Noncoordinate Regulation of Cardiac G_s Protein and β -Adrenergic Receptors by a Physiological Stimulus, Chronic Dynamic Exercise

H. Kirk Hammond, Lennart A. Ransnas, and Paul A. Insel

Veterans Administration Medical Center, San Diego, California 92161; and Departments of Medicine and Pharmacology, the University of California, San Diego, La Jolla, California 92093

Abstract

We used a physiological stimulus, chronic dynamic exercise, in pigs to examine resultant changes in chronotropic responsiveness to catecholamine and biochemical features of cardiac β -adrenergic receptors and the stimulatory guanine nucleotide-binding protein, G_s. Long-term treadmill running resulted in a substantial (44%) down-regulation of right atrial β -adrenergic receptors, but the dose of isoproterenol yielding a 50% maximal increase in heart rate was decreased by 57% (from 0.07 ± 0.03 to 0.03 ± 0.01 $\mu\text{g/kg}$; $P < 0.02$) despite this decrease in receptor number. This disparity between receptor number and physiological responsiveness suggested altered signal transduction. We therefore quantitated G_s in myocardial membranes obtained before and after chronic exercise in a competitive ELISA based on an antipeptide antibody developed to the α_s portion of G_s. We found a 42% increase in the amounts of G_s in right atrial membranes (from 11.4 ± 0.8 to 16.2 ± 2.0 pmol/mg; $P < 0.05$) and a 76% increase in the amounts of G_s in left ventricular membranes (from 15.6 ± 2.6 to 27.4 ± 5.2 pmol/mg; $P = 0.02$) after chronic running. These data suggest that in the heart physiological perturbations can result in changes in the levels of G_s, that G_s and β -adrenergic receptor number are not coordinately regulated, and that G_s may contribute to altered adrenergic responsiveness independently of changes in β -adrenergic receptor number.

Introduction

The detailed molecular mechanisms by which hormones and neurotransmitters regulate cardiovascular function are not precisely known. Specifically, the relationships between myocardial β -adrenergic receptor expression and physiological responsiveness to catecholamines are poorly defined. It is generally accepted that these receptors interact with a stimulatory guanine nucleotide-binding protein, G_s, and thereby activate ade-

nylate cyclase. However, the extent to which changes in cardiac responses to catecholamines result from changes in β -adrenergic receptors or postreceptor components is not well known.

For example, previous studies have indicated that long-term treadmill running of pigs results in diminished maximal heart rates during exercise and isoproterenol stimulation and a down-regulation of right atrial β -adrenergic receptors (1). In the current studies we have extended these observations and find that, in spite of this down-regulation of β -receptors, the dose of isoproterenol yielding a 50% maximal heart rate response, the ED₅₀, is decreased after training. Because this result suggested an increased efficiency of signal transduction despite down-regulation of receptor number, we asked whether the paradoxical findings of decreased receptor number and decreased ED₅₀ might be explained by changes in the amount of G_s. We hypothesized that in the heart the amount of G_s might influence physiological responsiveness to catecholamines independently of changes in β -adrenergic receptors. To quantitate the amount of G_s, we used a recently described ELISA assay that uses an anti-peptide antibody directed against a unique sequence in the α_s subunit of G_s (2).

Methods

Animals. Five sexually mature Yucatan miniswine (*Sus Scrofa*; three males, two females) weighing 26 ± 5 kg, were studied before and after chronic dynamic exercise. Thoracotomy was performed to obtain biopsies of the left ventricle and right atrium. Venous catheters were placed for pharmacological testing. After recovery from surgery animals underwent pharmacological and exercise testing to determine heart rate response, maximal work rate, and maximal oxygen consumption. Details of surgical procedures and physiological responses to exercise have been reported previously (1). When exercise and pharmacological tests were complete animals underwent 7–9 wk of treadmill running (60 min/d, 5 d/wk, 9% grade, 100 m/min) and physiological and pharmacological tests were repeated. Finally, animals underwent terminal thoracotomy (initial surgical conditions were duplicated) and samples of the right atrium and left ventricle were obtained. Tissue was excised in the region of the initial biopsy sites, excluding scar tissue. Samples were cleansed of blood and immediately frozen (-70°C). Pharmacological testing and exercise bouts were completed 72–96 h before terminal thoracotomy.

Testing of chronotropic responsiveness. After recovery from surgery all animals underwent pharmacological testing using (–)isoproterenol. Tests were conducted in early morning hours in a quiet, dark laboratory (21°C). Response to isoproterenol was assessed by graded, bolus injection of (–)isoproterenol (0.01 – 2.0 $\mu\text{g/kg}$ i.v.), and heart rate response was recorded until there was no further increase (usually 30 s). The 6-s period showing the largest increase in heart rate was defined as the maximal response. The heart rate was allowed to return to baseline

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Address reprint requests to Dr. H. Kirk Hammond, Veterans Administration Medical Center, V-111-A, 3350 La Jolla Village Drive, San Diego, CA 92161.

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between each dose of (–)isoproterenol. Injections were given until there was a < 5% increase in heart rate; the preceding dose was chosen as the one that caused a maximal response. The relation between change in heart rate and the logarithm of the dose of (–)isoproterenol was examined by linear regression analysis. Two or three tests conducted on separate days were combined into a single linear regression in the control and trained state in each animal. The dose of isoproterenol yielding ED_{50} was calculated from the linear regression equation.

To control for possible training-associated changes in reflex withdrawal of vagal tone occurring in response to isoproterenol-induced vasodilatation, isoproterenol dose-response tests were repeated after the administration of atropine sulfate (0.075 mg/kg i.v., bolus, with an additional 0.5 mg i.v., each 5 min for duration of test).

β -Adrenergic receptor binding studies. β -Adrenergic receptors were identified using the radioligand [125 I]iodocyanopindolol in saturation isotherm experiments conducted on crude myocardial membrane preparations as previously described (1).

Quantitation of G_S . Quantitation of G_S was performed using an ELISA based on an antipeptide antibody generated to the α_s portion of G_S as previously described (2). Myocardial biopsy samples were available from four animals. In all, 11 different samples were examined: 5 from the atria and 6 from the ventricle, 5 before and 6 after chronic exercise. For preparation of membranes, frozen tissue was powdered and then homogenized in glass-glass homogenizing tubes (Kontes Co., Vineland, NJ). An extraction to remove contractile protein, modified from Baker et al. (3), was performed. The membrane homogenate was diluted with 5 vol of buffer (Tris, EGTA, pH 7.5), an equal vol of 1.0 M KCl was added, and samples were incubated at 4°C for 20 min and then centrifuged (48,000 g for 20 min). The pellet was then suspended in buffer at a concentration of about 2.0 mg/ml. Protein concentration was determined by the method of Lowry (4) using BSA as standard.

The ELISA assay was conducted using supernatant fractions prepared from sodium cholate-extracted membranes. Membranes were extracted with 1% sodium cholate in 20 mM Tris-HCl (pH 8.0), 25 mM NaCl, and 1 mM EDTA (TEN) for 1 h on ice. The extract was centrifuged in a microfuge at 43,000 g for 1 h at 4°C and the supernatant fraction was diluted with 4 vol of TEN buffer containing TWEEN-20 so that the final TWEEN-20 concentration was 1%. Extracts were incubated with a 1:2,000 dilution of the G_S antibody for 1 h and then applied to a peptide-coated microtiter plate (2). In studies conducted to validate this antibody, immunoblot experiments indicated that only α_s and not α subunits from other G proteins (α_i , α_o) were detected by the antibody (2). Immunoblot experiments with cardiac membrane preparation confirmed the detection of a 45-kD/52-kD protein, presumably α_s (data not shown), as previously reported for other cell types (2).

Data analysis. Data are expressed as mean $\pm$ 1 SD. Specific measurements made before and after training were compared using *t* testing for paired data. The null hypothesis was rejected when $P < 0.05$ (two-tailed). The Pearson product-moment correlation coefficient (*r*) is reported as a measure of the strength of association between change

Figure 1. Isoproterenol-stimulated heart rate as a function of isoproterenol dose. Typical data for one animal before and after training are presented in the form of a double reciprocal plot. The x-intercept represents the negative inverse of the ED_{50} . This value was 0.11 μ g/kg in the control and 0.04 μ g/kg in the trained state.

Figure 2. The dose of isoproterenol required for a 50% of maximal heart rate response (ED_{50}) was reduced in all animals after the training period. Closed circles with error bars represent mean $\pm$ SD. Data from five animals in the control period and after exercise training are shown.

in heart rate and logarithm of the dose of (–)isoproterenol determined by linear regression analysis.

Results

Characterization of the model. We substantiated that the exercise protocol produced a training effect by measuring maximal systemic oxygen consumption, heart rate, and work rate before and after the training period. These data, which have been previously reported (1), showed a 25% increase in maximal oxygen consumption, a 29-beat/min decrease in basal heart rate, and a 100% increase in maximal work rate. Thus, animals subjected to this protocol showed substantial improvement in measures of cardiovascular performance.

Isoproterenol-stimulated chronotropic response. Figs. 1 and 2 show results of dose-response studies examining the ability of graded, bolus doses of intravenous (–)isoproterenol to increase heart rate. In all five animals, after training, less isoproterenol was required to yield a 50% maximal heart rate response (0.07 ± 0.03 vs. 0.03 ± 0.01 μ g/kg; $P < 0.02$). Mean correlation coefficients ($r = 0.95 \pm 0.04$) for the linear regressions relating logarithm of the (–)isoproterenol dose with heart rate response establish that the relationship is linear. Tests were repeated after administration of atropine sulfate to control for possible changes in reflex activation. Again, in all five animals, after training, less isoproterenol was required to yield a 50% maximal heart rate response (0.06 ± 0.03 vs. 0.03 ± 0.02 μ g/kg; $P < 0.05$). The maximal changes in isoproterenol-stimulated heart rate responses (i.e., maximal-basal) did not differ before and after the training period (119 ± 9 vs. 109 ± 27 ; $P = \text{NS}$). However, in these animals we observed an 18% decrease in isoproterenol-stimulated maximal heart rate and a 9% decrease in heart rate at maximal exercise after the training period (1).

β -Adrenergic receptor binding studies. As previously noted (1), animals subjected to the exercise protocol showed a 44%

Figure 3. Radioligand binding using [125 I]iodocyanopindolol (ICYP) demonstrates a significant decrease in β -adrenergic receptor number in right atrial (RA) but not left ventricular (LV) membranes after exercise training. The error bars represent mean $\pm$ 1 SD. Data from five animals are shown.

Figure 4. Quantitation of the stimulatory guanine nucleotide-binding protein (G_s) by a competitive ELISA in right atrial (RA) and left ventricular (LV) membrane preparations in control and exercise-trained pigs. Assays were performed on membranes from four animals, each membrane sample assayed in quadruplicate. The error bars represent mean $\pm$ 1 SD.

decrease in β -adrenergic receptor number in the right atrium but no change in β -receptor number in the left ventricle after training (Fig. 3).

Quantitation of G_s . The amount of G_s was substantially increased in right atrial and left ventricular membranes after the training period (Fig. 4). The mean increase in right atrial membranes was 42% (11.4 ± 0.8 vs. 16.2 ± 2.0 pmol/mg; $P < 0.05$), while in left ventricular membranes the mean increase was 76% (15.6 ± 2.6 vs. 27.4 ± 5.2 pmol/mg; $P = 0.02$).

Discussion

The key findings of this work are that a normal physiological perturbation, chronic dynamic exercise, is associated with changes in the amount of G_s protein in cardiac membranes, and that β -receptor number and G_s are not coordinately regulated in the heart.

The disparity between β -adrenergic receptor number and physiological responsiveness, as defined by the ED_{50} for isoproterenol-stimulated heart rate, suggests that the level of G_s in cardiac membranes is a critical determinant of physiological responsiveness to catecholamines. Exercise training appears to be associated with a more efficient transduction mechanism resulting in an enhanced response to β -adrenergic stimulation by a reduced number of β -adrenergic receptors. Thus, in the setting of increased amounts of G_s , reduction in receptor number may be adequately compensated so that physiological responsiveness is maintained or even increased.

Although it is commonly observed that changes in β -adrenergic receptor number correlate with changes in responsiveness of the heart to catecholamines (5–7), discordancy between receptor number and physiological responsiveness has been described in several physiological and pathophysiological circumstances. For example, using treadmill running in rats, Takeda and colleagues described a down-regulation of left ventricular β -receptors but an increased responsiveness in papillary isometric responsiveness to catecholamine stimulation (8). Similarly, Crampes and colleagues (9) found that adipocytes from trained humans exhibit enhanced lipolysis in response to catecholamine stimulation despite no change in adipocyte β -receptor number. Our finding of noncoordinate regulation of β -receptor number and G_s provides a possible mechanism that may apply to other hormone- and neurotransmitter-stimulated systems in which receptor number and physiological responsiveness are discordant. For example, Longabaugh and co-workers reported myocardial β -receptor up-regulation but a decrease in adenylate cyclase activity in a canine model of pressure-overload hypertrophy with heart fail-

ure (10). Using cholera toxin those investigators measured a 59% reduction in G_s levels in myocardial membranes from these dogs (10). These data from a pathological state confirm our own data from a physiological setting, that levels of G_s may provide a key mechanism to account for changes in adrenergic responsiveness.

It is noteworthy that the cholera toxin-catalyzed ADP-ribosylation assay used by Longabaugh and colleagues (10) to quantitate membrane-associated G_s yielded 7.1 ± 0.1 pmol G_s per mg canine ventricular membrane protein, a value $\sim$ 50% lower than what we find with the ELISA technique (15.6 ± 2.6 pmol/mg). These differences may be due to the assays used, since our assay measures the amount of the protein itself rather than the contribution of other biochemical factors (endogenous ADP ribosylation, NADase, accessibility of substrates and cofactors), but a species difference is also possible.

Since chronic dynamic exercise is known to result in mild myocardial hypertrophy, it is possible that increased levels of G_s may be the result of changes in membrane yield rather than the reflection of a primary change in G_s itself. However, the ratios of heart weight to body weight, the best assessment for myocardial hypertrophy, were not increased during the training period (1). Moreover, the left ventricle, which experiences the greatest stimulus for hypertrophy, showed no change in β -adrenergic receptor number during the study, but showed a substantial increase in the amount of G_s . On the other hand, the atrium, which is less likely to experience substantial hypertrophy, showed decreases in β -adrenergic receptor number and increases in the amounts of G_s . Taken with the physiological data, these data suggest a primary physiologically induced increase in G_s .

We previously reported that training-induced right atrial β -adrenergic receptor down-regulation was associated with decreased isoproterenol-stimulated maximal heart rate and a reduction in the slope of the line relating logarithm of isoproterenol dose and change in heart rate (1). The current findings of decreased ED_{50} for isoproterenol-stimulated heart rate, which implies increased responsivity, suggests that slope, maximal response, and ED_{50} may reflect different features of receptor-mediated responses. In the current study and in another study of triiodothyronine-induced hyperthyroidism in pigs (11) we find that quantities of G_s and the ED_{50} for isoproterenol-stimulated heart rate are inversely related, and that right atrial β -adrenergic receptor number is directly related to isoproterenol-stimulated maximal heart rate and the slope of the dose-response relationship. Current work in our laboratory is directed toward elucidating the relationships between receptor number, G_s , and physiological measures of responsivity *in vivo*.

The observation that the amount of G_s is not a static entity but can be changed by physiological perturbations is intriguing because it implies a potentially important means to regulate cardiac responsiveness to catecholamines and presumably other neurotransmitters and hormones. It should prove interesting to determine whether other physiological and pathophysiological settings are associated with such changes and to explore mechanisms by which exercise training increases the amount of G_s in the heart.

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