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Exercise training and β-blocker treatment ameliorate age-dependent impairment of β-adrenergic receptor signaling and enhance cardiac responsiveness to adrenergic stimulation

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Leosco D, Rengo G, Iaccarino G, Filippelli A, Lymperopoulos A, Zincarelli C, Fortunato F, Golino L, Marchese M, Esposito G, Rapacciuolo A, Rinaldi B, Ferrara N, Koch WJ, Rengo F. Exercise training and β-blocker treatment ameliorate age-dependent impairment of β-adrenergic receptor signaling and enhance cardiac responsiveness to adrenergic stimulation. *Am J Physiol Heart Circ Physiol* 293: H1596–H1603, 2007. First published June 8, 2007; doi:10.1152/ajpheart.00308.2007.—Cardiac β-adrenergic receptor (β-AR) signaling and left ventricular (LV) responses to β-AR stimulation are impaired with aging. It is shown that exercise and β-AR blockade have a favorable effect on cardiac and vascular β-AR signaling in several cardiovascular diseases. In the present study, we examined the effects of these two different strategies on β-AR dysregulation and LV inotropic reserve in the aging heart. Forty male Wistar-Kyoto aged rats were randomized to sedentary, exercise (12 wk treadmill training), metoprolol (250 $mg \cdot kg^{-1} \cdot day^{-1}$ for 4 wk), and exercise plus metoprolol treatment protocols. Ten male Wistar-Kyoto sedentary young rats were also used as a control group. Old trained, old metoprolol-treated, and old trained plus metoprolol-treated rats showed significantly improved LV maximal and minimal first derivative of the pressure rise responses to β-AR stimulation (isoproterenol) compared with old untrained animals. We found a significant reduction in cardiac sarcolemmal membrane β-AR density and adenylyl cyclase activity in old untrained animals compared with young controls. Exercise training and metoprolol, alone or combined, restored cardiac β-AR density and G-protein-dependent adenylyl cyclase activation in old rats. Although cardiac membrane G-protein-receptor kinase 2 levels were not upregulated in untrained old compared with young control rats, both exercise and metoprolol treatment resulted in a dramatic reduction of G-protein-receptor kinase 2 protein levels, which is a further indication of β-AR signaling amelioration in the aged heart induced by these treatment modalities. In conclusion, we demonstrate for the first time that exercise and β-AR blockade can similarly ameliorate β-AR signaling in the aged heart, leading to improved β-AR responsiveness and corresponding LV inotropic reserve.

aging; G-protein-coupled receptor kinase 2; β-adrenergic receptor desensitization; heart failure

A LARGE BODY OF EVIDENCE HAS accumulated over recent years suggesting an impairment of cardiovascular β-adrenergic receptor (β-AR) signaling and function in several chronic conditions such as heart failure (6, 36), hypertension (15, 16), and ventricular hypertrophy (3). β_1-AR downregulation and desensitization/uncoupling of both β_1- and β_2-subtypes represent the main alterations of β-AR signaling found in such diseases (4, 5, 7, 34). A critical role in promoting these molecular abnormalities has been ascribed to the increased expression and activity of G-protein-coupled receptor kinase 2 (GRK2) in the heart and/or in vascular tissues (9, 12, 15, 16, 19, 20, 36, 45). In human heart failure, cardiac GRK2 upregulation has been implicated in the uncoupling of cardiac β_1- and β_2-ARs from G proteins and appears to contribute to disease progression (19, 20, 36). In addition, in hypertensive humans and in animal models of hypertension, an increase in vascular smooth muscle GRK2 activity has been observed with concurrent β-AR dysfunction (12, 15, 16). When GRK2 expression is increased in the myocardium, there is a severely diminished inotropic reserve when β-ARs are stimulated (23). Consistent with a pathogenic role in heart failure (36), when GRK2 is inhibited by expression of a peptide inhibitor known as the βARKct, there is increased cardiac function (23), and the βARKct has rescued several animal models of heart failure (14, 18, 35, 40).

Importantly, similar alterations in vascular and cardiac β-AR function have been shown in physiological aging as several studies have shown that the cardiac response to β-AR stimulation decreases with age (10, 25, 31, 38, 46, 47). Of note, similar findings were obtained in vascular reactivity studies in the aorta of old rats (8) and in the dorsal hand and saphenous veins of elderly humans (33). Exercise training exerts several well-described positive effects on the cardiovascular system, and regular physical activity might balance the age-related decline of cardiovascular function (13, 43). In the elderly, exercise training improves left ventricular (LV) performance, increases maximal stroke volume, and restores the age-impaired vasorelaxation by lowering peripheral vascular resistances at rest and during exercise. All of these effects correlate with enhanced cardiovascular β-AR responsiveness (2, 28, 42). Exercise is also shown to ameliorate β-AR function in experimental studies conducted in aged rat myocardium (37).

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A significant "resensitization" of impaired β-ARs has been also recognized as an important mechanism by which β-blockers may ameliorate cardiac function and myocardial inotropic reserve in heart failure (24, 29, 41). These drugs upregulate β-ARs levels and can normalize elevated GRK levels (22). The purpose of this study was to determine whether exercise and β-blocker treatment, alone or combined, exert a positive action on age-dependent impairment of cardiac signaling and LV responses to β-AR stimulation. In this regard, we examined the effects of these two different strategies on specific cellular mechanisms critical to regulation of β-AR function. Specifically, we measured cardiac β-AR density, GRK2 protein expression, and adenylyl cyclase (AC) activity to explore the molecular pathways at receptor and postreceptor levels that can be altered in the aged rat with exercise and/or chronic β-blocker therapy. Our study might add further information on the positive effects of physical activity and β-blockers on age-related changes in cardiac β-AR signaling. This represents an important issue in the clinical setting because of the high prevalence in the elderly of cardiovascular syndromes, such as hypertension and heart failure, which are characterized by a severe impairment of cardiac and vascular β-AR pathway.

MATERIALS AND METHODS

Animal population and study protocol. The study protocol was designed in accordance to the *Guiding Principles in the Care and Use of Animals* outlined by the American Physiological Society and was approved by the ethics committee of our institution. Forty male Wistar-Kyoto aged rats (Charles River Laboratories, Calco, Italy) (24 mo old, weight = 601 ± 28 g) were housed in groups of two animals per cage at controlled room temperature (22–28°C) with 12:12-h light-dark cycles and controlled access to food and water. Animals were acclimated to a treadmill exercise by walking at a speed of 10 m/day for 2 wk on a two-lane treadmill (type 50190, TAKRAF, Schmalkalden, Germany). After this period, rats were numbered from 1 to 40. Afterward, numbers were consecutively drawn and assigned alternatively to exercise, sedentary, metoprolol, and exercise plus metoprolol treatment protocols. In this way, we obtained four groups, each composed of 10 animals. In the training group, rats underwent a training program that lasted 12 wk, consisting of 5 days/wk of treadmill exercise for 45 min/day with a running speed of 17 m/min. This protocol allowed us to obtain a relative exercise intensity of ~70–85% of maximal oxygen uptake (1, 27). In the untrained group, rats walked once per week for 10 min/day to maintain treadmill familiarity. In the metoprolol group, rats were housed in sedentary conditions for a period of 10 wk and then treated with the selective β_1-AR blocker metoprolol for a period of 4 wk. Metoprolol was dissolved in drinking water and administered at a daily dose of 250 mg/kg. In the exercise plus metoprolol group, training protocol was performed as described above, and metoprolol was administered in the last 4 wk of the training program at a daily dose of 250 mg/kg. Maximal exercise capacity was evaluated before protocols were started and at 12 wk after start of experimental procedures. Each evaluation was performed twice in each rat, in separated tests, and the average score was considered for analysis. The protocol for the maximal exercise capacity test consisted of walking at 10 m/min for 5 min followed by 2 m/min increases in speed every 2 min until the rat reached exhaustion. Rats were considered exhausted when they failed to stay off of a shock bar. The grade of the treadmill was set at 15°. Ten Wistar-Kyoto sedentary young rats (4 mo old, weight = 384 ± 10 g) were also included in the study and were used as the control group.

In vivo hemodynamic measurements. At the end of sedentary, training, and drug treatment protocols, all animals underwent in vivo cardiac hemodynamic evaluation. Rats were sedated with ketamine (50 mg/kg) and xylazine (0.5 mg/kg), and venous access was obtained by way of the right external jugular vein. A 2-0 French micromanometer was placed into the LV through the left carotid artery, and LV pressure was obtained with custom data-acquisition software at a sampling rate of 200 Hz. Data were acquired at baseline and after infusion of isoproterenol at 0.1, 0.5, and 1.0 $mg \cdot kg^{-1} \cdot min^{-1}$. The LV pressure data were then analyzed on a PowerLab system (Colorado Springs, CO) with custom software to derive the maximal and minimal first derivative of the pressure rise (LV $+dP/dt$ and LV $-dP/dt$, respectively), as well as heart rate and peak systolic pressure.

Preparation of tissue fractions. At the time the rats were killed, the rat circulatory system was perfused with 20 ml of cold saline, the heart was excised, and the atria were trimmed away. The wet weight was determined. Aliquots of LV (200–300 mg) were obtained from rat hearts of aged sedentary, trained, metoprolol-treated, and trained plus metoprolol-treated groups as well as from young sedentary controls. LV sections were homogenized in 15 vol of 250 mM sucrose, 5 mM EDTA, and 5 mM Tris·HCl (pH 7.5) at 4°C. To avoid protein degradation, protein inhibitors (2 μM leupeptin, 100 μM benzamindine, and 100 μM PMSF) were added to the mixture. The samples were centrifuged at 800 *g* for 15 min at 4°C to clear the homogenate of cellular debris and nuclei. Subsequent supernatants were filtered through cheesecloth and centrifuged at 25,000 *g* for 30 min at 4°C. The final supernatant was set aside for GRK2 analysis, and the pelleted membranes were resuspended (~1 mg/ml) in ice-cold β-AR binding buffer (75 mM Tris·HCl, pH 7.5, 12.5 mM $MgC1_2$, 2 mM EDTA). Membranes were aliquoted and stored at 80°C until use in biochemical assays.

β-AR radioligand binding. Membrane fractions from LV were used for β-AR radioligand binding studies using the nonselective β-AR antagonist ligand ^{125}I-labeled cyanopindolol as previously described (28). Maximal binding was measured with a saturating amount of ^{125}I-labeled cyanopindolol on 25 μg of membrane protein. Binding was allowed to occur for 1 h at 37°C. Nonspecific binding was determined in the presence of 10 μM alprenolol. Reactions were conducted in 100 μl of binding buffer at 37°C for 1 h and then terminated by vacuum filtration through glass fiber filters. All assays were performed in triplicate, and receptor density (in fmol) was normalized to milligrams of membrane protein.

AC assays. Cardiac membranes were prepared by homogenizing rat left ventricles in ice-cold lysis buffer [20 mM Tris·HCl (pH 7.4), 250 mM sucrose, 1 mM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 0.1 mM PMSF]. The samples were centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was centrifuged at 100,000 *g* for 2 h at 4°C. The pellet was resuspended in a buffer containing 20 mM Tris·HCl (pH 7.4) and 1 mM EDTA up to final protein concentration of 4.5–5 mg/ml. Membranes were aliquoted and stored at 80°C. Aliquots of the cardiac membranes (20 μg total protein) were incubated for 15 min at 37°C; the incubation mixture contained 1.6 mM [α-^{32}P]ATP (20–40 $counts \cdot min^{-1} \cdot pmol^{-1}$), 0.5 mM ATP, 0.1 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724), 20 mM creatine phosphate, 100 U/ml creatine phosphokinase, and 25 mM Tris·HCl buffer (pH 7.4) under basal or in the presence of isoproterenol (10 μM) + GTP (100 μM), β-γ-imidoguanosine 5′-triphosphate [Gpp(NH)p] (10 μM), and forskolin (100 μM). Next, 250 μl of a solution containing 1.5 mM cAMP and 0.05 μCi [^{3}H]cAMP in 10 mM Tris·HCl buffer (pH 7.4) were added, and the reaction was terminated by placing the samples in boiling water for 3 min. The reaction mixture of each sample was applied to a column of neutral alumina (0.4 × 15 cm, prepared by pouring dry alumina into Pasteur pipettes). Each column was washed with 3 ml of 10 mM Tris·HCl buffer (pH 7.4). The effluent was collected in a scintillation vial. For the assay of column fractions, 100 μM forskolin and 5 mM $MnCl_2$ were added. In the various assays, 5 mM $MgCl_2$ was used. Blanks were prepared by incubating the samples in the absence of enzyme (23).

Table 1. *Effects of age and exercise on hemodynamic data and physical characteristics*

	Young Untrained	Old	
		Untrained	Trained
Body weight, g	384±10	601±12*	555±18†‡
LW, mg	1,110±40	1,560±30*	1,620±20†
LW/body weight, mg/g	2.88±0.3	2.58±0.4*	2.91±0.6§[a]
Heart rate, beats/min	342±7	324±6*	280±16†‡
SBP, mmHg	119±2	129±3*	113±4§

Values are means ± SE. LW, left ventricular wet weight; SBP, systolic blood pressure. *Age effect significant ($P < 0.01$) between young and old untrained rats. †Age effect significant ($P < 0.01$) between young and old trained rats. ‡Training effect significant ($P < 0.05$) between old untrained and trained rats. §Training effect significant ($P < 0.05$) between old untrained and trained rats. [a]$P < 0.01$ for age-training interaction.

Protein immunoblotting. For protein immunoblotting of GRK2, membrane fractions were prepared as described above. GRK was immunoprecipitated from 200 μg of protein from clarified extracts with 1:2,000 of a monoclonal anti GRK1/2 (C5/1) antibody (22) and 35:l of a 50% slurry of protein A agarose conjugate agitated for 1 h at 4°C. Cytosol fractions or immune complexes were resolved on 10% polyacrylamide Tris-glycine gels and transferred to nitrocellulose. The 80-kDa GRK protein was visualized with a commercially available polyclonal antibody (SC-562; Santa Cruz) and a standard chemiluminescence technique (ChemiDoc System; Bio-Rad Laboratories, Hercules, CA).

Statistical analysis. A two-factor (age, training) ANOVA was performed on data presented in Table 1. Hemodynamics and physical characteristics of animal population were compared by use of one-way ANOVA, followed by a Bonferroni post hoc analysis. Dose-response curves to isoproterenol were analyzed by a two-way ANOVA for repeated measures with Bonferroni post hoc analysis. β-AR density, AC activity, and GRK2 protein levels from different groups were compared by a one-way ANOVA analysis. Significance was set at $P < 0.05$, and all data are reported as means ± SE.

RESULTS

Effects of age, training, and β_1-AR blockade on hemodynamic data and physical characteristics. Table 1 shows the effects of age, training, and age-training interaction on hemodynamic data and physical characteristics of young untrained and old trained and untrained rats. Heart rate and systolic blood pressure were significantly affected by aging and training. Aging and training were also significantly associated with greater body and LV weights; in addition, a significant age-training interaction on the reduction of LV-to-body weight ratio was found. As shown in Table 2, metoprolol and training plus metoprolol significantly reduced heart rate and systolic blood pressure in old treated rats. A significant reduction of LV weight and LV-to-body weight ratio was found in the metoprolol-treated group. These data confirm previous findings on the ability of β-blockade to reduce LV hypertrophy, which is a common finding associated with aging (26).

Effects of training and β_1-AR blockade on maximal exercise capacity in an old rat population. Before training, metoprolol, and training plus metoprolol protocols were started and after randomization, exercise levels were higher in young than in older rats as expected (Fig. 1). Training but not metoprolol induced a significant increase in maximal exercise capacity in old animals (Fig. 1). No additional effect on maximal exercise capacity was observed in old trained animals treated with metoprolol. Interestingly, at the end of the 12-wk training protocol, exercise levels were similar in old trained and trained plus metoprolol-treated rats and young untrained animals.

Exercise training and β_1-AR blockade in older rats improve LV inotropic responses to β-AR stimulation. In old rats that were either trained or untrained, metoprolol treated, or trained plus metoprolol treated, basal LV +dP/d*t* values were not statistically different from those recorded in young control rats (Table 3). These data indicate that aging per se is not associated with significant changes in resting LV contractility in healthy animals. Interestingly, training and β-AR blockade, alone or combined, did not significantly affect basal cardiac contractility in aged rats. Basal LV −dP/d*t* was significantly reduced in old rats of all groups compared with young control rats (Table 3). This confirms previous data on the impairment of LV relaxation occurring with age (44).

Old untrained rats displayed a significantly impaired contractile response to β-AR stimulation compared with young untrained controls, as indicated by their severely diminished isoproterenol-induced LV +dP/d*t* increases (Table 3, Fig. 2*A*). Interestingly, old trained and old metoprolol-treated rats showed significantly improved contractile responses to isoproterenol compared with old untrained animals. No additional

Table 2. *Physical and hemodynamic effects of sedentary, training, metoprolol, and training plus metoprolol treatment protocols in old animal population*

	Young Untrained	Old				
		Untrained	Trained	Metoprolol	Trained Plus Metoprolol	*P*
Body wt, g	384±10	601±12	555±18	570±15	545±21	<0.01 OT and OTM vs. OU <0.01 YU vs. all
LW, mg	1,110±40	1,560±30	1,620±20	1,480±20	1,580±25	<0.05 OT vs. OU <0.05 OM vs. OT and OU <0.01 YU vs. all
LW/body wt, mg/g	2.88±0.3	2.58±0.4	2.91±0.6	2.59±0.4	2.89±0.6	<0.01 OT and OTM vs. OU <0.01 OM vs. OT <0.01 YU versus OU and OM
HR, beats/min	342±7	324±6	280±16	224.8±7.6	230±18	<0.01 OM and OTM vs. OT and OU <0.01 YU vs. all
SBP, mmHg	119±2	129±3	113±4	98.3±3.0	100±6	<0.05 OM and OTM vs. OT, OU, and Y <0.05 YU vs. OU

Values are means ± SE. YU, young untrained; OT, old trained; OU, old untrained; OM, old metoprolol treated; OTM, old trained plus metoprolol.

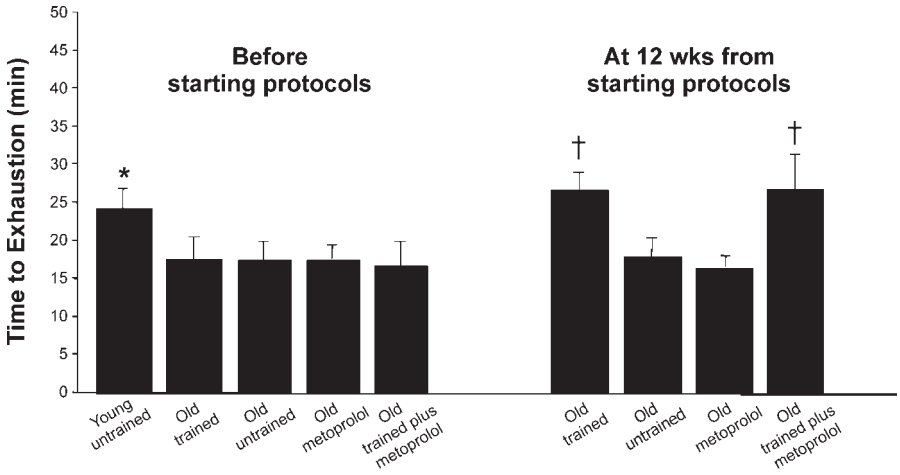

Fig. 1. *Left*: maximal exercise capacity evaluated in all animal groups at baseline, after randomization, and before study protocols were started. *$P < 0.01$ young controls vs. old trained, untrained, metoprolol, and training + metoprolol study groups. *Right*: maximal exercise capacity evaluated in old rats at the end of sedentary, training, metoprolol, and training plus metoprolol study protocols. †$P < 0.01$ old trained and old trained + metoprolol-treated vs. old untrained and metoprolol-treated rats. Values are means ± SE ($n = 10$ for each group).

effect on isoproterenol-stimulated LV +dP/d*t* was observed in old trained plus metoprolol-treated rats. At the highest isoproterenol dose, the increase in LV contractility was comparable between old trained and young controls, suggesting a complete restoration of cardiac inotropic reserve induced by exercise. In old metoprolol and trained plus metoprolol-treated rats, LV +dP/d*t* responses to maximal isoproterenol stimulation were slightly lower than in young controls but dramatically higher than in old untrained animals. In old untrained rats, LV −dP/d*t* responses to isoproterenol were significantly reduced compared with those obtained in young sedentary controls (Table 3, Fig. 2*B*). Although data do not clearly indicate the positive effects of different treatment protocols on LV relaxation properties (Table 3), the analysis of LV −dP/d*t* delta responses to isoproterenol showed that training, metoprolol, and training plus metoprolol significantly affected LV diastolic properties in old rats (Fig. 2*B*). In fact, in the treated old groups, values were higher than those observed in untrained old rats. Together, these results indicate that both pharmacological and nonpharmacological approaches (β-blocker and exercise) improve the inotropic reserve of the aged heart and ameliorate LV relaxation properties. As expected, heart rate and systolic blood pressure responses to isoproterenol infusion were significantly blunted by metoprolol treatment in old rats and heart rate responses to β-AR stimulation were significantly increased in trained old rats compared with results shown in untrained old animals (Table 3, Fig. 3, *A* and *B*).

Exercise training and β-AR blockade rescue aging-induced reduction of cardiac β-AR density and AC activity. As shown in Table 4, aging is associated with cardiac β-AR downregulation, as indicated by the significant reduction in sarcolemmal membrane β-AR density in aged rat hearts compared with young control ones. Importantly, exercise training, metoprolol, and training plus metoprolol restored cardiac β-AR density to

Table 3. *Left ventricular +dP/dt and −dP/dt dose responses to isoproterenol*

	Old Untrained	Young Untrained	Old Trained	Old Metoprolol	Old Trained Plus Metoprolol	*P*
			Left ventricular +dP/dt, mmHg/s			
Basal Isoproterenol	4,900±350	5,300±370	4,700±420	4,800±371	4,350±420	NS
0.1 μg·kg^{-1}·min^{-1}	5,074±330	6,350±440	4,705±335	5,516±380	4,755±325	<0.01 OU vs. YU
0.5 μg·kg^{-1}·min^{-1}	5,948±345	8,683±470	6,933±390	6,800±410	7,133±440	<0.01 OU vs. all
1.0 μg·kg^{-1}·min^{-1}	5,856±336	10,350±345	9,333±438	8,800±450	8,733±468	<0.01 OU vs. all
			Left ventricular −dP/dt, mmHg/s			
Basal Isoproterenol	−4,490±213	−5,966±312	−3,933±324	−3,966±345	−3,950±300	<0.005 YU vs. all
0.1 μg·kg^{-1}·min^{-1}	−4,940±250	−7,016±400	−5,100±301	−5,350±330	−5,225±380	<0.01 YU vs. all
0.5 μg·kg^{-1}·min^{-1}	−5,090±280	−7,266±320	−5,616±256	−5,783±221	−5,700±280	<0.01 YU vs. all <0.05 OT, OM, and OTM vs. OU
1.0 μg·kg^{-1}·min^{-1}	−5,110±300	−7,081±400	−5,733±237	−5,316±221	−5,383±350	<0.01 YU vs. all <0.05 OT vs. OU
			Heart rate, beats/min			
Basal Isoproterenol	304±19	341±17	280±15	224±18	220±19	<0.01 OM and OTM vs. all
0.1 μg·kg^{-1}·min^{-1}	333±24	376±25	300±31	240±19	234±22	<0.01 OM and OTM vs. all
0.5 μg·kg^{-1}·min^{-1}	347±23	395±25	333±22	240±21	231±20	<0.01 OM and OTM vs. all
1.0 μg·kg^{-1}·min^{-1}	358±30	425±32	375±18	253±14	245±17	<0.01 OM and OTM vs. all
			Systolic blood pressure, mmHg			
Basal Isoproterenol	129±5	119±8	113±4	98±7	100±4	<0.005 OM and OTM vs. all
0.1 μg·kg^{-1}·min^{-1}	128±7	121±8	116±8	105±5	105±6	<0.005 OM and OTM vs. all
0.5 μg·kg^{-1}·min^{-1}	130±5	125±14	119±13	108±7	110±5	<0.005 OM and OTM vs. all
1.0 μg·kg^{-1}·min^{-1}	140±7	123±15	121±13	109±4	111±6	0.005 OM and OTM vs. all

Values are means ± SE.

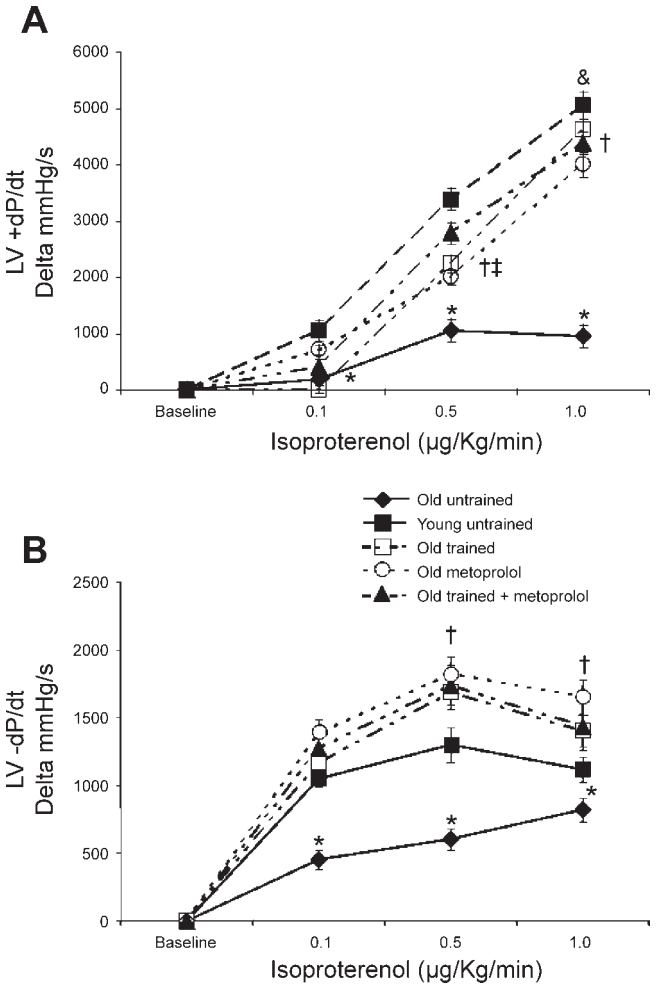

Fig. 2. Left ventricular (LV) maximal and minimal first derivative of the pressure rise (+dP/d*t* and −dP/d*t*, respectively) dose-response curves to isoproterenol. Isoproterenol was administered at ascending doses of 0.1, 0.5, and 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Baseline represents infusions with vehicle. Delta values represent change from baseline. *A*: LV +dP/d*t* responses to isoproterenol. *$P < 0.01$ old untrained vs. young untrained; †$P < 0.01$ old trained, metoprolol-treated, and trained + metoprolol-treated groups vs. old untrained; ‡$P < 0.01$ old trained, metoprolol-treated, and trained + metoprolol-treated vs. young untrained group; &$P < 0.05$ young untrained vs. old metoprolol. *B*: LV −dP/d*t* responses to isoproterenol. *$P < 0.01$ old untrained vs. young untrained; †$P < 0.01$ old trained, metoprolol-treated, and trained + metoprolol-treated groups vs. old untrained. Values are means ± SE ($n = 7$ for each group).

the levels of young control rats. In association with a loss in β-AR density in aged rat hearts, myocardial samples from untrained old rats displayed a severe reduction in basal cAMP levels compared with young control rat hearts and an almost complete inability to increase cAMP production in response to isoproterenol + GTP stimulation and Gpp(NH)p stimulation (Table 4, Fig. 4). To evaluate whether the decrease with age in the activation of AC was also correlated with a reduced number of AC catalytic units, we further assessed stimulation of AC by forskolin. Interestingly, the sensitivity for forskolin activation was also reduced in old untrained rats. As with β-AR density, both exercise training and metoprolol, alone or combined, induced, in old rat hearts, an almost complete restoration of cAMP levels under basal conditions and in response to isoproterenol + GTP and to Gpp(NH)p (Table 4, Fig. 4). In contrast, AC activation by forskolin was not favorably affected by different treatment strategies. Together, these data indicate that training and metoprolol are able to correct the age-related impairment of G-protein dependent mechanisms of AC activation but not those limited by the number of AC catalytic units.

Exercise training and β-AR blockade downregulate GRK2 in old rat hearts. As shown in Fig. 5, although cardiac membrane GRK2 levels are not altered in untrained old rats compared with young control rats, both exercise training and metoprolol, alone or combined, resulted in a dramatic reduction of cardiac GRK2 protein levels in old rats, which is a further indication of β-AR signaling amelioration in the aged heart induced by these treatment modalities.

DISCUSSION

In this study, we demonstrate for the first time that two different treatment strategies, such as exercise training and

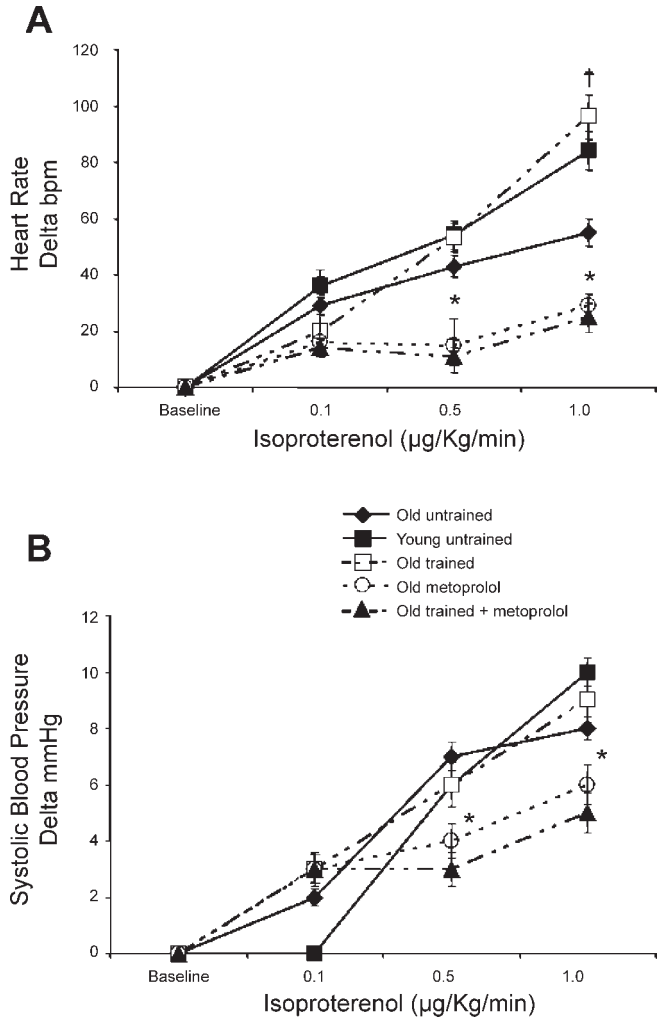

Fig. 3. Heart rate and systolic blood pressure dose-response curves to isoproterenol. Isoproterenol was administered at ascending doses of 0.1, 0.5, and 1.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Baseline represents infusions with vehicle. Delta values represent change from baseline. *A*: heart rate responses to isoproterenol. bpm, Beats/min. *$P < 0.01$ old metoprolol-treated and trained + metoprolol-treated groups vs. all; †$P < 0.01$ old trained and young untrained vs. old untrained. *B*: Systolic blood pressure responses to isoproterenol. *$P < 0.01$ old metoprolol-treated and trained + metoprolol-treated groups vs. all. Values are means ± SE ($n = 7$ for each group).

Table 4. *Effects of training, metoprolol treatment, and training plus metoprolol treatment on cardiac membrane β-AR density and adenylyl cyclase activity*

	β-AR Density, fmol/mg membrane protein					
	Old Untrained	Young Untrained	Old Trained	Old Metoprolol	Old Trained Plus Metoprolol	*P*
	29.6±2.4	53.1±5.1	48.8±4.9	47.5±6.0	51.4±7.4	<0.01 OU vs. all
	Rate of cAMP production, pmol·mg^{-1}·min^{-1}					
No addition	14.8±3.7	40.5±2.8	34.3±2.8	35.6±2.2	38.6±3.1	<0.001 OU vs. all
Iso (10 μM) + GTP (100 μM)	12.6±3.6	54.8±3.3	61.8±3.2	56.5±4.5	59.6±4.2	<0.001 OU vs. all
Gpp(NH)p (10 μM)	24.2±5.4	50.4±6.0	52.4±9.1	58.1±6.5	49.8±7.2	<0.01 OU vs. all
Forskolin (100 μM)	41.2±6.1	95.6±12	53.4±9.0	55.6±11	50.8±10	<0.01 YU vs. all

Values are means ± SE. Gpp(NH)p, β-γ-imidoguanosine 5′-triphosphate; Iso, isoproterenol.

selective β_1-AR blockade by metoprolol, alone or combined, favorably alter β-AR signaling in the aged heart. This improvement occurred via increased cardiac β-AR density and reversal of receptor desensitization and uncoupling from AC stimulation. The latter was due to a reduction of cardiac GRK2 levels due to both exercise and metoprolol. These molecular changes in the myocardial β-AR system led to improved β-AR responsiveness and corresponding inotropic reserve in the aged rat heart.

β-ARs are members of the family of G-protein-coupled receptors that are critical in the regulation of myocardial inotropy, lusitropy, and chronotropy (6, 36). Cardiac and vascular β-AR dysfunction represent common findings in several cardiovascular diseases (6, 7, 34, 36); interestingly, similar alterations of cardiac and vascular β-AR function have also been shown to be associated with physiological aging (8, 10, 25, 31, 33, 38, 46, 47). It appears that chronic β-AR stimulation by increased amounts of circulating catecholamines represents a common pathophysiological mechanism that progressively leads to β-AR dysfunction in the failing myocardium and in the aging heart. The role of β-AR downregulation in the determination of aging-related cardiac β-AR dysfunction is somewhat controversial (37, 48); however, receptor desensitization and uncoupling are well-characterized phenomena in aged myocardium (28, 37, 47, 48). It has been shown that increased levels of cardiac GRK2 play a key role in dysregulation of the β-AR pathway in human and animal models of heart failure (19, 20, 34, 45). This kinase phosphorylates G-protein-coupled receptors and leads to functional uncoupling of the receptors from downstream effector systems (34). Pre-

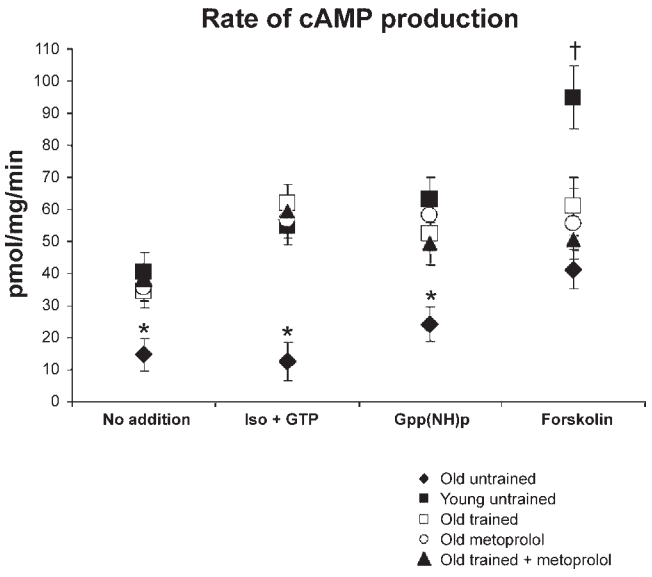

Fig. 4. Adenylyl cyclase (AC) activity expressed as rate of cAMP production. AC activity was assessed in crude cardiac membranes extracted from LV of old untrained, trained, metoprolol-treated, and trained + metoprolol-treated rats and young untrained animals. Shown is cAMP production with no addition and after stimulation by isoproterenol (Iso; 10 μM) + GTP (100 μM), β-γ-imidoguanosine 5′-triphosphate [Gpp(NH)p; 10 μM], and forskolin (100 μM). *$P < 0.001$ old untrained vs. all. †$P < 0.001$ young untrained vs. all. Values are means ± SE ($n = 5$ for each group).

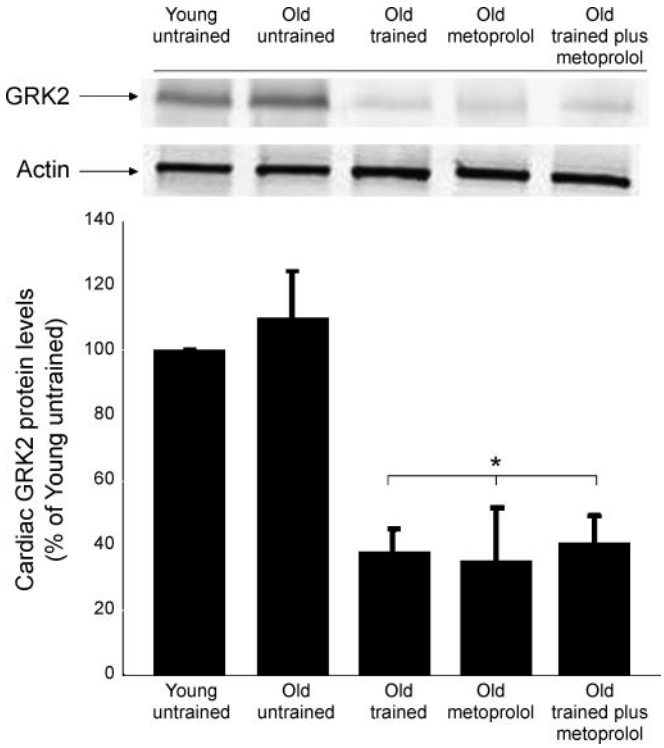

Fig. 5. Cardiac G-protein-receptor kinase 2 (GRK2) protein levels. Levels of expression of GRK2 in cardiac membranes were assessed by protein immunoblotting. Shown is the average data for 5 preparations isolated from old untrained, trained, metoprolol-treated, and trained + metoprolol-treated rats and young untrained animals. GRK2 densitometry values are expressed as percentage of young untrained controls. *Inset*: representative protein immunoblot from 1 preparation for each rat groups. Actin protein level is used as a loading control. *$P < 0.001$ old trained, metoprolol-treated, and trained + metoprolol-treated groups vs. old and young untrained. Values are means ± SE ($n = 5$ for each group).

vious observations from our group (28) showed that aging was associated with a significant reduction of vascular β-AR responses due to receptor downregulation and desensitization. Furthermore, endothelial GRK2 upregulation was shown to be associated with β-AR dysfunction in aged rat carotids (28). Importantly, as now shown in myocardium in this study, our group (28) previously found that exercise training improves vascular β-AR reactivity by reducing GRK2 activity.

At the cardiac level, the molecular mechanisms responsible for age-related β-AR dysfunction have not been clearly elucidated. In an earlier study, it was reported that the positive inotropic effects of both β_1- and β_2-AR stimulation were markedly decreased with age in rat ventricular myocytes, and this was accompanied by decreases in both β-AR subtype densities and a reduction in membrane AC activity (48). In this study, neither GRK2, GRK5, nor G_i proteins seemed to contribute to the age-associated cardiac β-AR desensitization (48). Accordingly, in the present study, cardiac levels of GRK2 were not different among old and young rats; however, we hypothesize that, despite comparable protein concentrations in both age groups, the net effect of GRK2 on β-AR desensitization and uncoupling may be higher in older myocytes, which have a marked reduction of membrane β-AR density. More importantly, both treatment strategies used in this study, training and β-blockade, alone or combined, significantly decreased cardiac GRK2 levels in aged rat hearts, and this was associated with a significant increase of basal and β-AR-stimulated, G-protein-dependent AC activation. The observation that forskolin-stimulated AC activity was still depressed after treatment protocols indicates that training and β-AR blockade, alone or combined, were not able to favorably affect the age-dependent defect of AC catalytic units (39, 48). This finding seems to further support our hypothesis of a key role of GRK2 in determining β-AR dysfunction in the aged heart.

Of note, other studies support the hypothesis that the actions of GRKs are extremely important in modulating myocardial adrenergic signaling and cardiac function in physiological conditions as well as in disease states (34, 36). In this regard, it is important to note that it has been shown that β-AR blockade by selective and nonselective agents, such as atenolol and carvedilol, is able to improve cardiac β-AR signaling by significantly reducing cardiac GRK2 levels in healthy mice (21, 22). In addition, inhibition of GRK2 activity in myocytes due to expression of the βARKct peptide enhances contractility in healthy mice (23) and improves β-AR inotropic reserve in failing myocardium (14, 18, 35, 40). These studies confirm that GRK2 can modulate β-AR signaling and control cardiac function even in physiological conditions (34). This seems to be the case also of aged myocardium as demonstrated in this study by the enhanced cardiac contractile responses to β-AR stimulation in trained old and in metoprolol-treated rats.

It is noteworthy that, in this study, no additional effects on β-AR signaling and cardiac inotropism were observed in old animals when training and metoprolol treatment were combined. This finding could be probably ascribed to the fact that training and β-AR blockade were already able to induce a complete restoration of β-AR pathway and cardiac β-AR responsiveness when adopted separately.

An important observation of this study includes the ability of selective β_1-AR blockade to reverse receptor desensitization by upregulating cardiac membrane β-AR levels and decreasing GRK2 levels. This represents a "paradox" of β-blocker therapy and may explain, at least in part, how long-term application of negative inotropic compounds can increase cardiac index, exercise capacity, and survival. To the best of our knowledge, this study demonstrates for the first time that β-blockade has resensitization activity on β-ARs in the aging heart.

Despite similar biochemical responses, training and β-AR blockade had different effects on maximal exercise capacity, which significantly increased in old trained rats but remained unchanged in old metoprolol-treated rats. These findings are in accordance with those reported in previous human studies indicating that long-term treatment with β-AR antagonists does not increase exercise capacity in patients with heart failure, despite improvements in hemodynamics at rest and LV ejection fraction (17, 32). A plausible explanation for this phenomenon is that, in heart failure, as well as with aging, the causes of exercise intolerance are multifactorial and cannot be exclusively reconducted to the decrease of cardiac performance. Abnormalities of ventilatory function, endothelial dysfunction with altered vascular adaptations, and abnormalities of skeletal muscle including atrophy and abnormal muscle metabolic responses are all factors that may account for the reduced exercise capacity in heart failure. Exercise has been shown to be more effective than β-blockers in favorably affecting these extracardiac components in heart failure (11, 30).

The results of this study could have important clinical implications because of the high prevalence of cardiovascular disease in the elderly, including hypertension and heart failure, which are known to be associated with severe β-AR dysfunction. In these patients, the negative effect of the disease on cardiac and vascular β-AR pathway may be exacerbated by the physiological receptor deterioration occurring with age and may explain, at least in part, the worse prognosis of cardiovascular diseases in the geriatric population. The results of this and other studies in this field may be reason to promote the implementation of β-blocker therapy in such high-risk populations thanks to the positive action of this class of drugs in restoring β-AR signaling and function.

In conclusion, our data indicate that age-related cardiac β-AR dysfunction is a reversible phenomenon and that both pharmacological and nonpharmacological interventions, such as β-blockers and exercise, respectively, may induce a significant improvement of signaling at receptor and postreceptor levels, including at the critical level of GRK2 activity. This also adds novel information on the molecular mechanisms by which these two different strategies exert their favorable action in the treatment of cardiovascular diseases in the elderly population.

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