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# Differential changes of myocardial β-adrenoceptor subtypes and G-proteins in dogs with right-sided congestive heart failure

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**Abstract**

We have shown previously in dogs with right heart failure that the reduction of myocardial β-adrenoceptor density occurs only in the failing right ventricle, while cardiac inotropic responses to β-adrenergic stimulation are reduced in both the right and left ventricles. The purpose of the present study was to determine whether a post-receptor defect in the guanine nucleotide-binding regulatory proteins (G-proteins) existed which would explain, at least in part, the adrenergic subsensitivity in both ventricles of the heart failure dogs. Using both immunoblotting technique and the bacterial toxin-mediated ADP ribosylation assays, we found that the stimulatory G-protein ($G_s$) was reduced in both ventricles of the heart failure dogs. In contrast, there were no changes in the inhibitory G-protein ($G_i$). In addition, receptor subtype analysis showed that only $\beta_1$-adrenoceptors were reduced in the failing right ventricle of the heart failure animals. This study demonstrated that the reduction of β-adrenoceptors in right heart failure was chamber-specific whereas the reduction of $G_s$ was non-selective, occurring in both ventricles of right heart failure dogs. The findings further suggest that the reduction of $G_s$ probably was caused by systemic neurohormonal activation, independent of local ventricular stress.

*Keywords:* G-protein; β-Adrenoceptor density; Congestive heart failure; Norepinephrine

## 1. Introduction

Failing myocardium is characterized by a decreased inotropic response to β-adrenergic stimulation (Bristow et al., 1982; Fan et al., 1987; Fowler et al., 1986; Liang et al., 1991). These changes could be explained in part by a reduction of β-adrenoceptor number (Bristow et al., 1982; Brodde, 1991; Fan et al., 1987; Fowler et al., 1986). However, the decrease in inotropic response to isoproterenol also may occur in the failing myocardium without changes in β-adrenoceptor density in rats with myocardial infarction (Cherng et al., 1994). Guanine nucleotide-binding regulatory proteins (G-proteins), which transduce signals from β-adrenoceptors to adenylyl cyclase, can possibly play a pivotal role in the regulation of the β-adrenoceptor-G-protein-adenylyl cyclase system. Indeed, studies have shown that there is a decrease in the stimulatory G-protein ($G_s$) and/or an increase in the inhibitory G-protein ($G_i$) in animals and humans with congestive heart failure (Brodde et al., 1995; Bristow and Feldman, 1992; Feldman et al., 1988; Urasawa et al., 1992). However, the mechanisms by which heart failure leads to the changes in myocardial β-adrenoceptor and G-proteins have not been fully understood.

Using a right heart failure dog model, we have shown previously that β-adrenergic sensitivity is reduced in both the failing right ventricle and the unloaded left ventricle. However, myocardial β-adrenoceptor number is reduced only in the failing right ventricle (Fan et al., 1987). We proposed to employ this animal model to investigate the chamber-specific changes of β-adrenoceptor subtype number and G-protein changes in heart failure. In the right heart failure dogs, the right ventricle with an elevated filling pressure is susceptible to both local and systemic factors. In contrast, the left ventricle while unloaded is under the influence of the same systemic factors as the right ventricle. By observing the changes in both ventricles, we could determine whether β-adrenoceptor and G-proteins are differently regulated. Our results suggest that, unlike the myocardial β-adrenoceptor density which is decreased in the failing right ventricle, changes in G-pro-

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teins are seen in both ventricles, probably produced by some systemic factors in heart failure.

## 2. Materials and methods

### 2.1. Animal models

Adult Mongrel dogs (17–32 kg) were used for the study. The animal preparation has been previously described (Fan et al., 1987; Higgins et al., 1972). In brief, right heart failure was produced by a two-stage sterile thoracotomy using pentobarbital sodium (25 mg/kg) general anesthesia. During the first surgery, a right thoracotomy was performed to rupture the anterior chordae tendineae of the tricuspid valves and to insert a Tygon catheter (1.02 mm i.d.; Norton, Plastics and Synthetics Division, Akron, OH, USA) in the right atrium. Two weeks later, a left thoracotomy was performed for placement of a silicon rubber balloon occluder (R.E. Jones, Silver Spring, MD, USA) around the main pulmonary artery, Tygon catheters in the left atrium, main pulmonary artery and descending aorta, and an implantable Konigsberg micromanometer (Konigsberg Instruments, Pasadena, CA, USA) into the apex of the left ventricle. After additional 2 weeks of recovery, the pulmonary occluder was inflated progressively with the balloon volume adjusted once or twice a week for 3 weeks to produce a stable right atrial pressure of 12–14 mm Hg. The animals developed resting tachycardia and ascites. Sham-operated animals were studied concurrently for comparison. These dogs underwent two thoracotomies identical to that described above, except that neither tricuspid valve avulsion nor pulmonary artery constriction was performed.

Final hemodynamic studies were carried out 6 weeks after the beginning of pulmonary artery constriction in right heart failure dogs or 8 weeks after the left thoracotomy in the sham-operated dogs. The animals were then killed 2 days later by lethal doses of pentobarbital sodium (> 100 mg/kg) and the hearts were excised immediately. The right and left ventricular free walls were separated and weighed.

The study was approved by the University of Rochester Committee on Animal Resources and conformed with the Guiding Principles in the Use and Care of Animals approved by the American Physiological Society and with the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals'.

### 2.2. Hemodynamic measurements

Animals were acclimated to the laboratory environment and trained to lie quietly on a table for the final hemodynamic studies. The pulmonary artery occluder was deflated in the right heart failure dogs and the chronically implanted Tygon catheters were connected to pressure transducers and an eight-channel Brush model 480 recorder (Gould, Instrument Systems Division, Cleveland, OH, USA) for measuring right atrial, left atrial and aortic pressures. The Konigsberg micromanometer was connected to the recorder and an electronic differentiator for measuring left ventricular pressure and the first derivative of pressure rise (dP/dt). A Millar catheter (Millar Instrument, Houston, TX, USA) was inserted via an external jugular vein under local anesthesia to measure right ventricular pressure and dP/dt. Cardiac output was measured by injecting indocyanine green (Cardio-Green, Hynson, Westcott and Dunning, Baltimore, MD, USA) into the pulmonary artery and sampling the arterial blood for dye concentrations using a Gilford model 140 cardiac output system (Gilford Instrument Laboratories, Oberlin, OH, USA).

Final hemodynamic studies were begun after at least 1 h had elapsed after insertion of the Millar catheter. Resting systemic hemodynamics were measured in triplicate over a 15-min baseline period. The data were averaged and the means were used for statistical analysis.

### 2.3. Membrane preparation

Myocardium from ventricular free walls was taken for measuring adenylyl cyclase activity, β-adrenoceptor density and G-proteins. The muscle blocks were trimmed, minced and homogenized in an ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 22°C). The homogenate was centrifuged at $40\,000 \times g$ for 15 min at 4°C. The resultant pellets were stored at −70°C for assays. The protein yield of the pellets was determined using bicinchonic acid (BCA kit; Pierce, Rockford, IL, USA), with bovine serum albumin as a standard.

### 2.4. Adenylyl cyclase activity

Adenylyl cyclase activity was measured in a 0.4 ml incubation mixture containing 50 mM Tris-HCl, pH 7.5, 0.4 mM EGTA, 0.5 mM 2-isobutyl-1-methylxanthine, 2 mM magnesium chloride, 5 mM phosphocreatine, 15 U creatine phosphokinase and approximately 35 μg membrane protein. The reaction was initiated by adding 50 μl ATP to give a final concentration of 1 mM and was terminated 10 min later by immersing the reaction tube in a boiling water bath for 3 min. The samples were assayed for cAMP by the competitive protein-binding technique (Tovey et al., 1974). Isoproterenol ($10^{-8}$–$10^{-4}$ M) and 5′-guanylylimidodiphosphate ($10^{-8}$–$10^{-4}$ M) were used to stimulate adenylyl cyclase activity by their actions on the β-adrenoceptors and the β-adrenoceptor-coupled G-proteins, respectively (Rodbell, 1980). The reaction for isoproterenol was performed in the presence of 0.1 mM GTP.

### 2.5. Myocardial β-adrenoceptor assay

Myocardial β-adrenoceptor density was measured by specific binding of [$^{125}$I]iodocyanopindolol (2200 Ci/mmol; New England Nuclear, Boston, MA, USA) to β-adrenoceptors. Approximately 6 μg of cardiac membrane was incubated with varying concentrations of radiolabeled iodocyanopindolol (5–250 pM) in 50 mM Tris-HCl, pH 7.4, 120 mM sodium chloride and 5 mM potassium chloride. Non-specific binding was determined by parallel incubation of samples containing 100 μM *l*-isoproterenol. Incubations were done in triplicate for 1 h at 37°C in a final volume of 0.25 ml. Ice-cold Tris-HCl buffer was added to the sample at the end of the incubation. The membrane was filtered and washed 3 × through Whatman GF/B filter on a Brandel cell harvester (Biomedical Research and Development Laboratories, Gaithersberg, MD, USA). β-Adrenoceptor-specific binding was assessed by measuring the difference of bound iodocyanopindolol between the samples with or without *l*-isoproterenol. Myocardial β-adrenoceptor density and apparent dissociation constant ($K_d$) for iodocyanopindolol were calculated using the AccuFit saturation two-site program developed by Hawkin (Lundon Software, Chargrin Falls, OH, USA).

### 2.6. β-Adrenoceptor subtype assay

Myocardial $\beta_1$- and $\beta_2$-subpopulations were identified by analysis of displacement curves using the highly selective $\beta_1$-adrenoceptor antagonist CGP 20712A (Ciba-Geigy Pharmaceuticals, Basel, Switzerland) (Dooley et al., 1986). The assay was carried out in the same way as β-adrenoceptor-binding measurement with 50 pM [$^{125}$I]iodocyanopindolol and 14 different concentrations of CGP 20712A, ranging from $10^{-10}$ to $2 \times 10^{-4}$ M. The bottom of the competition curve was regarded as the non-specific binding, which was essentially the same as that found with parallel incubation of samples containing 100 μM *l*-isoproterenol. The displacement curve were analyzed for $\beta_1$- and $\beta_2$-adrenoceptor densities and the estimates of the affinity of the β-adrenoceptor subtypes for the unlabeled CGP 20712A ($K_{d1}$ and $K_{d2}$), using the AccuFit competition program (Lundon Software).

### 2.7. Measurement of G-proteins

Immunoblotting and bacterial toxin-mediated [$^{32}$P]ADP ribosylation were used to measure the G-proteins. For immunoblotting, the membrane pellets were resuspended in a sample buffer containing 0.32 M sucrose, 1 mM EDTA, pH 7.4, supplemented with 0.5 mM phenylmethylsulfonyl fluoride and 1 μg/ml leupeptin. Membrane fractions containing 20 μg of protein were added to an equal volume of 2 × loading buffer (0.1 M Tris-HCl, pH 6.8, 20% glycerol, 4% sodium dodecyl sulfate, 10% β-mercaptoethanol and 10 μg/ml phenoblue), heated at 95°C for 5 min and loaded onto a 4.5% polyacrylamide stacking gel and a 10% polyacrylamide/0.1% sodium dodecyl sulfate resolving gel. The gel system was run at 10 mA for 15 h and the proteins were transferred to polyvinylidene difluoride membranes in a transfer buffer containing 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (pH 11) and 10% methanol at 4°C for 80 min at 500 mA constant current. The membranes were then placed in a blocking buffer (50 mM Tris-HCl, pH 7.4, 0.9% sodium chloride, 1 mg/l thimerosal, supplemented with 1% non-fat milk) for 1 h and then probed with anti-G-protein α-subunit antibodies (anti-$G_{i\alpha}$: AS/7, New England Nuclear, Boston, MA; anti-bovine $G_{s\alpha}$: Upstate Biotechnology, Lake Placid, NY, USA). Both antibodies are rabbit polyclonal immunoglobulin G in origin. The anti-$G_{s\alpha}$ antibody recognizes both the 45 kDa and 52 kDa protein forms of $G_{s\alpha}$ antigen. The probed membranes were then washed with the blocking buffer for 4 ×, each for 10 min, and incubated with a horse-radish-peroxidase conjugated goat anti-rabbit immunoglobulin G antibody (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA). The membranes were washed and reacted with 3,3′,5,5′-tetramethylbenzidine membrane peroxidase substrate (Kirkegaard and Perry Laboratories). The signal intensity of the blue precipitates was quantified by videodensitometry using a Bio-Image system (Millipore, Ann Arbor, MI, USA). Four different amounts (5, 10, 20 and 30 μg) of a protein standard (membrane preparation from the myocardium of one dog from the control group) were run with each experiment to generate a reference standard curve by plotting videodensitometric readings against the protein amount. The G-protein contents of the unknown sample were then calculated by interpolation, using the reference standard curve, and expressed in micrograms of standard protein per microgram of sample protein.

The cholera toxin-mediated [$^{32}$P]ADP ribosylation of the $G_s$ was carried out as described previously (Longabaugh et al., 1986). Enriched sarcolemmal membrane was prepared according to the method of Jones and Besch (1984). The sarcolemmal fractions (10 μg protein) were mixed with membranes prepared (40 μg protein) from a $G_s$-deficient variant S49 mouse lymphoma cell line cyc$^-$ in 250 μl of buffer (100 mM Tris-HCl, 5 mM magnesium chloride and 1 mM EDTA, pH 7.4). Cholera toxin was preactivated in a 50 mM HEPE buffer (pH 7.5) for 30 min at 37°C. To the membrane protein was added 100 μl of assay buffer (250 mM potassium phosphate, pH 7.0, 40 mM phosphocreatine, 20 mM thymidine, 113 U/ml creatine phosphokinase, 10 mM ATP, 0.5 mM GTP and 2.5 mM NADP) and 100 μl of the preactivated cholera toxin. The reaction was initiated by adding 50 μl of [$^{32}$P]NAD (50 μM, 10–50 Ci/mmol). Reactions were carried out for 90 min and quenched by the addition of an equal volume of an ice-cold buffer (100 mM Tris-HCl, pH 7.4, 5 mM magnesium chloride, 1 mM EDTA and 1 mM NAD).

The pertussis toxin-mediated ADP ribosylation of $G_i$

was carried out according to the method of Bokoch et al. (1984). Membrane fractions were resuspended in a 2% cholate solution for 60 min immediately before ADP ribosylation. The membrane was then diluted in a 20 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1 mM dithiothreitol and 0.05% Lubrol PX (Sigma, St. Louis, MO, USA). The reaction was initiated by adding 10 μl of the solubilized sarcolemma to 26 μl of a reaction buffer containing 3 μg/ml pertussis toxin and 1 μM [$^{32}$P]NAD and then incubated at 30°C for 60 min. The reaction was stopped by adding 3 volumes of the resuspension buffer.

The samples of cholera or pertussis toxin ADP ribosylation were run on polyacrylamide/0.1% sodium dodecyl sulfate gel electrophoresis. The protein bands specifically labeled by the toxins were excised from gels by using autoradiogram as templates, then counted in a liquid scintillation counter. The molar amount of $G_s$ or $G_i$ was calculated on the basis of specific activity of [$^{32}$P]NAD used in each experiment. Background interference was evaluated by carrying out the same reactions without cholera or pertussis toxin.

### 2.8. Data analysis

All results are expressed as mean ± S.E.M. The statistical significance between groups was determined using two-tailed Student's *t*-test for unpaired data. A $P < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Baseline characteristics of right heart failure

Table 1 shows the baseline characteristics of the right heart failure dogs. Right heart failure was evidenced by a higher right atrial pressure. The right heart failure dogs also had increased body weight, higher resting heart rate, decreased cardiac output and depressed right ventricular dP/dt. In addition to hemodynamic changes, the right ventricular weight to body weight ratio was also markedly increased in the right heart failure dogs indicating right ventricular hypertrophy. Left atrial pressure and left ventricular dP/dt was decreased in right heart failure dogs while there was no difference in mean aortic blood pressure between the two groups.

Table 1
Baseline characteristics of the right heart failure and sham-operated dogs

| | Right heart failure | Sham-operated |
|---|---|---|
| Body weight (kg) | 25.1 ± 0.8 [a] | 22.9 ± 0.5 |
| Heart rate (beats/min) | 130 ± 5 [a] | 110 ± 7 |
| Mean aortic pressure (mm Hg) | 109 ± 4 | 118 ± 4 |
| Right atrial pressure (mm Hg) | 12.0 ± 1.0 [b] | 5.0 ± 0.4 |
| Left atrial pressure (mm Hg) | 7.0 ± 0.7 [a] | 9.0 ± 0.6 |
| Cardiac output (l/min) | 2.75 ± 0.22 [b] | 4.67 ± 0.24 |
| RV dP/dt (mm Hg/s) | 563 ± 47 [a] | 717 ± 51 |
| LV dP/dt (mm Hg/s) | 2473 ± 140 [b] | 3373 ± 138 |
| RV/body weight (g/kg) | 2.03 ± 0.11 [a] | 1.74 ± 0.06 |

Values are mean ± S.E.M. $n = 18$ in each group of animals. LV, left ventricle; RV, right ventricle. [a] $P < 0.05$, [b] $P < 0.001$, compared to sham-operated dogs, as determined by two-tailed Student's *t*-test.

Table 2
Basal and peak-stimulated adenylyl cyclase activities (pmol/mg protein/min) in the right and left ventricles of the right heart failure and sham-operated dogs

| | Right heart failure | Sham operated |
|---|---|---|
| *Right ventricle* | | |
| Basal | 29 ± 2 [a] | 53 ± 5 |
| Isoproterenol | 68 ± 6 [a] | 137 ± 5 |
| 5′-Guanylylimidodiphosphate | 153 ± 16 [b] | 204 ± 16 |
| *Left ventricle* | | |
| Basal | 30 ± 2 [a] | 55 ± 4 |
| Isoproterenol | 69 ± 3 [a] | 132 ± 3 |
| 5′-Guanylylimidodiphosphate | 139 ± 9 [a] | 200 ± 12 |

Values are mean ± S.E.M. $n = 18$ in each group of animals. The basal adenylyl cyclase activity was measured in the presence of 0.1 mM GTP. The peak-stimulated adenylyl cyclase activities shown above were those obtained with $10^{-4}$ M of isoproterenol or 5′-guanylylimidodiphosphate. [a] $P < 0.001$, [b] $P < 0.05$, compared to sham-operated dogs, as determined by two-tailed Student's *t*-test.

### 3.2. Adenylyl cyclase activity

As expected, isoproterenol and 5′-guanylylimidodiphosphate produced dose-dependent increases in right ventricular and left ventricular adenylyl cyclase activity in both right heart failure and sham-operated animals. A plateau adenylyl cyclase activity was reached at the highest concentration employed ($10^{-4}$ M). Table 2 shows that basal right ventricular and left ventricular adenylyl cyclase activities were reduced in right heart failure. The peak adenylyl cyclase activity produced by the two adenylyl cyclase stimulants were also reduced in both ventricles of the right heart failure dogs.

### 3.3. Total β-adrenoceptor measurement

The iodocyanopindolol-binding assay demonstrated a saturable and highly specific binding pattern. Total right ventricular β-adrenoceptor density was reduced in the right heart failure dogs, compared to the sham-operated dogs (Table 3). In contrast, myocardial β-adrenoceptor density did not differ significantly in the left ventricle between the right heart failure (132 ± 11 fmol/mg) and sham-operated (143 ± 10 fmol/mg) animals. Table 3 shows there was no difference in dissociation constant ($K_d$) in the right ventricle between the two experimental groups. Nor did $K_d$ differ significantly in left ventricles of the two groups (31 ± 3 vs. 32 ± 2 nM).

Table 3
Total β-adrenoceptor density and subtypes in the right ventricles of the right heart failure and sham-operated dogs

| | Right heart failure ($n = 18$) | Sham-operated ($n = 14$) |
|---|---|---|
| *Total β-adrenoceptors* | | |
| Density (fmol/mg) | 61 ± 7 [a] | 128 ± 10 |
| $K_d$ (nM) | 26 ± 2 | 31 ± 3 |
| *$\beta_1$-Adrenoceptors* | | |
| Density (fmol/mg) | 28 ± 4 [a] | 96 ± 8 |
| $K_{d1}$ (nM) | 3.9 ± 06 | 4.3 ± 0.7 |
| *$\beta_2$-Adrenoceptors* | | |
| Density (fmol/mg) | 34 ± 3 | 28 ± 2 |
| $K_{d2}$ (μM) | 25 ± 5 | 19 ± 6 |

Values are mean ± S.E.M. $K_d$, apparent dissociation constant for iodocyanopindolol. $K_{d1}$, estimate of affinity of the $\beta_1$-adrenoceptors for CGP 20712A; $K_{d2}$, estimate of affinity of the $\beta_2$-adrenoceptors for CGP 20712A. [a] $P < 0.001$, compared to sham-operated dogs, as determined by two-tailed Student's $t$-test.

### 3.4. β-Adrenoceptor subtype measurement

The iodocyanopindolol-binding curve became biphasic in the presence of the $\beta_1$-adrenoceptor-specific antagonist CGP 20712A (Fig. 1). The competition curve was fit into a two-site binding model. CGP 20712 exhibited $\beta_1$-adrenoceptor selectivity. The ratio of the $K_{d1}$ and $K_{d2}$ for the two different receptors was in the order of $10^{3-4}$. Fig. 1 shows that the $\beta_1$-adrenoceptor fraction was significantly reduced in the right ventricle of right heart failure dogs. $\beta_1$-Adrenoceptor density was reduced in the failing right heart, while the $\beta_2$-adrenoceptor density remained relatively unaffected (Table 3). The reduction of total β-adrenoceptor in the right ventricle of right heart failure dogs was due to a selective down-regulation of the $\beta_1$-adrenoceptors. The affinity of the β-adrenoceptor subtypes for the antagonist CGP 20712A did not differ between the sham-operated and right heart failure animals (Table 3).

### 3.5. Immunoblotting for G-proteins

Fig. 2 shows typical immunoblot analysis of membranes using the anti-$G_{s\alpha}$ and anti-$G_{i\alpha}$ antibodies. The anti-$G_i$ antibody recognized a protein of 41 kDa, whereas the anti-$G_s$ antibody identified both a 45 kDa and a 52 kDa protein. The 45 kDa protein was the predominant isoform in the heart and was used for quantification. The reference curves generated by different loadings of the membrane protein from one of the sham-operated dogs showed a good linear correlation between the videodensitometric readings and the amount of loading. The $G_s$ content was reduced in both the right and left ventricles of the right heart failure dogs compared to the sham-operated dogs. In contrast, the $G_i$ did not change significantly in either ventricle of the right heart failure dogs (Table 4).

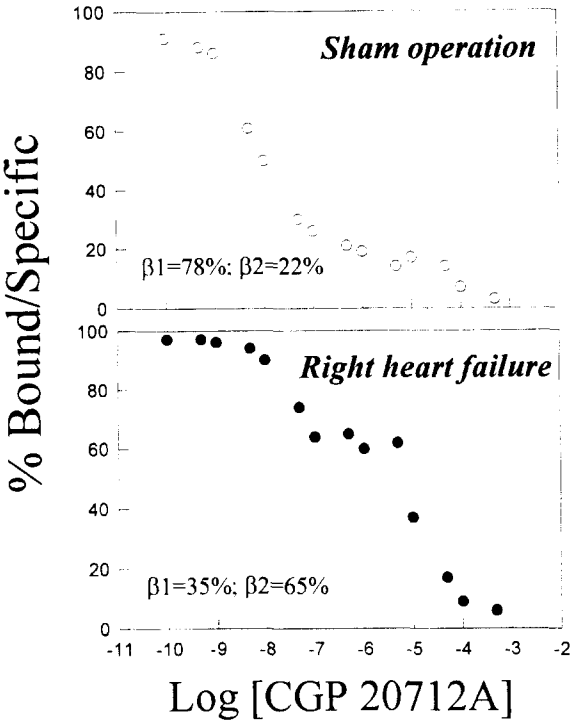


Fig. 1. Representative β-adrenoceptor competition assays utilizing [$^{125}$I]iodocyanopindolol and the $\beta_1$-adrenoceptor-specific antagonist CGP 20712A in the right ventricular myocardial membrane preparation of a sham-operated animal (upper panel) and a right heart failure animal (bottom panel). The assays identified two β-adrenoceptor subtypes and showed that the ratio of the $\beta_1$-adrenoceptors to $\beta_2$-adrenoceptors was reduced in right heart failure.

### 3.6. [$^{32}$P]ADP ribosylation by cholera and pertussis toxin

Cholera toxin catalyzed the ADP ribosylation of a 45 kDa protein ($G_s$) in the canine cardiac sarcolemma. The $G_s$ was reduced in both right and left ventricles of the right heart failure dogs compared to the sham-operated dogs (Table 4). Pertussis toxin catalyzed the ADP ribosylation of two major bands with molecular weight of 39 and 41 kDa, which corresponding to the pertussis toxin substrates

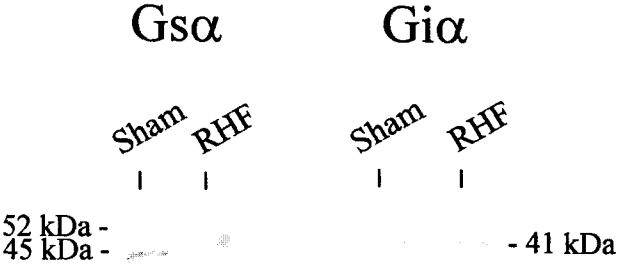


Fig. 2. Western blot detection of $G_{s\alpha}$- and $G_{i\alpha}$-proteins in right ventricular membrane protein preparations from a sham-operated dog (Sham) and a right heart failure dog (RHF). Numbers to the right and left of the figure indicate apparent molecular masses (kDa) based on mobility of a protein standard. The $G_{s\alpha}$ antibody detected two isoform proteins (45 and 52 kDa). The 45 kDa isoform was the predominant isoform in the dog heart.

Table 4
Myocardial G-protein contents in the right heart failure and sham-operated dogs

| G-protein | Ventricle | Right heart failure | Sham-operated |
|---|---|---|---|
| *Immunoblotting (μg/μg; n = 18)* | | | |
| $G_s$ | LV | 0.85±0.06 [a] | 1.06±0.06 |
| | RV | 0.70±0.04 [b] | 0.96±0.05 |
| $G_i$ | LV | 0.99±0.10 | 0.89±0.06 |
| | RV | 1.11±0.10 | 1.05±0.07 |
| *ADP ribosylation (pmol/mg; n = 8)* | | | |
| $G_s$ | LV | 1.4±0.3 [c] | 4.9±0.6 |
| | RV | 2.1±0.5 [a] | 4.3±0.7 |
| $G_i$ | LV | 13±4 | 10±1 |
| | RV | 12±5 | 13±4 |

Values are mean±S.E.M. LV, left ventricle; RV, right ventricle. [a] $P < 0.05$, [b] $P < 0.001$ and [c] $P < 0.01$, compared to sham-operated dogs, as determined by two-tailed Student's *t*-test.

$G_o$ and $G_i$, respectively. The 41 kDa band was used for the measurement of $G_i$. The $G_i$ level was not significantly changed in either ventricle of the right heart failure dogs (Table 4).

## 4. Discussion

The right heart failure animal model employed in this study has been extensively studied (Fan et al., 1987; Greenberg et al., 1973; Higgins et al., 1972; Liang et al., 1989, 1991). The right heart failure dogs exhibit increased body weight, tachycardia, increased right atrial pressure, decreased cardiac output, decreased dP/dt of both ventricles and a normal left atrial pressure. As shown in our prior study (Fan et al., 1987), myocardial β-adrenoceptor density was reduced in the right ventricle of right heart failure dogs in the present study. Results of the present study also indicate that the reduction is limited to the $\beta_1$-adrenoceptor subtype. The findings are consistent with earlier reports in human heart failure by Bristow et al. (1986, 1989).

Our present study further showed that cardiac $G_s$ was reduced in right heart failure. Like other tissues (Mumby et al., 1986), the dog heart contains two forms of the $G_\alpha$ with apparent molecular weights of 45 and 52 kDa. The 45 kDa protein is the predominant isoform in the dog heart. Robishaw et al. (1986) showed that the mRNAs encoding these protein arise from a single gene by internal alternative RNA splicing.

In contrast to the chamber-specific reduction of myocardial $\beta_1$-adrenoceptors in right heart failure, $G_s$ was reduced in both ventricles of the right heart failure dogs. These changes of $G_s$ probably accounted, at least in part, for the decreased production of cAMP as stimulated by isoproterenol and 5′-guanylylimidodiphosphate which were present in both ventricles of the right heart failure dogs. The reductions of maximum production of cAMP by isoproterenol, 5-guanylylimidodiphosphate and forskolin in right heart failure have been reported previously (Fan et al., 1987; Liang et al., 1991). In contrast, the effect of manganese which increases cAMP production by a direct action on the catalytic unit of the adenylyl cyclase is unaffected in right heart failure (Fan et al., 1987; Liang et al., 1991). The findings suggest that the primary defect in the cardiac β-adrenoceptor-coupled adenylyl cyclase system in right heart failure occurs proximal to the catalytic adenylyl cyclase.

Changes in myocardial G-proteins have been demonstrated in patients and animals with heart failure. Most studies in human heart failure have shown that the cardiac $G_{i\alpha}$ isoforms are increased at both the protein and mRNA levels (Brodde et al., 1995; Bristow and Feldman 1992; Feldman et al., 1988; Neumann et al., 1988). $G_{s\alpha}$ is relatively unchanged in human heart failure (Brodde et al., 1995). However, unlike in human heart failure $G_{i\alpha}$ is either unchanged (Hammond et al., 1992) or decreased in animal models of heart failure (Katoh et al., 1990; Ping and Hammond, 1994). Furthermore, a decrease in $G_{s\alpha}$ and mRNA has been shown in a majority of experimental animals with heart failure (Hammond et al., 1992; Katoh et al., 1990; Longabaugh et al., 1988; Ping and Hammond, 1994; Roth et al., 1993). Our findings in right heart failure dogs are consistent with the studies in other animal models of congestive heart failure. The differences in results might be related to the different experimental conditions or animals. Feldman et al. (1988) reported that the increase in cardiac $G_i$ was observed only in dilated but not in ischemic cardiomyopathy. Differences in the assay conditions may also contribute to the discrepancies. For example, the ADP ribosylation method has been shown to underestimate the endogenous amount of G-proteins (Ransnas and Insel, 1988), because of incomplete bacterial toxin catalyzed reaction. The immunoblotting method, on the other hand, measures antigenicity without considering the functional integrity of the proteins. In the present study, we employed both the functional and immunoblotting techniques, showing a reduction in myocardial $G_s$ in right heart failure. However, the $G_s$ as measured by the ADP ribosylation reaction showed a larger percent reduction than that as measured by immunoblotting. We speculate that $G_s$ might have lost its functional integrity, but retained its usual antigenicity during the relatively short period of right heart failure. It is possible that the functional alterations of the $G_s$ occur early in right heart failure, while the change of $G_s$ antigenicity requires a longer exposure to heart failure.

It has been postulated that the changes of G-proteins in congestive heart failure are caused by neurohormonal activation. Horn et al. (1988) reported that $G_s$ was reduced in the mononuclear leukocytes from patients with New York Heart Association Class III or IV heart failure. The findings suggest that the change in $G_s$ is caused by a circulating humoral factor, and as shown in the left ventricle of our right heart failure dogs, is not limited to the failing

myocardium. One could speculate that the change is caused by an increase in sympathetic activity as evidenced by the increased plasma norepinephrine in right heart failure (Fan et al., 1987; Liang et al., 1989, 1991). This is supported by the observations that chronic norepinephrine infusion reduced adenylyl cyclase activation and activity of the GTP-coupling protein $G_s$ in normal dogs (Vatner et al., 1988) and that chronic β-adrenoceptor blockade prevented the reduction of cAMP produced by 5′-guanylylimidodiphosphate and forskolin (Liang et al., 1991). Chronic exposure to norepinephrine also has been shown to increase the myocardial $G_i$ level in cultured neonatal rat muscle cells (Reithmann et al., 1989) as well as in intact rats (Urasawa et al., 1992).

In summary, our study demonstrated that total β-adrenoceptor density was reduced in the right but not the left ventricle of right heart failure dogs and the reduction of β-adrenoceptor was limited to the $\beta_1$-adrenoceptor subtype. In contrast, $G_s$ was reduced in both ventricles while $G_i$ remained unchanged. We speculate that the changes in $G_s$ is related to adrenergic activation, and that the reduction of $G_s$ may play a role in the β-adrenergic desensitization.

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