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Effect of Exercise Training on Antioxidant System in Brain Regions of Rat

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SOMANI, S. M., R. RAVI AND L. P. RYBAK. *Effect of exercise training on antioxidant system in brain regions of rat.* PHARMACOL BIOCHEM BEHAV **50**(4) 635-639, 1995.—The purpose of this investigation was to determine whether any alterations in antioxidant enzyme activities and levels of glutathione (GSH) in brain regions occurred following exercise training. Sprague-Dawley rats were given exercise training on a treadmill for 7.5 weeks and sacrificed 18 h after the last exercise along with the sedentary control rats. Different brain regions—cerebral cortex (CC), brainstem (BS), corpus striatum (CS), and hippocampus (H)—were isolated; GSH, oxidized glutathione (GSSG), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were determined. The exercise training increased SOD activity significantly (130% of sedentary control) in BS and in CS. SOD activity in H was the lowest of all four brain regions. Different brain regions showed GSH-Px activity in decreasing order for CS < BS < CC < H. GSH levels were 43% less in BS than CC and CS. The ratio of GSH/GSSG significantly increased from 6.8 to 8.3 in CC, and from 9.4 to 13.5 in BS as a result of exercise training. Different brain regions contained different activities of antioxidant enzymes, as well as GSH and GSSG levels, which were preferentially altered as a result of exercise training to cope with oxidative stress.

Exercise Brain regions Superoxide dismutase Glutathione

EXERCISE increases the intake of oxygen in the body. The potential harm of oxygen derives from its ability to be converted to reactive intermediates such as superoxide anions, singlet oxygen, and hydroxyl radicals. These species are produced as a result of oxidation (electron transfer) processes occurring in the mitochondria. Approximately 4-5% of oxygen consumed will form superoxide anions, which can be readily dismuted [by superoxide dismutase (SOD)] to hydrogen peroxide and singlet oxygen. Hydrogen peroxide is then converted by either catalase (CAT) or glutathione peroxidase (GSH-Px) to H_2O and $^1O_2$. Reduced glutathione (GSH) consumed in the GSH-Px reaction is converted back to its reduced form by glutathione reductase (GR). When the production of reactive oxygen intermediates exceeds the ability of the antioxidant system to eliminate them, oxidative stress results (16).

The antioxidant enzymes SOD, CAT, and GSH-Px and the ratio of GSH to oxidized glutathione (GSSG) are critical for protection against oxyradical toxicity. These antioxidant enzymes and GSH levels are much lower in the CNS and brain as compared to erythrocytes and peripheral tissues (19). Glutathione in the reduced state plays an important role in cellular protection against damage from free radicals and oxyradicals (34). The turnover of GSH in brain is considered to be slower than that in liver and kidney. The half-life of GSH in rat brain was esimated to be 70 h (7). The half-life of GSH in the mouse kidney was estimated to be 29 min (27).

Glutathione deficiency leads to mitochondrial damage in the brain (13). The brain stem and spinal cord are more vulnerable to free radical injury because of low levels of GSH compared to cerebrum and cerebellum (18). Low levels of glutathione in six regions of brain compared to levels in the liver were reported for young adult, middle-aged, and aged rats (22). The exposure of brain mitochondria to oxidative stress resulted in glutathione depletion and the formation of glutathione-protein mixed disulfide (23). Substantia nigra neurons showed a particular vulnerability to oxidative stress (5). Thus, these findings suggested that the CNS may be more vulnerable to oxyradical toxicity, or may have different coping mechanism(s) to deal with oxidative stress. Recently, Somani (28) reviewed the influence of exercise-induced oxidative stress on the CNS.

Various investigators have determined the effect of exercise on the free-radical scavenging enzymes (antioxidant enzymes) in different tissues: liver (3); skeletal muscle (15); cardiac muscle (1,25,33); lung (26); red blood cells (6,12); muscle, liver, and blood (17); and heart subcellular fractions (31). Maximal

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activities of antioxidant enzymes correlate with aerobic capacity (VO^{2max}) among various tissues (14).

The oxygen consumption rate increases 10–15-fold during exercise. Brain uses 20% of the oxygen consumed by man. The high consumption of oxygen due to exercise may enhance the free radicals in the brain. Brain could be more susceptible to lipid peroxidation damage under such oxidative stress. The high concentrations of polyunsaturated fatty acids in this organ make it more susceptible to liquid peroxidation damage (11). Antioxidant enzyme adaptation is considered to be one of the fundamental changes in skeletal muscle in response to exercise training, much the same as mitochondrial oxidative enzyme adaptation (14). However, very little information is available concerning the effect of exercise training on the status of antioxidant enzymes and GSH in brain regions.

To delineate more clearly the effects of exercise on CNS, this study sought to resolve some important question: whether exercise training elicits adaptative changes in antioxidant status, and whether such changes are differentially expressed within subregions of the brain.

MATERIALS AND METHODS

Animals, Exercise Training, and Sacrifice

Male Sprague–Dawley rats weighing 145–163 g (152 ± 3.4 SEM average weight) were obtained from Harlan Industries (Indianapolis, IN). Rats were fed ad lib with Rodent Laboratory Chow (Ralston Purina, Indianapolis, IN). Feed consisted of protein (23.4%) and fat (4.5%) balanced with carbohydrate, fibers, vitamins, and minerals. Rats were maintained on a 12 L : 12 D photoperiod.

Rats were randomly assigned to two groups designated as sedentary control and exercise training.

Sedentary control. Five rats were put on a nine-channel, motor-driven treadmill (custom-built at SIU-SM) for 5 days/week over 7.5 weeks and given 2 m/min exercise for 5 min for equivalent handling. These rats were maintained similar to the exercise-trained group.

Exercise training. Five rats were given exercise training on a nine-channel, motor-driven treadmill for 5 days/week over 7.5 weeks using an incremental exercise program to obtain progressive levels of exercise (Table 1). During this program of exercising, the speed (meters per minute), angle of inclination (degree), and the duration (minutes) of exercise were varied to obtain different levels of exercise intensity. The exercise protocol was designed so that a more demanding exercise task confronted each animal as each week of training elapsed. In the first 2 weeks, the conveyor belt speeds were 8.2, 15.2, and 19.3 m/min, and the angle of inclination was 6°. The exercise duration for each speed was 5 min (total time 15 min) for the first week and 10 min at each speed (total time 30 min) the second week. In the third and fourth weeks of exercising, speeds were increased to 19.3, 26.8, and 30.3 m/min. The duration of exercise was 10 min at each speed (total time 30 min). The angle of inclination was 6° during the third week and 9° in the fourth week. The final 3.5 weeks of exercising involved increasing the angle of inclination to 10°, and the duration of exercise was 10 min at each belt speed (total time 30 min). VO^{2max} was determined at the beginning, midpoint, and end of the training protocol, using the Omnitech Oxyscan Analyzer as reported previously by us (29,30). The rate of oxygen consumption, respiratory exchange ratio, and heat production increased with an increase in exercise intensity. At the end of 7.5 weeks, the rats scheduled to be sacrificed were exercised in the evening.

TABLE 1

EXERCISE TRAINING OF MALE SPRAGUE-DAWLEY RATS

Wk	Belt speed (m/min)	Angle of inclination (degrees)	Duration at each speed (min)
1	8.2,15.2,19.3	6	5
2	8.2,15.2,19.3	6	10
3	19.3,26.8,30.3	6	10
4	19.3,26.8,30.3	9	10
5	26.8,30.3,35.4	9	10
6	26.8,30.3,54.4	10	10
7	26.8,30.3,54.4	10	10
8	26.8,30.3,54.4	10	10

Animals weighed 160–200 g on the first day of exercise and 300–350 g at sacrifice.

Sacrifice. The exercise training protocol was stopped 18 h before sacrifice. Both groups of rats (sedentary control and exercise training) were weighed every week. Sedentary control rats at sacrifice weighed 319–343 g (331 ± 5.1, mean ± SEM), whereas exercise training rats weighed 295–338 g (315.4 ± 9.03). There was no statistically significant difference in the weights of these two groups. Five exercise-trained as well as five sedentary control rats were sacrificed by decapitation between 1000 and 1100 h to minimize circadian cycle effects (4). The brain was immediately removed and freed from blood; and the cortex, striatum, brainstem, and hippocampus were dissected on ice, frozen in liquid nitrogen, and stored at −80°C until analysis.

Tissue Preparation for Enzyme Assays

All tissues were homogenized in EDTA phosphate buffer by sonication and centrifuged at 40,000 rpm for 30 min in a Beckman Ultra-Centrifuge (Palo Alto, CA). Then, 0.5 ml of the supernatant solution was passed through a 5-ml Sephadex G10 column. The elutant was collected from the column by centrifugation, and 0.5 ml of buffer was passed through the column. The total volume of the elutant was approximately 1 ml. This tissue extract was used for each enzyme assay.

Determination of GSH and GSSG by HPLC

High performance liquid chromatography was used to determine reduced GSH and GSSG in tissues (8,24). Frozen brain regions were thawed in solution containing 10% perchloric acid (PCA) and 1 mM bathophenanthroline disulphonic acid (BPDS), and were homogenized. The PCA acid extract of the tissue was centrifuged and internal standard (γ-glutamyl glutamate) was added to the supernatant. The solution was then made alkaline (pH 8–9) by the addition of KOH-$KHCO_3$ buffer, in which immediate S-carboxy methylation occurred with iodoacetic acid. This mixture was left overnight in 2,4-dinitrofluorobenzene to obtain DNP derivatives that were stable products, and analyzed on an HPLC at 365 nm using a variable wavelength detector.

Determination of Enzyme Activity

SOD. Superoxide dismutase was determined as described by Nishikimi et al. (21) and Fried (10). Three hundred microli-

ters of the tissue extract was added to 1100 μl of the reaction mixture [200 mM phosphate buffer, pH 7.8, 2 mM EDTA: 0.41 mM nitroblue tetrazoline (NBT); 1.1 mM phenzine methosulphate; and 1 mg bovine serum albumin], and incubated at room temperature for 10 min. Then, 100 μl of NADPH (50 mM) was added to the extract plus reaction mixture, and was read at 540 nm. SOD activity was estimated from the percent NBT reduction with sample and using the plot of percent NBT reduction with increasing concentrations of standard reference SOD (Sigma Chemical, St Louis, MO). Increasing amounts of SOD (0.30–3.0 U) linearly inhibited the NBT reduction. Activity was expressed as standard SOD units per milligram of protein.

GSH-px. Glutathione peroxidase was determined by a modified version of Flohe and Gunzler (9) at 37°C. All reaction mixtures were dissolved in 0.05 M, pH 7.0, 0.1 mM EDTA phosphate buffer. A reaction mixture of 100 μl phosphate buffer, 100 μl 0.01 M glutathione (reduced form), 100 μl 1.5 mM NADPH, 100 μl glutathione reductase (5 mg/μl of 120 U/mg), and 500 μl of the tissue extract was incubated at 37°C for 10 min. Then, 100 μl of 12 mM *t*-butyl hydroperoxide was added to the tissue reaction mixture and measured at 340 nm for 180 s on a Beckman DU 7400 spectrophotometer. The molar extinction coefficient of 6.22 mM cm^{-1} was used to determine the activity of GSH-Px. One unit of activity is equal to 1 mM of NADPH oxidized/min per mg protein.

Protein. Protein in the tissues was measured by the Bradford method (2) using a Beckman DU 7400 spectrophotometer.

Statistics

The data were expressed as mean ± SEM for five rats. Statistical analyses were carried out using paired independent Student's *t*-test. Significance was determined at 0.05.

RESULTS

Figure 1 shows the effect of exercise training on SOD in different regions of the brain. Regional differences in the SOD activity were observed in sedentary control rats. The highest activity was found in the CC followed by BS and CS. The hippocampus had the lowest activity of all four regions. This activity was almost 50% that of CC. SOD activity increased significantly ($p < 0.05$), from 38.45 ± 4.43 to 50.11 ± 7.48 U/mg protein in BS, and from 35.67 ± 4.66 to 46.53 ± 3.20 in CS. Because of exercise training, SOD activity slightly increased in H, whereas there was no increase in activity in CC.

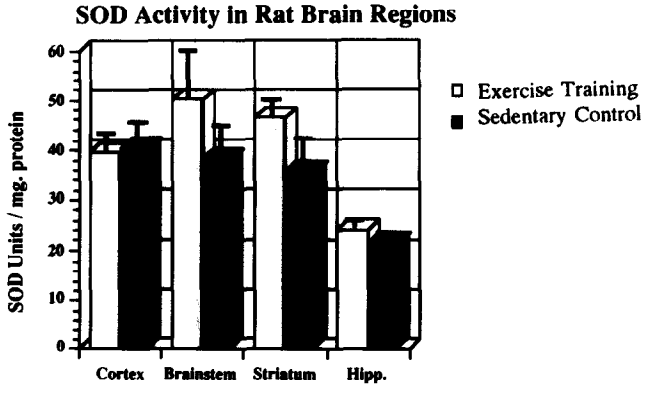

FIG. 1. Superoxide dismutase (SOD) activity in the cortex, brain stem, striatum and hippocampus (Hipp.) of exercise training at 7.5 weeks on treadmill □ and sedentary control ■ Sprague–Dawley rats. The data are expressed as means ± SEM ($n = 5$).

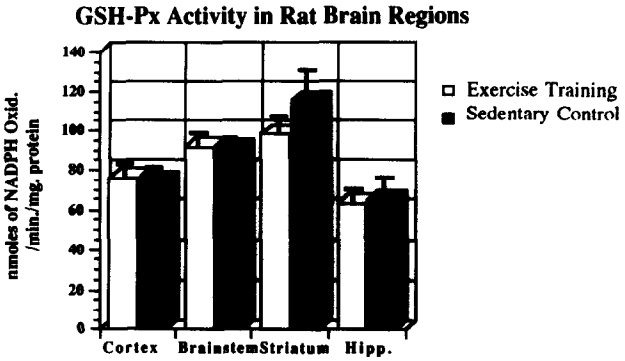

FIG. 2. Glutathione peroxidase (GSH-Px) activity in the cortex, brain stem, striatum, and hippocampus (Hipp.) of exercise training at 7.5 weeks on □ treadmill and ■ sedentary control Sprague–Dawley rats. The data are expressed as means ± SEM ($n = 5$).

Figure 2 shows that in sedentary control rats, GSH-Px activity was highest in striatum followed by BS, CC, and H. This enzyme activity in H was 55% that in CS. The GSH-Px activity showed no significant changes in brain regions as a result of exercise training. GSH-Px activity slightly decreased in CS following exercise training.

Figures 3 a and b show the concentrations of GSH and GSSG in different regions of the brain in sedentary control and exercised rats. GSH was highest in CC and CS and lowest in BS. However, GSSG level was highest in CC, followed by CS and BS. Hippocampus is a small tissue; only two enzymes could be determined, and not the GSH and GSSG. GSH content in BS was 322 ± 18 pmol/mg tissue, which increased to 352 ± 14 pmol/mg tissue after exercise training. GSH in CS and CC were in the range of 540–580 pmol/mg tissue, and these did not change as a result of exercise training. However, the ratio of GSH/GSSG was altered in the BS and CC after exercise training.

Table 2 shows the ratio of GSH/GSSG in different regions of brain in sedentary control and exercise groups. Cortex and BS showed significantly higher GSH/GSSG ratio after exercise training. This ratio did not change significantly in CS.

DISCUSSION

We have previously reported that the brain regions (CC, BS, CS, and H) involved with control of motor, autonomic, and cognitive functions were affected by the administration of subacute physostigmine and exercise. The responsiveness of these brain regions to different stressors is a function of the level of ongoing cholinergic acitvity; and elevation in ACh levels due to AChE inhibition may have long-term effects on the regulation of ChAT and AChE activities through a negative feedback mechanism (32).

Regional variations were observed in cerebral GSH and GSSG levels and GSH-Px and SOD activity in sedentary control rats. These changes were enhanced after exercise training in specific brain regions. The brain regions of sedentary control rats showed that a) GSH levels were low in BS as com-

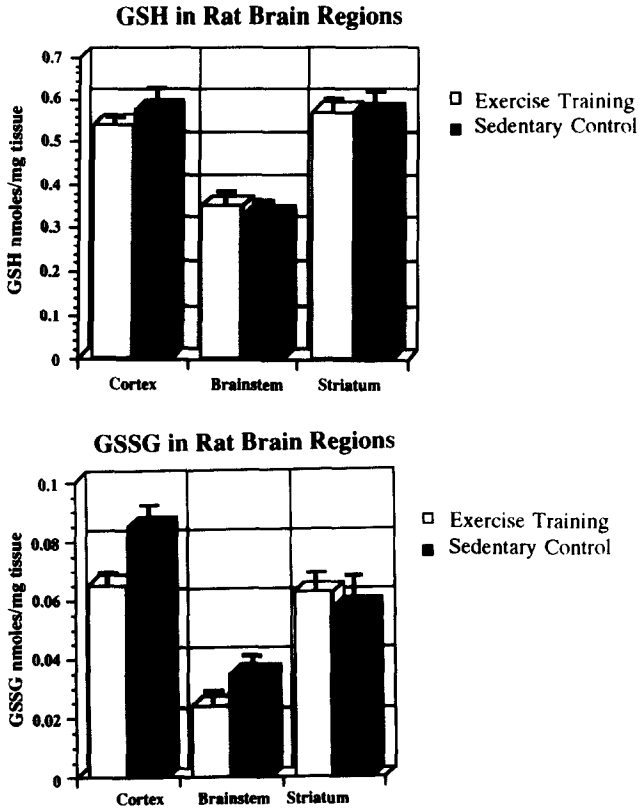

FIG. 3. Levels of (A) glutathione (GSH) and (B) disulfide of glutathione (GSSG) in the cortex, brain stem, and striatum of exercise training at 7.5 weeks on □ treadmill and ■ sedentary control Sprague-Dawley rats. The data are expressed as means ± SEM (n = 5).

pared to CC and CS (the latter two were comparable); b) GSSG levels were also lower in BS as compared to CC and CS; c) the GSH/GSSG ratio was found in the decreasing order: CS > BS > CC. In sedentary control rats, GSH-Px activity was found in the decreasing order: CS > BS > CC > H; however, SOD activity was in the order: CC > BS > CS > H. This is an interesting pattern in sedentary control in comparison with changes in exercise. BS and CS seem to be more sensitive to oxidative stress. SOD activity within these regions increased as a result of exercise training. Exercise did not seem to produce significant alterations in GSH-Px activity in these brain regions, but SOD activity increased significantly following exercise training.

An examination of glutathione levels in each specific brain region showed that the CC of sedentary controls had higher levels of GSH (578 ± 29) than other regions, which suggests that this area may deal with oxidative stress better than other regions. The exercise training did not significantly change GSH levels in this region. CC showed a significant decrease ($p < 0.05$) in GSSG following exercise, indicating that GSH to GSSG conversion did not occur due to exercise training. It seems that exercise training results in a better redox ratio, and this region seems to be more capable of coping with oxidative stress because of the highest concentration of GSH. SOD and GSH-Px activity was not altered because of exercise training in this region.

The levels of GSH were significantly lower in BS as compared to CC and CS, thereby potentially predisposing it to greater oxidative stress. However, GSH levels slightly increased as a result of exercise training. GSSG levels significantly decreased in BS, however, indicating a turnover caused by exercise training. The GSH/GSSG ratio (13.5) was the highest in the BS of the exercise-trained rats compared to sedentary controls (9.4). This suggests that exercise training may contribute to BS's being more resistant to oxidative stress. This region also showed increased activity of SOD due to exercise training, possibly by enzyme induction from exercise. The oxidative stress seems to be predominantly by production of superoxide radicals, which are scavenged by SOD. GSH-Px activity in BS is identical in exercised and sedentary controls. The lack of change in GSH-Px activity further suggested that no significant peroxidation had taken place. These results suggested that this region is highly benefited by exercise training. Because the brain stem is a very vital area for autonomic function (sleep and cardiovascular regulation), exercise training may prove to be beneficial specifically to this region and its functions, including those of controlling heart rate and blood pressure (20). This could be a part of an improved cardiovascular function that accompanies exercise training. The GSH level and the SOD activity in the CS region are comparable to the CC region of sedentary control rats, although CS is much smaller than CC. However, the CS region has a significantly higher GSH-Px activity than CC. SOD activity increased and GSSG levels increased slightly after exercise training in the CS region. The metabolic considerations of striatum (basal ganglia) in terms of O_2 consumption by these tissues on a per-gram basis exceeds that of CC. The highest GSH-Px activity and GSH concentration may help this part of the brain to cope with any oxyradicals formed as a result of a high consumption of oxygen.

The H has the lowest level of GSH-Px and SOD among all the four regions studied. Exercise training increased SOD activity in this region which scavenges superoxides formed as a reult of excess consumption of O_2 during exercise.

In summary, BS and CS showed significant increases in SOD enzyme activity (130%), and CC showed no change in this enzyme activity as a result of exercise training. It seems that exercise training causes more oxidative stress in the BS and CS regions, or has a better ability to induce antioxidant enzymes to cope with the superoxides formed. BS and CS may be more sensitive to oxidative stress, thereby showing enzyme induction, whereas the CC region might have acquired more resistance to oxidative stress because of a higher level of GSH. Different brain regions show GSH-Px activity in the decreasing order: CS > BS > CC > H. Although GSH-Px shows variations in various brain regions, it does not seem to play a major role during exercise training, nor does it seem to affect GSH-Px activity in these four brain regions. GSH level was 43% less in BS than in CC. Exercise training altered GSH to GSSG ratio in BS and in CC, whereas this ratio did not change

TABLE 2

RATIO OF GSH/GSSG IN VARIOUS REGIONS OF BRAIN

	Sedentary control	Exercise training
Cortex	6.8	8.3*
Brain stem	9.4	13.5*
Striatum	10.01	9.4

*Significantly higher at $p < 0.05$.

in CS. The ratio of GSH to GSSG significantly increased from 6.8 to 8.3 in CC and from 9.4 to 13.5 in BS as a result of exercise training. The benefit of exercise training seems to be evident, as GSSG levels decreased and GSH levels remained unaltered.

In conclusion, exercise training altered SOD activity and the GSH to GSSG ratio differentially in different brain regions to cope with oxidative stress.

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