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FULL-LENGTH ORIGINAL RESEARCH

Swimming training prevents pentylenetetrazol-induced inhibition of Na^+, K^+-ATPase activity, seizures, and oxidative stress

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Summary

Purpose: In the present study we decided to investigate whether physical exercise protects against the electrographic, oxidative, and neurochemical alterations induced by subthreshold to severe convulsive doses of pentyltetrazole (PTZ).

Methods: The effect of swimming training (6 weeks) on convulsive behavior induced by PTZ (30, 45, and 60 mg/kg, i.p.) was measured and different electrographic electroencephalography (EEG) frequencies obtained from freely moving rats. After EEG recordings, reactive oxygen species (ROS) generation, nonprotein sulfhydryl (NPS), protein carbonyl, thiobarbituric acid-reactive substances (TBARS), superoxide dismutase (SOD), catalase (CAT), Na^+, K^+-ATPase activity, and glutamate uptake were measured in the cerebral cortex of rats.

Results: We showed that physical training increased latency and attenuated the duration of generalized seizures induced by administration of PTZ (45 mg/kg). EEG recordings showed that physical exercise decreased the spike amplitude after PTZ administration (all doses). Pearson's correlation analysis revealed that protection of physical training against PTZ-induced seizures strongly correlated with NPS content, Na^+, K^+-ATPase activity, and glutamate-uptake maintenance. Physical training also increased SOD activity, NPS content, attenuated ROS generation per se, and was effective against inhibition of Na^+, K^+-ATPase activity induced by a subthreshold convulsive dose of PTZ (30 mg/kg). In addition, physical training protected against 2′,7′-dichlorofluorescein diacetate (DCFH-DA) oxidation, TBARS and protein carbonyl increase, decrease of NPS content, inhibition of SOD and catalase, and inhibition glutamate uptake induced by PTZ.

Conclusions: These data suggest that effective protection of selected targets for free radical damage, such as Na^+, K^+-ATPase, elicited by physical training protects against the increase of neuronal excitability and oxidative damage induced by PTZ.

KEY WORDS: Seizure, Oxidative damage, Physical exercise, Pentylenetetrazol, Na^+, K^+-ATPase.

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Epilepsy is a common and chronic neurologic disorder constituting a large group of neurologic diseases, with an incidence of 0.5–1% in the general population (Andrade & Minassian, 2007). Many studies have suggested that a cascade of biologic events, including generation of reactive oxygen species (ROS), underlie the development and propagation of epilepsy (Patsoukis et al., 2005). In fact, oxidative stress in the central nervous system (CNS) has been shown in several rodent models of experimental epilepsy, such as the amygdala kindling model (Frantseva et al., 2000), the kainic acid model (Gluck et al., 2000), the pentylenetetrazol (PTZ) kindling model (Gupta et al., 2003), and in acute PTZ-induced seizure (Patsoukis et al., 2004).

PTZ is a blocker of the chloride ionophore complex to the γ-aminobutyric acid $(GABA)_A$ receptor (Huang et al., 2001) that has convulsant effects after repeated or single-dose administration and also affects several neurotransmitter systems, such as the GABAergic and glutamatergic systems (Jensen et al., 1991; Psarropoulou et al., 1994; Thomsen, 1999; Walsh et al., 1999). In addition, previous studies from our group have demonstrated that striatal injection of PTZ-induced convulsive activity is accompanied by ROS generation and inhibition of local Na^+, K^+-ATPase activity (Oliveira et al., 2004; Ribeiro et al., 2005; Fighera et al., 2006). These experimental data reinforce the assumption that inhibition of some selected targets for free radicals increases cellular excitability (Jamme et al., 1995; Danbolt, 2001; Prigol et al., 2007).

Recently, a substantial body of evidence has suggested that regular exercise has the capacity to beneficially effect certain brain functions and plays an important preventive and therapeutic role in oxidative stress–associated diseases, including ischemic heart disease, type 2 diabetes, Alzheimer's disease, and Parkinson's disease (Kiraly & Kiraly, 2005; Lazarevic et al., 2006; Belardinelli et al., 2007; Khedr et al., 2007). Accordingly, studies have shown that animals and humans clearly undergo significant adaptive responses to regular endurance exercise that involve greatly increased endurance capacity, which is permitted by dramatic mitochondrial biogenesis, reduction of oxidant production, and increase of antioxidant defenses (Packer & Cadenas, 2007; Sachdev & Davies, 2008). In fact, data on the effect of physical exercise on the brain indicate that, under certain conditions, physical exercise can attenuate oxidative stress-related damage in the brain causing improved brain function (Alessio et al., 1988; Radak et al., 2001; Cotman & Engesser-Cesar, 2002). Furthermore, regular physical exercise plays a preventive role against lifestyle-dependent diseases, and molecular mechanisms behind this favorable effect could be linked to redox homeostasis, a free radical–related adaptation mechanism (Radak et al., 2008).

Although the favorable effect of physical exercise on general health is unquestionable, fitness programs in patients with epilepsy are still a matter of controversy. Clinical investigations have demonstrated that there is a reduction in the number of seizures after physical training programs (Denio et al., 1989; Nakken et al., 1990; Eriksen et al., 1994); however, a significant number of patients with epilepsy believe that physical exercise increases likelihood of a seizure and are advised by family, friends, and even their physicians to avoid exercise (Steinhoff et al., 1996). Therefore, epileptic patients leading sedentary lives have shown greater body weight, and significantly poorer muscle strength and respiratory capacity than people taking part in regular exercise (Jalava & Sillanpaa, 1997).

On the other hand, experimental findings in animal models of epilepsy, such as temporal lobe epilepsy and kindling development, reveal that physical exercise increases the amount of stimulation necessary to reach the convulsive threshold (Arida et al., 1998), attenuates the frequency of seizures, and decreases susceptibility to subsequently evoked seizures in the pilocarpine model of epilepsy (Arida et al., 1999; Setkowicz & Mazur, 2006). In addition, it has been demonstrated that physical exercise promotes positive plastic changes in the hippocampal formation of rats with epilepsy (Arida et al., 2007a).

These facts clearly indicate that physical exercise may ameliorate the course of epileptic activity in the brain. However, very little information is available regarding the exact role of free radicals in its development and the improvement induced by physical exercise in an experimental model of epilepsy induced by PTZ. Therefore, in the present study we aimed to investigate whether physical exercise protects against the electrographic, oxidative, and neurochemical alterations induced by subthreshold, moderate, and severe convulsive doses of PTZ (30–60 mg/kg).

Materials and Methods

Animal and reagents

All experiments involving the animals were conducted in conformance with the policy statement of the American College of Sports Medicine. In the present study 90-day-old male Wistar rats, weighing 250–300 g at the beginning and 400–450 g at the end of the experimental period were used. During this period, the animals were maintained in a controlled environment (12:12 h light–dark cycle, 24 ± 1°C, 55% relative humidity) with free access to food (Guabi, Santa Maria, Brazil) and water. Animal utilization protocols followed the Official Government Ethics guidelines and were approved by the University Ethics Committee. All efforts were made to reduce the number of animals used, as well as to minimize their suffering. All other reagents were purchased from Sigma (St Louis, MO, U.S.A.).

Adaptation to the water

All the rats were adapted to the water before the beginning of the experiment. The adaptation consisted of keeping the animals in shallow water at 32°C between 9:00 and 11:00 a.m. The adaptation period proceeded during the entire experimental period. The purpose of the adaptation was to reduce stress without promoting a physical training adaptation.

Training protocol and lactate threshold assay

The use of swimming rats as a model of exercise presents advantages over treadmill running, since swimming is a natural ability of the rats and this avoids the selection of animals, which is necessary in experimental protocols using treadmill running (Arida et al., 1999). For exercise training, the rats were randomly assigned to the following groups: trained/saline (0.9% NaCl, 1 ml/kg, i.p., n = 8), trained/PTZ (30 mg/kg; n = 8), trained/PTZ (45 mg/kg; n = 8), and trained/PTZ (60 mg/kg; n = 8). The training period lasted 6 week and consisted of 60-min daily sessions five times per week. The training tank used for this study was 80 cm in length, 50 cm in width, and 90 cm in depth, and swimming was always performed in water at a temperature of 32°C between 9:00 and 11:00 a.m. During the first week of training, all animals underwent a swimming adaptation period without weights. After the swimming adaptation period, the rats were subjected to swimming training with a work load (5% of body weight) to improve endurance (Gobatto et al., 2001). At the same time of the training session, sedentary rats were placed in a separate tank with shallow water (5 cm in depth) at 32°C, 5 days/week without the work load (5% of body weight). After 6 weeks, sedentary rats received saline (0.9% NaCl, 1 ml/kg, i.p., n = 8–10) or PTZ (30, 45 or 60 mg/kg, i.p., n = 8–10 in each group) and were used as controls.

After 6 weeks of training, a test protocol was used to determine the lactate threshold (LT) in sedentary (n = 6) and trained rats (n = 6). The LT test was carried out according to the protocol described by Marquezi et al. (2003) and consisted of swimming exercises with progressive overload through weights attached to the animal's tail, corresponding to 4%, 5%, 6%, 7%, and 8% of body weight of each animal for 3-min periods, separated by 1-min resting periods. During the resting periods, 25-μl blood samples were collected from the tail vein into heparinized capillary tubes for determination of lactate concentration. The LT for each animal was calculated based on the point of inflection of the graph when plotting lactate concentration against the corresponding exercise workload.

Surgical procedure

All animals were submitted to surgery 24 h after the last session of training. In brief, the rats were deeply anesthetized with Equithesin (1% phenobarbital, 2% magnesium sulfate, 4% chloral hydrate, 42% propylene glycol, 11% ethanol; 3 ml/kg, i.p.). Two screw electrodes were placed bilaterally over the parietal cortex, along with a ground lead positioned over the nasal sinus. The electrodes were connected to a multipin socket fixed to the skull with acrylic cement. The experiments were performed 5 days after surgery.

Seizure evaluation

Seizures were monitored in all animals by electrocorticographic recording. On the day of the experiments, each animal was transferred to an acrylic glass cage (25 × 25 × 40 cm) and allowed to adapt for 20 min before electroencephalography (EEG) recording. The rat was then connected to the lead socket in a swivel inside a Faraday's cage, and the EEG was recorded using a digital encephalographer (Neuromap EQSA260, Neuromap LTDA, Itajubá, MG, Brazil). EEG signals were amplified, filtered (0.1–70.0 Hz, bandpass), digitalized (sampling rate 256 Hz), and stored in a personal computer for off-line analysis. Routinely, a 10-min baseline recording was obtained to establish an adequate control period. After baseline recording, the sedentary and trained animals received an injection of saline (0.9% NaCl, 1 ml/kg, i.p.) and/or PTZ (30, 45 and 60 mg/kg, i.p.). The animals were observed for the appearance of generalized tonic–clonic convulsive episodes for 20 min according to Ferraro et al. (1999), who describes clonic convulsions as episodes characterized by typical partial clonic activity affecting the face, head, vibrissae, and forelimbs. Generalized convulsive episodes were considered as generalized whole-body clonus involving all four limbs and tail, rearing, and wild running and jumping, followed by sudden loss of upright posture and autonomic signs, such as hypersalivation and defecation, respectively. During the 20-min observation period, the latencies for the first generalized tonic–clonic convulsions were measured. EEG recordings were visually analyzed for seizure activity, which were defined by the occurrence of the following alterations in the recording leads (McColl et al., 2003): isolated sharp waves (≥1.5 × baseline); multiple sharp waves (≥2 × baseline) in brief spindle episodes (≥1 s ≥ 5 s); multiple sharp waves (≥2 × baseline) in long spindle episodes (≥5 s); spikes (≥2 × baseline) plus slow waves; multispikes (≥2 × baseline, ≥3 spikes/complex) plus slow waves; and major seizure (repetitive spikes plus slow waves obliterating background rhythm, ≥5 s). For quantitative analysis of EEG amplitude, we averaged EEG amplitude over the 20-min of observation.

Sample processing

After the behavioral evaluation (20 min after PTZ administration), the animals were killed by decapitation and their brain was exposed by removing the parietal bone. After decapitation, the whole brain was removed

and placed in ice-cold Krebs Cerebral Henseleit buffer containing 124 mM NaCl, 5 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 23 mM $NaHCO_3$, 3 mM HEPES, and 10 mM D-glucose, and the pH was adjusted to 7.4 with 95% O_2/5% CO_2. Cerebral cortex slices (0.4 mm) were obtained by transversal cuts using a McIlwain chopper. The time duration between decapitation and preparation of the slices was 2–3 min, and during this period the brain was immersed in the ice-cold medium.

Estimation of ROS generation

Generation of ROS was estimated with the fluorescent probe, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), as described by Ali et al. (1992). Briefly, the slice from cerebral cortex was homogenized in 2.5 ml of saline solution (0.9% NaCl). Aliquots of 2.5 ml were incubated in the presence of DCFH-DA (5 μM) at 37°C for 60 min. The DCFH-DA is enzymatically hydrolyzed by intracellular esterases to form nonfluorescent DCFH, which is then rapidly oxidized to form highly fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. DCF fluorescence intensity is proportional to the amount of ROS that is formed. Fluorescence was measured using excitation and emission wavelengths of 480 and 535 nm, respectively. A calibration curve was established with standard DCF (0.1 nM to 1 μM), and ROS levels were expressed as percentages of control.

Nonprotein sulfhydril (NPS) levels

The levels of NPS in slices of cerebral cortex were determined in the presence of 50 mM Tris–Cl, pH 7.4. Free –SH groups were determined according to Ellman and Lysko (1967). Incubation at 37°C was initiated by the addition of the thiol compounds. Aliquots of the reaction mixture (100 μl) were checked for the amount of –SH groups at 412 nm after 90–120 min of addition of color reagent 5′5′-dithio-bis (2-nitrobenzoic) acid (DTNB).

Measurement of protein carbonyl

For the protein carbonyl assay, a slice from the cerebral cortex was homogenized in 10 volumes (w/v) of 10 mM Tris–HCl buffer pH 7.4 using a glass homogenizer, and its carbonyl protein content was determined by the method described by Levine et al. (1990) adapted for brain tissue (Oliveira et al., 2004).

Measurement of thiobarbituric acid-reactive substances (TBARS) content

For the TBARS assay, a slice from cerebral cortex was homogenized in ultra-purified water, and the TBA reagent (15% of trichloroacetic acid, 0.375% of thiobarbituric acid, and 2.5% v/v of HCl) was added. After 30 min of incubation, samples were centrifuged (3000***g***, 15 min) and then TBARS levels were measured at 532 nm (Ríos & Santamaría, 1991).

Superoxide dismutase (SOD) and catalase (CAT) activity

To verify SOD and CAT activity, a slice from the cerebral cortex was adequately homogenized in 40 volumes (w/v) with Tris–HCl 10 mM (pH 7.4), and an assay was performed according to the methods of Misra and Fridovich (1972) and Aebi (1984) respectively. The SOD activity was expressed as units/g of protein, and CAT activity was expressed in units (1 U decomposes 1 μmol of H_2O_2 per minute at pH 7.0 at 25°C).

Measurement of Na^+, K^+-ATPase activity

The measurement of Na^+, K^+-ATPase activity was performed in the same kind of homogenate used for determination of the protein carbonyl content. The enzyme assay was performed according to Wyse et al. (2000).

Glutamate uptake

For the glutamate uptake measurement, the slices of cerebral cortex were previously maintained in a pregassed (carbogen) artificial cerebrospinal fluid for 15 min containing (in mM): 137 NaCl, 0.63 Na_2HPO_4, 4.17 $NaHCO_3$, 5.36 KCl, 0.44 KH_2PO_4, 1.26 $CaCl_2$, 0.41 $MgSO_4$, 0.49 $MgCl_2$, and 5.55 glucose (pH 7.2). Glutamate uptake was performed according to Frizzo et al. (2002) with few modifications. Briefly, uptake was carried out at 35°C by adding 100 μM of unlabeled glutamate and 1 μM [^{3}H] glutamate. The reaction was stopped after 7 min by washing two times with 1 ml cold buffer, immediately followed by addition of 0.5 N NaOH, which was kept overnight. Sodium independent uptake was determined by using choline chloride instead of sodium chloride, which was subtracted from the total uptake to obtain the sodium dependent uptake. Incorporated radioactivity was determined with a Packard scintillator (TRI CARB 2100 TR).

Protein determination

Protein content was measured colorimetrically by the method of Bradford (1976) using bovine serum albumin (1 mg/ml) as a standard.

Statistical analysis

Statistical analysis was carried out by one- or two-way analysis of variance (ANOVA), and only F-values of $p < 0.05$ are presented. Post hoc analysis was carried out, when appropriate, by the Student–Newman–Keuls test. All data are expressed as mean ± SEM.

Results

Effects of physical exercise on lactate threshold and body weight

In the present investigation, we showed a significant increase in total body weight in sedentary versus trained rats along the 6 weeks of swimming training [$F(1,14) = 15.09$; $p < 0.05$; Fig. 1A]. In addition, statistical analysis

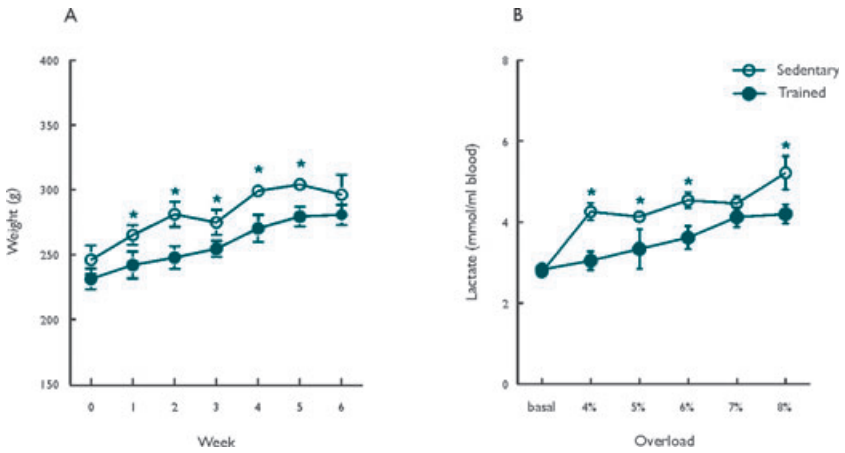

Figure 1.
Effect of 6 weeks of swimming training on body weight (**A**) and lactate threshold assay (**B**). *p < 0.05 compared with sedentary group (F test for simple effect). Data mean + SEM for n = 6 in each group.
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showed a clear stabilization of the blood lactate concentration in the trained group when compared with the sedentary group for the lactate threshold assay [$F(1,14) = 10.89$; $p < 0.05$; Fig. 1B] indicating that the training program increased aerobic resistance of the animals.

Effects of physical training on PTZ-induced behavioral convulsions and epileptiform EEG activity

Figs 2A, B show the effect of physical training on the latency and duration of generalized tonic–clonic convulsions induced by PTZ (45 or 60 mg/kg). Partitioning of the sum of squares into trend components revealed that administration of PTZ induced convulsive activity linearly with the dose given. The 6 weeks of swimming training increased the latency for the first convulsive episode induced by the moderate convulsive dose of PTZ (45 mg/kg) [$F(2,44) = 1,33$; $p < 0.05$; Fig. 2A] and attenuated the duration of convulsive episodes induced by PTZ (45 and 60 mg/kg) [$F(2,44) = 10,75$; $p < 0.05$; Fig. 2B].

Electroencephalographic recordings revealed similar EEG signals between the trained and sedentary groups before and after saline administration (Fig. 3A, panels I–II), suggesting that the protocol of physical training used here did not elicit detectable alterations in surface basal EEG. Injection of PTZ at 30 mg/kg caused only minor behavioral and EEG alterations (Fig. 3B, panel III), but injection of PTZ (45 or 60 mg/kg) caused the appearance of generalized tonic–clonic seizures characterized by the appearance of 2–3 Hz high-amplitude activity in the recording leads (Figs 3C, D, panels V and VII, respectively). Of note, physical training decreased the occurrence of EEG seizure activity induced by all doses of PTZ (Figs 3B, C, and D, panels IV, VI, and VIII, respectively). In addition, statistical analysis showed that swimming training had no effect on baseline EEG amplitude (Fig. 3E) but decreased the increase in EEG wave amplitude induced by administration of all doses of PTZ [$F(3,44) = 4,55$; $p < 0.05$; Fig. 3F].

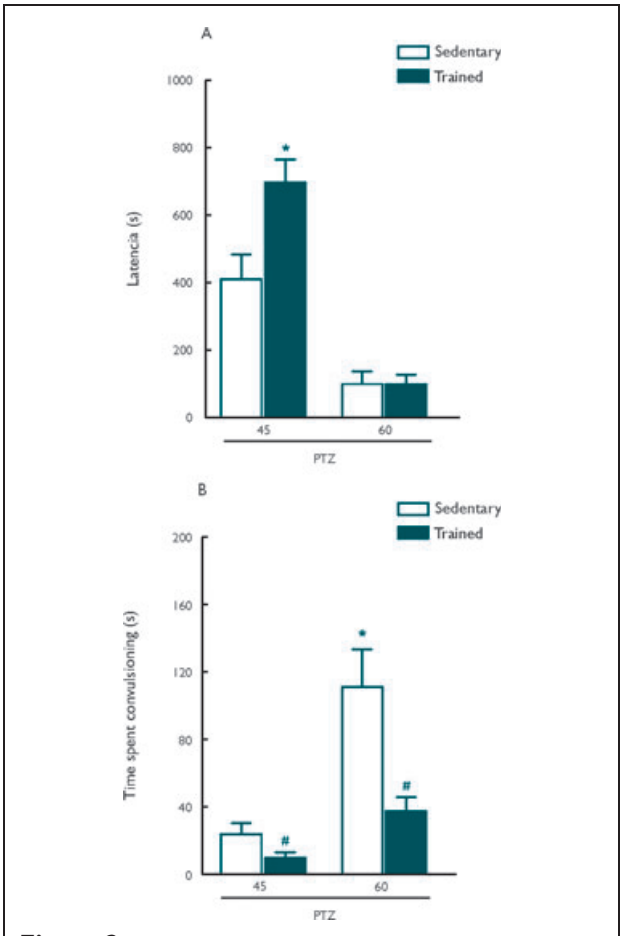

Figure 2.
Effect of physical training on latency (**A**) and duration (**B**) of generalized tonic–clonic convulsions induced by PTZ (45 and 60 mg/kg, i.p.). *p < 0.05 compared with sedentary-PTZ group (45 mg/kg, i.p.). #p < 0.05 compared to sedentary-PTZ group (45 and 60 mg/kg, i.p.) Data are mean + SEM for n = 8–10 in each group.
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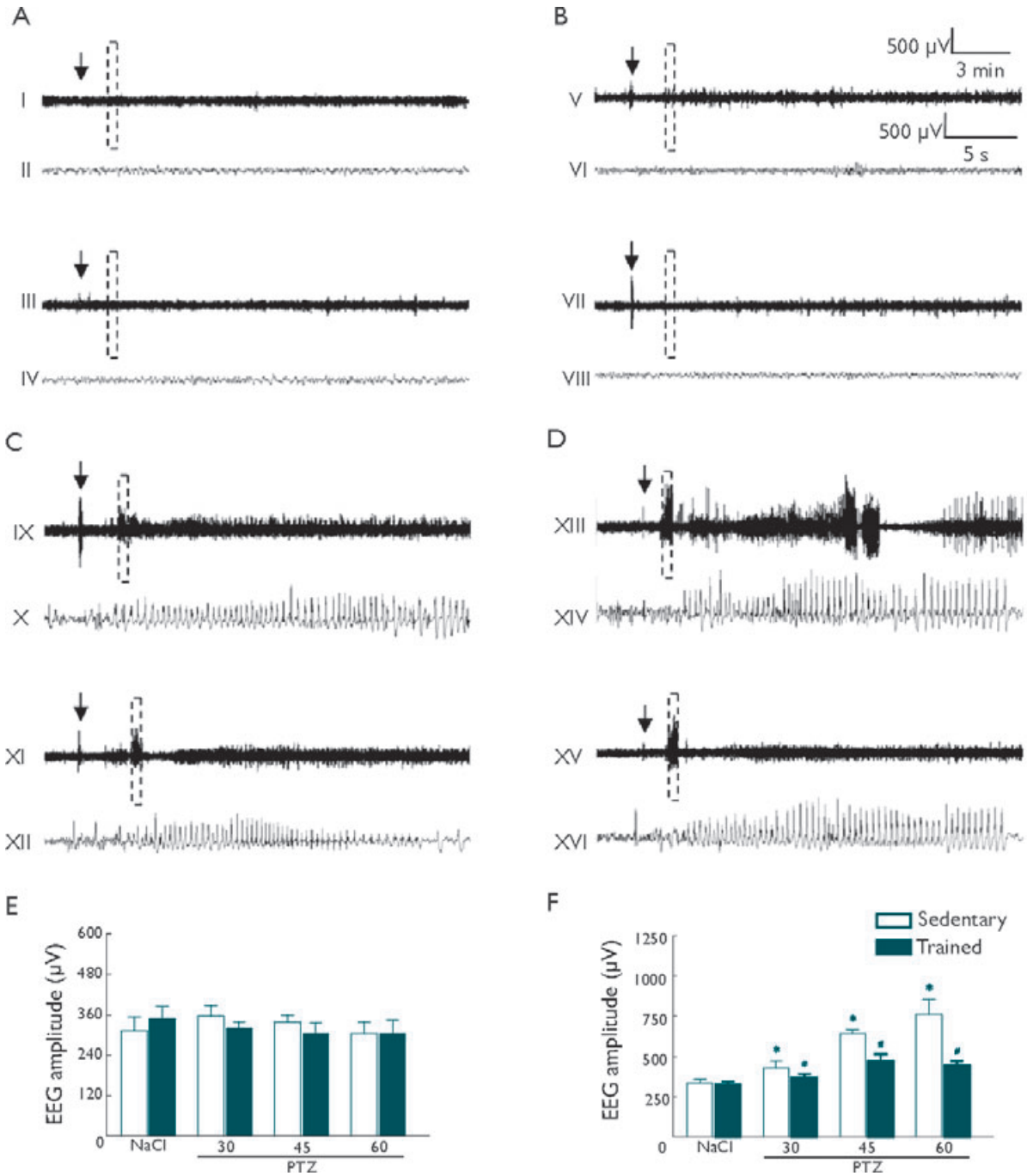

Figure 3.
Representative electroencephalographic recordings demonstrate the discharge sequence observed in sedentary (A-I) and trained group (A-II) after systemic administration after saline (0.9% NaCl, 1 ml/kg, i.p.). Typical electroencephalographic recordings variations after administration of the subthreshold to severe convulsive dose of PTZ (30–60 mg/kg, i.p.) in sedentary (B-III; 3C-V; 3D-VII) and physical training group (3B-IV; 3C-VI; 3D-VIII). The arrow indicates NaCl or PTZ (30, 45 or 60 mg/kg, i.p.) administration. Calibration bars 500 μV and 3 min. Figs. 3E, F show the effect of physical training on EEG amplitude before (**A**) and after saline (0.9% NaCl, 1 ml/kg, i.p.) or PTZ (30–60 mg/kg, i.p.) (**B**). *$p < 0.05$ compared with NaCl group. $^{\#}p < 0.05$ compared to sedentary-PTZ group (30–60 mg/kg, i.p.) Data are mean + SEM for n = 8–10 in each group.
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Table 1. Effect of swimming training on 2′,7′-dichlorofluorescein (DCF) oxidation, protein carbonyl, and TBARS content after injection of PTZ (30, 45, and 60 mg/kg, i.p.)

	DCFH-DA oxidation (% of control)		Protein carbonyl content (nmol/mg protein)		TBARS content (nmol MDA/mg protein)	
Treatment (mg/kg)	Sedentary	Trained	Sedentary	Trained	Sedentary	Trained
NaCl	100 ± 5.0	−16.33 ± 8.2[a]	6.6 ± 0.7	6.01 ± 0.2	57.45 ± 17.7	40.10 ± 10.6
PTZ-30	50.01 ± 5.5[c]	10.21 ± 5.1[c]	7.7 ± 0. 6	5.2 ± 0.4	63.37 ± 10.7	55.90 ± 14.4
PTZ-45	51.96 ± 6.1[c]	11.16 ± 4.8[b]	8.9 ± 0.6[c]	6.2 ± 0.3[b]	68.9 ± 15.6	54.2 ± 10.5
PTZ-60	49..47 ± 4.2[c]	10.05 ± 4.3[b]	10.4 ± 0.6[c]	6.0 ± 0.5[b]	118.2 ± 20.5[c]	66.2 ± 13.9[b]

Data are mean ± SEM for n = 8–10 in each group. MDA, malondialdeide.
[a]p < 0.05 compared to NaCl-sedentary group.
[b]p < 0.05 compared with sedentary-PTZ groups (30–60 mg/kg, i.p.) (Student–Newman–Keuls test).
[c]p < 0.05 compared to NaCl-sedentary group.

Epileptiform activity–induced alteration in ROS generation and the antioxidant status in cerebral cortex: effects of physical training

Several parameters that indicate the antioxidant status and the generation of oxidative stress in the cell were determined in cortical homogenates. These include DCFH-DA oxidation, lipid peroxidation (TBARS), protein carbonyl, NPS content, and CAT and SOD activities.

The effect of injection of PTZ (30, 45, and 60 mg/kg, i.p.) and swimming training on DCFH-DA oxidation, protein carbonyl, and lipid peroxidation (TBARS) is showed in Table 1. Statistical analysis revealed that physical training attenuated DCFH-DA oxidation [$F(1,64) = 35.17$; $p < 0.05$] per se, and prevented the increase in DCFH-DA oxidation induced by all doses of PTZ [$F(1,30) = 5.17$; $p < 0.05$]. This training protocol also protected against TBARS [$F(1,64) = 14.98$; $p < 0.05$] and protein carbonyl increase [$F(3,64) = 2.17$; $p < 0.05$] induced by higher doses of PTZ (45 and 60 mg/kg).

In addition, statistical analysis showed that physical training increased SOD activity [$F(1,64) = 31.69$; $p < 0.05$; Fig. 4A] per se, and protected against its inhibition after administration of the fully convulsant dose of PTZ (60 mg/kg) [$F(3,64) = 3.90$; $p < 0.05$]. Moreover, administration of PTZ at 45 or 60 mg/kg induced a significant decrease in CAT activity [$F(3,64) = 4.89$; $p < 0.05$], an effect prevented by physical training [$F(3,64) = 2.73$; $p < 0.05$; Fig. 4B]. Furthermore, physical training increased NPS content [$F(1,64) = 22.24$; $p < 0.05$] per se, and protected against the decrease in NPS induced by higher doses of PTZ (45 and 60 mg/kg) [$F(3,64) = 5.87$; $p < 0.05$] (Fig. 4C).

Effects of physical training on PTZ-induced glutamate uptake and inhibition of Na^+,K^+-ATPase activity

Considering that alterations in the redox state of regulatory sulfhydryl groups in selected targets, such as Na^+, K^+-ATPase (Morel et al., 1998) and glutamate transporters

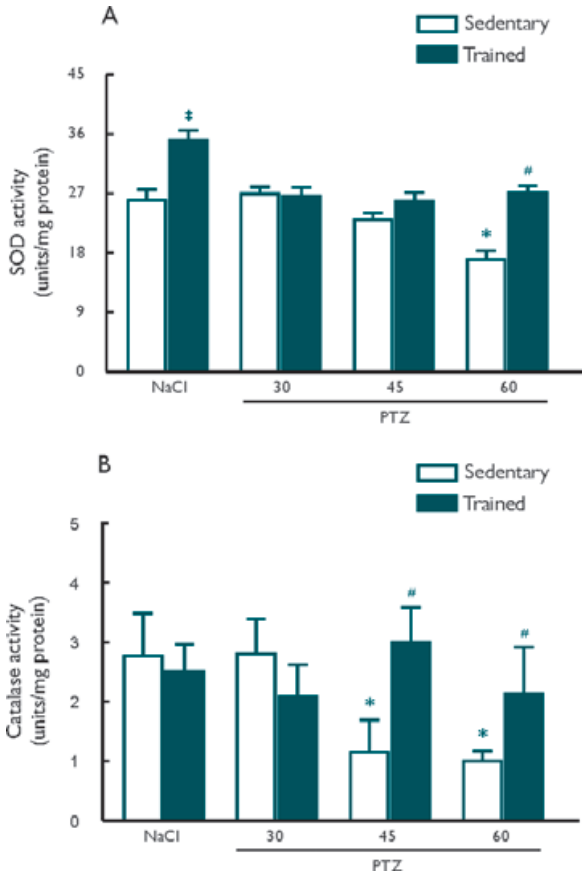

Figure 4.
Effect of physical training and PTZ injection on SOD (**A**) and catalase (**B**) activity ex vivo. Data are mean ± SEM for n = 8–10 in each group. ‡p < 0.05 compared to NaCl-sedentary group. #p < 0.05 compared with sedentary-PTZ groups (45–60 mg/kg, i.p.). *p < 0.05 compared to NaCl-sedentary group. (Student–Newman–Keuls test).
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Table 2. Effect of swimming training on nonprotein sulfhydryl groups, Na^+, K^+-ATPase activity and, [^{3}H] Glutamate uptake after injection of PTZ (30, 45, and 60 mg/kg, i.p.)

	Free –SH group content (μmol/mg protein)		Na^+, K^+-ATPase activity (nmol Pi/mg protein/min)		[^{3}H] Glutamate uptake (pmol/mg protein/min)	
Treatment (mg/kg)	Sedentary	Trained	Sedentary	Trained	Sedentary	Trained
NaCl	1.21 ± 0.02	2.79 ± 0.02[a]	141.3 ± 13.0	156.4 ± 12.9	101.3 ± 13.0	89.20 ± 15.0
PTZ-30	1.09 ± 0.06	2.02 ± 0.02	108.8 ± 8.3[c]	139.9 ± 10.0[b]	81.27 ± 8.3	90.25 ± 13.9
PTZ-45	0.96 ± 0.01[c]	2.16 ± 0.01[b]	88.8 ± 7.8[c]	131.1 ± 10.1[b]	69.80 ± 7.8	73.10 ± 10.1
PTZ-60	0.47 ± 0.02[c]	1.53 ± 0.02[b]	79.7 ± 6.2[c]	153.6 ± 8.7[b]	37.62 ± 6.3[c]	64.5 ± 8.7[b]

Data are mean ± SEM for n = 8–10 in each group.
[a] $p < 0.05$ compared to NaCl-sedentary group.
[b] $p < 0.05$ compared with sedentary-PTZ groups (30–60 mg/kg, i.p.) (Student–Newman–Keuls test).
[c] $p < 0.05$ compared to NaCl-sedentary group.

(Trotti et al., 1997), increases cellular excitability and facilitates the appearance or propagation of convulsions (Ames, 2000), we investigated the effect of physical training and PTZ administration on Na^+, K^+-ATPase activity and [^{3}H]-glutamate uptake (Table 2). Statistical analysis showed that administration of PTZ at all doses used decreased Na^+, K^+-ATPase activity [$F(3,64) = 4.72$; $p < 0.05$], and that physical training was protective against Na^+, K^+-ATPase activity inhibition induced by PTZ [$F(3,64) = 1.14$; $p < 0.05$]. Moreover, statistical analysis showed that administration of PTZ at 60 mg/kg decreased [^{3}H]-glutamate uptake [$F(3,64) = 3.85$; $p < 0.05$)] and that physical training prevented this effect [$F(3,64) = 1.27$; $p < 0.05$].

Correlation analysis of the duration of convulsive episodes with biochemical parameters

Correlation analysis (Pearson's correlation analysis) revealed that the duration of convulsive episodes induced by severe convulsive dose of PTZ (60 mg/kg) did not correlate with TBARS production ($r = 0.418$; $p < 0.303$), protein carbonylation ($r = 0.302$; $p < 0.453$), or DCFH-DA oxidation ($r = 0.450$; $p < 0.263$) (data not shown). On the other hand, Pearson's correlation analysis revealed that the duration of convulsive episodes elicited by this dose of PTZ strongly correlated with free –SH-group oxidation ($r = 0.912$; $p < 0.007$); Na^+, K^+-ATPase activity ($r = 0.844$; $p < 0.05$); and glutamate-uptake inhibition ($r = 0.934$; $p < 0.001$) (Fig. 5). Furthermore, we showed a negative correlation between duration of convulsive episodes after injection of PTZ (60 mg/kg) with free –SH-group oxidation ($r = 0.931$ $p < 0.001$); Na^+, K^+-ATPase activity ($r = 0.812$; $p < 0.05$); and glutamate uptake inhibition ($r = 0.921$; $p < 0.001$) in trained rats.

Discussion

In the current study we have confirmed and extended our previous findings that PTZ elicits behavioral and electrographic seizures and increases reactive species generation (ROS) in vivo (Oliveira et al., 2004; Ribeiro et al., 2005; Fighera et al., 2006), and have shown for the first time that a 6 weeks of swimming training protocol affords significant alteration in the antioxidant status and is effective in attenuating convulsions and neurochemical alterations elicited by PTZ.

Concerns about increased seizure frequency and its potential for injury have led to overprotective measures by many health care professionals (Bjorholt et al., 1990; Arida et al., 2003; Wong & Wirrell, 2006). In this context, the protection exerted by physical training on epileptiform activity and neurochemical alterations induced by PTZ is of particular interest because PTZ-induced seizures are an important model of myoclonic and generalized tonic–clonic seizures, which are utilized in the routine test for screening anticonvulsants (Swinyard et al., 1987) and because it supports the idea that physical training may prevent seizures induced by different agents. In this context, a significant body of evidence has demonstrated that training performed before and during the kindling procedure slows amygdala kindling development (Arida et al., 1998,2007b) and decreases susceptibility (Setkowicz & Mazur, 2006) and frequency of spontaneous recurrent seizures in the pilocarpine model of epilepsy in rats (Arida et al., 1999; 2004). On the other hand, although there is convincing evidence of the positive role of physical exercise in reducing the frequency and severity of seizures in several models of epilepsy (McAuley et al., 2001; Sutoo & Akiyama, 2003; Howard et al., 2004), the mechanism underlying this protective effect has not been clearly investigated.

In the present study, we showed that 20 min after administration of moderate and severe doses of PTZ (45 and 60 mg/kg, i.p.), several parameters that indicate the anti-oxidant status and generation of oxidative stress in cortical homogenates were affected. The occurrence of DCFH-DA oxidation, lipid peroxidation (TBARS), increase in protein carbonyl, decrease in NPS content, and inhibition of catalase and SOD activity after single doses

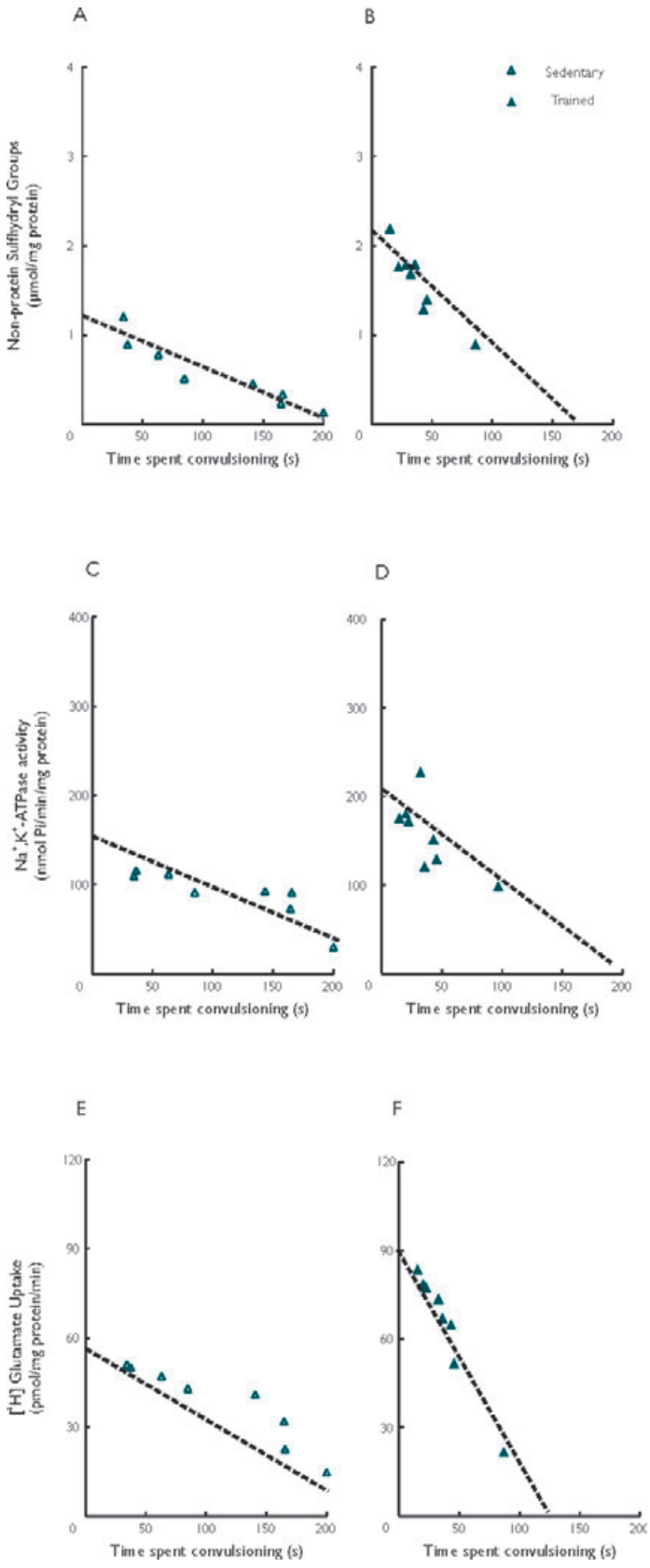

Figure 5.
Duration of convulsive episodes induced by PTZ (60 mg/kg, i.p.) correlates with free –SH-group oxidation (**A**); Na^+, K^+-ATPase activity (**C**); and glutamate-uptake inhibition (**E**) in sedentary group. Pearson's correlation coefficient also showed a negative correlation between duration of convulsive episodes after injection of PTZ (60 mg/kg, i.p.) with free –SH-group oxidation (**B**); Na^+, K^+-ATPase activity; (**D**) and glutamate-uptake inhibition (**F**) in a trained group of rats. Data are individual values for n = 7 in each group.
Epilepsia ©ILAE

of PTZ suggest that epileptic seizures elicited by this convulsant agent were accompanied by increase of oxidative stress. However, the cause–effect relationship between these events is difficult to postulate, since we found no correlation between duration of PTZ-induced convulsion and total protein carbonylation, TBARS production, or DCFH-DA oxidation. If such a cause–effect relationship between convulsions and these neurochemical parameters existed, a significant positive correlation between these variables should be found. Nevertheless, one might also consider the possibility that selected targets, which could not contribute significantly to the content observed, could be responsible for the convulsant action exerted by PTZ. In this context, it has been demonstrated that, in some cases, such as Alzheimer's disease, only selected proteins show increases in the levels of carbonylation (Castegna et al., 2002). With respect to the effects of the subconvulsive dose of PTZ (30 mg/kg, i.p.) in cerebral cortex, the data from the present study suggest that changes in this structure could be related to the specific function of PTZ as a selective blocker of the chloride ionophore complex (Psarropoulou et al., 1994; Walsh et al., 1999) or to cellular thiol homeostasis (Patsoukis et al., 2004) before the appearance of seizures.

On the other hand, the results presented in this report showed that the duration of convulsive episodes elicited by PTZ (60 mg/kg, i.p.) strongly correlated with free –SH-group oxidation; Na^+, K^+-ATPase activity; and glutamate-uptake inhibition, suggesting that convulsive activity and neurochemical parameters are closely linked events. These experimental findings also suggest that the significant correlation observed previously may be related to a thiol redox-affected function of *N*-methy-D-aspartate (NMDA) and $GABA_A$ receptors (Amato et al., 1999; Sanchez et al., 2000). Furthermore, the PTZ-induced excitability proposed in this report is in accordance with previously reported data showing that a single convulsive dose of PTZ results in significant changes in many parameters such $GABA_A$ receptor density and function (Psarropoulou et al., 1994; Walsh et al., 1999), whole brain hydroxyl radicals (Rauca et al., 1999), free fatty acids, and glutathione peroxidase activity in specific brain areas (Eraković et al., 2003).

In the current study, the electrographic and behavioral recordings indicate that physical training had an effect on the generation and duration of generalized seizures induced by PTZ (45 and 60 mg/kg, i.p.). These experimental findings suggest that important changes in the brain induced by physical training (Sutoo & Akiyama, 2003) might affect the susceptibility to seizurogenic stimuli or events followed by spontaneous epileptiform activity elicited by PTZ. In this context, considering that Na^+,K^+-ATPase enzyme plays a pivotal role in cellular ionic gradient maintenance and is particularly sensitive to reactive species (Morel et al., 1998; Petrushanko et al., 2006), we suggest that the maintenance of Na^+, K^+-ATPase activity induced by physical training might protect against enhance neuronal excitability induced by PTZ. In fact, in the present study we showed a strongly negative correlation between duration of PTZ-induced convulsive episodes with free –SH-group oxidation, Na^+, K^+-ATPase activity, and glutamate-uptake inhibition in trained group of rats.

The results presented in this report also showed that administration of a moderate to severe convulsive dose of PTZ (45 and 60 mg/kg, i.p.) inhibited Na^+, K^+-ATPase activity, whereas a severe convulsive dose of PTZ (60 mg/kg, i.p.) inhibited glutamate uptake. Although the exact mechanism through which PTZ reduces glutamate uptake is still unknown, it is tempting to propose that the reduction of glutamate uptake by PTZ could be related directly or indirectly to PTZ-induced oxidative stress. In this line of thinking, oxidation of regulatory sulfhydryl groups in glutamate transporters decrease its activity (Trotti et al., 1997) and a reduction in Na^+,K^+-ATPase activity also decreases glutamate uptake, since it depends on Na^+ gradients across cell membrane. This is particularly important considering that the activity of glutamate transporters can be impaired by several indirect mechanism, including ROS formation and reduction of Na^+,K^+-ATPase activity (Volterra et al., 1994; Nanitsos et al., 2004). However, further in-depth studies are necessary to definitively establish the mechanisms involved.

In the present study, we showed that physical training was effective against PTZ-induced TBARS formation, protein carbonylation, DCFH-DA, and free –SH-group oxidation. In addition, the present protocol of training protected against PTZ- induced inhibition of SOD and CAT activity. These results agree with a substantial body of evidence that suggests adaptive responses to regular and moderate endurance exercise involving an increase of antioxidant defenses, a reduction of basal production of oxidants, and a reduction of radical leak during oxidative phosphorylation (Packer & Cadenas, 2007). Accordingly, a recent study has demonstrated that moderate exercise significantly decreases the age-associated development of oxidative damage in mice, increases lifespan, prevents deterioration of mitochondrial function, and even improves behavioral performance (Navarro et al., 2004). Furthermore, Radak et al. (2006) suggest that exercise-induced production of ROS plays a role in the induction of antioxidants, and DNA repair and protein degrading enzymes, resulting in decreased incidence of oxidative stress.

Considering that regular exercise leads to the development of compensatory responses to oxidative stress (Salo et al., 1991; Viguie et al., 1993; Leeuwenburgh & Heinecke, 2001) and that failure of some selected targets, such as Na^+,K^+-ATPase, may increase cellular excitability and facilitate the appearance or propagation of convulsions (Patel et al., 2004), we suggest that the increase of antioxidant defenses and reduction of basal production of oxidants elicited by this physical training may protect

against Na^+, K^+-ATPase inhibition induced by PTZ. In fact, results presented in this report showed that this protocol of swimming training increased NPS content and SOD activity, protected against ROS generation per se, and was effective against Na^+, K^+-ATPase inhibition elicited by a subthreshold convulsive dose of PTZ (30 mg/kg, i.p.). Accordingly, the negative correlation between the duration of convulsive episodes after injection of PTZ (60 mg/kg, i.p.) with free –SH-group oxidation, Na^+, K^+-ATPase activity, and glutamate-uptake inhibition in a trained group of rats reinforce the assumption that physical training displays antioxidant properties and protects against the epileptiform activity–induced alterations in ROS generation in cerebral cortex.

With respect to the difference in body weight between sedentary and trained rats, we think that such difference may be explained by changes in body composition. For instance, a decrease in subcutaneous adipose tissue of trained rats may explain why body mass was smaller in this group. However, we have not determined body composition in the present study, and, therefore, this explanation remains speculative in nature, and further studies are necessary to determine the mechanisms involved.

In conclusion, the present study reports that PTZ administration induces convulsive behavior following excitotoxic damage in vivo and that previous physical training protects against these deleterious effects. The results showing specific molecular systems modulated by exercise also provide a framework to guide further studies to examine the mechanisms by which exercise alters neuronal functions. Therefore, these experimental findings suggest that physical training may be a new therapeutic approach to control acute and chronic excitotoxicity, including seizure activity.

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. In addition, we would like to state that all authors have seen and approved the study and that no part of the work has been published or is under consideration for publication elsewhere. Moreover, the present study was supported by government funding and has no financial or other relationship that might lead to a conflict of interest. We also would like to declare that all experiments were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996, and that the University Ethics Committee approved the respective protocols.

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