ATP to cAMP; GppNHp, nonhydrolyzable GTP analogue 5'-guanylimidodiphosphate; n, number of dogs in each group; LV, left ventricle; RV, right ventricle.

*p<0.01 vs. corresponding value for normal dogs.
model is characterized by a decrease in basal and β-adrenergic, GppNHp, and forskolin-stimulated adenylate cyclase activity. The rightward shift in the forskolin dose–response curve of adenylate cyclase stimulation was paralleled by a similar shift in the potency of forskolin to increase tension and dT/dt in the papillary muscle preparations. Unexpectedly, this decrease in adenylate cyclase activity was associated with a decrease in G_i protein content, and pertussis toxin treatment failed to normalize enzyme activity. An important reduction (35%) in the density of the β-adrenergic receptor was also found in the membranes of the failing heart. In addition to this decrease in β-adrenergic signal transduction, a decrease and rightward shift in myocardial response to α_1-adrenergic stimulation was also found. However, as opposed to β-adrenergic receptors, there was no decrease in α_1-adrenergic receptor density.

In the pacing-overdrive dog model of chronic cardiac failure, the heart has been shown to dilate and fail without developing myocardial hypertrophy and with only minor histological abnormalities. It has been likened to the cardiomyopathy caused by chronic tachycardia in humans. As demonstrated in this study, the heart of the pacing-overdrive dog fails not only because of the increase in wall stress that occurs as a result of ventricular wall thinning but also because of an important decrease in the intrinsic contractile state of the myocardium. Indeed, baseline total tension and dT/dt as well as maximum stimulation of tension and dT/dt by forskolin, isoproterenol, or phenylephrine were significantly depressed as compared with control.

**β-Adrenergic Signal Transduction**

The decrease in basal cAMP generation in membrane preparations from the pacing-overdrive hearts suggests that an intrinsic defect in the cAMP-generating capacity of adenylate cyclase may be one of the reasons for the decrease in intrinsic myocardial contractility. It would appear that the decrease in basal cAMP generation was not due to an increased metabolism of cAMP, because the addition of the phosphodiesterase inhibitor theophylline did not

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**TABLE 6. Mechanical Characteristics of Canine Papillary Muscles and Trabeculae**

<table>
<thead>
<tr>
<th></th>
<th>Tension (g/mm²)</th>
<th>dT/dt (g/mm²/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>7±0.7</td>
<td>26±3</td>
</tr>
<tr>
<td>Forskolin (5 μM)</td>
<td>11±1.1</td>
<td>61±8</td>
</tr>
<tr>
<td>Isoproterenol (20 μM)</td>
<td>12±1.0</td>
<td>75±7</td>
</tr>
<tr>
<td>Paced dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>4±0.4*</td>
<td>13±1*</td>
</tr>
<tr>
<td>Forskolin (5 μM)</td>
<td>7±0.8†</td>
<td>35±4†</td>
</tr>
<tr>
<td>Isoproterenol (20 μM)</td>
<td>8±0.9*</td>
<td>57±6†</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of dogs in each group; dT/dt, rate of tension development.

*p<0.01 and †p<0.05 vs. corresponding value in normal dogs.
modify the cAMP ratio between groups. A decrease in reactivity of this membrane-bound enzyme has been found to occur in at least two other animal models of heart failure: the failing guinea pig and failing dog heart induced by pressure overload.\(^4,5\)

Consistent with this hypothesis of an intrinsic defect in the catalytic unit of adenylate cyclase was the observation that both the potency (\(K_{\text{act}}\)) and efficacy (\(V_{\text{max}}\)) of forskolin to stimulate adenylate cyclase were decreased in the failing heart. However, because of the solubility problems encountered with forskolin, a true \(V_{\text{max}}\) was not obtained, and because the calculation of \(K_{\text{act}}\) is dependent on \(V_{\text{max}}\), \(K_{\text{act}}\) was underestimated in both groups (see "Materials and Methods"). Since the functional studies of papillary muscles were performed in the same hearts as the adenylate cyclase assay and since the dose–response curve of forskolin stimulation for the muscles of the pacing-overdrive dogs clearly demonstrates a rightward shift and a decrease in maximal response, there is reason to believe that, in the membrane preparations, the rightward shift observed in the forskolin dose–response curve of cyclase stimulation would have persisted even if the true \(V_{\text{max}}\) had been obtained.

The reason for this decrease in intrinsic adenylate cyclase activity remains speculative, and numerous possibilities exist. One possibility was an increased \(G_i\) activity. Studies performed on the failing human heart have found \(G_i\) content and activity to be increased and that this increase in \(G_i\) activity was partially responsible for the diminished adenylate cyclase activity.\(^32\) In the present study, ADP-ribosylation of \(G_i\) by prior treatment of cardiac membrane preparations with pertussis toxin failed to normalize basal and forskolin-stimulated adenylate cyclase activity. Because ADP-ribosylation of \(G_i\) by pertussis toxin caused a similar increase in adenylate cyclase activity, despite a 48% decrease in \(G_i\) content, it is possible that \(G_i\) was qualitatively more active in the pacing-overdrive dog. In any case, it would appear that, as opposed to human heart failure, an increase in \(G_i\) activity was not significantly contributing to decreased adenylate cyclase activity. A second possible reason for the decrease in adenylate cyclase activity was an alteration in the intrinsic activity of the enzyme or a change in the surrounding microenvironment. Adenylate cyclase is a membrane-associated enzyme, which has been shown to be dependent on membrane lipids.\(^33,34\) In this study, a second sarcolemmal bound enzyme, the \(Na^+\cdotK^+\)-ATPase pump was found to have decreased activity. In a similar model of heart failure, numerous membrane bound enzymes were found to be abnormal.\(^35\) Thus, it is possible that the decrease in adenylate cyclase activity found in this study may reflect a generalized dysfunction of membrane bound enzymes.

Coupling of \(G_i\) and \(G_s\) with adenylate cyclase was assessed using the agonist GppNHP, a nonhydrolyzable GTP analogue. \(V_{\text{max}}\) for this reaction was lower for both ventricles of the pacing-overdrive dog as compared with the control. The percent decrease of \(V_{\text{max}}\) was similar to the decrease in basal and forskolin-stimulated adenylate cyclase activity. These findings suggest that the decrease in cAMP generation found with GppNHP stimulation could be due to the defect found in adenylate cyclase response. The
derived $K_{act}$ for GppNHp stimulation of cAMP generation was similar in both groups, suggesting a normal coupling between $G_{i}$ and adenylate cyclase in the pacing-overdrive dog.

There was also a significant decrease in isoproterenol-induced cAMP generation but with no shift in the dose–response curve in the membrane preparations from the pacing-overdrive dogs. Functional studies of the papillary muscle response from normal and failing hearts were consistent with this observation, as the maximal increase in tension and in $dT/dt$ induced by isoproterenol was also decreased with no rightward shift in the dose–response curve. That the decrease in cAMP and tension generation with isoproterenol was similar to that observed for forskolin stimulation suggests that the intrinsic defect in adenylate cyclase may represent the major reason for a poor response to $\beta$-adrenergic receptor–mediated stimulation.

Radioligand binding studies with $[^{3}H]$DHA or $[^{125}I]$CYP revealed a 35–44% decrease in total $\beta$-adrenergic receptor density in both ventricles of the pacing-overdrive dog. Using the ligand $[^{125}I]$CYP, Frey et al. found a similar decrease in $\beta$-adrenergic receptor density and noted that this decrease was limited to the $\beta_{1}$-adrenergic receptor population. In failing human myocardium, a decrease in $\beta$-adrenergic receptor density is accompanied by a parallel decrease in cAMP production. Indeed, in these studies, there was no decrease in cAMP generation when $G_{i}$ or adenylate cyclase was directly stimulated. However, in our study, the percentage decrease of $V_{max}$ found in the presence of isoproterenol was quantitatively similar to that with GppNHp and forskolin, despite the 35% decrease in receptor density in the pacing-overdrive model. Such a discrepancy between catecholamine-stimulated adenylate cyclase activity and the number of $\beta$-adrenergic receptors has been previously reported in various cell systems. The reason for this finding remains uncertain. One possible explanation could be the existence of spare receptors in dog ventricular tissue, a receptor population in excess of that required to attain maximal stimulation of the adenylate cyclase response. At this time, there is no evidence that there is a specific one-to-one relation between the loss of receptors and the loss of hormonal responsiveness in dog ventricles, and it is still unknown which of the three components (receptor, $G_{i}$, or adenylate cyclase) represents the limiting factor in the generation of cAMP. It is noteworthy that a downregulation of $\beta$-adrenergic receptor density was observed despite an intrinsic alteration of the activity of the adenyl cyclase catalytic unit. This would suggest that even in the presence of reduced adenylate cyclase reactivity, increased sympathetic tone can still result in elevated cAMP production in cells, which in turn could contribute to the downregulation of the receptor. Alternatively, cAMP-independent mechanisms, which have been suggested to contribute to agonist-mediated downregulation in the cell system, could also be involved in the downregulation observed in the present study.

$\alpha$-Adrenergic Responsiveness

In addition to a decrease in the $\beta$-adrenergic responsiveness and a rightward shift in the forskolin dose–response curve, a decrease and rightward shift of the $\alpha_{1}$-adrenergic dose–response curve was also found. These results appear to be at odds with previous reports of no change in IP$_{3}$ production or an increase in myocardial $\alpha_{1}$-adrenergic responsiveness in heart failure. However, $\alpha_{1}$-adrenergic receptor density and affinity were found to be normal in this...
study, and this is only the second study evaluating myocardial responsiveness to α1-adrenergic stimulation; other studies10–12 have limited themselves to measuring receptor density and IP3 production. As for the study of Sen et al13 in cardiomyopathic hamsters, the increase in α1-adrenergic responsiveness in this species may not be representative of heart failure in general, because increased α1-adrenergic responsiveness before the development of myocardial lesions has been documented by Böhm et al.42 Indeed, hyperresponsiveness to adrenergic stimulation has been postulated to be a cause of the cardiomyopathy in this species.14

Thus, it would appear that, at least in this model of heart failure, a defect in α1-adrenergic responsiveness exists distal to the receptor. A decrease in α1-adrenergic responsiveness may be beneficial in heart failure in at least two ways. First, decreased α1-adrenergic responsiveness would be expected to decrease the risk of calcium overload, much as decreased β-adrenergic pathway downregulation is thought to do. Also, decreased α1-adrenergic responsiveness would at least theoretically serve to decrease the myocardial relaxation abnormalities well documented in heart failure.33

Finally, it is possible that the decrease in enzyme activity in this study was the result of myocardial necrosis. However, there are at least four reasons to believe that this was not so. First, this is a reversible model of heart failure44 with few pathological changes.16 Second, both β-adrenergic36 and α1-adrenergic receptor density are normal, suggesting that, if anything, only an insignificant decrease in myocardial membrane recovery occurred. Third, the decrease in enzyme activity was marked, as much as 50% in some cases, much more than could be accounted for without widespread necrosis. And fourth, the decrease in enzyme activity was accompanied by a rightward shift in the dose–response curve, a finding that cannot be explained by loss of myocardial tissue.

This study has one limitation. Control dogs were housed and fed along with paced dogs, but they were not sham-operated. The insertion of a pacemaker is minor surgery that is done under local anesthesia in humans. In dogs, light general anesthesia is used to avoid movement during surgery. The thorax is never opened. Although possible, it is unlikely that such a minor procedure significantly modified the major changes in myocardial adrenergic function found 4–6 weeks later.

In conclusion, our model differs markedly from all previously described models of heart failure. It may represent the animal counterpart of human heart failure caused by chronic tachycardia. One of the major findings in the present study is not only a decrease in β- and α-adrenergic responsiveness but also a rightward shift in forskolin and α-adrenergic dose–response curves. The primary defect to the stimulatory β-adrenergic pathway in the pacing-overdrive dog model of heart failure appears to be an intrinsic defect in adenylate cyclase activity. Forskolin dose–response curves for membrane and isolated papillary muscle preparations were both shifted significantly to the right, and V max was decreased. Adenylate cyclase response to GppNHp and β-agonist stimulation were lower in both ventricles of the pacing-overdrive model than in the controls; however, the percent decrease was similar to that found in basal and forskolin-stimulated adenylate cyclase activity. Radioligand binding studies revealed a 35% decrease in total β-adrenergic receptor density; nevertheless, this decrease did not result in a further decrease in the isoproterenol-stimulated cAMP generation. This decrease in adenylate cyclase activity cannot be attributed to an increase in G1 activity, since ADP-ribosylation of G1 with pertussis toxin did not restore adenylate cyclase activity to normal in membrane preparations from the pacing-overdrive dogs. The decrease in adenylate cyclase activity could be partially explained by a generalized membrane dysfunction, because Na+,K+-ATPase activity was decreased and previous studies35 in this model have found numerous abnormalities in membrane-bound enzymes. The decrease in contractility of the myocardium from the hearts of the pacing-overdrive dogs paralleled the decrease in basal and stimulated cAMP generation in the membrane preparations. We suggest that a decrease in adenylate cyclase reactivity contributes to the decrease in catecholamine-mediated cAMP production in the pacing-overdrive model of heart failure. This decrease in β-adrenergic responsiveness is accompanied by a decrease in α1-adrenergic responsiveness. This occurs despite no change in α1-adrenergic receptor density and suggests that a defect in secondary messenger or beyond also occurs in this model. These defects may be a reflection of more widespread dysfunction of sarcolemmal-bound enzymes and may play a role in the decrease in contractility and the development of heart failure in this model.

References

Beta- and alpha-Adrenergic Dysfunction in the Pacing-Overdrive Dog


31. Morgan DE, Tomlinson CW, Qayumi AK, Toleikis PM, McCombelle B: Evaluation of ventricular contractility indices in the dog with left ventricular dysfunction induced by rapid atrial pacing. *J Am Coll Cardiol* 1989;14:489–495


**Key Words:** rapid ventricular pacing, congestive heart failure, adenylate cyclase, β-adrenergic receptors, α-adrenergic receptors, contractility
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