Alterations in myocardial signal transduction due to aging and chronic dynamic exercise

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Roth, David A., Cynthia D. White, Deborah A. Podolin, and Robert S. Mazzeo. Alterations in myocardial signal transduction due to aging and chronic dynamic exercise. J. Appl. Physiol. 84(1): 177–184, 1998.—Normal aging without disease leads to diminished chronotropic and inotropic responses to catecholamine stimulation, resulting in depressed cardiac function with stress. The purpose of this study was to determine molecular mechanisms for decrements in adrenergic responsiveness of the left ventricle (LV) due to aging and to study the effects of chronic dynamic exercise on signal transduction. We measured β-adrenergic receptor (β-AR) density, adenylyl cyclase (AC) activity, and G-protein content and distribution in LV from 66 male Fischer 344 rats from three age groups that were either sedentary or treadmill trained (60 min/day, 5 days/wk, 10 wk at 75% of the maximal capacity). Final ages were 7 mo (young), 15 mo (middle-age), and 25 mo (old). There was no significant difference in β-AR density among groups as a function of age or training. AC production of adenosine 3',5'-cyclic monophosphate (cAMP) with the use of five pharmacological stimulations revealed that old sedentary myocardium had depressed basal, receptor-dependent, G-protein-dependent, and AC catalytic responses. Immunoblot analysis of stimulatory GTP-binding regulatory G proteins (G_i and G_s, respectively) showed 43% less total G_s with similar G_i content in hearts from old sedentary compared with middle-age sedentary rats. When compared with young sedentary animals, G_i content was 39 and 50% higher in middle-age sedentary and old sedentary myocardium, respectively. With age, there was a significant shift in the α-subunit of G_s distribution from cytosolic fractions of LV homogenates to membrane-bound fractions (8–12% redistribution in middle-age sedentary vs. old sedentary). The most significant training effect was a decrease in G_i content in hearts from old trained rats (23%), which resulted in values comparable with young sedentary rats and reduced the G_i/G_s ratio by 27% in old-rat LV. We report that age-associated reductions in cardiovascular β-adrenergic responsiveness correspond with alterations in postreceptor adrenergic signaling rather than with a decrease in receptor number. Chronic dynamic exercise partially attenuates these changes through alterations in postreceptor elements of cardiac signal transduction.

β-adrenergic receptors; GTP-binding proteins; adenylyl cyclase; adenosine 3',5'-cyclic monophosphate

IN RESPONSE TO A VARIETY OF STRESSORS (e.g., postural changes, hypoxemia, exercise, hypercapnia, valsalva), cardiovascular responsiveness declines with advancing age (3, 4, 10, 14–16). These reflex adjustments to physical and environmental stress are modulated by graded release of neurotransmitters and hormones that signal intracellular effectors to regulate cellular responses. Deterioration of β-adrenergic response of both the heart and the vasculature occurs with aging. For example, a decline in maximum heart rate, an increase in end-diastolic and end-systolic volume indexes, and decreased ejection fraction are seen in the cardiovascular response to exercise in older humans (16). Moreover, Guarnieri et al. (8) showed in isolated perfused intraventricular septa from rats that the rate of maximum force production in response to graded isoproterenol infusion was diminished in senescent myocardium. Also, the effects of increasing epinephrine and norepinephrine concentrations on isometric trabecular contractions showed a diminished maximal rate of tension development (dT/dt) with no increase in active tension in old compared with young adult muscles (17). However, there was no age difference in the response of active tension and dT/dt to increasing concentrations of calcium ion, showing that the intrinsic inotropic response to catecholamines is diminished in aged myocardium. These alterations may become pathophysiological in senescence and may involve changes in adrenergic signal transduction that result in reduced cardiac chronotropy and inotropy and in peripheral vasodilatory responses (7, 14–16).

Catecholamines are the major stimulatory agonists of cardiac function during acute stress. They are the first messengers of β-adrenergic signaling that integrate and amplify chemical signals from outside the sarcolemma to effectors within the myocyte. This signal-transduction pathway involves the sequential interactions of three heterogeneous plasma membrane-associated proteins: β-adrenergic receptors (β-ARs), G proteins, and adenylyl cyclase (AC). Agonist binding to β-AR causes interactions of receptors with coupled stimulatory GTP-binding regulatory G proteins (G_s), which, in turn, interact with membrane-bound catalytic subunits of AC to increase production of adenosine 3',5'-cyclic monophosphate (cAMP). Intracellular cAMP then stimulates cAMP-dependent protein kinase, which signals multiple effectors in the nucleus and sarcoplasmic reticulum, contractile proteins, and ion channels, all serving to increase inotropy in ventricular myocytes and both chronotropy and inotropy in atrial cells (7, 25). Neither an integrated nor mechanistic explanation of depressed cardiovascular function and catecholamine responsiveness with age has emerged and is the primary purpose of the present study.

Regularly performed aerobic exercise in humans and rats induces cardiovascular adaptations that slow or reverse many changes in structure and function associated with both aging and disease (37, 39, 40). These include greater cardiovascular functional capacity as
reflected in higher maximal stroke volume, ejection fraction, cardiac output, and maximal oxygen uptake, concomitant with decreased resting and exercise heart rate and peripheral resistance (3, 22). Chronic dynamic exercise has also been shown to alter sympathetic nervous system activity, catecholamine release, and adrenergic receptor density and responsiveness (1, 6, 19, 20–22, 26, 35). However, the varied changes reported in cardiac signal transduction with training have produced an inconsistent and confusing picture, with no definitive mechanistic explanations. To clarify these issues, we have not only examined all elements of the transduction pathway in aging myocardium but have extended our studies to include the independent and interactive effects of aging and exercise training.

The purpose of this study was to determine mechanisms for decrements in cardiac adrenergic regulation in senescence and to establish how attenuation of these impairments may be accomplished after exercise training. It was our overarching hypothesis that the primary mechanism for age-related decrements in cardiovascular response to physiological stress is depressed cAMP production due to alterations in myocardial signal transduction. We also hypothesized that chronic dynamic exercise would increase adrenergic responsiveness in senescent animals by positively affecting elements of the pathway. We have, therefore, examined the content and function of individual and integrated components of myocardial β-adrenergic signal transduction from a genetically homogeneous population of healthy animals across three age groups that were either sedentary or exercise trained by moderate-intensity treadmill running.

METHODS

Animals. Sixty-six male Fischer 344 rats were obtained from the National Institute on Aging at 4, 12, and 22 mo of age. The animals were housed in pairs in a climate-controlled room, at 25°C, on a 12:12-h light-dark cycle, with free access to Teklab rodent chow and water. Animals were cared for according to the “Guide for the Institutional Care and Use of Laboratory Animals” and monitored by a full-time veterinarian. One week after arrival, all animals performed a graded exercise test on a motor-driven treadmill up a 15% grade to familiarize them with treadmill running and handling. After the training period, all animals were tested for endurance capacity by running at 75% of the initial maximal capacity until exhaustion.

Tissue preparation. Terminal ages of the groups were 7, 15, and 25 mo. Three days after final exercise, testing animals were anesthetized (Nembutal, 60 mg/kg ip) and hearts were removed, rinsed of blood, trimmed of fat and connective tissue, dissected for chamber specificity, weighed, frozen in liquid nitrogen, and stored at −70°C. Frozen left ventricular (LV) tissue was homogenized to 5.0% (wt/vol) crude muscle homogenate with 10 mM phosphate buffer with protease inhibitor (10 mM KH₂PO₄, 5 mM MgCl₂, 6 H₂O, 5 mM EDTA 2Na, 1 mM ethylene glycol-bis-[β-aminoethyl ether]-N,N,N',N’-tetraacetic acid, and 50 KIU aprotinin). Myocardium was homogenized in two bursts at maximum speed, 45 s, over ice. A 200-µl aliquot of crude muscle homogenate was saved at −70°C while the remaining crude muscle homogenate was transferred to centrifuge tubes and spun at 45,000 g for 20 min at 4°C. A 1.3-ml aliquot of supernatant (S45) was stored at −70°C. The pellet fraction (P45) was resuspended in the same volume of phosphate buffer calculated above, by using several strokes of a 5-ml syringe, 18-gauge needle, then aliquoted and stored at −70°C.

Protein concentration and yield. Protein concentration was determined by using the protein dye-binding technique of Bradford (2), using bovine serum albumin (BSA) as standard, and was recorded at 595 nm. Linear regression analysis was used to calculate unknown sample protein concentrations from the BSA standard curve. Assessment of protein yield (calculated as both milligram protein per gram wet weight LV, and milligram protein per milliliter crude homogenate) was conducted to determine whether aging caused differences in protein yield due to fibrosis, edema, or focal hypertrophy.

Citrate synthase. Skeletal muscle biochemical adaptations due to exercise training were verified by soleus citrate synthase activity after the method of Srere (38).

Radioligand-binding studies. Myocardial β-AR density was evaluated by using [125I]iodocyanopindolol (ICYP) as radiolabeled ligand. Frozen LV samples from each group were powdered in a stainless steel mortar and pestle under liquid nitrogen, placed in tris(hydroxymethyl)aminomethane buffer, glass-glass homogenized, and contractile proteins were extracted with 0.5 M KC1, 20 min at −70°C. The pellet of a 45,000 g centrifugation (P45) was resuspended in buffer, filtered through Nitex cloth, and saturation isotherm experiments using eight concentrations of ICYP (5–700 pM) were performed by using 10 µM propranolol to determine nonspecific binding, as previously described (27–29). All experiments were performed in triplicate at 37°C and counted on a Wizard 1470 gamma counter. Data from myocardial β-AR assays were examined by Scatchard analysis and fit best with a single-component model. Maximal binding sites for ICYP were determined, and receptor density was reported in femtomoles per milligram protein. The equilibrium affinity constant was calculated and compared among samples.

Second-messenger studies. Biochemical analyses were performed on individual LV tissue homogenates from each experimental group. Frozen (−70°C) LV samples of −0.5 g were weighed, homogenized with phosphate buffer at 5.0% (wt/vol), then centrifuged at 45,000 g for 20 min at 4°C, and the pellet P45 was then resuspended in a precalculated volume of phosphate buffer. Second-messenger studies measured cAMP production in picomoles of cAMP formed per milligram protein per minute by AC under five conditions: basol (no additions), stimulation by 10 µM isoproterenol in the presence of 100 µM GTP, 100 µM 5’-guanylylimidodiphosphate [Gpp(NH)p], 10 mM fluoride ion (AlF), and 100 µM forskolin (all final concentrations), by using sequential-
column chromatography as described by Salomon et al. (31). We found that cAMP production under these conditions was linear with respect to time and protein concentration and that 3-isobutyl-2-methylxanthine (1.0 mM), adenosine deaminase (5 U/ml), or both in combination had no effect on basal or maximally stimulated cAMP production. Previous experiments established that AC does not contaminate S45 fractions in our preparations (9, 27).

Quantification of \( G_s \) and \( G_{i,2} \) by immunoblotting. Assessment of the stimulatory and inhibitory \( \alpha \)-subunits of the cardiac G-proteins (\( G_s \) and \( G_{i,2} \), respectively) was conducted by using standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting techniques, as previously described (27-29). Briefly, all sample homogenates were electrophoresed, transferred to nitrocellulose membranes, incubated with purified polyclonal rabbit anti sera primary antibodies, then identified by using \(^{125}\)I-protein A secondary antibodies, and autoradiographed. To quantify cardiac \( G_{i,2} \) purified fusion proteins were constructed as previously described, and both protein standards and sample bands at 45 and 52 kDa were removed for gamma counting. Similar procedures were performed on the 39-kDa band for assessment of \( G_{i,2} \). Bands were excised from the membranes and counted in a Wizard 1470 gamma counter, and linear regression analysis was used to quantify G-protein content.

Statistical analysis. A 2 \( \times \) 3 independent groups analysis of variance was used for multiple comparisons between aged and trained groups (P < 0.05). Newman-Keuls post hoc tests were performed to determine significant differences among groups. Data are presented as means ± SE.

### RESULTS

Physiological and biochemical characteristics of all groups at time of death are shown in Tables 1 and 2. Training resulted in significantly lower body weight in the young and middle-age trained animals compared with their sedentary counterparts, but this trend did not reach statistical significance in the old animals. Maximal running speed, endurance time to exhaustion, and soleus citrate synthase activities were significantly greater in trained vs. sedentary animals across all age groups.

Heart mass increased significantly with advancing age (Table 2). When heart weight and LV weight were normalized for age-related changes in body weight, significantly higher ratios were seen in the old sedentary animals compared with both young and middle-age sedentary animals. Training had no significant effect on LV mass, heart-to-body weight ratios, or LV-to-body weight ratios in young, middle-age, or old animals (Table 2).

Protein yield and \( \beta \)-AR density. There were no significant differences between or within groups when protein yield was compared (expressed as milligrams of protein per gram wet weight LV or milligram of protein per milliliter homogenate; data not shown). Similarly, neither age nor training had any effect on LV \( \beta \)-AR density or binding affinity (Table 2).

### Table 1. Final physiological and biochemical characteristics of sedentary and trained rats across age groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight, g</th>
<th>Maximum Run Speed, m/min</th>
<th>Endurance Run Time, min</th>
<th>Soleus Citrate Synthase Activity, ( \mu )mol·g(^{-1})·min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
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<tr>
<td>Trained (n = 9)</td>
<td>339.9 ± 6.0(^*)</td>
<td>57.4 ± 2.5(^*)</td>
<td>141.9 ± 6.0(^*)</td>
<td>43.9 ± 4.3(^*)</td>
</tr>
<tr>
<td>Sedentary (n = 12)</td>
<td>392.8 ± 9.4</td>
<td>37.7 ± 2.3</td>
<td>28.8 ± 2.0</td>
<td>29.2 ± 3.0</td>
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<tr>
<td>Middle-age</td>
<td></td>
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<tr>
<td>Trained (n = 9)</td>
<td>390.6 ± 7.5(^†)</td>
<td>44.6 ± 1.1(^†)</td>
<td>136.2 ± 5.5(^*)</td>
<td>31.1 ± 1.3(^†)</td>
</tr>
<tr>
<td>Sedentary (n = 12)</td>
<td>431.8 ± 5.9</td>
<td>29.3 ± 1.0(^†)</td>
<td>25.1 ± 1.7</td>
<td>26.1 ± 1.3</td>
</tr>
<tr>
<td>Old</td>
<td></td>
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</tr>
<tr>
<td>Trained (n = 6)</td>
<td>387.2 ± 9.8(^†)</td>
<td>29.5 ± 2.5(^†‡)</td>
<td>126.3 ± 12.5(^†‡)</td>
<td>21.2 ± 1.2(^†‡)</td>
</tr>
<tr>
<td>Sedentary (n = 5)</td>
<td>416.4 ± 19.8</td>
<td>19.3 ± 1.0(^†‡)</td>
<td>11.0 ± 2.1(^†‡)</td>
<td>16.2 ± 1.1(^†‡)</td>
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</table>

Values are means ± SE. Citrate synthase activity measured at 37°C. \(^*\)Significantly different from sedentary age cohort (P < 0.05). \(^†\)Significantly different from young animals of comparable training condition (P < 0.05). ‡Significantly different from middle-age animals of comparable training condition (P < 0.05).

### Table 2. Heart parameters, \( \beta \)-adrenergic receptor density, and binding affinity of sedentary and trained rats across age groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Weight, mg</th>
<th>LV Weight, mg</th>
<th>Heart Weight/Body Weight, mg/g</th>
<th>LV Weight/Body Weight, mg/g</th>
<th>( \beta )-AR Density, fmol/mg protein</th>
<th>Binding Affinity, pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trained (n = 9)</td>
<td>921 ± 17(^*)</td>
<td>673 ± 14</td>
<td>2.71 ± 0.02</td>
<td>1.98 ± 0.01</td>
<td>30.0 ± 1.0</td>
<td>52.3 ± 6</td>
</tr>
<tr>
<td>Sedentary (n = 12)</td>
<td>986 ± 24</td>
<td>701 ± 18</td>
<td>2.51 ± 0.03</td>
<td>1.78 ± 0.02</td>
<td>32.2 ± 1.7</td>
<td>57.1 ± 7</td>
</tr>
<tr>
<td>Middle-age</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Trained (n = 9)</td>
<td>1,047 ± 16(^†)</td>
<td>784 ± 16†</td>
<td>2.68 ± 0.03</td>
<td>2.02 ± 0.05</td>
<td>32.2 ± 1.4</td>
<td>61.2 ± 7</td>
</tr>
<tr>
<td>Sedentary (n = 12)</td>
<td>1,045 ± 13†</td>
<td>782 ± 13†</td>
<td>2.42 ± 0.02</td>
<td>1.81 ± 0.02</td>
<td>31.9 ± 1.3</td>
<td>62.8 ± 8</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Trained (n = 6)</td>
<td>1,083 ± 38(^†)</td>
<td>810 ± 22†</td>
<td>2.80 ± 0.05</td>
<td>2.09 ± 0.02</td>
<td>31.0 ± 1.8</td>
<td>66.7 ± 7</td>
</tr>
<tr>
<td>Sedentary (n = 5)</td>
<td>1,201 ± 38(^†)</td>
<td>877 ± 43†</td>
<td>2.91 ± 0.15†</td>
<td>2.12 ± 0.09†</td>
<td>30.2 ± 1.9</td>
<td>59.3 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( \beta \)-AR, \( \beta \)-adrenergic receptor; LV, left ventricular. \(^*\)Significantly different from sedentary age cohort (P < 0.05). \(^†\)Significantly different from young animals of comparable training condition (P < 0.05). ‡Significantly different from middle-age animals of comparable training condition (P < 0.05).
AC activity. AC activities with no pharmacological stimulation (basal) and all four pharmacological stimulations were similar among hearts from young and middle-age animals but were significantly diminished in both sedentary and trained old groups (Fig. 1). Basal AC activity in cardiac LV homogenates from old rats was \( \sim 30\% \) lower than that in young and middle-age rats in both training conditions. Similarly, \( \beta\)-AR-dependent 10 \( \mu \)M isoproterenol stimulation in the presence of 100 \( \mu \)M GTP showed that old rats had \( \sim 34\% \) lower AC activity than both young and middle-age rats in both sedentary and trained groups. Regardless of training status, LV homogenates from old animals had significantly lower 10 mM AlF \( (\sim 29\% \) and 100-\( \mu \)M Gpp(NH)p \( (\sim 36\% \))-stimulated AC activity (both G-protein dependent) than those from both the young and middle-age animals. An age-related decline in cAMP production \( (\sim 43\% \) was also found when AC activity was maximally stimulated through Gs and the catalytic subunit of AC by 100 \( \mu \)M forskolin, regardless of training status. Thus, whether stimulated through the \( \beta\)-AR, through Gs, or maximally through Gs and the catalytic subunit of AC, cAMP production was diminished in the old animals.

Training had no significant effect on basal-, receptor-mediated, or maximal stimulation of AC (Fig. 1) in any age group. AlF-stimulated CAMP production was \( \sim 34\% \) higher in young trained compared with their sedentary cohorts, but was not different in middle-age and old animals as a result of training.

Myocardial G proteins. Representative autoradiographs of cardiac Gi and Gs are seen in Fig. 2, A and B, respectively. Two species of Gs \( \alpha \) were found in P45 fractions at 52 and 45 kDa (Fig. 2 B); radioactivity from both bands was combined to quantitate both the P45 fraction and the total Gs \( \alpha \) content (Fig. 3). Results of quantitative immunoblotting established significant aging-induced alterations in both total Gs \( \alpha \) (Fig. 3) and Gi \( \alpha \) (Fig. 4) content. There were significant declines in total Gs \( \alpha \) content (cumulative S45 and P45 content) in LV from old animals when compared with young and middle-age animals, regardless of training status. Total Gs \( \alpha \) declined in old rats because of significant decreases in Gs \( \alpha \) in both P45 and S45 fractions (Fig. 3). Regardless
of training status, no significant differences in S45, P45, or total Gs between young and middle-age animals were found. A 43% decrease in total Gs occurred in sedentary old compared with middle-age animals, concomitant with a 61% decrease in Gs content in S45 fractions. Training had no effect on either Gs content or redistribution between these age groups. With age, there was a shift in the cellular distribution of Gs, such that 43% of total Gs was found in the S45 fraction in sedentary middle-age animals, compared with 30% in sedentary old animals, a pattern repeated in the trained animals (42 and 30%, respectively). Thus, regardless of training, increasing age resulted in the redistribution of LV Gs from the cytosolic to the sarcolemmal fraction.

Significant increases in Gi2 were found with age in sedentary groups (Fig. 4). Regardless of training status, both middle-age and old rat LV contained significantly greater Gi2 than did hearts from young rats. When young vs. middle-aged animals were compared, a 39 and 51% increase with age in Gi2 content was found for the sedentary and trained groups, respectively. Similar increments with age were seen when young and old animals were compared in the sedentary (50%) and

Fig. 2. Autoradiograms of immunoblots. A1: purified protein standards. Purified polyclonal antibodies specific for α2-subunit of inhibitory G protein (Gi2) were used against 4 concentrations of purified fusion protein (GST-Gi2F7) to generate a standard curve for quantitative immunoblotting. Similar curves were generated for Gi standards (GST-Gi2F7) and antibodies (not shown). A2: autoradiogram: Gi2. Polyclonal antibodies were used to identify and quantify Gi2 content in partially purified membrane fractions of transmural left ventricular (LV) samples from each of 6 groups of animals: old (O; 25-mo final age) and sedentary (S; OS); O and trained (OT); middle-age (M; 15-mo) and S (MS); M and T (MT); young (Y; 7-mo) and S (YS); and Y and T (YT). Protein (100 µg) from the P45 (P, particulate; sarcolemmal-associated) fraction was examined per lane. Autoradiogram of 6 representative animals from 1 blot is shown. B: autoradiograms: Gs. Purified polyclonal antibodies specific for Gs were used against partially purified membrane fractions of transmural LV samples from each of 6 groups of animals. Protein (100 µg) from P45 and S45 (S, soluble; cytosolic) fractions was examined for each preparation to evaluate both particulate and soluble (i.e., total) Gs activity. B1: sedentary myocardium; B2: trained myocardium. Two species of Gs are found only in P45 fraction (45- and 52-kDa). MW, molecular weight marker lanes.

Fig. 3. Distribution of Gs in LV of sedentary and trained young, middle-age, and old Fischer 344 rats. Cumulative bar height is total Gs content (S45 + P45); error bar is for SE of total Gs content (S45 + P45); SE for each fraction (S45, P45) is shown within respective bar. †Total Gs content significantly different from young animals regardless of training status (P < 0.05). ‡Total Gs content significantly different from middle-age animals regardless of training status (P < 0.05).

Fig. 4. Gi2 content in LV of sedentary and trained young, middle-age, and old Fischer 344 rats. *Significantly different from sedentary age cohort (P < 0.05). †Significantly different from young animals of comparable training status (P < 0.05).
trained (30%) state. When sedentary middle-age and old animals were compared, there was a modest 8% increase in $G_i$ content with age but a 14% decrease in $G_s$ content in the LV from old trained animals. Most striking was the finding that training significantly affected the old group, such that the old trained LV had 23% lower $G_i$ content than did their sedentary cohorts, indicating a significant training-induced diminution in LV $G_i$ content in senescent myocardium.

As shown in Fig. 5, significant increases in $G_i/G_s$ ratios were found across age groups regardless of training status. Threefold higher $G_i/G_s$ ratios were seen with age in the sedentary group, and more than twofold higher $G_i/G_s$ ratios were seen when young and old trained groups were compared. Although there were only small training effects on $G_i$ protein content in S45, P45, or total $G_i$ protein content within any of the three age groups, training decreased both $G_i$ content and the $G_i/G_s$ ratio in old trained animals. The 23% decline in LV $G_i$ content in the old trained animals resulted in levels comparable with those of young sedentary animals. As a result, the $G_i/G_s$ ratio decreased with training by 27% in the old group, indicating a significant training-induced reduction in the overall inhibitory influence on senescent myocardium.

DISCUSSION

The aim of this study was to determine age-related alterations in cardiac cAMP production through multiple interactive components that regulate adrenergic signal transduction. Furthermore, we investigated the plasticity of this system when challenged by the isolated and combined effects of chronic exercise training and aging. We report here that age-associated reductions in cardiovascular β-adrenergic responsiveness are the result of multiple changes in postreceptor adrenergic signaling rather than of lesions at any single step in the amplification cascade. Specifically, we found no changes in cardiac β-AR density but significant age-associated decreases in AC activity. These declines are due to modifications in both myocardial $G_s$ and $G_i$ contents and perhaps to the activity of the catalytic subunit of AC. Chronic dynamic exercise was shown to alter $G_i$ content in old LV, thereby altering the $G_i/G_s$ and positively affecting adrenergic signal transduction in senescent myocardium.

Heterologous desensitization occurs slowly over time, suggesting an adaptive response to prolonged agonist stimulation that ultimately results in diminished cardiac output. In isolated cardiac preparations, Guarneri et al. (8) and Lakatta et al. (17) reported reduced inotropic responses to catecholamine stimulation in LV from senescent rats. In addition, Sakai et al. (30) recently showed significant decrements in contractile response of isolated cardiac myocytes to elevated norepinephrine with increasing age. After chronic β-adrenergic stimulation, as seen in normal aging as well as in a variety of cardiac pathologies, decreased LV β-AR density, decrements in high-affinity binding sites, and uncoupling of β-ARs from $G_s$ have been shown in some, but not all, studies (14, 17, 33, 34). However, the majority of studies that have investigated β-AR density in LV have found no significant changes with age (1, 20, 21, 30, 32, 33, 35, 42), in agreement with our results. Several studies have demonstrated impaired coupling of the receptor-agonist complex and $G_s$, and concluded that this may be responsible for the observed age-induced decline in isoproterenol-stimulated cAMP production (4, 5, 23, 32). In this study, we report a 21–48% decline in basal, isoproterenol-, Gpp(NH)p-, AlF-, and forskolin-stimulated AC activity with age, findings similar to those of Scarpace et al. (35). Because β-AR density was unchanged with aging or training, it is possible that this receptor-linked decrease in AC activity is due to a decrease in β-AR high-affinity agonist binding, in accordance with studies in aging human leukocytes (5) and in rat LV (23). However, receptor-independent stimulation of AC via AlF, Gpp(NH)p, and forskolin all declined progressively with advancing age, findings similar to those of Scarpace et al. (35) but not in accordance with those of Bohm et al. (1). Therefore, our data suggest that $G$ proteins and/or the catalytic subunit of AC are the sites of depressed cAMP production in aging rat LV, and we have identified several mechanisms downstream from the β-AR that reduce the activation of AC and diminish the rate of cAMP production.

Whereas rapid changes in G-protein expression do not appear to acutely regulate adrenergic responsiveness (24), slow alterations in protein expression, as might be expected from chronic agonist stimulation and heterologous desensitization, do seem to be an important mechanism for modulating responsiveness and cardiac function (14, 17, 24, 25, 27). β-AR-independent activation of AC by AlF and Gpp(NH)p was reduced 28% and 42%, respectively, in LV from old compared with young sedentary animals. This decrease in G-protein-mediated cAMP production was accompanied by a 43% decrease in total $G_s$ content, findings that were positively correlated with age-related declines seen with fluoride stimulation ($r = 0.81$) and Gpp(NH)p stimulation ($r = 0.88$). This supports the
hypothesis that G-protein-mediated mechanisms may be responsible, in part, for decreased adrenergic sensitivity in aging rat LV. These findings are in contrast to several studies that reported no age-associated changes in myocardial Gs content (1, 13, 35, 36, 42). This conflict may be explained, in part, by several experimental differences seen between studies, including rat strain and gender, as well as measurements made previously in particulate fractions only (28). To quantify alterations in Gs content, we examined both cytosolic and particulate fractions of all partially purified preparations and found that decreased total Gs content was accompanied by a significant age-associated shift in Gs location. It has been established that Gs subunits are found in light fractions as well as in the sarcolemma (10–12, 27–29), suggesting that alterations may not be detected by simply measuring one cellular locale. Old sedentary rat LV showed 48% less total Gs and 57% less cytosolic Gs, compared with young rats, suggesting a cytosolic “de-pot” of Gs, perhaps associated with the cytoskeletal matrix, for rapid recruitment to the sarcolemma (11, 24, 25, 28). Blunted physiological responses to β-adrenergic stimulation have been reported with age in a variety of animal and cell models (14–17, 21). It is, therefore, possible that age-induced redistribution of Gs from cytosol to membrane may be one compensatory mechanism to enrich sarcolemmal Gs in attempts to restore AC activation and adrenergic responsiveness.

cAMP dynamics in the myocardium play a pivotal role in ventricular performance. Mechanisms of AC inhibition are more complex than Gs stimulation, involving both Gi and common βγ-subunits. Compared with LV from young sedentary animals, we report a 39 and 50% increase in Gi content in sedentary middle-age and old animals, respectively. However, after training, old LV demonstrated only a 30% higher Gi content compared with young LV, indicating a significantly reduced age-related increase in Gi content as a result of training. Interestingly, we found a significant 23% reduction in Gi content with training in old myocardium compared with sedentary cohorts, which partially reversed the increased Gi content seen with increasing age. These results are similar to those reported recently by Bohm et al. (1), who reported a 72% higher Gi content in old compared with young rat LV, and that training reduced Gi content by 30–35% in young and old animals. It is also interesting to note that Johnson et al. (13) have recently reported increased steady-state levels of Giα2 mRNA in aging LV, but increased protein content (assayed by immunoblotting) was not found.

Because cardiac Gi is coupled to both M2-type muscarinic-cholinergic receptors and to adenosine-A1 receptors (7, 25), it is possible that alterations in these receptor densities and/or receptor-Gi interactions could be responsible for alterations in AC activation with both aging and training. However, Bohm et al. (1) found no significant alterations in M2-muscarinic-cholinergic receptors due to aging or training. In addition, the effects of aging and training on adenosine-A1 receptors in the LV are not presently known and may be an important determinant of signal transduction in senescent myocardium.

Age-associated declines in total cardiac Gs content were seen only in old animals regardless of training status. In contrast, Gi content in sedentary LV was shown to increase throughout the three age groups, with a significant training-induced decrease in Gi content found only in the old animals. Thus there was a steady increase in Gi/Gs with age in both the trained and sedentary groups (Fig 5). Within the sedentary group, Gi/Gs increased almost threefold with age. This increase with age was only twofold within the trained group, with no significant reductions in Gi/Gs due to training in the young and middle-age animals. Hearts from trained old animals, however, had 27% diminished Gi/Gs when compared with sedentary age cohorts, indicating a training-induced diminution in the inhibitory influence of Gi proteins in senescence.

In addition to increased Gi content and Gi/Gs in sedentary aging myocardium, reduced cAMP production may be the result of decrements in AC catalytic activity. We found significantly depressed cAMP production in old rat LV that was invariant with training. Shu and Scarpace (36) have recently shown in Fischer 344 rats that diminished cAMP production was a result of progressive deactivation of AC by forskolin, concomitant with a 41% decrease in tritiated forskolin binding in senescent myocardium. This group also reported that exercise training increased AC activation in senescent LV but decreased AC stimulation in young myocardium, such that the age-related decline in signal transduction was no longer significant, suggesting that exercise training itself may directly influence the amount of active AC catalytic units per milligram protein (35). We observed that forskolin stimulation was compromised to the same extent as Gpp(NH)p when young and old animals were compared, suggesting that in addition to the age-associated alterations in G proteins discussed above changes in AC function, number, and/or isoform expression (41) may also contribute to decreased catecholamine responsiveness in aged myocardium (34–36, 41). Taken together, these findings suggest that expression and function of cardiac G proteins and AC are important loci of both aging- and training-induced modifications in myocardial signal transduction.

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