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Aerobic Physical Training Protects the Rat Brain Against Exercise-Heat Related Oxidative Damage through the Increased Expression of HSP70

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We explored the mechanism underlying the improvement of tolerance to exercise-heat stress through physical training at a moderate temperature. Rats were randomly divided into an aerobic exercise (AE) group and a control (C) group. Rats in the former group were subjected during 5 weeks for AE in a cool environment (20°C). The oxidative damage level in the brain to AE rats after both 1-h-long heat exposure and exhaustive exercise in heat and the rate of core temperature rise were lower than those in the control ($P < 0.05$). The expression level of HSP70 in the motor cortex and the exhaustion time in group AE were found to be significantly greater compared to those in group C ($P < 0.05$). Our results indicate that the enhanced expression of HSP70 may be an important factor for physical training in a cool environment, and this factor improves tolerance to exercise-heat stress.

Keywords: aerobic physical training, oxidative damage, brain, temperature conditions, exercise-heat stress, HSP70.

INTRODUCTION

During physical exercising at a high temperature, both air temperature and physical activity itself may put extra stress on the organism. These two risk factors may work in conjunction to affect the capacity to perform prolonged physical activity [1, 2]. Routinely, exercising in heat may present a serious health risk for athletes and other practitioners.

It has been recognized that physical training in a cool environment might improve an individual's tolerance to exercise-heat stress [3, 4]. It was reported that fit subjects could adapt to heat acclimation more rapidly than non-fit subjects [5], although the beneficial gains obtained from physical training in a cool environment are less than those obtained from heat acclimation training [6].

Numerous studies have demonstrated that increased oxidative stress following exercise in a hot environment creates a significant danger for the CNS. Such stress profoundly influences the brain functions and leads to alteration of the brain activity. This may lead to failure of the CNS, in particular preventing an adequate central drive to spinal motor mechanisms [7, 8]. Failure to drive the motoneurons adequately can play a dominant role in modulating exercise performance under heat conditions. Hence, one may assume that physical training in a cool environment may decrease the oxidative damage to the brain during rest and exercise under heat conditions. This may in part account for the improved tolerance to exercise-heat stress, but such a presumption has not been explored so far.

In this study, we observed the effect of aerobic exercise (AE) on oxidative stress in the motor cortex of the rat brain immediately after 1-h-long heat exposure or exhaustive exercise at a high temperature. In order to investigate a possible underlying mechanism of exercise affecting the oxidative stress level, we also observed the expression levels of heat stress protein 70 (HSP70) in rats performing AE and control animals. The purpose of the study was to explore the mechanisms by which physical training in a cool environment may improve tolerance to exercise-heat stress from the point of view of the CNS.

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METHODS

Male Wistar rats (Shandong Lukang Pharmaceutical Co., China) weighing 270 ± 30 g were used for the experiments. All rats were randomly divided into an AE group and a control (C) group. Each group were further divided into three subgroups, a 1-h-long heat exposure (1h HE) subgroup, an exhaustive exercise (EE) subgroup, and an HSP70 Western blotting (WB) study subgroup, with each group consisting of 8 rats. The animals were maintained on a 12/12 h light/dark cycle and given food and water *ad libitum*. The room temperature was kept at 20 ± 1°C.

Rats in the AE group were subjected to treadmill aerobic physical training with progressive loading for 5 weeks (6 days/week) [9]. The running speed from the first to the fifth week was 10, 15, 18, 21, and 21 m/min, while the training time from the first to the fifth week was 10, 20, 30, 40, and 40 min, respectively. The physical AE was carried out under room temperature. Rats of the C group were kept sedentary for 5 weeks.

Three electric heaters (3000 W) and one air conditioner were used to keep the temperature in the thermo chamber at 38–40°C. The chamber used for providing exercise-heat stress was separated from that where rats lived. After finishing 5 weeks of physical AE or sedentary lifestyle conditions, all rats of the 1h HE and EE subgroups were carried into the artificial heat chamber. Rats of the 1h HE subgroup were subjected to 1-h-long heat exposure without exercise, while rats of the EE subgroup were subjected to vigorous exercise at the speed of 21 m/min till exhaustion. The criteria of exhaustion were the following: the running posture changing from a stomp style into a prostrate style, staying in the rear part of the treadmill stationary, and no motor response to slight sound/light/direct current stimulation [10]. The time to exhaustion was recorded.

During exhaustive exercise, rats were taken off from the treadmill, and the rectal temperature was measured through a digital thermometer every 15 min; the whole episode took less than 15 sec.

Rats of the 1h HE and EE subgroups were anesthetized with an overdose of chloral hydrate immediately after finishing the 1-h-long heat exposure or exhaustive heat exercise. Following this, rats were decapitated; their brains were removed, washed in cold normal saline, and gently blotted with paper; then the brains were stored in an ultra-low temperature freezer (–80°C) for future use. For detecting the oxidative stress manifestations, the whole brain was taken out of the freezer; after thawing, the motor cortex was separated on an ice-cold glass plate according to the rat brain stereotaxic atlas [11]. Thereafter, the hydroxyl radical scavenging capacity (HRSC), malondialdehyde (MDA) level, superoxide dismutase (SOD) activity, xanthine oxidase (XOD) activity, and glutathione peroxidase (GPX) level in the brains were detected. The assays were performed with standard techniques using commercialized kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China).

Detection of HSP70 was carried out using the Western blotting method. The procedure was as follows. The tissue homogenate was centrifuged at 10,000g for 15 min, and the supernatant was used for HSP70 detection after determination of the total protein concentration in the sample through the BCA method. Twenty micrograms of protein were separated by SDS-polyacrylamide gel electrophoresis and transferred overnight to nitrocellulose membranes; nonspecific binding of antibodies was blocked with 5% non-fat dry milk. Membranes were then probed with an anti-HSP70 polyclonal antibody (1:400, Wuhan Boster Biological Technology, China). After three 10-min-long washes in Tris-buffered saline (TBS) with 0.5% Tween 20, HRP-labeled secondary antibody (1:2000, Wuhan Boster Biological Technology, China) was applied for 1 h. After three 10-min-long washes in TBS+0.5% Tween 20 and three 5-min-long washes in TBS, the ECL system was used to visualize the immunoreactive bands. Films were scanned and quantitatively analyzed using Quantity One software.

All numerical data are presented below as means ± ± s.d. One-way ANOVA with repeated measures was performed for comparison of the rectal temperature during exhaustive exercise. For statistical analysis of other data, the Student's *t*-test was performed. In intergroup comparisons, $P < 0.05$ was considered an index of statistical significance.

RESULTS

The results showed that the HSP70 expression level in the brain of rats of the AE group was significantly higher (on average, by 52%) than that in the C group ($P < 0.05$; Fig. 1), indicating that

5-week-long aerobic physical training considerably enhanced the expression of HSP70 in the CNS.

The data in Table 1 depict the oxidative stress level in the brains of rats of different groups immediately after 1-h-long heat exposure. As is shown in Table 1, both XOD activity and MDA level in the brains of the AE rats were lower after such exposure than those of the C group ($P < 0.05$). At the same time, the HRSC, SOD, and GSH-PX levels in the AE group were all significantly higher than those in the C group ($P < 0.05$).

Table 2 shows the oxidative stress indices in the brains of rats of different groups immediately after exhaustive exercise under heat conditions. The results demonstrate characteristics quite comparable, in fact, with those shown in Table 1. The XOD activity and MDA level in the AE group were lower than those in the C group ($P < 0.05$). Simultaneously, the HRSC, SOD, and GSH-PX levels of the AE group were significantly higher than those in the C group ($P < 0.05$).

As is shown in Fig. 2, exhaustive exercise in the heat resulted in increases in the rectal temperature of rats of both AE and C groups. Due to such exercise, it reached, at the point of exhaustion, nearly the same average values, 40.75 ± 0.21°C and 40.82 ± ± 0.35°C, respectively. At the 30, 45, and 60 min time points, the rectal temperature in rats of the AE group were, however, significantly lower than the respective values in the C group ($P < 0.05$).

As Fig. 3 shows, the mean exhaustion time of rats of the AE group (76.58 ± 14.21 min) was significantly greater than that in the C group (68.86 ± ± 10.04 min) during exhaustive exercise under heat conditions.

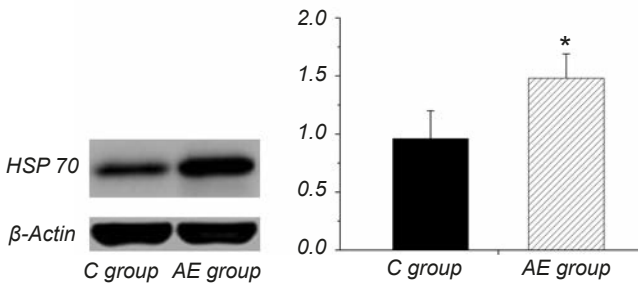


Fig. 1. Averaged HSP70 expression levels in the brains of rats of different groups ($n = 8$ in each group). *$P < 0.05$ *vs.* group C. Means ± s.d. are shown.

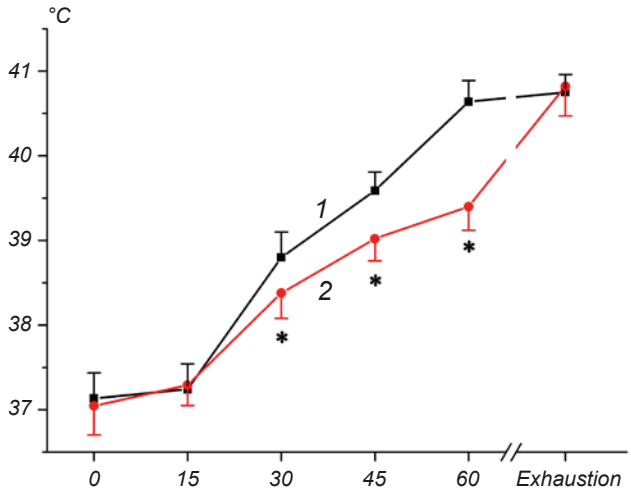


Fig. 2. Dynamics of the rectal temperature in rats of groups C (1) and AE (2) during exhaustive exercise in heat ($n = 8$ in each group). *$P < 0.05$ *vs.* group C.

Table 1. Oxidative Stress Manifestations in the Brains of Rats of Different Groups after 1-h-long Heat Exposure ($n = 8$)

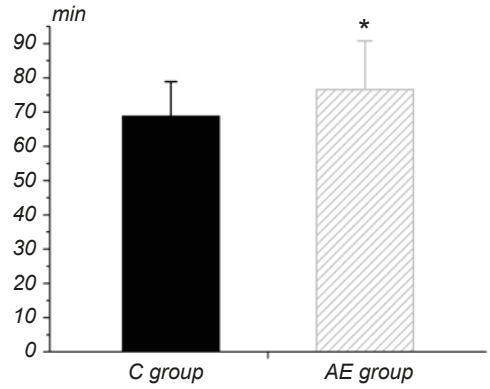
	XOD (U/g prot.)	MDA (nmol/mg prot.)	HRSC (U/mg prot.)	SOD (U/mg prot.)	GSH-PX (activity units)
group C	29.82±4.11	2.78±0.31	19.36±3.06	87.27±6.24	2.45±0.31
group AE	19.18±2.01*	1.98±0.37*	23.69±4.12*	110.40±7.57*	4.18±0.26*

Footnotes. XOD is xanthine oxidase, MDA is malondialdehyde, HRSC is hydroxyl radical scavenging capacity, SOD is superoxide dismutase, and GSH-PX is glutathione peroxidase. *P < 0.05 vs. group C. Means ± s.d. are shown.

Table 2. Oxidative Stress Manifestations in the Rat Brains of Different Groups after Exhaustive Exercise in Heat ($n = 8$)

	XOD (U/g prot.)	MDA (nmol/mg prot.)	HRSC (U/mg prot.)	SOD (U/mg prot.)	GSH-PX (activity units)
group C	35.56±5.09	4.38±0.41	14.21±3.44	58.19±5.62	1.65±0.26
group AE	24.25±3.05*	2.46±0.36*	17.47±3.79*	89.87±4.76*	2.38±0.35*

Footnote. Designations are similar to those in Table 1.



F i g. 3. Average time (min) until exhaustion in rats of different groups during exhaustive exercise in heat (n = 8 in each group). $^{*}P < 0.05$ *vs.* group C.

DISCUSSION

The concept that training in a cool environment helps to improve the performance in heat has been widely accepted, but the underlying mechanisms remain not fully understood. Aoyagi [3] showed that the possible mechanism in physical training in cool environments that improve performance in heat might include: (i) improved aerobic fitness and, thus, a greater cardiovascular reserve; (ii) a decreased energy cost of a given intensity of exercise; (iii) an enhanced sweating response at a given percentage of maximal effort; (iv) a slower increase in the body temperature, and (v) reduced cardiovascular stress largely due to changes in the autonomic nervous system.

Free radicals are atoms or groups of atoms with an odd number of unpaired electrons. The chief danger comes from the damage that ensues when such redicals react with important cellular components, such as DNA or components of the cell membrane. As is known, the brain, a relatively small part of the human organism, uses 20% of inspired oxygen [12]. In other mammals, this proportion is smaller but still very high. Because of the high rate of oxidative metabolic activity, as well as of the relatively low antioxidant capacity, the brain is regarded as the tissue most vulnerable to oxidative damage [13, 14]. Bailey et al. [8] reported that an acute bout of maximal exercise might increase the amount of free radicals in the brain and finally increase blood-brain barrier (BBB) leakage. Kiyatkin et al. [15] reported that hyperthermia might also result in an increase in BBB leakage through the increase in oxidative stress levels. Since the BBB plays an important role in maintaining a stable environment in the CNS by tightly regulating the exchange of molecules between the CNS and peripheral circulation, BBB leakage may allow the entry or exit of oxidizing species that can affect metabolism and functioning of the brain and, consequently, influence a wide range of homeostatic mechanisms [16]. The effects of exercise in heat, the exercise itself, and the environmental air temperature may be accumulative factors effectively working together to aggravate the generation of free radicals and, therefore, BBB breakdown, which might disturb normal brain functioning and finally contribute to the development of central fatigue [17]. Within recent years, failure to drive the spinal motor mechanisms adequately as a consequence of central fatigue has become one of the major mechanisms explaining how exercise-heat stress affects exercise performance, besides depletion of muscle glycogen or cardiovascular and fluid balance factors [18]. Thus, finding methods to successfully reduce the oxidative stress levels in the brain throughout exercise in heat may be of great importance for improving physical performance under heat conditions.

In our study, it was found that the exhaustion time in the AE group was significantly longer than that of the C group during exhaustive exercise in the heat environment (Fig. 3). The results indicate that a 5-week aerobic physical training program in a cool environment improves exercise performance in heat. This further demonstrates the validity of physical training in cool environments in improving tolerance to exercise-heat stress. The oxidative stress level in the rat motor cortex of the examined groups showed a significant difference. Immediately after 1-h-long heat exposure, the XOD and MDA in the motor cortex of the AE group were both lower than those of the C group. At the same time, the HRSC, SOD, and GSH-PX indices in group AE were all higher than those in the C group. The results observed immediately after exhaustion demonstrated similar characteristics. Since the MDA and XOD indices reflect mostly the intensity of oxidative damage, the SOD, HRSC, and GPX indices reflect the antioxidant capacity. The results of our study strongly imply that 5 weeks of aerobic physical training in a cool environment provides considerable protection of the motor cortex of rats against oxidative damage during rest and exercise in heat. Since the motor cortex is closely related to

motor activity, it may be presumed that the reduced oxidative stress level in this cerebral structure may reduce the damage it inflicts to brain functions and, thus, improves tolerance to exercise-heat stress.

The mechanism responsible for the perceived reduction of oxidative stress due to AE in a cool environment may include two factors. First, the dynamic changes of rectal temperature during exhaustive exercise in heat showed that the rectal temperature of rats from the AE group at the 30, 45, and 60 min time points were all significantly lower than those in group C. Thus, long-lasting (5 weeks) AE in a cool environment can reduce the rate of rectal temperature rise. Previous studies [19] showed that the oxidative stress levels in the brain demonstrate a strong positive correlation with the core temperature of the organism. Therefore, the lower rectal temperatures shown during rest or exhaustive exercise in the heat may be indicative of a lower oxidative level. Second, Western blotting showed that aerobic physical training in a cool environment significantly enhanced the expression in HSP70. This result is consistent with previous reports [20]. The increase in HSP70 expression might be related to the stress from exercise, since cellular stress resulting from hyperthermia, physical injury, or strong exercise can all induce an increase in HSP70 expression dramatically. This general coordinated activation of HSP70 expression is called the heat shock response [21].

In our study, 5 weeks of AE in a cool environment worked as a stress enhancer, fundamentally increasing the HSP70 expression in the rat brains. It has been well documented [22, 23] that HSP70, as other molecular chaperones, can facilitate protein folding, prevent protein aggregation, or target improperly folded proteins to the specific degradative pathways. Through these pathways, HSP70, similarly to other powerful antioxidant and repair proteins, can intervene in the oxidative damage caused by oxidative radicals in the brain and, thus, can improve physical performance in heat.

In summary, our findings show that long-lasting AE training in a cool environment enhanced the HSP70 expression in the rat brain and decreases the rate of increase in the core temperature. This may result in a lower oxidative stress level in the brain during rest or exhaustive exercise under heat conditions. The decreased oxidative damage may be an important factor in physical training in cool environments and may improve tolerance to exercise-heat stress.

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All experiments were conducted in accordance with the recommendations of the European Convention on "Protection of vertebrate animals used for experimental scientific purposes, May 31, 1986".

The authors, D. Wang, J. W. Ripley-Gonzalez, and Y. Hu, declare the absence of any conflict in commercial or financial relations, relationships with organizations or persons that in any way could be related to the study, and also in interrelations of the co-authors.

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