Exercise restores β-adrenergic vasorelaxation in aged rat carotid arteries

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1Chair of Geriatrics and 2Chair of Internal Medicine, Department of Clinic Medicine, Cardiovascular, and Immunological Sciences, University of Naples “Federico II,” 80131 Naples; and 3Department of Gerontology, Geriatrics, and Metabolic Diseases, II University of Naples, 80131 Naples, Italy

Submitted 14 January 2003; accepted in final form 6 March 2003

Leosco, Dario, Guido Iaccarino, Ersilia Cipolletta, Domenico De Santis, Eliana Pisani, Valentina Trimarco, Nicola Ferrara, Pasquale Abete, Daniela Sorrentino, Franco Rengo, and Bruno Trimarco. Exercise restores β-adrenergic vasorelaxation in aged rat carotid arteries. Am J Physiol Heart Circ Physiol 285: H369–H374, 2003. First published March 13, 2003; 10.1152/ajpheart.00019.2003.—Aging is associated with alterations in β-adrenergic receptor (β-AR) signaling and reduction in cardiovascular responses to β-AR stimulation. Because exercise can attenuate age-related impairment in myocardial β-AR signaling and function, we tested whether training could also exert favorable effects on vascular β-AR responses. We evaluated common carotid artery responsiveness in isolated vessel ring preparations from 8 aged male Wistar-Kyoto (WKY) rats trained for 6 wk in a 5 days/wk swimming protocol, 10 untrained age-matched rats, and 10 young WKY rats. Vessels were preconstricted with phenylephrine (10−6 M), and vasodilation was assessed in response to the β-AR agonist isoproterenol (10−10, 3 × 10−8 M), the α2-AR agonist UK-14304 (10−9,10−6 M), the muscarinic receptor agonist ACh (10−9,10−6 M), and nitroprusside (10−8,10−6 M). β-AR density and cytoplasmic β-AR kinase (β-ARK) activity were tested on pooled carotid arteries. β-AR expression was assessed in two endothelial cell lines from bovine aorta and aorta isolated from a 12-wk WKY rat. β-AR, α2-AR, and muscarinic responses, but not that to nitroprusside, were depressed in untrained aged vs. young animals. Exercise training restored β-AR and muscarinic responses but did not affect vasodilation induced by UK-14304 and nitroprusside. Aged carotid arteries showed reduced β-AR number and increased β-ARK activity. Training counterbalanced these phenomena and restored β-AR density and β-ARK activity to levels observed in young rat carotids. Our data indicate that age impairs β-AR vasorelaxation in rat carotid arteries through β-AR downregulation and desensitization. Exercise restores this response and reverses age-related modification in β-ARs and β-ARK. Our data support an important role for β-ARK in vascular β-AR vasorelaxation.

agin.; beta-adrenergic receptor; exercise

THE ROLE OF β-ADRENERGIC RECEPTOR (β-AR) in the regulation of the cardiovascular system is widely recognized (7). During the past years, the implications of impaired β-AR signaling in the pathophysiology of several cardiovascular disorders were explored in animals as well as in humans. Data from these studies indicate that changes in β-AR function are associated with heart failure (6, 38), hypertension (13, 14), and hypertension complicated with ventricular hypertrophy (2).

Upregulation of β-AR kinase 1 (β-ARK), also known as the G protein-coupled receptor kinase 2 (29) has been recognized as one of the mechanisms responsible for β-AR dysfunction (29). Transgenic mice with cardiac overexpression of β-ARK show a depressed contractile response to isoproterenol (Iso) (22). In the failing human heart the uncoupling of cardiac β1- and β2-ARs from G proteins has been attributed to increased levels of myocardial β-ARK (35). Abnormalities of β-AR signaling due to increase in β-ARK activity have been observed in both human (14) and animal (10, 13) hypertension.

Interestingly, similar alterations in vascular and cardiac β-AR function were found with physiological aging (8, 24). In the rat myocardium, the age-related decline in β-AR responsiveness was ascribed to β-AR desensitization and uncoupling of β-ARs from stimulating G proteins (30). Similarly, vascular reactivity studies conducted in the aorta of aged rats (4) or in the dorsal hand vein and saphenous vein of elderly humans (26) showed a reduced vasorelaxant response to β-AR agonists such as Iso. The recently demonstrated β-AR desensitization in aged rat aorta due to β-ARK upregulation has been proposed as one of the molecular mechanisms responsible for age-related β-AR dysfunction and decreased vasorelaxation (31).

Regular physical activity may counteract the decline of cardiovascular function observed with age (11, 33). Improvements in left ventricular performance, including higher maximal stroke volume, ejection fraction, and cardiac output and lower resting and exercise peripheral vascular resistances, are beneficial effects of exercise in the elderly related to enhanced cardiovascular β-AR responsiveness (1, 32).

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The ability of exercise to attenuate the age-related alterations in β-AR function was demonstrated in experimental studies conducted on aged rat myocardium (30). Whether physical activity may improve β-AR responsiveness by modulating β-ARK levels in aging vasculature has not yet been explored. To investigate this issue, we conducted a study exploring the vasorelaxant responses to β-AR stimulation of rat carotids from aged trained and untrained animals. Vascular reactivity data were correlated with exercise-induced changes in vascular β-ARK activity. Because β-AR vasorelaxation in the rat carotid is largely endothelium dependent, we confirmed for the first time that β-ARK is indeed expressed in the endothelium.

**METHODS**

Endothelial cell culture and β-ARK expression. Two endothelial cell lines from bovine aorta (BAEC; American Type Culture Collection) and aorta isolated from a 12-wk-old Wistar-Kyoto (WKY) rat as previously described (20) were assessed for β-ARK expression. Cells were cultured to confluence in DMEM-10% FBS, washed twice in phosphate-buffered saline, and then Dounce homogenized with an insulin syringe in 25 mM Tris·HCl (pH 7.5), 5 mM EDTA, 5 mM EGTA, 10 μg/ml leupeptin, 20 μg/ml aprotinin, and 1 mM PMSF. Soluble cytosol fractions were separated from membrane fractions by centrifugation. Alternatively, cells were solubilized with ice-cold 50 mM Tris·HCl (pH 8.0), 5 mM EDTA, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 10 mM NaF, 5 mM EGTA, 10 mM sodium pyrophosphate, and 1 mM PMSF and β-ARK was immunoprecipitated from 200 μg of protein from clarified extracts with 1:2,000 of a monoclonal anti-βARK1/2 (C5/1) antibody (19) and 35 μl 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 agitated for 1 h at 4°C (19) and 35 μl of binding buffer (25 mM Tris·HCl (pH 7.5), 5 mM EDTA, 5 mM EGTA, 10 μg/ml leupeptin, 20 μg/ml aprotinin, and 1 mM PMSF). Cytosolic and membrane fractions were separated by serial centrifugation (19).

Receptor binding on pooled rat carotid membranes was performed with the nonselective β-AR ligand [125I]-labeled cyanopindolol. 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. β-ARK activity was tested by rhodopsin phosphorylation assays on rat carotid cytosolic extracts as previously described (19).

**Statistical analysis.** All values are presented as means ± SE. A one-way ANOVA was performed to separately test the main effects of age and exercise training in adult and sedentary and trained aged rats. Dose-response curves to all drugs used were analyzed by a two-way ANOVA for repeated measures with Bonferroni’s post hoc analysis. β-AR density and β-ARK activity values from different groups were compared.

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**Fig. 1.** β-Adrenergic receptor (β-AR) kinase 1 (β-ARK) expression in endothelial cells. To test the expression of the kinase we performed Western blot of cytosolic extracts from bovine (lane 1) and rat (lane 2) aorta endothelial cells and immunoprecipitation of cytosolic β-ARK from bovine (lane 4) and rat (lane 5) endothelial cells in culture. As positive control we used purified bovine β-ARK (lanes 3 and 6). Arrow indicates 80-kDa molecular mass marker.
by a one-way ANOVA analysis. A \( P \) value <0.05 was considered statistically significant.

RESULTS

\( \beta \)-ARK is expressed in endothelium. Although \( \beta \)-ARK expression has been postulated to be ubiquitous, the presence of \( \beta \)-ARK in the endothelium has never been demonstrated before. In rat and bovine vascular endothelium we showed \( \beta \)-ARK expression by Western blot on whole cell lysate or cytosol immunoprecipitation (Fig. 1).

Effect of age and training on whole body and left ventricle weight. Whole body and left ventricle weight and left ventricle-to-body weight ratio of study groups are shown in Table 1. Both trained and untrained aged animals had significantly greater body and left ventricular weights than young rats. A significant training effect was observed between untrained and trained aged rats, trained being leaner than untrained aged rats. Accordingly, the left ventricle-to-body weight ratio was smaller in aged trained than young and aged untrained animals.

ISO-induced vasorelaxation was lower in untrained aged than in young and trained aged animals (Fig. 2A);

Table 1. \textit{Body and left ventricular weights in young and untrained and trained aged rats}

<table>
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<tr>
<th></th>
<th>Young</th>
<th>Untrained</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>385 ± 6</td>
<td>601 ± 6*</td>
<td>572 ± 8†‡</td>
</tr>
<tr>
<td>LW, mg</td>
<td>1.11 ± 0.02</td>
<td>1.56 ± 0.03*</td>
<td>1.61 ± 0.02†</td>
</tr>
<tr>
<td>LW/BW, mg/g</td>
<td>2.88 ± 0.05</td>
<td>2.60 ± 0.47*</td>
<td>2.83 ± 0.07§</td>
</tr>
</tbody>
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Values are means ± SE. BW, body weight; LW, left ventricular wet weight. *Age effect significant at \( P < 0.001 \) between young and untrained aged rats. †Age effect significant at \( P < 0.01 \) between young and trained aged rats. ‡Training effect significant at \( P < 0.01 \) between untrained and trained aged rats. §Training effect significant at \( P < 0.05 \) between untrained and trained aged rats.

Fig. 2. Effects of aging and training on vasorelaxant responses of common carotid arteries from Wistar-Kyoto (WKY) rats. Vasorelaxation was tested in response to the \( \beta \)-AR agonist isoproterenol (A), isoproterenol + \( N^\text{N} \)-monomethyl-L-arginine (L-NMMA) (B), the muscarinic agonist ACh (C), the \( \alpha_2 \)-AR agonist UK-14304 (D), and the endothelium-independent vasodilator nitroprusside (E). Open circles, young carotids; black circles, untrained aged carotids; gray circles, trained aged carotids. *\( F = 4.598, P < 0.0001; †F = 2.409, P < 0.05; ‡P = 5.593, P < 0.01 \) (2-way ANOVA).
differences between groups were abolished by L-NMMA to demonstrate that the age-impaired vasorelaxation in untrained aged animals was mainly related to a defect in the endothelium-mediated component of vasodilation induced by Iso (Fig. 2B).

ACh-induced vasodilation was lower in untrained aged than in trained aged and young animals (Fig. 2C). These data indicate that exercise induces a favorable effect on muscarinic response.

In contrast, exercise was not able to restore the $\alpha_2$-AR-mediated vasorelaxation to UK-14304, which was depressed in untrained and trained aged animals compared with young animals (Fig. 2D). Responses to nitroprusside were not different between groups (Fig. 2E). This demonstrates that the endothelium-independent component of vasodilation was unaffected by age and physical conditioning.

$\beta$-AR density and $\beta$-ARK activity. Carotid $\beta$-AR density was significantly affected by age as demonstrated by its reduction in untrained aged compared with young animals. Exercise increased $\beta$-AR density to values not statistically different from those measured in young rats (Fig. 3A).

$\beta$-ARK activity in cytosol carotid extracts from young, untrained aged, and trained aged animals was assessed by rhodopsin phosphorylation. In untrained aged animals total carotid $\beta$-ARK activity was increased nearly twofold over that recorded in young animals. Physical exercise significantly reduced the kinase activity to levels similar to those observed in young animals (Fig. 3B).

DISCUSSION

This study shows that aging is associated with a significant reduction of vascular $\beta$-AR response caused by receptor downregulation and desensitization with functional consequences of the senescent vasculature. Physical training shows a favorable effect on vascular reactivity of aged rat carotids and corrects age-impaired vascular $\beta$-AR vasodilation by increasing $\beta$-AR density and reducing cytoplasmic $\beta$-ARK activity.

To our knowledge, this is the first study to investigate the relationship between abnormalities of the $\beta$-AR pathway and impaired vasorelaxation in aged rat carotid artery. Our data clearly indicate that carotid responsiveness to $\beta$-AR stimulation is blunted in aged sedentary rats compared with young rats and that the reduced vasorelaxation is associated with a decrease of $\beta$-AR density in whole artery preparation. The presence of $\beta$-AR downregulation in the senescent vasculature is controversial because different studies exploring the age-related changes in vascular $\beta$-AR pathway provide opposite results (15, 37). Our study confirms that the vascular $\beta$-AR number is reduced in aged sedentary rats and extends previous evidences by showing that exercise training corrects this abnormality.

Our data also point to $\beta$-ARK upregulation as a possible mechanism of $\beta$-AR dysfunction in aged rat carotids. The important role of increased cardiac $\beta$-ARK in the pathophysiology of $\beta$-AR signaling has been consolidated in animal and human models of heart failure (29). At the vascular level, increased $\beta$-ARK protein expression was demonstrated in spontaneously hypertensive rats, suggesting that increased $\beta$-ARK activity might account for the impairment of $\beta$-AR-mediated vasodilation observed in hypertension (13, 14). Consistent with this, a recent report showed impaired $\beta$-AR vasorelaxation and hypertension in transgenic mice with selective overexpression of $\beta$-ARK in vascular smooth muscle cells (10). Our results indicate that vascular $\beta$-ARK is increased and participates in the deterioration of vasodilation to $\beta$-AR stimulation also in aging. The present study extends a previous report showing the relationship between the increase in $\beta$-ARK activity and the decline in $\beta$-AR-mediated signaling occurring with age (31) and for the first time demonstrates that exercise reduces vascular $\beta$-ARK activity and exerts a beneficial effect on $\beta$-AR vasorelaxation.

We also provide a demonstration that $\beta$-ARK is expressed in the endothelium and suggest that endothelial $\beta$-ARK has physiological implications in the control
of the vascular tone. Indeed, in rat carotids β-AR vasorelaxation is largely endothelium dependent (20). The physiological relevance of endothelial β-ARs is supported by their distribution in the vasculature. Evidence is mounting that β-AR vasorelaxation is largely endothelium dependent in a wide range of vascular regions that actively participate in the determination of total peripheral resistances (9, 16, 23). Furthermore, in vivo studies in cat hindlimb (12), canine coronary artery (27), and newborn pial arteries (28) suggest that the endothelium dependence of β-AR vasorelaxant responses is generalized.

Although the role of β-ARK in β-AR phosphorylation and desensitization is well recognized, several studies show a possible implication in the regulation of other receptor signaling. To this regard, the recent observation of cross talk between M₃ muscarinic receptors and β-AR in terms of receptor phosphorylation and desensitization (3, 36) and the demonstration that β-ARK may phosphorylate M₃ and M₂ muscarinic receptors (18) provide a possible explanation for our results on the reduced ACh-mediated vasorelaxation in carotids of untrained aged animals. The reduction in vascular β-AR activity obtained in trained aged rats could also contribute to improve muscarinic receptor responsiveness and explain our and previous results on the enhanced ACh-mediated vasodilation after training (5, 21).

Several studies suggest that the main mechanism by which exercise improves impaired vasodilation is increased endothelial NO bioavailability (17, 35). Our data are only in apparent contrast with these previous observations. In fact, we agree on the crucial role of untrained aged animals. The reduction in vascular β-AR activity and impaired β-AR-mediated gene transfer to the vascular endothelium of the carotid artery, is able to correct impaired β-AR vasorelaxation in hypertensive rats (20).

The favorable effects of exercise on vascular age-related changes in β-AR responsiveness and in the postreceptor adrenergic pathway reported in this study are similar to those previously observed in aged rat myocardium (33). In fact, chronic dynamic exercise attenuates the age-determined alterations in postreceptor elements of cardiac signal transduction. This suggests the potential role of physical activity in a global cardiovascular improvement in β-AR function.

Our study may contribute to a better understanding of the mechanisms underlying the physiological aging of the cardiovascular system, providing important insights about the clinical manifestation of cardiovascular disease in the elderly population. β-AR downregulation and desensitization phenomena in senescent vasculature may partly explain the reduced cardiovascular adaptations to different stressors occurring with age and may contribute to the vulnerability of the cardiovascular system typical of the aging process. Changes in β-AR signaling could explain the progression with age of endothelial dysfunction, which is a common finding in pathological conditions such as coronary and peripheral artery disease, hypertensive status, and heart failure.

It seems reasonable to affirm that physiological aging is characterized by an altered vascular β-AR reactivity, which may be one of the molecular backgrounds for the development of cardiac and vascular diseases. The ability of physical activity to counteract the biochemical abnormalities related to endothelial dysfunction and impaired vascular reactivity suggests its potentially useful role in the prevention, treatment, and prognosis of cardiovascular diseases in the elderly.

This work was funded in part by a grant of the Ministry of Education, University, and Scientific Research, 2001, Rome, Italy.

REFERENCES


