ARTIGO ORIGINAL DOI: 10.5007/1980-0037.2011v13n2p106

Preventive effects of physical exercise on the inhibition of creatine kinase in the cerebral cortex of mice exposed to cigarette smoke

Efeitos preventivos do exercício físico sobre a inibição da creatina quinase em córtex cerebral de camundongos expostos à fumaça de cigarro

Daiane Bittencourt Fraga [1]
Renata Tiscoski Nesi [2]
Giselli Scaini [1]
Bruna Tramontin De-Nês [1]
Patricia Fernanda Schuck [1]
Emilio Luiz Streck [1]
Ricardo Aurino Pinho [1]

Abstract – Recent studies have shown the health benefits of physical exercise, increasing the oxidative response of muscle. However, the effects of exercise on the brain are poorly understood and contradictory. The inhibition of creatine kinase (CK) activity has been associated with the pathogenesis of a large number of diseases, especially in the brain. The objective of this study was to determine the preventive effects of physical exercise in the hippocampus and cerebral cortex of mice after chronic cigarette smoke exposure. Eight to 10-week-old male mice (C57BL-6) were divided into four groups and submitted to an exercise program (swimming), 5 times a week, for 8 weeks. After this period, the animals were passively exposed to cigarette smoke for 60 consecutive days, 3 times a day (4 Marlboro red cigarettes per session), for a total of 12 cigarettes. CK activity was measured in cerebral cortex and hippocampal homogenates. Enzyme activity was inhibited in the cerebral cortex of animals submitted to the inhalation of cigarette smoke. However, exercise prevented this inhibition. In contrast, CK activity remained unchanged in the hippocampus. This inhibition of CK by inhalation of cigarette smoke might be related to the process of cell death. Physical exercise played a preventive role in the inhibition of CK activity caused by exposure to cigarette smoke.

Key words: Physical exercise; Tobacco; Brain metabolism.

1 Universidade do Extremo Sul Catarinense. Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde. Criciúma, SC. Brasil

2 Universidade do Extremo Sul Catarinense. Laboratório de Fisiologia e Bioquímica do Exercício. Programa de Pós-Graduação em Ciências da Saúde. Criciúma, SC. Brasil.

Recebido em 08/12/09
Revisado em 08/02/10
Aprovado em 16/09/10

Resumo – *O exercício físico aeróbico tem demonstrado benefícios em pesquisas recentes, uma vez que aumenta a resposta oxidativa muscular, porém os efeitos do exercício sobre o cérebro são pouco conhecidos e bastante contraditórios. A inibição da atividade da enzima creatina quinase (CK) está relacionada à patogênese de um grande número de doenças, especialmente no cérebro, e que a disfunção mitocondrial leva ao dano na síntese de ATP. Este trabalho tem como objetivo verificar os efeitos preventivos do exercício físico no hipocampo e córtex cerebral de camundongos submetidos à exposição crônica da fumaça de cigarro. Foram utilizados 24 camundongos C57BL-6 machos, com idade de entre 8-10 semanas, divididos em 4 grupo, foram submetidos a um programa de exercício (natação), cinco vezes por semana, durante 8 semanas, após esse período os animais foram expostos passivamente à fumaça de cigarro por 60 dias consecutivos, 3 vezes ao dia totalizando em 12 cigarros, 4 cigarros por vez, da marca Marlboro vermelho. A atividade enzimática da CK foi determinada em hipocampo e córtex cerebral. Os resultados mostraram que a atividade da enzima CK foi inibida no córtex cerebral dos animais submetidos à inalação da fumaça do cigarro, porém o exercício conseguiu prevenir esta alteração. A atividade da CK não foi alterada no hipocampo dos animais. Essa inibição da CK pela inalação da fumaça do cigarro pode estar relacionada com processos de morte celular. O exercício preventivo mostrou um papel protetor sobre a inibição dessa enzima.*

Palavras-chave: *Exercício físico; Tabaco; Metabolismo cerebral.*

INTRODUCTION

Cigarette smoke shows a high potential to change brain physiology and biology[1]. Nicotine has the capacity to cause addiction and is responsible for a larger number of deaths than other psychoactive drugs[2]. Neuroadaptation to nicotine involves biological substrate tolerance and physical dependence. In this respect, the changes that occur in synaptic membrane composition, especially in postsynaptic intracellular events, have been suggested as the basis for the neuroadaptation to psychoactive drugs abuse[3].

Studies have shown that nicotine interferes with cell metabolism and homeostasis[4]. A recent study showed that regular physical exercise exerts a protective effect against pulmonary oxidative damage in mice exposed to cigarette smoke[5]. The main target of these metabolic alterations is the central nervous system, with the observation of neuropsychiatric alterations caused by nicotine[6].

Cigarette smoke is an important source of the toxic metal cadmium, a contaminant of tobacco[7]. Studies have shown that cadmium inhibits enzymatic systems and that it is also able to modify metalloenzymes by modulating the affinity of sulfhydryl groups present in these enzymes. Moreover, cadmium interferes with oxidative phosphorylation, membrane calcium channels and Ca^{2+}-ATPases, prevents DNA repair (the same mechanism as induced by tumors), and inhibits catalase activity. Cadmium can also cause neurotoxicity when reaching the central nervous system, directly affecting neurotransmitter systems, generating free radicals, reducing glutathione levels, causing oligodendrocyte death, and compromising mitochondrial metabolism[8].

Organ-specific enzymes are used for the assessment of tissue injury in various diseases. Creatine kinase (CK) is an enzyme widely used as a reliable marker for the assessment of myocardial, muscle and brain damage[9]. CK catalyzes the reversible phosphorylation of creatine by ATP, with the transfer of the high-energy phosphate moiety being an important step in various processes in the body[10].

Mitochondria are the main site of creatine phosphate synthesis in tissues characterized by high energy requirements[11]. The creatine/phosphocreatine system is important for the initiation of intense exercise before glycogenolysis is activated[12]. Recent studies using animal models and human studies have been carried out in order to understand the neurobiological basis of the benefits of physical exercise[13]. However, the mechanisms underlying the molecular and neurophysiological effects remain poorly understood[14-17].

Studies have shown that moderate exercise produces a series of beneficial effects in aging mice, such as increased survival, improved performance in behavioral tasks of neuromuscular function and exploratory activities, and a decrease in markers of oxidative damage to the mitochondrial membranes in organs such as the brain and heart[18].

Within this context, the aim of the present study was to evaluate the effects of chronic exposure to cigarette smoke on CK activity in the hippocampus and cerebral cortex of mice subjected to physical exercise.

METHODOLOGICAL PROCEDURES

Animals

Eight to 10-week-old male mice (C57BL-6) obtained from the Central Animal House of Universidade do Extremo Sul Catarinense, Santa Catarina, Brazil, were used in this study. The animals were housed in groups of four and maintained on a 12-h light/12-h dark cycle, with commercial mouse chow and water being available *ad libitum*.

Experimental design

The animals were divided randomly into four groups (n=6): group 1 was not exposed to cigarette smoke and was not submitted to physical exercise (sham); group 2 was exposed to cigarette smoke; group 3 was submitted to physical exercise; group 4 was exposed to cigarette smoke and physical exercise. The study was performed in accordance with National Institutes of Health guidelines and was approved by the Ethics Committee of Universidade do Extremo Sul Catarinense, Santa Catarina, Brazil.

Physical exercise protocol

The first task was swimming trainings in a heated swimming pool adapted for animals, where the mice are forced to swim. All animals were acclimatized for one week in a swimming pool measuring 120 x 60 x 50 cm. The volume of water was 37.5 cm^3. After this period, the animals that received the treatment were submitted to the exercise program five times per week (for 8 weeks), adapted from Pellegrin and colleagues[19].

Cigarette smoke exposure

The animals were submitted to the passive inhalation of a total of 12 cigarettes per day (four cigarettes per session, three times a day) for 60

consecutive days. Cigarettes of the commercial Marlboro brand were used for this protocol[18]. The animals were placed in an acrylic chamber (30 x 40 x 25 cm). This chamber is interconnected with a conventional inhalation equipment by a hospital aspiration plumb (16 mm in diameter). The cigarette is placed in another plumb on the opposite side of the inhalation equipment and the cigarette smoke enters the acrylic chamber. Each cigarette burns for 6 minutes, with a 1-minute interval between cigarettes. The protocol was carried out 7 days a week, for 8 weeks, always at the same times.

Tissue and homogenate preparation

Twenty-four hours after the last inhalation, the animals were killed by cervical dislocation. The brain was removed and the hippocampus and cerebral cortex were separated and homogenized in SETH buffer (1:10, w/v), pH 7.4, containing 250 mM sucrose, 2 mM EDTA, 10 mM Trizma base, and 50 IU/mL heparin. The homogenates were centrifuged at 800 × *g* for 10 min and the supernatants were stored at -70° C until the time for determination of enzyme activity. Protein content was determined by the method of Lowry and colleagues[20] using bovine serum albumin as standard.

Creatine kinase activity

CK activity was measured in brain homogenates pretreated with 0.625 mM lauryl maltoside. The reaction mixture contained 60 mM Tris-HCl, pH 7.5, 7 mM phosphocreatine, 9 mM $MgSO_4$ and approximately 0.4–1.2 µg protein in a final volume of 100 µL. After pre-incubation for 15 min at 37 °C, the reaction was started by the addition of 3.2 mmol ADP plus 0.8 mmol reduced glutathione. The reaction was stopped after 10 min by the addition of 1 µmol p-hydroxymercuribenzoic acid. The creatine formed was estimated according to the colorimetric method of Hughes (1962). The color was developed by the addition of 100 µL 2% α-naphthol and 100 µL 0.05% diacetyl in a final volume of 1 mL and read spectrophotometrically after 20 min at 540 nm. The results are expressed as units/min/mg protein.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by the Duncan test when F was significant. All analyses were performed using the Statistical Package for the Social Sciences (SPSS). Differences were considered to be significant when $P<0.05$.

RESULTS

It is well established that cigarette smoke exposure induces cell modifications[22]. Studies have shown that cigarette smoke causes an increase in the production of reactive oxygen species and DNA damage and induces cell apoptosis[23]. In the present study, we evaluated the effect of chronic exposure to cigarette smoke on CK activity in the cerebral cortex and hippocampus of mice, and the protective effect of physical exercise on CK inhibition caused by cigarette smoke. We observed that chronic cigarette smoke exposure inhibited CK activity in the cerebral cortex (Figure 1).

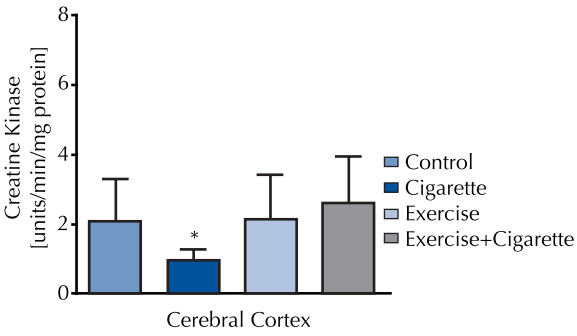

Figure 1: Creatine kinase activity in the cerebral cortex of mice 60 days after physical training and chronic cigarette smoke exposure. Data were analyzed by the Duncan test when F was significant. Values are expressed as unit per minute per mg protein. * p<0.01: Significant difference when compared to the control, exercise and exercise plus chronic cigarette exposure groups.

Furthermore, the inhibition of CK activity was prevented by physical exercise when compared to the control group. On the other hand, CK activity in the hippocampus was not affected by exposure to cigarette smoke or physical exercise (Figure 2).

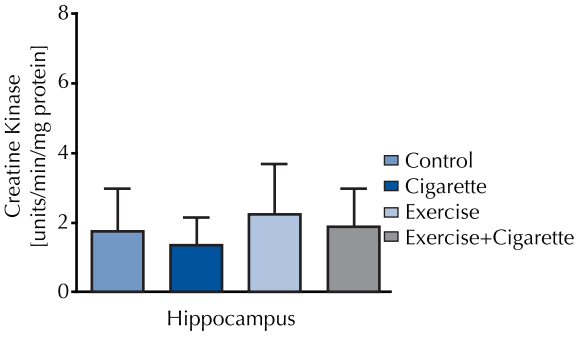

Figure 2: Creatine kinase activity in the hippocampus 60 days after physical training and chronic cigarette smoke exposure. Data were analyzed by the Duncan test when F was significant. Values are expressed as unit per minute per mg protein.

DISCUSSION

Nicotine has been shown to interfere with cell metabolism, especially in the brain because of

high metabolic activity that leads to a high oxygen consumption rate (35 mL/min/kg) and of the high amount of oxidizable polyunsaturated fatty acids in the cellular membranes[4,6,24]. Furthermore, Rezyvani and Levin[25] showed that nicotine facilitates hippocampal synaptic activity and increases hippocampal long-term potentiation. Therefore, nicotinic treatments directed at specific receptor subtypes and nicotinic co-treatment with drugs affecting the interaction with transmitter systems may provide cognitive benefits for different syndromes of cognitive impairment. However, the use of nicotine has dual and conflicting effects on oxidative stress and neuroprotection depending on dose of the drug used and its underlying mechanisms. Generally, a high dose of nicotine induces neurotoxicity and stimulates oxidative stress[4].

A recent study demonstrated that regular physical exercise protects against pulmonary oxidative damage in mice exposed to cigarette smoke[5], suggesting a beneficial effect of physical exercise. In the present study, CK activity was significantly decreased in the cerebral cortex of animals exposed to cigarette smoke when compared to the control group. On the other hand, CK activity in the hippocampus was not altered by cigarette smoke exposure, suggesting that the cerebral cortex is more susceptible to the effects of cigarette smoke than the hippocampus. In this respect, CK activity has been shown to be inhibited in the brain and heart of Wistar rats exposed to cigarette smoke[26]. This effect might be the result of oxidative damage to the enzyme since cigarette smoke exposure has been shown to increase the generation of free radicals and membrane permeability, resulting in brain and heart cell damage[26]. Elsayed and Bendich[27] demonstrated that cigarette smoke also causes mitochondrial DNA damage provoked by oxidative compounds. In addition, cigarette smoke elicits an inflammatory response induced by phagocytes through the activation of polymorphonuclear cells that contain myeloperoxidase and NADPH oxidase, enzymes that catalyze the production of free radicals[27]. Cigarette smoke also contains nitric oxide, a molecule that reacts with superoxide anion, forming peroxynitrite (ONNO-).

The present study also showed that regular physical exercise has a protective effect against CK inhibition caused by cigarette smoke exposure. In this respect, studies have demonstrated that regular physical exercise improves mitochondrial oxidative function, increasing electron flow and ATP synthesis[28]. This improvement in mitochondrial function is probably due to the adaptation of antioxidant defenses, preventing oxidative damage and cell death caused by cigarette smoke[29,30].

CONCLUSION

In conclusion, this study demonstrated that CK activity in the cerebral cortex was inhibited after exposure of mice to cigarette smoke and that regular physical activity was able to prevent this inhibition. However, the mechanisms involved in these events remain obscure. Taken together, the present data suggest a protective role of moderate and regular physical exercise against the damage in brain energy metabolism caused by cigarette smoke exposure in mice.

REFERENCES

1. Benowitz NL. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther 2008;83(4):531-40.
2. Dome P, Lazary J, Kalapos MP, Rihmer Z. Smoking, nicotine and neuropsychiatric disorders. Neurosc Biobehav Rev 2010;34(3):295-342
3. Kenny PJ, Markou A. Conditioned nicotine withdrawal profoundly decreases the activity of brain reward systems. J Neurosci 2005;25(26):6208-12.
4. Barros DM, Galhardi FG, Ferreira RJL, Guterres LB, Dickel O, Geracitano LA, et al. The benefits and drawbacks of nicotine exposure in the cortex and hippocampus of old rats. Neurotoxicology 2007;28(3):562-8.
5. Menegali BT, Nesi RT, Souza PS, Silva LA, Silveira PC, Valença SS, Pinho RA. The effects of physical exercise on the cigarette smoke-induced pulmonary oxidative response. Pulm Pharmacol Ther 2009;22(6):567-73.
6. Mansvelder HD, McHegee DS. Cellular and synaptic mechanisms of nicotine addiction. J Neurobiol 2002;53(4):606-23.
7. Goyer RA. Casarett and Doull´s Toxicology: the basic science of poisons. In: Klassen CD, editors. New York: McGraw-Hill; 1996. p. 691-712.
8. Cannino G, Ferruggia E, Luparello C, Rinaldi AM. Cadmium and mitochondria. Mitochondrion 2009;9(6):377-84.
9. Bell RD, Khan M. Cerebrospinal fluid creatine kinase-BB activity: a perspective. Arch Neurol 1999;56(11):1348-52.
10. Wallimann T, Wyss M, Brdiczka D, Nicolay K, Eppenberger H.M. Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the phosphocreatine circuit' for cellular energy homeostasis. Biochem J 1992;281(1):21-40.
11. Lipskaya TY. Mitochondrial creatine kinase: properties and function. Biochemistry 2001;66(10):1098-111.
12. Meyer RA, Sweeney HL, Kushmerick MJ. A simple analysis of the phosphocreatine shuttle. Am J Physiol 1984;246(5):C365-C377.

13. Cotman CW, Engesser-Cesar C. Exercise enhances and protects brain functions. Exercise and Sport Sciences Review 2002;30(1):75-9.
14. Boveris A, Navarro A. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. Free Radic Biol Med 2008;44(2):224-9.
15. Dworak M, Diel P, Voss S, Hollmann W, Struder HK. Intense exercise increases adenosine concentrations in rat brain: implications for a homeostatic sleep drive. Neuroscience 2007;150(4):789-95.
16. Navarro A, Gomez C, Cepero-López JM, Boveris A. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol 2004;286(3):R505-R511.
17. Sarbadhikari SN, Saha AK. Moderate exercise and chronic stress produce counteractive effect on different areas of the brain by acting through various transmitter receptor subtypes: a hypothesis. Theor Biol Med Model 2006;23(3):1-18.
18. Valença SS, Castro P, Pimenta WA, Lanzetti M, Silva SV, Barja-Fidalgo C, Koatz, C. Porto VL. Light cigarette smoke-induced emphysema and NFkappaB activation in mouse lung. I J Exp Pathol 2006;87(5):373-81
19. Pellegrin M, Berthelot A, Houdaver C, Gaume V, Laurant P. New insights into the vascular mechanisms underlying the beneficial effect of swimming on the endothelial vasodilatador function in apolipoprotein E-deficient mice. Atheriosclerosis 2007; 190(1):35-42.
20. Lowry OH, Rosebrough NJ, Farr AL. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193(1):265-7.
21. Hughes BP. A method for estimation of serum creatine kinase and its use in comparing creatine kinase and aldolase activity in normal and pathologic sera. Clin Chim Acta 1962;7:597-604.
22. Kovacic P, Cooksy A. Iminium metabolite mechanism for nicotine toxicity and addiction: Oxidative stress and electron transfer. Med Hypotheses 2005;64(1):104-11.
23. Cardellach F, Alonso JR, López S, Casademont J, Miró O. Effect of smoking cessation on mitochondrial respiratory chain function. J Toxicol Clin Toxicol 2003;41(3):223-8.
24. Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant, lipoic acid. Free Radic Biol Med 1996;22(1-2):359-78.
25. Rezvani AH, Levin ED. Cognitive effects of nicotine. Biol Psychiatry 2001;49(3): 258-67.
26. Ambarasi K, Vani G, Balakrishna K, Shyamala D. Creatine kinase isoenzyme patterns upon chronic exposure to cigarette smoke: protective effect of Bacoside A. Vascul Pharmacol 2005;42(2):57-61.
27. Elsayed NM, Bendich A. Dietary antioxidants: potential effects on oxidative products in cigarette smoke. Nutrition Res 2000;2(3):551-67.
28. Navarro A. Mitochondrial enzyme actives as biochemical markers of aging. Mol Aspects Med 2004;25(1-2):37-48.
29. Den Hoed M, Hesselink MK, Van Kranenburg GP, Westerterp KR. Habitual physical activity in daily life correlates positively with markers for mitochondrial capacity. J Appl Physiol 2008;105(2):561-8.
30. Ussher M, Aveyard P, Coleman T, Straus L, West R, Marcus B, et al. Physical activity as an aid to smoking cessation during pregnancy: Two feasibility studies. BMC Public Health 2008;8:328-32.

Address for correspondence
Emilio Luiz Streck
Universidade do Extremo Sul Catarinense – UNESC.
Programa de Pós-Graduação em Ciências da Saúde – PPGCS
Laboratorio de Bioquímica do Exercício
Av Universitária 1106, Bairro Universitário
88802-250. Criciúma, SC.
E-mail: emiliostreck@gmail.com