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EFFECT OF EXHAUSTIVE ENDURANCE EXERCISE ON CARDIAC CATECHOLAMINE METABOLISM IN TRANSGENIC MICE

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ABSTRACT

Purpose. Neurotrophin-3 (NT-3) is required for proliferation, differentiation and survival of sympathetic neurons. A NT-3 transgenic mutant mouse strain was used to determine whether disruption of one NT-3 gene can maintain increased cardiac sympathetic response during stress of exhaustive endurance exercise.

Methods. Ten NT-3 mutant (+/-) and 10 wild type (+/+) adult mice were randomly assigned into four groups: mutant sedentary control (MUT, +/-, CON), mutant exercise (MUT, +/-, EX), wild type sedentary control (WT, +/+, CON) and wild type exercise (WT, +/+, EX). Exercise groups ran until exhaustion on a motor-driven treadmill, while control groups remained in their cages. Mice were euthanized immediately after running or resting. Left ventricles (LV) were excised and homogenized in 0.1M percholoric acid and catecholamine concentrations were measured by HPLC. Norepinephrine (NE), 3-methoxy-4-hydroxyphenylglycol (MHPG), dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) concentrations were measured in the LV.

Results. Data showed that stress of exercise could uncover an effect of NT-3 gene disruption on the LV NE concentration and its turnover. Exercising WT mice demonstrated higher cardiac sympathetic activity. NE and MHPG concentrations and MHPG/NE ratios significantly increased in the LV of WT-EX. Mice with disruption of one NT-3 gene (MUT, +/-, knockout mice) could not maintain increased sympathetic response at the same level. There was no significant increase in NE and MHPG concentrations or MHPG/NE ratios in MUT (+/-) mice under stress of exercise. There were no significant differences in the concentration of dopamine's metabolite DOPAC in the LV among any group.

Conclusion. MUT (+/-)-knockout mice could not maintain increased cardiac sympathetic response of the LV during exhaustive endurance exercise at the same level as wild type mice.

Key words: neurotrophin-3, knockout mice, exercise, cardiac norepinephrine

Neurotrophins are trophic factors required for the proliferation, differentiation and survival of vertebrate neurons (16). Neurotrophin-3 (NT-3) has the most diverse and complex roles, functioning through multiple receptors and cellular mechanisms in different types of neurons (9). Of particular significance to the present report are the

findings that NT-3 homozygous null mutant (MUT, -/-) show loss of sensory and sympathetic neurons in the peripheral nervous system (8). Heterozygous mutant MUT (+/-) mice have no visible phenotypic abnormalities, are fertile, and exhibit much less impairment of sympathetic nervous system (SNS) than (-/-) littermates (6, 8, 24). In further investigation of the cardiac sympathetic function in heterozygous mutant MUT (+/-) mice, exercise stress might be a useful experimental tool. A significant elevation in the sympathetic function (increase of NE concentration or NE turnover) occurs in the rat myocardium during acute stress of exercise (4,11,12, 17, 18). Therefore, a NT-3 transgenic mutant mouse strain was used to determine whether disruption of one NT-3 gene can maintain increased sympathetic response during stress of exhaustive endurance exercise.

We sought to test the consequences of NT-3 gene disruption on cardiac sympathetic response during exercise. We employed an NT-3 knockout strain, which have one copy of the mouse NT-3 gene inactivated (MUT, +/-), to study whether the performance of the SNS (cardiac catecholamines response) of these mice is impaired compared to wild type (+/+), normal mice during exhaustive treadmill running.

The specific purpose of the present study was to examine the effect of exhaustive endurance exercise on norepinephrine (NE), 3-methoxy-4-hydroxyphenolglycol (MHPG) (a major metabolite of NE), dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) content of the left ventricle in both wild type mice and MUT (+/-) mice.

Methods

Transgenic mice

The NT-3 mutant strain used was developed on a 129 background by insertion of a partially deleted NT-3 coding exon for the NT-3 gene (7, 24). The Animal Care and Use Committees at Northeastern Ohio Universities (NEOUCOM) in accordance with NIH Guidelines for the Care and Use of Laboratory Animals approved all experimental procedures. Mice were housed at the NEOUCOM in plastic cages, fed and watered *ad libitum* and maintained on 12 h: 12 h light/dark cycles. NT-3 +/- mice were mated to produce offspring. Mice were genotyped as +/+, +/- by PCR amplification and 2% agarose gel electrophoresis of tail DNA. Oligonucleotide primers used for genotyping mice were oIMR130, oIMR131, and oIMR132. Two separate reactions were run for each sample using oIMR130 in combination with oIMR131 and oIMR132, which amplified products from the wild type and targeted alleles, respectively (Jackson Laboratory IMR protocol) (24).

Exercise protocol

Ten MUT (+/-) knockout and 10 wild type (+/+) adult (male and female) mice were randomly assigned into the following four groups: MUT (+/-)-sedentary control (n = 5), MUT (+/-)-exercise (n = 5), wild type-sedentary control (n = 5) and wild type-exercise (n = 5). No statistically significant gender differences in cardiac norepinephrine concentrations, resting heart rate, MAP, or sympathetic tonus were noted for age/NT-3 (+/-) genotype-matched mice (24). Hence, data from males and females were combined.

From our experience with the rodents we observed that running would not last long and the animals often refused to run if running activity is initiated without previous familiarization to the treadmill. For this reason the special attention was given for proper preparation of the mice for the exercise task. “WT” and “MUT” groups of mice were familiarized with the treadmill over a one week period. During familiarization period, the animals in exercise groups were

submitted to walking on the treadmill for 10 minutes at the speed of 10 meters per minute for the five days. The non-exercised animals were placed on the treadmill for the same amount of time as the exercise groups, but without running during same five days. Each animal was tested individually to ensure continual monitoring. Each animal was carefully monitored during the exercise session from the beginning to its end. No electric shock aversion was used.

The exercise groups performed progressive endurance running on a motor-driven treadmill until exhaustion, and the control groups remained in their cages. Treadmill activity was initiated at 6 m•min^{-1} and 4% inclination, then increased to 12m•min^{-1} 10 min later, and subsequently increased to 18 m•min^{-1} 10 min later. This speed was maintained until exhaustion. Exhaustion was defined as the point when animals were unable to keep pace with the treadmill for one-minute (2). The mice were euthanized by a rapid decapitation immediately after either running or resting. Left ventricles were excised and homogenized in 0.1M (4˚C) percholoric acid and NE, MHPG, DA and DOPAC concentrations were measured by HPLC.

Statistical analysis and gender differences

Mice were grouped according to genotype, sedentary or exercise. One-way ANOVA procedures were conducted to identify significant differences among the four groups. Statistical significance was tested at p = 0.05.

Results

No significant group differences were found in body weight and the heart weight/ body weight ratio (Table 1). Table 2 illustrates the changes in measured variables of catecholamine metabolism in the left ventricles of mice during the experiment. The results showed that the left ventricular concentration of NE and its major metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) significantly increased in response to stress imposed by running to exhaustion in the wild type (+/+) mice, compared to the sedentary wild type (+/+) control. By contrast, the concentration of NE and MHPG in the LV of exercised MUT (+/-) knockout mice did not differ significantly as compared to the MUT (+/-) sedentary control (Table 2). There was a significant increase of the MHPG/NE ratio in the left ventricle of the wild type mice in response to exercise. However, there was no significant difference in the MHPG/NE ratio between the MUT (+/-) knockout mice after exercise to exhaustion compared to the sedentary control MUT (+/-) knockout mice (Table 2). There were no significant differences in the concentration of dopamine's metabolite DOPAC in the LV among any

Table 1. *Physical characteristics of wild type (WT, +/+) and heterozygous mutant (MUT, +/-) mice*

Group	N	TRT	Age (wk)	BW	HT/BW
WT (+/+)	5	CON	11.09 ±1.02	22.2 ±1.3	5.24 ±1.3
WT (+/+)	5	EX	11.00 ±.90	18.8 ±.90	5.62 ±.26
MUT (+/-)	5	CON	10.86 ±.77	20.5 ±.80	5.26 ±.42
MUT (+/-)	5	EX	11.52 ±.93	22.0 ±1.3	5.49 ±.56

BW = body weight; TRT = treatment group; HT/BW = heart weight/ body weight ratio;
N = number of animals; CON = sedentary control; EX = exercise; WT = wild type;
MUT (+/-) = NT-3 knockout mice.
Values presented are Mean±SD.

Table 2. *Effects of acute treadmill running on cardiac norepinephrine (NE), dopamine (DA), 3-methoxy-4-hydroxyphenolglycol (MHPG) and dihydroxyphenylacetic acid (DOPAC) (pg/mg wet weight) of MUT (+/-) and wild type (+/+) mice*

Group	NE Ratio	MHPG/NE	MHPG	DOPAC	DA
WT-CON (+/+) (n = 5)	1018.5±70.2	.0452±.016	42.03±12.4	12.10±3.90	40.10±7.90
WT-EX (+/+) (n = 5)	1390.1±122.3*	.2282±.071*	282.10±106.6*	32.02±10.20	37.33±6.65
MUT-CON (+/-) (n = 5)	1302.78±42.50	.0394±.006	51.52±7.7	9.88±1.62	31.9±6.9
MUT-EX (+/-) (n = 5)	1432.17±95.77	.0755±.0222	107.27±13.4	16.82±1.99	57.1±9.6**

WT-CON = wild type (+/+) sedentary control
WT-EX = wild type (+/+) exercise
MUT-CON = MUT knockout (+/-) sedentary control
MUT-EX = MUT knockout (+/-) exercise
* significance at p=0.05 for comparing wild type (+/+) sedentary control and wild type (+/+) exercise
** significance at p=0.05 for comparing MUT knockout (+/-) type sedentary control and MUT knockout (+/-) exercise

groups. Finally, there was an elevation in the concentration of DA in the LV between the sedentary and exercised groups of MUT (+/-) knockout mice (Table 2).

Discussion

Of the catecholamines, cardiac NE has been the primary subject in exercise studies (4, 11, 12,17, 18, 19). NE is recognized as an important sympathetic neuromediator, with significant roles in the regulation of normal cardiac functions and metabolism. Several studies have shown a variety of myocardial NE responses to acute bouts of exercise among rats. They include a significant increase in NE in the blood and heart during the first hours of exercise (4, 11, 12,17, 18, 19), followed by a plateau in NE levels and a gradual decline in NE in these tissues after several hours (6-12hrs) of swimming or treadmill running (11, 15, 19).

Exercise to exhaustion produced an increase in left ventricular NE concentration in wild type mice, which reflects an elevation in sympathetic activity during physical exertion. Our findings are in agreement with existing data (4, 11, 12, 17) obtained in the studies using acute endurance exercise for rats. Since norepinephrine is a powerful regulator of cardiac contractile activity, metabolism and has an advantageous affect on the heart in relation to exercise (18), a higher concentration of cardiac NE in wild type mice may play an integral role in acute adaptation to exercise stressor. Increases in cardiac NE content during exercise may affect the changes in signaling cascade such as increase in cAMP content (10, 21) and activity of the cardiac cAMP-dependent protein kinase (13). Previously, it was shown that an increase in cAMP content and in cAMP-dependent phosphorylation may in turn stimulate Ca^{++} flux across the sarcolemma, the rate of Ca^{++} uptake by the sarcoplasmic reticulum (1, 14), and lipoprotein lipase activity in the cardiac mu-

scle of rats (3, 20). All of these variables are important for the cardiac adaptation to exercise.

Concerning the major NE metabolite, we found that myocardial MHPG concentrations significantly increased in wild type mice as a result of exercise. An MHPG/NE ratio is a useful index of cardiac sympathetic activity characterizing NE turnover for studies in the rat model. Elevated MHPG/NE ratios after running to exhaustion of wild type mice in our study apparently reflected the increase in NE turnover that accompanies elevated sympathetic activity in exercise more precisely than changes in NE levels or MHPG levels alone. It is of interest that the MHPG/NE ratio failed to change significantly in MUT (+/-) knockout mice during exercise. The fact that NE turnover was not increased in the myocardium of the MUT (+/-) knockout mice is probably a sign of decreased release of NE by the sympathetic nerves at the increased demands - exercise to exhaustion. It is of interest also that in another knockout model of mice, b-adrenoreceptors knockout, despite the total absence of b1-and b2-adrenoreceptors, their resting functions and metabolism (heart rate, blood pressure, and metabolic rate) were normal and there was a normal chronotropic response to the b-adrenoreceptor agonist isoproterenol. However, stress of exercise revealed significant impairments in the chronotropic range, vascular reactivity, and metabolic rate of b-adrenoreceptors knockout mice (5, 22, 23).

There is no available data in the literature regarding DA and DOPAC content in the LV of MUT (+/-) mice either at rest or during exercise. DOPAC concentrations did not differ significantly in either the MUT (+/-) knockout or wild type mice during exercise. Because DOPAC is a major metabolite of DA, the absence of changes in its concentration in the LV after exercise is evidence that exercise did not alter the catabolism of DA in both groups of exercised mice.

To the best of our knowledge, this is the first investigation in which diminished response in LV concentration of NE, and impaired NE turnover have been observed during exhaustive endurance exercise in heterozygous mutant MUT (+/-) knockout mice. In summary, our study showed that stress of exercise could uncover an effect of inactivation of one copy of the mouse NT-3 gene on the LV NE concentration and its turnover. In response to exercise stress, wild type mice demonstrated higher cardiac sympathetic activity. NE and MHPG (a major metabolite of NE) concentrations signnificantly increased in the LV of the exercised wild type mice. MHPG/NE ratio, which characterizes NE turnover, was also significantly increased in the LV of the exercised wild type mice. The mice with disruption of one NT-3 gene (MUT (+/-) knockout mice) failed to show comparable responses. It is concluded that heterozygous mutant NT-3 (+/-) knockout mice (MUT (+/-) could not maintain increased cardiac sympathetic response of the LV during exhaustive endurance exercise at the same level as wild type mice.

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