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EVIDENCES THAT MATERNAL SWIMMING EXERCISE IMPROVES ANTIOXIDANT DEFENSES AND INDUCES MITOCHONDRIAL BIOGENESIS IN THE BRAIN OF YOUNG WISTAR RATS

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Abstract—Physical exercise during pregnancy has been considered beneficial to mother and child. Recent studies showed that maternal swimming improves memory in the offspring, increases hippocampal neurogenesis and levels of neurotrophic factors. The objective of this work was to investigate the effect of maternal swimming during pregnancy on redox status and mitochondrial parameters in brain structures from the offspring. Adult female Wistar rats were submitted to five swimming sessions (30 min/day) prior to mating with adult male Wistar rats, and then trained during the pregnancy (five sessions of 30-min swimming/week). The litter was sacrificed when 7 days old, when cerebellum, parietal cortex, hippocampus, and striatum were dissected. We evaluated the production of reactive species and antioxidant status, measuring the activities of superoxide-dismutase (SOD), catalase (CAT) and glutathione-peroxidase (GPx), as well as non-enzymatic antioxidants. We also investigated a potential mitochondrial biogenesis regarding mitochondrion mass and membrane potential, through cytometric approaches. Our results showed that maternal swimming exercise promoted an increase in reactive species levels in cerebellum, parietal cortex, and hippocampus, demonstrated by an increase in dichlorofluorescein oxidation. Mitochondrial superoxide was reduced in cerebellum and parietal cortex, while nitrite levels were increased in cerebellum, parietal cortex, hippocampus, and striatum. Antioxidant status was improved in cerebellum, parietal cortex, and hippocampus. SOD activity was increased in parietal cortex, and was not altered in the remaining brain structures. CAT and GPx activities, as well as non-enzymatic antioxidant potential, were increased in cerebellum, parietal cortex, and hippocampus of rats whose mothers were exercised. Finally, we observed an increased mitochondrial mass and membrane potential, suggesting mitochondriogenesis, in cerebellum and parietal cortex of pups subjected to maternal swimming. In conclusion, maternal swimming exercise induced neurometabolic programing in the offspring that could be of benefit to the rats against future cerebral insults.

Key words: metabolic programing, oxidative/nitrative stress, redox status, antioxidants, mitochondrial biogenesis, maternal swimming exercise.

INTRODUCTION

Regular physical activity may prevent the onset of a large range of pathological conditions, from heart diseases, and psychiatric disorders, to ischemia and neurodegenerative diseases (Dishman et al., 2006; Powell et al., 2011; Wolff et al., 2011). A recent review from Vina et al. (2012) reports the benefits of exercise promoting health and lifespan, and proposes an effective dose to obtain the best effect of exercise. Whether it is difficult to find the best level of exercise in health people, the better level of physical activity to persons with some special need, such as pregnant women, is much more distant to close. The American College of Obstetricians and Gynecologists recommends 30 min/day of moderate-intensity exercise, all days of the week, similar to the British recommendations (Artal and O'Toole, 2003). For pregnant women, aquatic exercise is preferable, in order to maintain the blood flow with nutrients to the fetus, protect the joints and against falls, as well as control of the body temperature (Hartmann and Bung, 1999; Artal and O'Toole, 2003; Lynch et al., 2003).

Exercise benefits are mediated by the metabolic adaptation elicited, which could be identified including in the CNS, such as increments in neurogenesis and neurotrophic factors, improvement of cognitive function (Vina et al., 2012), raises in glycogen stores (Dalsgaard, 2006; Matsui et al., 2012), glucose uptake, oxidative capacity, and the activity of the electron transport chain (Dishman et al., 2006; Kinni et al., 2011), besides promoting mitochondrial biogenesis (Steiner et al., 2011;

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Abbreviations: ABAP, 2,2′-azo-bis(2-amidinopropane); BDNF, brain-derived neurotrophic factor; CAT, catalase; DCF, dichlorofluorescein; GSH, -reduced glutathione; GPx, glutathione-peroxidase; H_2DCFDA, 2′,7′-DCF diacetate; mtDNA, mitochondrial DNA; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NFκB, nuclear factor κ B; NO, nitric oxide; Nrf2, nuclear erythroid 2 p45-related factor 2; PGC-1α, peroxisome-proliferator-activated receptor-c coactivator-1α; PND, postnatal day; ROS, reactive oxygen species; RNS, nitrogen reactive species; SOD, superoxide-dismutase; TAR, total antioxidant reactivity; TRAP, total radical-trapping antioxidant potential.

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Zhang et al., 2012). Although the brain maintains its oxygen supplement virtually unchanged during exercise, the cellular adaptations to oxidative stress might be observed, such as upregulation of antioxidant enzymes and DNA repair enzymes, increasing the resistance to oxidative stress (Radak et al., 2001, 2007, 2008).

The effect of exercise during pregnancy has been studied in a few animal models, suggesting promising changes in the CNS of offspring. Pups, delivered from rat dams subjected to involuntary swimming during pregnancy, were evaluated in the Morris water maze task. They presented an improvement in spatial memory allied to the rise in the number of cells in the hippocampus, CA1, and dentate gyrus (Akhavan et al., 2008). In agreement, Lee et al. (2006) showed that maternal swimming improves memory, measured by inhibitory avoidance test, and increases neurogenesis and brain-derived neurotrophic factor (BDNF) in the hippocampus of 29 postnatal day (PND) rats. Recently, Dayi et al. (2012) reported that maternal running during pregnancy increased the number of neurons in the hippocampus of pups, which have better scores in the Morris water maze, indicating a positive effect of maternal exercise on spatial memory even in adult pups.

The mechanism by which exercise elicited the brain metabolic adjustment is still unknown. Memory improvement appears to be related to BDNF increment, neuroplasticity, and neurogenesis (Lee et al., 2006; Kim et al., 2007). Akhavan et al. (2008) revealed that noradrenergic and serotonergic systems collaborate with the mnemonic positive effects of maternal exercise. Furthermore, energy metabolism might be altered by exercise in brain regions associated to motor performance, suggesting a connection between muscle and brain (Dishman et al., 2006). Park et al. (2013) reported that treadmill (40 min daily/3 weeks) performed by mice during pregnancy improves brain mitochondrial function in the offspring, in a mechanism related to adaptive counterresponse to eliminate reactive species. In addition, it has been showed that redox-sensitive transcription factors, such as CREB, could also mediate exercise changes in the brain (Radak et al., 2007, 2008). Redox status seem to have a fundamental role in exercise effect, reducing oxidative damage markers and increasing the antioxidant network in the brain, although the literature is not unanimous (Radak et al., 2007). Taking these data into account, we assessed the effect of maternal swimming on the redox status, mitochondrial mass, and membrane potential, measured in the litter's cerebellum, parietal cortex, hippocampus, and striatum. In order to accomplish the landscape of the redox status in offspring brain, we evaluated dichlorofluorescein (DCF) oxidation, mitochondrial superoxide, total nitrite levels, antioxidant enzyme activities (superoxide-dismutase (SOD), catalase (CAT), and glutathione-peroxidase (GPx)), and non-enzymatic antioxidant potential. Mitochondrial mass and mitochondrial membrane potential were determined using the probes MitoTracker® Green and Red, respectively.

EXPERIMENTAL PROCEDURES

Animals and reagents

Twenty female and 10 male Wistar rats (adult, 90 days of age) were obtained from the Central Animal House of Departamento de Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil. Animals were maintained on a 12/12-h light/dark cycle in an air-conditioned constant temperature (22 ± 1 °C) colony room. During the exercise protocol we housed five female rats per cage, except for mating (one male per two females). One day before the birth of the pups, we isolated the pregnant dams (one/cage). After the delivery, we leave the mother and offspring together, until the euthanasia of pups, on the 7th day of life. Rats had free access to a 20% (w/w) protein commercial chow and water. The experiments were approved by the local Ethics Commission (Comissão de Ética no Uso de Animais/Universidade Federal do Rio Grande do Sul – CEUA/UFRGS) under the number 19481, and followed the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996). We further attest that all efforts were made to minimize the number of animals used and their suffering.

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA, except MitoTracker® and MitoSOX®, obtained from Invitrogen.

Experimental design

The experimental design is presented in Fig. 1. Female Wistar rats were subjected to the swimming protocol 1 week previous to mating the males, in order to habituate to the aquatic environment. In mating, one male rat was placed in contact with two females for 12 h. Pregnancy was diagnosed by the presence of a vaginal plug. The pregnant rats were submitted to the swimming exercise protocol during all of the pregnancy. From the 20th day after the onset of pregnancy, the rats were observed twice a day (at 9 and 18 h), to verify the litter's birth. The day corresponding to the birth of offspring is defined as postnatal day (PND) 0. Table 1 shows reproductive data, where we could see that control- and swimming-exercised rat dams have approximately the same number of implantations and the number of pups delivered. The sex ratio was 0.60 to control litter against 0.49 to maternal swimming exercise litter, and the viability index in the PND 7 was 96.7% for control and 100% for the litter subjected to maternal swimming exercise. We observed a modest reduction in body weight in rats subjected to maternal swimming exercise, on PND1 ($p < 0.001$) and PND7 ($p < 0.05$). We used one pup for each offspring for each technique, in order to eliminate the litter effect (total = 85 animals from both sex).

The litter was left with the mother until PND7, when the rats were weighted and decapitated without anesthesia. Cerebellum, parietal cortex, hippocampus, and striatum were dissected, and stored at −80 °C until