Exercise Training Fails to Modify Arterial Baroreflex Sensitivity in Ovariectomized Female Rats

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Exercise Training Fails to Modify Arterial Baroreflex Sensitivity in Ovariectomized Female Rats. Tohoku J. Exp. Med., 2007, 211 (4), 339-345 — In men, exercise training attenuates age-related reduction in baroreflex sensitivity, which is related to cardiovascular health. It is unknown, however, if this holds true for post-menopausal women. We examined the effects of exercise training on baroreceptor-heart rate (HR) reflex sensitivity in ovariectomized (OVX) and sham-operated (SO) Wistar-Kyoto rats. At the age of 8 weeks, OVX and SO rats were assigned to either sedentary or exercise-trained group. Exercise training was performed on a treadmill 5 days per week. At the age of 20 weeks, baroreflex sensitivity in response to increases in blood pressure (BRSinc) and decreases in blood pressure (BRSdec) were evaluated by injections of phenylephrine and sodium nitroprusside, respectively. Both BRSinc and BRSdec were significantly reduced in sedentary OVX rats compared with sedentary SO rats. Exercise training decreased resting HR and BRSdec, but had no effect on BRSinc in SO rats. In OVX rats, exercise training decreased resting HR but modified neither BRSdec nor BRSinc. We conclude that withdrawal of female sex hormones in normotensive female rats is associated with reduced baroreflex sensitivity in response to both increase and decrease in blood pressure and that exercise training fails to modulate the decline of BRSinc associated with withdrawal of female sex hormones. To maintain high level of BRSinc in post-menopausal women, hormone replacement therapy may be needed. —— baroreflex sensitivity; female sex hormone; exercise

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The baroreflexes actively buffer the blood pressure responses to pressor or depressor stimuli, and thus play an important role in decreasing blood pressure variability. In addition, baroreflex sensitivity in response (BRS) to increase in blood pressure (BRSinc), which is predominantly controlled by cardiac vagal activity, is an important physiological determinant of electrical stability in the heart (Schwartz et al. 1992), and reduced BRSinc is associated with an increased risk of life threatening arrhythmia and sudden cardiac death during acute myocardial ischemia (Billman et al. 1982; Cerati et al. 1991). BRSinc is reduced in patients with hypertension, coronary heart disease or congestive heart failure (Parati et al. 2001). Apart from these pathological conditions, BRSinc is
known to decline with advancing age even in healthy subjects (Monahan et al. 2000). Indeed, the incidence of sudden cardiac death increases with age (Becker et al. 1993). In men, reduced central arterial compliance in which arterial baroreceptors are located is thought to play an important mechanistic role in age-related decrease in BRSinc (Monahan et al. 2001). However, the age-related change in BRSinc seems to be not uniform between men and women. BRSinc in postmenopausal women are lower than those in age-matched men, while women with hormone replacement therapy (HRT) have a similar level of BRSinc as men (Huikuri et al. 1996). In an animal study, it has been shown that enhancement of BRSinc by physiological level of estrogen is mediated via central nervous system (Mohamed et al. 1999). Thus reduced BRSinc observed in postmenopausal women may be brought about through both decreased arterial compliance and altered central autonomic integration.

In normotensive men, habitual exercise attenuates age-related decrease in BRSinc by maintaining central arterial compliance to relatively favorable levels (Monahan et al. 2001). Since the age-related reduction in arterial compliance is also attenuated in healthy women by habitual exercise (Tanaka et al. 1998), it can be inferred that the age-related decrease in BRSinc is prevented to some degree by habitual exercise in post-menopausal women. On the other hand, it is unknown whether exercise training could ameliorate the reduction in BRSinc caused by altered central autonomic integration associated with withdrawal of female sex hormones. However, it has been shown that exercise training has potency to modulate intrinsic properties of several autonomic-related nucleus in central nervous system (Jackson et al. 2005; Mueller and Hasser 2006). Moreover, it is well known that exercise training increases BRSinc in pathological conditions such as hypertension (Somers et al. 1991), myocardial infarction (La Rovere et al. 2002), and heart failure (Liu et al. 2002). The purpose of this study was, therefore, to determine the effects of exercise training on baroreflex function in ovariectomized normotensive female rats. We also examined the effects of ovariectomy and/or exercise training on baroreflex tachycardiac response, which is largely regulated by cardiac sympathetic nerve activity.

**Methods**

**Animal care, exercise training**

Female Wistar-Kyoto rats were obtained from Charles River, Astugi, Japan. At the age of 8 weeks, they were anesthetized with ether, and a small abdominal incision was made with the use of sterile techniques. Ovariectomy was performed on 20 rats, and 20 rats underwent a sham operation (SO) without ovariectomy. These rats were further assigned to either sedentary or exercise training group, yielding four groups: sedentary, SO control group (C, \(n = 10\)); exercise-trained SO group (EX, \(n = 10\)); sedentary, ovariectomized group (OVX, \(n = 10\)) and exercise-trained, OVX group (OVX + EX, \(n = 10\)). The rats were fed standard laboratory chow and water ad libitum while housed at a controlled temperature (23°C) with 12-hr light-dark cycle. Exercise training was performed on a motor treadmill 5 days per week for 12 weeks from 8 weeks of age. Rats ran at 20 m/min, 0 grade incline, for 60 min a day as previously described (Minami et al. 2003). All experimental procedures were in accordance with institutional guidelines.

**Measurement of arterial pressure**

At the age of 20 weeks, after the last training session, under ether anesthesia, arterial catheter and two venous catheters were implanted into the femoral artery, femoral vein, and right jugular vein, respectively. The free ends of these catheters were brought subcutaneously to the back of the neck. These catheters were filled with heparinized saline (100 U/ml). The rats were returned to individual cages and allowed to recover 2 days after surgery. Two OVX and three OVX + EX rats were excluded from the experiment because of unsuccessful catheterization or catheter troubles. Arterial pressure was monitored from the femoral artery catheter with a strain-gauge transducer (LIFE KIT DX-360, NIHON KOHDEN, Tokyo) and amplifier (MacLab Bridge Amp ADInstruments Pty Ltd., Castle Hill, NSW, Australia). Phasic pressure, mean arterial pressure (MAP) and heart rate (HR) were recorded at a sampling rate of 200/s by a data acquisition system and laboratory computer (MacLab 8 analog-to-digital converter and Macintosh computer).
Measurement of arterial baroreflex

The rats were studied in their home cages under unrestraint after at least 30-min period of habituation. Intravenous injections of 1-50 μl phenylephrine (0.5 mg/kg/ml = 0.5-25 μg/kg) and sodium nitroprusside (1 mg/kg/ml = 1-50 μg/kg) were given through a jugular and femoral vein catheter, respectively, to produce a series of graded steady-state increases and decreases in MAP. At least three increases in MAP (from 5 to 30 mm Hg) or decreases in MAP (from -5 to -30 mmHg) and reflex changes in HR were plotted against each other, and regression lines were obtained by the least-squares method. These linear regression slopes were taken to represent baroreflex sensitivity during increases (BRSinc) and decreases (BRSdec) in blood pressure. At the end of the study, pentobarbital (60 mg/kg, i.v.) was given, and heart and uterus were removed to measure the left ventricular weight and uterus body weight.

Data analysis

All results were expressed as means ± s.e. Data analyses were performed by Fisher’s least significant difference test for multiple comparisons after one way-ANOVA. Differences were considered significant when \( p < 0.05 \).

RESULTS

There were no significant differences in resting MAP among the groups, however resting HR in EX and OVX + EX were significantly and similarly reduced compared with sedentary rats (Table 1). Linear MAP-HR relations in response to increases in blood pressure and decreases in blood pressure were presented in Fig. 1, and the parameters of BRS were shown in Table 1. Both BRSinc and BRSdec in OVX were significantly reduced compared with C. BRSdec in EX were significantly reduced compared with C, however

![Fig. 1. Average linear regression lines obtained from the data on MAP-HR relation separately induced by the phenylephrine and nitroprusside injections in C and OVX (A), C and EX (B), OVX and OVX + EX (C). The linear regression slopes were taken to represent BRS.](image)

**Table 1. Baroreflex parameters in linear MAP-HR relationships.**

<table>
<thead>
<tr>
<th></th>
<th>C (n = 10)</th>
<th>EX (n = 10)</th>
<th>OVX (n = 8)</th>
<th>OVX + EX (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>102 ± 2</td>
<td>100 ± 2</td>
<td>101 ± 1</td>
<td>101 ± 2</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>297 ± 5</td>
<td>267 ± 4’</td>
<td>292 ± 5</td>
<td>272 ± 4’</td>
</tr>
<tr>
<td>BRSinc (bpm/mmHg)</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>2.0 ± 0.3’</td>
</tr>
<tr>
<td>BRSdec (bpm/mmHg)</td>
<td>5.8 ± 0.3</td>
<td>4.9 ± 0.3’</td>
<td>4.6 ± 0.2’</td>
<td>4.1 ± 0.4’</td>
</tr>
</tbody>
</table>

Values are means ± s.e.

C, sedentary sham-operated (SO) control; EX, exercise-trained SO rats; OVX, sedentary ovariectomized rats; OVX + EX, exercise-trained OVX rats; MAP, resting mean arterial pressure; HR, resting heart rate; BRSinc, BRS in response to increases in blood pressure; BRSdec, BRS in response to decreases in blood pressure. * \( p < 0.05 \) vs C
there was no difference in BRSinc between C and EX. There were no significant differences in both BRSinc and BRSdec between OVX and OVX + EX.

Physiological parameters were shown in Table 2. OVX and OVX + EX rats exhibited a significant body weight gain and uterus atrophy compared with C and EX, respectively. Body weight gain in EX and OVX + EX were slightly but significantly greater than that in C and OVX, respectively. Left ventricular to body weight ratio (LV/BW) in OVX were significantly smaller that that in C. Exercise training slightly but significantly increased LV/BW in sham-operated rats, but had no effect on LV/BW in ovariectomized rats.

**DISCUSSION**

**Baroreflex bradycardia**

In the present study, BRSinc was reduced in OVX compared with C. This finding is in accordance with previous studies (He et al. 1998; Pamidimukkala et al. 2003). In those studies, 17β-estradiol replacement increased the depressed BRSinc in OVX. Thus it has been considered that withdrawal of estrogens is responsible for depressed BRS in OVX. Mohamed et al. (1999) have demonstrated that estrogen-induced facilitation of baroreflex function is centrally mediated. Supporting this, it has been shown that estrogen receptors have been identified in brain centers involved in cardiovascular regulation (Mitra et al. 2003). Estrogens may also improve BRSinc by increasing distensibility of large artery where baroreceptors are located, via enhanced endothelial vasodilation as short-term effects (Polderman et al. 1993; Lieberma et al. 1994; Sudhir et al. 1996) or structural vascular changes as long-term effects (Nichols et al. 1983; Vargas et al. 1993; Fischer and Swain 1997). In the present study, however, it is unlikely that 12-week withdrawal of female sex hormones modulated BRSinc via possible effects on arterial distensibility, because, in a human study withdrawal of hormonal therapy for 4 weeks decreased distal but not central arterial compliance in postmenopausal women (Waddell et al. 1999).

In the present study, exercise training was not able to improve the reduced BRSinc in OVX rats. Although exercise training has potency to modulate intrinsic properties of autonomic-related nucleus in central nervous system (Jackson et al. 2005; Mueller and Hassar 2006), altered central autonomic integration induced by withdrawal of estrogen may not respond to exercise training. This finding also suggests that the mechanism(s) responsible for reduced BRSinc in OVX are different from those in pathological conditions (Andresen et al. 1980; Mircoli et al. 2002; Gao et al. 2005), in which exercise training has been consistently reported to increase BRSinc (Somers et al. 1991; La Rovere et al. 2002; Liu et al. 2002).

Failure of exercise training to improve the reduced BRSinc in OVX rats may have a clinical relevance. Of the reduced BRSinc observed in

<table>
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<th>OVX (n = 8)</th>
<th>OVX + EX (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g), 8 wks</td>
<td>156 ± 1</td>
<td>159 ± 1</td>
<td>155 ± 2</td>
<td>157 ± 2</td>
</tr>
<tr>
<td>BW (g), 20 wks</td>
<td>238 ± 2</td>
<td>253 ± 2*</td>
<td>272 ± 3*</td>
<td>283 ± 4†</td>
</tr>
<tr>
<td>Uterus (mg)</td>
<td>92 ± 6</td>
<td>91 ± 9</td>
<td>28 ± 2†</td>
<td>23 ± 1‡</td>
</tr>
<tr>
<td>LV (mg)</td>
<td>525 ± 9</td>
<td>594 ± 8*</td>
<td>541 ± 6</td>
<td>577 ± 8*</td>
</tr>
<tr>
<td>LV/BW</td>
<td>2.20 ± 0.03</td>
<td>2.35 ± 0.04*</td>
<td>1.99 ± 0.03</td>
<td>2.02 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± s.e. BW, body weight; LV, left ventricle weight. † p < 0.05, vs C. ‡ p < 0.05, vs OVX. † ‡ p < 0.05, vs EX.
postmenopausal women, the component associated with the age-related reduction in arterial distensibility could be prevented to some degree by habitual exercise (Tanaka et al. 1998), however the component elicited by altered central autonomic integration may not be restored by exercise training. Thus, to maintain high level of BRSinc in post-menopausal women, hormone replacement therapy may be necessary.

Exercise training decreased resting HR in OVX rats similarly as in SO rats. Although we did not examine intrinsic HR determined by cardiac pacemaker free of the effects of cardiac autonomic nervous system, low- or moderate-intensity exercise as employed in this study does not change intrinsic HR (Gava et al. 1995; Minami et al. 2003). Thus resting bradycardia observed in exercise training rats might be due to changes in autonomic tone, either an increase in vagal activity, or a decrease in sympathetic activity, or both. In this regard, Davy et al. (1996) have shown that physically active postmenopausal women have lower resting HR and higher heart rate variability, a marker of tonic cardiac vagal activity (Malliani et al. 1991).

It has been considered that increases in both tonic vagal activity and reflex vagal activity (BRSinc) play an important role in reducing susceptibility to ventricular fibrillation and sudden cardiac death (Moolgard et al. 1991; Farrel et al. 1992; Hull et al. 1994; La Rovere et al. 2001). Although epidemiological study has indicated that HRT protects women from sudden cardiac death (Sourander et al. 1998), a combination of exercise training and HRT may be superior to each intervention alone in terms of preventing sudden cardiac death by maintaining both tonic and reflex vagal activity to favorable levels.

**Baroreflex tachycardia**

In the present study, BRSdec was reduced in OVX compared with C. This finding is inconsistent with previous studies demonstrating that ovariectomy does not affect baroreflex tachycardiac responses (Mohamed et al. 1999; Pamidimukkala et al. 2003). Reasons for inconsistent results between our and previous studies are not clear, however a difference in the methods evaluating baroreflex tachycardia may be related. We evaluated baroreflex HR control by using “steady-state” method in which alternations in blood pressure from resting were maintained for 20–30 sec to allow the sympathetics to respond maximally (Head 1994). In the previous studies, baroreflex-mediated tachycardiac responses were evaluated using bolus injections of nitroprusside. That technique does not provide sufficient time for full change of the sympathetic nervous system (Coleman 1980). It has been shown that physiological levels of estrogens increase baroreflex sensitivity of muscle sympathetic nerve activity in young as well as postmenopausal women (Minson et al. 2000; Hunt et al. 2001). Taken together, physiological levels of estrogens may increase the capacity to cope with blood pressure reduction, and thus decrease blood pressure variability.

It has been well shown that exercise training reduces baroreflex tachycardiac responses (DiCarlo and Bishop 1988; Collins and DiCarlo 1997). In agreement with previous studies, BRSdec in EX was reduced compared with C. On the other hand, there was no significant difference in BRSdec between OVX and OVX + EX, although resting HR in OVX + EX were significantly reduced compared with OVX. These findings suggest that female sex hormones play some role in exercise-training induced attenuation of baroreflex tachycardia. Supporting this, Chen and DiCarlo (1996) have demonstrated that exercise training reduces the gain of baroreflex tachycardiac response in female rats but not in male rats. It is known that exercise training attenuates the gain of the arterial baroreflex regulation of sympathetic nerve activity at central nervous system (Chen et al. 1995). Moreover, DiCarlo and Bishop (1990) demonstrated that the reduced gain of the arterial baroreflex regulation of sympathetic nerve activity in trained rabbits was restored by cardiac afferent blockade. This finding suggests that exercise training enhances an inhibitory influence of cardiac afferents, resulting in attenuation of arterial baroreflex regulation of cardiovascular systems. In the present study, LV/BW ratio in EX was slightly but significantly greater than that in C,
while there was no difference in LV/BW ratio between OVX and OVX + EX. It is known that ovariectomy decreases cardiac function associated with change in myosin isoenzymes (Schaible et al. 1984). It has also been shown that exercise training induces greater cardiac hypertrophy in female mice than in male mice (Konhilas et al. 2004). It is possible that some differences in cardiac or central adaptation to exercise training between SO and OVX rats result in different modification of baroreflex tachycardiac responses.

In conclusion, withdrawal of female sex hormones was associated with reduced BRsinc and BRsdec. Exercise training was not able to restore reduced BRsinc in OVX rats while decreasing resting HR similarly as in SO rats. Exercise training reduced BRsdec in SO rats but not in OVX rats.

Acknowledgments

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References


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