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Voluntary exercise delays heart failure onset in rats with pulmonary artery hypertension

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Submitted 9 April 2015; accepted in final form 18 May 2015

Natali AJ, Fowler ED, Calaghan SC, White E. Voluntary exercise delays heart failure onset in rats with pulmonary artery hypertension. Am J Physiol Heart Circ Physiol 309: H421–H424, 2015.—Increased physical activity is recommended for the general population and for patients with many diseases because of its health benefits but can be contraindicated if it is thought to be a risk for serious cardiovascular events. One such condition is pulmonary artery hypertension (PAH). PAH and right ventricular failure was induced in rats by a single injection of monocrotaline (MCT). MCT rats with voluntary access to a running wheel ran on average 2 km/day. The time for half the animals to develop heart failure signs (median survival time) was 28 days (exercise failure group), significantly longer than sedentary animals (sedentary failure group, 23 days). The contractility of single failing myocytes in response to increasing demand (stimulation frequency) was significantly impaired compared with that in both sedentary control and exercising control myocytes. However, myocytes from exercising MCT rats, tested at 23 days (exercise + MCT group), showed responses intermediate to the control (sedentary control and exercising control) and failing (sedentary failure and exercise failure) groups. We conclude that voluntary exercise is beneficial to rats with heart failure induced by PAH, and this is evidence to support the consideration of appropriate exercise regimes for potentially vulnerable groups.

e speculate, given as a reason to limit physical activity in patients with PAH (5, 9). Although exercise has been shown to benefit patients with PAH (3, 16) this has not yet been incorporated into treatment guidelines (15).

An animal model study of PAH found treadmill running exercise was beneficial to rats with stable PAH but detrimental to those with progressive PAH, decreasing survival time (11). The interpretation of these data could be that sufferers of severe PAH will not benefit from exercise. However, there are multiple types of exercise modes and regimes used in rodents with respective strengths and weaknesses (17, 20, 24). The aim of our study was to test whether an alternative exercise regime might prove beneficial in rats destined to develop RV failure induced by PAH and thus give an alternative view of the potential role of exercise in PAH.

METHODS

Male Wistar rats (200 g) had either free access to a running wheel that logged rotations and thus daily running distance (designated as exercise animals, E) or no access to wheels (designated as sedentary animals, S) [see White and colleagues (18, 22)]. Animals were introduced to the running wheels 2 days before treatment with monocrotaline (MCT) or saline.

Animals received either a single intraperitoneal injection of 60 mg/kg MCT to induce RV failure (F) or an equivalent volume of saline as control (C) [see Benoist et al. (1, 2)]. Rats were euthanized upon showing signs of heart failure (e.g., weight loss, dyspnea, piloerection) or on equivalent days for control animals. In addition to these four groups (SC, EC, SF, EF), a fifth group was given access to running wheels, injected with MCT but taken at the median end-point day (±1 day) of SF animals for temporal comparison. This group was designated as exercise + MCT (EM). The median survival time for SF and EF groups represented the time after MCT treatment when more than 50% of the group reached the heart failure end point (see Fig. 1).

Following euthanasia, the heart and lungs were excised, blotted dry, and weighed. The heart was then attached to a Langendorff-retrograde perfusion system, and single RV myocytes were isolated as previously described (1, 2, 14). Each group had N = 6 animals. Experiments were conducted in accord with Health Research Extension Act (public law 99-158, 1985 “Animals in Research”) and UK Home Office regulations, and local ethical approval was obtained.

Myocytes were placed in a bath on the stage of an inverted microscope and superfused with a Tyrode solution containing (in mM) 137 NaCl, 5.4 KCl, 0.33 NaH2PO4, 0.5 MgCl2, 5 HEPES, 5.6 glucose, and 1.8 CaCl2 (pH 7.4) with 5 N NaOH. Individual myocytes were selected for study if they had a clear, regular striated (sarcomer) pattern, did not spontaneously contract in the absence of external

NEW & NOTEWORTHY

In rats that developed pulmonary artery hypertension, voluntary wheel running exercise delayed both progression to right heart failure and deterioration of the myocytes’ response to increased demand. Our observations suggest that appropriate exercise regimes may be useful in the treatment of pulmonary artery hypertension.

REGULAR PHYSICAL ACTIVITY (exercise) is known to have multiple beneficial health effects and is recommended to the general population and many patients including those with heart disease (10). However, conditions exist where exercise is contraindicated because of the possibility of provoking serious cardiopulmonary events. Pulmonary artery hypertension (PAH) results in right ventricular (RV) failure, and the impaired ability of the RV to increase stroke volume during exercise is...
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stimulation, and responded to 1-Hz stimulation with a single twitch. Myocytes were stimulated to contract, and demand progressively increased by increasing stimulation frequency from 1 to 7 Hz. Cells were field stimulated at the required frequency by external platinum electrodes, and the resultant cell shortening was measured via analysis of a video image of the cell using an Ionoptix camera and software (Ionoptix, Milton, MA). Cell shortening was expressed as percentage of resting cell length. All experiments were performed at 37°C.

Statistics. Data are presented as means ± SE. P < 0.05 was considered significant. Survival was tested by a Mann-Whitney test. Running distances (on representative days 1, 8, 15, and 22) were compared by two-way repeated-measures analysis of variance (ANOVA), contraction-frequency relationships by two-way ANOVA, and animal weights and organ weights by one-way ANOVA. ANOVAs were followed by pairwise Tukey correction tests. Proportions were tested by χ²-test. Numbers of rats, hearts, and myocytes used in each experiment are given in the relevant table and figure legends.

RESULTS

Figure 1 shows survival for SF and EF animals. Although all animals in these groups developed heart failure signs, the median survival time for EF animals (28 days) was significantly longer than SF animals (23 days, P < 0.05), indicating a benefit of exercise. There was no correlation between total running distance or daily running distance before heart failure onset and survival time.

All animals given access to running wheels ran on them. The EF group and most EM animals ran on average 2 km/day, 22 days after injection of MCT (Fig. 2). There were no significant differences in the daily running distances of the three exercising groups (EC, EF, EM, P > 0.05). Overall, running distances were greater on days 15 and 22 (P < 0.001) and day 8 (P < 0.05) than on day 1. Mean daily distances were increased in EC and EM groups by 1 to 2 animals running more than others, hence, the larger means ± SE in these groups (Fig. 2). Daily running distance in the EF group began to fall 2 to 3 days before the observation of heart failure signs.

Whole animal and organ weights showed that failing animals displayed the characteristic increase in heart-weight-to-body weight and RV-to-left ventricular weight ratios that is indicative of RV hypertrophy. MCT-treated animals with voluntary access to exercise wheels, taken before the onset of failure (EM), had values intermediate between the control (SC, EC) and failing (SF, EF) groups (Table 1).

When stimulated to contract, SF myocytes displayed a steep negative contraction-frequency relationship and were the only group to show statistically greater cell shortening at 1 and 3 Hz vs. 7 Hz (P < 0.001). In contrast, over the range of 1–7 Hz, SC, EC, and EM myocytes displayed a biphasic relationship (Fig. 3A). There were no statistical differences in the magnitude of shortening at 1 Hz among the five groups, but at 3–7 Hz, statistical differences were seen between control (SC, EC) and failing (SF, EF) groups. The EM group was intermediate in response to the control and failing groups (Fig. 3A). All cells were able to entrain to a stimulation frequency of 1–5 Hz and contract at those frequencies, but some cells were not able to entrain at 7 Hz and did not respond to each stimulus. The proportion of cells able to entrain at 7 Hz was significantly different between the groups (P < 0.001) with the smallest proportions found in the two failing groups, SF and EF (Fig. 3B).

DISCUSSION

Male MCT-treated rats voluntarily used running wheels, an interesting observation given that lethargy can be a reported characteristic of MCT treatment. The study was performed in male rats for consistency with previous studies (1, 2). The daily running distance of 2 km/day is less than we have previously reported in female rats (18, 22); even so, this improved survival and functional characteristics of myocytes of MCT-treated animals.

The organ parameters for SF and EF animals were not different from each other, indicating that although exercise

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Fig. 1. Survival, measured in days to heart failure signs, was significantly shorter in sedentary failure (SF) than in exercise failure (EF) rats with voluntary access to running wheels. P < 0.05, Mann-Whitney test, N = 6 each group.

Fig. 2. Mean ± SE daily running distance in groups with voluntary access to running wheels for exercise control (EC) rats, monocrotaline (MCT)-treated rats that reached heart failure signs end point (EF), and MCT-treated rats taken at the median ±1 end-point day of sedentary MCT-treated rats (EM). Animals were injected with MCT or saline on day 1, running distances were compared at days 1, 8, 15, and 22 of treatment and were significantly greater on day 8 (P < 0.05) and days 15 and 22 (P < 0.001) than day 1. There were no significant differences between the groups on days 1, 8, 15, or 22. Two-way repeated-measures ANOVA. N = 6 in each group until animals reach designated end points from day 21 onward.

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00262.2015 • www.ajpheart.org
Table 1. Whole animal and organ parameters

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>EC</th>
<th>SF</th>
<th>EF</th>
<th>EM</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>323.17 ± 5.64</td>
<td>286.17 ± 7.75*</td>
<td>269.67 ± 10.54*</td>
<td>288.83 ± 12.19</td>
<td>271.50 ± 4.26*</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.16 ± 0.06</td>
<td>1.15 ± 0.04</td>
<td>1.39 ± 0.06</td>
<td>1.64 ± 0.11*#&amp;</td>
<td>1.26 ± 0.04</td>
</tr>
<tr>
<td>RV weight, g</td>
<td>0.27 ± 0.04</td>
<td>0.21 ± 0.01</td>
<td>0.44 ± 0.05*#&amp;</td>
<td>0.45 ± 0.02*#A</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td>LV weight, g</td>
<td>0.58 ± 0.04</td>
<td>0.54 ± 0.07</td>
<td>0.48 ± 0.04</td>
<td>0.63 ± 0.05</td>
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<tr>
<td>Lung weight, g</td>
<td>2.32 ± 0.16</td>
<td>2.02 ± 0.17</td>
<td>2.97 ± 0.38*#&amp;</td>
<td>2.73 ± 0.15</td>
<td>2.16 ± 0.22</td>
</tr>
<tr>
<td>Ratio of heart weight to body weight, mg/g</td>
<td>3.56 ± 0.14</td>
<td>4.03 ± 0.10</td>
<td>5.19 ± 0.24*#&amp;</td>
<td>5.70 ± 0.16*#&amp;</td>
<td>4.66 ± 0.19*#</td>
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<tr>
<td>Ratio of RV weight to LV weight, mg/mg</td>
<td>0.46 ± 0.05</td>
<td>0.41 ± 0.06</td>
<td>0.95 ± 0.12*#&amp;</td>
<td>0.75 ± 0.06&amp;</td>
<td>0.59 ± 0.06</td>
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<tr>
<td>Ratio of lung weight to body weight, mg/g</td>
<td>7.16 ± 0.44</td>
<td>7.13 ± 0.76</td>
<td>11.24 ± 1.66&amp;</td>
<td>9.55 ± 0.68</td>
<td>8.02 ± 0.90</td>
</tr>
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</table>

Values are means ± SE; N = 6 in each group. Monocrotaline-treated animals with voluntary access to exercise wheels taken before the onset of failure (EM) have values intermediate between the sedentary and exercise control (SC, EC) and failing (SF, EF) groups. RV, right ventricle; LV, left ventricle. *P < 0.05 vs. SC; #P < 0.05 vs. EM; &P < 0.05 vs. EC. One-way ANOVA.

delayed the onset of failure signs, the characteristics of these animals on reaching that end point were similar. In the EM group, the mean values for all parameters, except left ventricular weight, were intermediate between the C and F groups, indicating that exercise delayed the progression of organ remodeling in PAH.

An inability to respond to an increase in demand is a defining characteristic of heart failure (8). In RV failing myocytes, this is manifested as a steep negative contraction-frequency relationship and a smaller proportion of cells able to respond to high frequency (7 Hz) stimulation compared with the control groups. EM myocytes (i.e., MCT-treated myocytes before the onset of failure) displayed the characteristics of control cells, indicating an improvement of contractile function relative to myocytes from animals that had developed failure.

Positive effects of treadmill running have previously been reported when exercise was begun 2 wk before MCT administration (4, 21). However, this pretraining might improve resistance to the conversion of MCT into the active agent. Another MCT study showed that whereas exercise had beneficial effects in animals with stable PAH, in those with progressive PAH exercise had detrimental effects on cardiac hemodynamics, caused increased RV inflammation, and decreased survival times (11). In that study exercise began 2 wk after MCT treatment; once PAH had developed, exercise volume was 2 km/wk (at 13.3 m/min; 0.5 maximum oxygen consumption) under an enforced continuous treadmill running regime with a noxious reinforcement stimulus (11).

In contrast, our study, using the same heart failure end point, found a beneficial effect of voluntary exercise (2 km/day) in progressive PAH. However, the studies are not direct comparisons. Though voluntary running can attain speeds of 60 m/min, the running is intermittent and carried out in short bursts (6, 19). In addition, we began voluntary exercise before development of PAH. This design was chosen because voluntary running distances take several days to build up, and the development of PAH in this model is rapid. We were therefore concerned that a later exercise starting date would give little time for any exercise benefits to accrue. This is supported by our observation that daily running distance began to fall 2 to 3 days before the observation of heart failure signs.

Exercise mode may influence study outcomes. With enforced regimes, the loss of control over locomotion and use of a reinforcement stimulus can trigger stress responses (17, 20, 24). Furthermore, continuous running is an unnatural mode of locomotion for rodents (17, 24). High-intensity, treadmill-running exercise does produce physiological training responses...
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(12), but at lower intensity levels, these responses may not outweigh the negative effects of stress. It is acknowledged that stress has a negative effect on heart failure prognosis (7, 13), and enforced exercise is not used with humans.

In patients with left ventricular heart failure, the greatest benefits of exercise are seen with high-intensity training (23). MCT-treated animals were still exercising when heart failure signs developed. This raises the possibility of experimentally increasing voluntary exercise volume and intensity using reward techniques or wheel loading to enhance the effects of exercise. This may allow exercise to be introduced to MCT rats at a later time point to better model the use of exercise as a treatment in patients with established PAH.

Our observations do show that voluntary exercise slowed the development of severe PAH and RV failure. This is in accord with the wide-ranging health benefits of increased physical activity in both healthy and diseased populations and suggests that these extend even to severe PAH. Extrapolating these animal data to humans, we conclude that no group should be considered beyond the beneficial effects of an appropriate exercise regime.

GRANTS

This study was funded by a Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil Scholarship (to A. J. Natali) and a University of Leeds PhD studenthip (to E. D. Fowler).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


