Amelioration of depressed cardiopulmonary reflex control of sympathetic nerve activity by short-term exercise training in male rabbits with heart failure

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Pliquet, R. U., K. G. Cornish, K. P. Patel, H. D. Schultz, J. D. Peuler, and I. H. Zucker. Amelioration of depressed cardiopulmonary reflex control of sympathetic nerve activity by short-term exercise training in male rabbits with heart failure. J Appl Physiol 95: 1883–1888, 2003.—The reflex regulation of sympathetic nerve activity has been demonstrated to be impaired in the chronic heart failure (CHF) state compared with the normal condition (Liu JL, Murakami H, and Zucker IH. Circ Res 82: 496–502, 1998). Exercise training (Ex) appears to be beneficial to patients with CHF and has been shown to reduce sympathetic outflow in this disease state (Hambrecht R, Hilbrich L, Erbs S, Gielen S, Pfehn E, Schoene N, and Schuler G. J Am Coll Cardiol 35: 706–713, 2000). We tested the hypothesis that Ex corrects the reduced cardiopulmonary (CP) reflex response to volume expansion in the CHF state. Normal, normal with Ex, CHF, and CHF with Ex (CHF-Ex) groups (n = 10–21) of male New Zealand White rabbits were studied. CHF was induced by chronic ventricular pacing. Rabbits were instrumented to record left ventricular end-diastolic pressure (LVEDP), left ventricular end-diastolic diameter (LVEDD), and renal sympathetic nerve activity (RSNA). Ex substantially restored both CP reflex sensitivity and baseline RSNA in CHF animals. Thus Ex beneficially affects reflex regulation in CHF, thereby lowering resting sympathetic nerve activity.

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NEUROHUMORAL ACTIVATION, INCLUDING sympatoexcitation relates to the severity of disease and overall prognosis in the chronic heart failure (CHF) condition (5, 12, 29). In CHF, exercise training (Ex) may relieve the symptomatic burden and improve overall prognosis (2). Hence, Ex represents a lifestyle recommendation for patients with moderate CHF in whom training protocols can still be performed (26). The reason Ex is salutary in CHF may relate to endothelial cell (16) and skeletal muscle function (14) as well as to an overall cardiorespiratory conditioning that may, in turn, affect sympatoexcitation. Several reflexes have been investigated in relation to the exercise-related cardiorespiratory conditioning in CHF. The proposed reflex conditioning in CHF by Ex includes the chemosensitive ergoreflex of peripheral muscles (25) and the arterial baroreflex (18). An improvement in baroreflex function after Ex was paralleled by a decrease in sympatoexcitation (18). Because abnormal reflex regulation in the CHF state may trigger sympatoexcitation, including increased catecholamine spillover into the blood (30), interventions that augment reflex function may be effective in counteracting neurohumoral activation. This concept was supported by serum epinephrine concentrations that were found to be lower in exercise-trained CHF patients (15).

Inputs from mechanoreceptors in the ventricles and atria comprising the primary afferent inputs of the cardiopulmonary reflex have been found to be desensitized in the CHF state (30). Because Ex may augment the input from cardiopulmonary mechanoreceptors (8, 9), we hypothesized that Ex would condition and restore the attenuated cardiopulmonary reflex in the setting of CHF. A restored cardiopulmonary reflex may contribute to a normalization of sympathetic control of the circulation in CHF. The aim of this study was to assess cardiopulmonary reflex function in a model of CHF with and without Ex compared with the normal.
EXERCISE AND CARDIOPULMONARY REFLEX IN HEART FAILURE

METHODS

Animal model. The study design and experiments were reviewed and approved by the University of Nebraska Medical Center Institutional Animal Care and Use Committee and conformed to guidelines for the care and use of experimental animals of the American Physiological Society and the National Institutes of Health.

Fifty-six male New Zealand White rabbits weighing between 3.0 and 3.5 kg were subjected to two surgeries: first, to implant cardiac pacing leads and sonographic crystals 5 wk before experiments and, second, to attach renal sympathetic nerve electrodes and to insert a jugular venous catheter 3 days before experiments as described previously (20). In brief, in both surgeries, the trachea was intubated and the rabbits were anesthetized with isoflurane. In the first surgery, a left thoracotomy was performed in the third interscapular space. After the pericardium was opened, a pair of 5-MHz, 2-mm piezoelectric crystals were sutured to the epicardial surface of the left ventricle across the base of the short axis. A pacing electrode was sutured to the epicardium of the left ventricle. A reference electrode was secured to the left atrium. The chest was closed and evacuated. Rabbits were allowed to recover for 2 wk before entering into the study. Rabbits were assigned to one of four groups: group I was a normal control (Norm) group, group II was exercise-trained normal (Norm-Ex) rabbits, group III was CHF control (CHF) rabbits, and group IV was exercise-trained CHF (CHF-Ex) animals. Rapid pacing over 3 wk (100 beats/min over resting heart rate up to 340 beats/min) with use of a pacemaker of our own design was carried out in groups III and IV starting 2 wk after recovery from the initial surgery. Ex for groups II and IV was started after 2 wk of recovery from surgery.

In the second surgery, the left or right kidney was approached via a flank incision, and the renal artery and nerves were identified. After a small portion of several nerves was freed, wire electrodes were placed around the nerves as described earlier (20). The electrodes and ground wire were secured with silicone gel (Wacker Sil-Gel). All wires were tunneled subcutaneously and exited in the midscapular region. Finally, jugular venous and left ventricular catheters (via the left carotid artery) were implanted.

Biochemistry tests. Blood samples (3 ml) were taken 20 min after the pacemaker was turned off (or at a similar time in the nonpaced rabbits) when the rabbits were calm. Samples were drawn just before each volume-expansion experiment. Blood sample tubes were immediately placed on ice and centrifuged for 10 min at 4°C at 13,000 rpm. Plasma samples were stored at −70°C until analyzed. Plasma concentrations of norepinephrine were determined by using a radioenzymatic assay (24) (obtained in kit form; Amersham Biosciences, Piscataway, NJ).

Sympathetic nerve activity. Renal sympathetic nerve activity (RSNA) recordings were conditioned by a preamplifier and band-pass filter (model P 55, Grass) that was connected to a data-acquisition device (Powerlab, ADInstruments) as described previously (27). In brief, the signal was amplified, rectified, filtered, and displayed on a storage oscilloscope and computer monitor. RSNA was calculated as spike frequency (spikes/s) by using a discriminator that was set 10% above the noise within the amplitude of sympathetic bursts. The relationship between changes of RSNA to a given absolute left ventricular end-diastolic pressure (LVEDP) or left ventricular end-diastolic diameter (LVEDD) were plotted and analyzed. All parameters were averaged over 1 min during baseline conditions or over each 3 s during dynamic changes (e.g., volume-expansion or stimulation conditions). Baseline sympathetic nerve activity (spikes/s) was calculated as the percentage of maximum inducible RSNA frequency (% of maximum). Stimuli used to elicit peak sympathetic nerve activity were either sodium nitroprusside (SNP; 50 μg/kg) for transient hypotension or a puff of smoke to the face. Whereas SNP acts by unloading the baroreceptors, smoke irritates trigeminal afferents (11) as a pressure-independent way to raise sympathetic nerve activity. These techniques have been used previously by this laboratory and correlate qualitatively with integrated sympathetic nerve activity (19).

Ex protocol. Rabbits assigned to an exercise protocol (Norm-Ex, CHF-Ex) were subjected to a 5-min warm-up period on a treadmill at 4 m/min at zero grade. They were then exercised for 30 min at 18–20 m/min. A final cool-down period of 5 min at 4 m/min was performed, whenever possible. This exercise protocol was maintained for 3 wk and was carried out 6 days/wk. During that time, the CHF-Ex group was constantly paced as were CHF rabbits.

Experimental protocol. Conscious rabbits were trained to sit in a plastic box in a dimly lit, quiet laboratory. The external pacemaker was disconnected from the pacing leads, and ECG electrodes were connected to the pacing leads to obtain heart rate. After a 30-min stabilization period, baseline heart rate and baseline RSNA were determined as described in Sympathetic nerve activity. After return to baseline levels, intravenous volume expansion was carried out by infusion of 35 ml of a 6% dextran 70 (37°C) solution in normal saline at a rate of 5 ml/min with an infusion pump. Hemodynamic, dimensional, and neural data were continuously recorded. The relationships between the changes in RSNA and cardiac dimensions or left ventricular filling pressures during volume expansion were determined by linear regression of data derived from 3-s bins acquired after each 5-ml volume infused. The slopes of these relationships served as indexes of cardiopulmonary reflex sensitivity.

Statistical analysis. The data for each group are expressed as means ± SE. Differences among groups were assessed by using a one-way ANOVA for repeated measures. Post hoc analysis consisted of the Tukey-Kramer multicomparisons test. Comparisons between changes of parameters before and after volume expansion were evaluated by using the two-tailed, paired t-test after a normality test was passed. A value of P < 0.05 was considered statistically significant.

RESULTS

Ex resulted in an increase in skeletal muscle mass in trained animals as shown by weights of the vastus lateralis muscle [Norm: 10.5 ± 1.1 g, Norm-Ex: 11.8 ± 0.4 g (P < 0.05 vs. CHF), CHF 8.0 ± 0.6 g, and CHF-Ex 12.6 ± 1.0 g (P < 0.01 vs. CHF)]. However, body weights on the day of the experiment and left ventricular mass as determined postmortem remained unchanged among groups (body weight and body-weight-to-left ventricular weight ratio in Norm: 3.0 ± 0.1 kg, 1.6 ± 0.1 kg; Norm-Ex: 3.3 ± 0.1 kg, 1.6 ± 0.1 kg; CHF: 3.2 ± 0.1 kg, 1.5 ± 0.0 kg; CHF-Ex: 3.3 ± 0.1 kg, 1.6 ± 0.1 kg). Hemodynamics. All hemodynamics were measured after the pacemaker was turned off for ∼30 min. Sonometric readings for cardiac dimensions, including LVEDD and derivatives thereof such as the percent
fractional shortening (%FS) and the maximum first derivative of diameter shortening (−dD/dt\textsubscript{max}), were obtained and indicated cardiac dilation and loss of systolic ventricular function during pacing. Hemodynamic (heart rate, systolic blood pressure, LVEDP) and sonometric findings (change in LVEDD during the course of study, %FS, −dD/dt\textsubscript{max}) are summarized in Table 1. In the nonexercised CHF group, resting heart rate was found to be higher compared with all other groups. In addition to these objective criteria for demonstrating heart failure, clinical signs such as pulmonary edema, ascites, and cachexia, were commonly observed.

Whereas CHF animals showed markedly increased LVEDP and a decreased systolic function as evident by lower −dD/dt\textsubscript{max} compared with Norm, CHF-Ex animals were not statistically different from Norm animals for the same parameters.

Catecholamines. Mean plasma concentrations of norepinephrine, epinephrine, and dopamine are shown in Table 2. Whereas there was a significant difference between Norm and CHF animals for the norepinephrine and epinephrine data, dopamine concentrations only tend to be elevated in the CHF group but were not significantly elevated compared with the Norm group. Although the catecholamine data for the CHF-Ex group tended to be lower than the CHF group, these data did not reach statistical significance. Interestingly, however, there were no statistical differences between the CHF-Ex group and either the Norm or Norm-Ex groups.

Cardiopulmonary reflex. Cardiopulmonary reflex sensitivity is displayed as the linear regression slope for the relationship between changes in RSNA and LVEDP (examples for Norm and CHF animals are shown in Fig. 1; mean data are shown in Fig. 2A) and additionally, for the relationship between changes in RSNA and LVEDD (Figs. 1 and 2B) during volume expansion. As can be seen, RSNA fell in a linear fashion during volume expansion, and the slopes of the relationships were markedly blunted in the CHF state.

Table 1. Baseline hemodynamics in Norm, Norm-Ex, CHF, and CHF-Ex rabbits

<table>
<thead>
<tr>
<th></th>
<th>Normal (n = 21)</th>
<th>Norm-Ex (n = 13)</th>
<th>CHF (n = 10)</th>
<th>CHF-Ex (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, min</td>
<td>218.5 ± 5.7</td>
<td>214.3 ± 11.9</td>
<td>249.7 ± 7.1\textsubscript{a,d}</td>
<td>220.9 ± 4.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>103.1 ± 4.1</td>
<td>107.1 ± 5.5</td>
<td>95.6 ± 7.4</td>
<td>101.7 ± 4.7</td>
</tr>
<tr>
<td>dD/dt\textsubscript{max}, cm/s</td>
<td>-13.5 ± 0.8</td>
<td>-10.9 ± 1.0</td>
<td>-9.2 ± 1.1\textsuperscript{a}</td>
<td>-11.4 ± 0.7\textsuperscript{a}</td>
</tr>
<tr>
<td>%FS</td>
<td>8.4 ± 0.5</td>
<td>8.2 ± 1.0</td>
<td>4.6 ± 0.3\textsuperscript{b,e}</td>
<td>5.9 ± 0.5\textsuperscript{a}</td>
</tr>
<tr>
<td>ΔLVEDD, mm</td>
<td>-0.1 ± 0.2</td>
<td>0.1 ± 0.3</td>
<td>2.9 ± 0.5\textsuperscript{b-c}</td>
<td>1.8 ± 0.4\textsuperscript{b-e}</td>
</tr>
<tr>
<td>LVEDP, mmHg</td>
<td>4.6 ± 1.5</td>
<td>5.2 ± 1.3</td>
<td>10.7 ± 1.7\textsuperscript{a}</td>
<td>6.0 ± 1.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Measurements were taken 30 min after the pacemaker was turned off. Norm, normal control; Norm-Ex, exercise-trained normal; CHF, chronic heart failure control; CHF-Ex, exercise-trained chronic heart failure; BP, blood pressure; −dD/dt\textsubscript{max}, first derivative of dimension representing maximum shortening velocity; %FS, percent fractional shortening; ΔLVEDD, change in left ventricular end-diastolic diameter after 3 wk of pacing or not pacing; LVEDP, left ventricular end-diastolic pressure. \textsuperscript{*}P < 0.05, \textsuperscript{b}P < 0.01, \textsuperscript{c}P < 0.001 compared with Norm. \textsuperscript{d}P < 0.05, \textsuperscript{e}P < 0.01, \textsuperscript{f}P < 0.001 compared with Norm-Ex. \textsuperscript{g}P < 0.05 compared with CHF.

Table 2. Catecholamine values in Norm, Norm-Ex, CHF, and CHF-Ex rabbits

<table>
<thead>
<tr>
<th></th>
<th>Norm</th>
<th>Norm-Ex</th>
<th>CHF</th>
<th>CHF-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>268.2 ± 27.1</td>
<td>547.2 ± 62.8</td>
<td>1143 ± 319.4\textsuperscript{*}</td>
<td>740.4 ± 236.3</td>
</tr>
<tr>
<td>Dopamine, pg/ml</td>
<td>52.6 ± 27.5</td>
<td>28.3 ± 15.1</td>
<td>108.1 ± 36.7</td>
<td>42.0 ± 7.6</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>41.6 ± 7.7</td>
<td>80.7 ± 20.3</td>
<td>290.4 ± 189.2\textsuperscript{*}</td>
<td>107.5 ± 33.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. \textsuperscript{*}P < 0.05 compared with Norm.
after SNP. Ex reduced resting sympathetic nerve activity in CHF rabbits as determined by both methods. However, Ex did not further lower RSNA in rabbits with normal cardiac function.

DISCUSSION

The data obtained in this study confirm our laboratory’s previous finding that Ex is associated with less sympathoexcitation (14) in male rabbits with pacing-
induced CHF. Second, we have demonstrated here, for the first time, in a conscious model of CHF that the sympathoinhibitory cardiopulmonary reflex sensitivity is enhanced after Ex in animals with CHF. These data support the concept that Ex is beneficial in the CHF state (26) because it ultimately lowers the detrimental sympathetic drive through changes in reflex function slowing down the vicious cycle leading to further cardiac dysfunction.

**Exercise and hemodynamics in heart failure.** The CHF state was documented by cardiac dilation increased cardiac filling pressures and reduced contractility. However, the dilation process was significantly less for animals in the CHF-Ex group, indicative of a slower progression of the heart failure process. Specifically, $-d/d_{max}$ and LVEDP returned toward normal values, whereas %FS and the change in LVEDD over 3 wk of ventricular pacing were statistically less for Norm animals than the CHF counterparts. The lower heart rate in the CHF-Ex group may be due to both lowering sympathetic excitation and restoration of vagal function (21). Left ventricular weight was found to be similar among groups, suggesting that this exercise protocol did not result in left ventricular hypertrophy in the time frame of this study. Interestingly, in the CHF group, the loss of body mass due to peripheral cachexia outweighed the increase in weight due to ascites and edema. Body weight as such does not differentiate between these two phenomena.

**Hormonal milieu.** Plasma catecholamines tended to be lower for CHF-Ex rabbits compared with the sedentary CHF group. Although there was no statistical difference between these groups, it is important to note that there was also no statistical difference between the CHF-Ex group and the Norm and Norm-Ex groups, whereas the CHF group exhibited significantly higher levels of norepinephrine and epinephrine compared with the Norm group. Dopamine tended to be lower in the CHF-Ex group, but the data did not reach statistical significance in any group. Taken together with the decrease in resting RSNA, these data suggest a decrease in sympathoexcitation in CHF-Ex rabbits. A similar finding has been demonstrated in patients with CHF (3, 15). The smaller effect on catecholamines compared with RSNA may reflect contributions from other vascular beds that are not normalized to the degree that sympathetic outflow to the kidneys were. Furthermore, because the present study used male rabbits solely, possible gender differences of Ex in CHF need to be explored in further studies.

**Cardiopulmonary reflex and sympathetic tone.** The relationships of RSNA and LVEDP or RSNA and LVEDD were used as measures of reflex sensitivity for the sympathoinhibitory cardiopulmonary reflex. Although plotting RSNA vs. volume infused would have served the same purpose, the use of changes in cardiac wall distension (LVEDD) (13) and in filling pressures (LVEDP) (22) emphasizes the physiological stimuli for the cardiopulmonary reflex. Because there were no relevant changes in mean blood pressure for the individual experiments of CHF and CHF-Ex animals, we do not consider the baroreflex as a mediator of changes in RSNA under these conditions (22). The increased venous return provided by volume expansion predominantly changes pressure in the low-pressure circulation as determined by the LVEDP serving as the reflex stimulus.

The effects of Ex on sympathetic nerve activity may reflect a generalized normalization of all known cardiovascular reflexes. Besides the restoration of the cardiopulmonary reflex sensitivity, as shown here, our laboratory previously demonstrated enhanced arterial baroreflex sensitivity in CHF-Ex rabbits (18). Furthermore, it has also been demonstrated that Ex reduces an augmented peripheral sympathoexcitatory chemoreflex sensitivity (31) and enhances Bezold-Jarisch reflex responses (33) in experimentally induced CHF. Alternatively, differences in the input and expression of somatic reflexes from skeletal muscle may be involved in the response to Ex (25); however, there are no data to support the latter notion.

The mechanisms by which Ex in heart failure alter reflex function and sympathetic tone are not completely understood. Adverse changes in CHF, such as increased inducible nitric oxide synthase (NOS) and cytokine expression (1, 14), may be reversed by exercise implying a better prognosis (7). Conversely, endothelial NOS is augmented by Ex (6), possibly caused by enhanced arteriolar shear stress via a hydrogen peroxide pathway (23). It is conceivable that an increased vascular nitric oxide availability and a possible concomitant decrease in oxidant stress may affect afferent nerve fibers (e.g., baroreflex afferents), thereby altering vagal afferent function (32). DiCarlo et al. (10) have shown an increase in NADPH diaphorase-staining neurons in the hypothalamus indicative of an increase in NOS I activity in hypertensive rats subjected to a daily exercise regime. Alternative explanations may relate to brain natriuretic peptide (BNP) (17). Even though exogenous BNP lowers sympathoexcitation in CHF (4), plasma BNP is inversely correlated to exercise capacity. However, as a limitation of the present study, BNP was not determined. A possible role of BNP as a mediator of exercise-induced outcomes in CHF still remains uncertain. Finally, a previous study from this laboratory (17) showed an exercise-related reduction in plasma angiotensin II in CHF. That is, in addition to the direct effects of reflex changes, hormones, including angiotensin II (28) and endothelin (20), likely participate in the Ex-induced changes in CHF. Therefore, according to the evolving concept of neurohumoral activation in CHF, other hormonal pathways need to be investigated with regard to Ex effects in CHF patients.

In summary, Ex reduces resting sympathetic nerve activity and plasma catecholamines in CHF rabbits. In addition, cardiopulmonary reflex sensitivity was shown to be improved after Ex. Overall, the concerted action of cardiovascular reflex conditioning by Ex is proposed to be one mechanism of beneficially affecting neurohumoral stimulation as well as the rate of disease progression in the CHF state.
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DISCLOSURES

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REFERENCES


