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Exercício físico agudo e permeabilidade da barreira-hematoencefálica

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RESUMO

INTRODUÇÃO: Exercício físico de grande intensidade é comum em diversos desportos. Hoje em dia, mais do que nunca, a atividade física é reconhecida e aceite como uma estratégia comportamental para melhorar os parâmetros de saúde. Embora o exercício extenuante cause stress oxidativo no músculo e noutros tecidos, não se encontram na literatura certezas sobre se este pode ou não gerar stress oxidativo nas células cerebrais. Por outro lado, substâncias dopantes ganharam grande popularidade em desportos de competição, apesar do facto de poderem ser tóxicas para vários órgãos, incluindo o cérebro. Algumas substâncias, como as anfetaminas, podem facilmente atingir o cérebro atravessando a barreira hemato-encefálica (BHE), e podem também ter um efeito direto na função da BHE. Sabe-se que esta barreira protege o cérebro contra moléculas nocivas e organismos patológicos, e é também responsável por manter a homeostase. A sua disfunção pode ter consequências graves, tais como o dano irreversível de células cerebrais, o que por sua vez pode originar alterações neurológicas e psiquiátricas. Os principais responsáveis pela integridade desta barreira, são as junções intercelulares oclusivas e as junções aderentes, que controlam a via paracelular através da BHE. As junções aderentes são constituídas por proteínas transmembranares, como a claudina e a ocludina, e por proteínas intracelulares, como *zonula occludens.* A claudina-5, a proteína de menor peso molecular (24 kDa), é a principal e primeira responsável pela integridade da BHE, e a ocludina (64 kDa) é responsável por um suporte adicional. Tendo em conta que não há nenhuma informação sólida sobre a relação entre o exercício físico e estas proteínas, o presente trabalho teve como objetivo estudar as alterações destas proteínas durante um episódio de exercício físico agudo.

MÉTODOS: Neste estudo foram utilizados ratos Wistar, machos e jovens adultos com 8 semanas de idade. Metade dos animais foram submetidos a um programa agudo de exercício físico (corrida forçada num tapete rolante durante 35 min, com 20 cm / s de velocidade e com 15º de inclinação - grupo de exercício), e a outra metade não foi submetida ao protocolo de exercício (grupo controlo). Os ratos do grupo experimental foram sacrificados imediatamente após o exercício, e os ratos do grupo controlo foram sacrificados no minuto anterior. Em seguida, as possíveis alterações nos níveis proteicos da ocludina e claudina-5 no córtex frontal, estriado e hipocampo foram analisados pela técnica de *western blot*.

RESULTADOS: Em relação à claudina-5 não se verificaram diferenças significativas entre os grupos controlo e exercício nas três regiões cerebrais estudadas. Nos níveis de ocludina também não existiram diferenças significativas, quer no córtex frontal quer no hipocampo. No entanto, houve uma diminuição significativa dos níveis de ocludina no estriado, no grupo de exercício quando comparado com o grupo controlo.

DISCUSSÃO: Os nossos resultados sugerem que pode haver um aumento da permeabilidade da BHE durante o exercício físico agudo. Isto pode levar à entrada no cérebro de substâncias nocivas, e consequentemente prejudicar a sua função normal, nomeadamente a nível do sistema dopaminérgico estriatal.

PALAVRAS-CHAVE: Barreira hemato-encefálica, Exercício físico agudo, Ocludina, Claudina-5, Junções oclusivas.

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ACUTE PHYSICAL EXERCISE AND BLOOD-BRAIN BARRIER PERMEABILITY

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Abstract

Introduction: Sports with intensive physical activity are performed around the world. Nowadays, more than ever, physical activity is widely accepted as a behavioral strategy to enhance health. Although exhaustive exercise causes oxidative stress in muscle and other tissues, references are conflicting whether or not strenuous exercise could generate oxidative stress in the brain. On the other hand, doping substances have gained a great popularity in competition sports, despite the fact that they can be toxic to the brain. Some drugs, like amphetamines, can easily reach the brain by crossing the blood-brain barrier (BBB) but can also have a direct effect in BBB function. It is known that this barrier protects the brain against hazardous molecules and pathological organisms, and is also responsible for maintaining the homeostasis. Its dysfunction can lead to several and harmful consequences, such as irreversible damage of brain cells, that may originate neurological and psychiatric abnormalities. The intercellular junctions, tight (TJs) and adherens junctions (AJs), are the main responsible for the barrier integrity since they control the paracellular pathway across BBB. TJs are constituted by transmembrane proteins, like claudin and occludin, and by intracellular proteins, like zonula occludens

(ZO) proteins. Claudin-5, the smallest protein (24 kDa), is responsible for the primary seal of the BBB, and occludin (64 kDa) is responsible for additional support. Taking into account that there is no solid information regarding exercise and these TJs proteins, the present work aims to study alterations of these proteins during an acute physical exercise.

Methods: Young adult male Wistar rats (8 weeks old) were used. Half of them were subjected to an acute exercise program (forced running in a treadmill for 35 min, with a 20 cm/s speed and a 15º of inclination - exercise group), and the other half was not subjected to exercise (control group). The rats of the experimental group were sacrificed just after the exercise, being the rats of the control group sacrificed the minute before. Then, the possible alterations in the protein levels of occludin and claudin-5 in frontal cortex, striatum and hippocampus were analysed by western blot.

Results: We concluded that regarding claudin-5 there were no significant differences between exercise and control groups in the three cerebral regions studied. In addition, there were no significant changes of occludin protein levels in the frontal cortex and hippocampus. However, there was a significant decrease of occludin protein levels in the striatum in the exercise group when compared with the control.

Discussion: Our results may suggest an increase of the BBB permeability during the acute physical exercise, which may lead to the entry of harmful substances into the brain and consequently impair the normal brain function, in particular the striatal dopaminergic system.

KEYWORDS: Blood-Brain Barrier, Acute physical exercise, Occludin, Claudin-5, Tight Junctions.

ABBREVIATIONS:

AJs (Adherens Junctions)

BBB (Blood-Brain Barrier)

CNS (Central Nervous System)

CREB (cAMP Response Element-Binding)

DL (Law by Decree)

IL (Interleukin)

ROS (Reactive Oxygen Species)

RT (Room Temperature)

SEM (Standard Error of Mean)

TJs (Tight Junctions)

1. INTRODUCTION

Nowadays, people are practicing a huge variety of sports which involve acute intense physical activity and it is widely accepted that sports offer many benefits, being a strategy to enhance good health and better cognitive performance (Kramer et al., 2006). However, in response to acute physical exercise, cognitive impairments were documented (Aguiar et al., 2010). It is also known that acute intense physical activity can induce oxidative stress imbalance in several tissues (Aguiar et al., 2010).

In competition sports, dopant substances, in a significant number, are sometimes taken by athletes in order to improve performances (Clarkson and Thompson, 1997). These substances can be harmful to the brain. As an example, methanphetamine (METH) is a psychostimulant drug that has a negative impact in brain function. Specifically, METH compromises the capacity of the brain to generate new neurons, and thus decreasing the endogenous brain repair resources (Silva et al., 2010). Moreover, it leads to BBB dysfunction allowing the entry of pathogens into the brain parenchyma and then contributing to brain damage (Silva et al., 2010). Fortunately, humans have barriers to protect their brain from hazardous substances, making a separation between blood and cerebral constituents. Brain barriers are very important to neuronal signaling within the CNS, which requires a stable and optimal extracellular environment. BBB regulates the flux of components between the blood and the brain, while it protects the CNS from chemical insult and damage (Abbott et al., 2010).

The BBB is formed by brain capillary endothelial cells, as well as surrounding pericytes and astrocytic foot processes (Nierwinska et al., 2008). The endothelial cells are connected by a junctional complex formed by tight junctions (TJs) and adherens junctions (AJs) (Ballabh et al., 2004). TJs are primarily responsible for the extremely

low permeability and high electrical resistance of BBB. Continuous strands of TJs are responsible for the almost complete sealing of the paracellular cleft between adjacent lateral endothelial membranes (Gloor et al., 2001). These cell-cell junctional complexes are located in the apical region of lateral plasma membranes and prevent macromolecules from paracellular diffusion driven by their concentration gradient (Gloor et al., 2001).

TJs at the BBB are composed of both transmembrane and intracellular molecules. The transmembrane components include the junctional adhesion molecules, occludin and claudins (a family with 24 members identified so far) (Ballabh et al., 2004), while the intracellular proteins comprise primarily the zonula occludens (ZO) – 1, -2 and -3 proteins (Furuse et al., 1999). The ZO proteins are accessory proteins that link transmembrane proteins of the TJs to the actin cytoskeleton (Wolburg et al., 2002).

Claudins are 24 kDa phosphoproteins with four transmembrane domains. They are the major component of TJs and are localized exclusively at TJs strands. These proteins bind homotypically to claudins on adjacent endothelial cells to form the primary seal of the TJ (Furuse et al., 1999). The carboxy terminal of claudins binds to cytoplasmic proteins including ZO-1, -2, -3 proteins (Furuse et al., 1999). The most described claudins in the brain are claudin-1 and-5 (Wolburg et al., 2002).

Occludin is a 65 kDa phosphoprotein that shows no amino acid sequence similar to the claudins. It has four transmembrane domains, a long COOH-terminal cytoplasmic domain, and a short NH2- terminal cytoplasmic domain. The two extracellular loops of occludin and claudin originating from neighboring cells form the paracellular barrier of TJ (Ballabh et al., 2004). The cytoplasmic domain of occludin is associated with ZO proteins. Occludin is a major transmembrane protein localized at the tight junction and is present at high levels, distributed continuously at cell-cell contacts

in brain endothelial cells. It appears to be a regulatory protein that can alter paracellular permeability in different tissues (Hirase et al., 1997). It acts as an additional support structure linked to the intracellular actin by intracellular proteins.

The BBB can be disrupted in different conditions involving injury and/or inflammation of the CNS, like multiple sclerosis, ischemic stroke, encephalitis, tumors, neurodegenerative diseases, like Parkinson´s and Alzheimer´s diseases, epilepsy and AIDS-related dementia (Martins et al., 2011).

Some recent studies report alterations of the BBB related to physical activity. Astrocyte proliferation in rats is increased after a running exercise, strengthening the neurovascular unit, which may protect BBB function following brain injury (Li et al., 2005). Another report suggests that there is a disruption of the BBB after prolonged exercise in warm environments (Watson et al., 2006) and after forced swimming exercises (Nierwinska et al., 2008). However, all these studies are performed for prolonged moderate exercise. Studies are needed to evaluate the alterations in acute physical exercise. In fact, the impact of the acute physical activity in the BBB integrity is yet unknown.

Thus, the purpose of our study was to investigate the effect of the acute physical activity in the BBB permeability, by analyzing the possible alterations of the claudin-5 and occludin protein levels in different brain regions.

2. EXPERIMENTAL PROCEDURES

2.1.ANIMALS

The experiments were carried out with 8 male Wistar rats (weighing 249–274 gr, with an average of 262gr), aged eight weeks (Charles River Laboratories, Inc, Barcelona, Spain) that were housed under standard 12-h light/dark cycle at room temperature (RT) of 21±1 °C, with ad libitum access to food and water. Two trial groups were formed with four animals each as follows: one experimental group with an acute exercise program and the control group, without an exercise program. Rats of the experimental group were sacrificed (cervical dislocation) right after the end of the exercise program, and the control group was sacrificed the minute before of each experimental rat. All the procedures of our experience were made according to the European Community guidelines for the use of animals in laboratory (86/609/EEC) and also the Portuguese law for the care and use of experimental animal (Law by decree - DL- no. 129/92), minimizing that way the animal suffering and reducing the number of animals used.

2.2.EXERCISE PROGRAM

Running exercises were performed between 11:00 a.m. and 15:00 p.m. In this study the experimental group was submitted to our exercise protocol, while the control group was not submitted to this protocol. For this exercise protocol, we used a treadmill (Panlab, model LE 8706; program SeDaCom 32-Version 1.10), composed by a superior acrylic structure, which divides the rug in two bands (that way we can submit two rats at the same time to the exercise). There is also a grid on the posterior part emitting electric shocks, forcing them to run and to keep the exercise program. The exercise protocol

consists on forcing the experimental group to run until exhaustion (defined as the animal touching the electrified grid at the rear of the treadmill five times in 2 minutes), with a 20cm/s speed, and with a 15º slope. The average exhaustion time was 35 min +/- 5 min. Rats were sacrificed by cervical dislocation immediately after the exercise program and the control rats were sacrificed the minute before each exercise rat.

2.3.WESTERN BLOT ANALYSIS

Right after the exercise program, the animals were sacrificed by cervical dislocation, the brain was removed and the hippocampi, frontal cortex and striata were dissected on an ice-cold surface. The isolated tissues were homogenized in RIPA buffer (150 mM NaCl, 5 mM EGTA, 50 mM Tris, 1% (v/v) Triton, 0.1% SDS and 0.5% sodiumdeoxycholate), supplemented with protease inhibitor cocktail tablets (Roche Applied Sciences, Germany) in the ratio of 1 tablet/10 ml RIPA buffer. The homogenates were centrifuged at 14,000×g for 10 min, collecting the supernatants after this process. The protein concentration was determined by the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Northumberland, UK). Protein samples (25 µg – occludin and 200 µg – claudin-5) were separated by electrophoresis on 8–12% SDS-PAGE, and transferred electrophoretically onto a PVDF membrane (Millipore, Madrid, Spain) after the separation. Membranes were blocked for 1h at RT in blocking solution, PBS containing 0.1% (v/v) Tween-20 (PBST) and 5% (w/v) non-fat dried milk, and incubated with primary antibodies overnight at 4°C as follows: claudin-5 (1:100; Zymed Lab) or occludin (1:250; Zymed Lab). The membranes were washed 3×20 min with PBST, and then were incubated with alkaline phosphatase-conjugated secondary antibodies (1:20,000; Amersham, GE Healthcare Life Science, USA) for 1 h at RT. The proteins were detected using the Enhanced Chemifluorescence (ECF) reagent

(Amersham) on a Storm 860 Gel and Blot Imaging System (Amersham, GE Healthcare Life Science, Buckinghamshire, UK). β-Actin was used as loading control, so the blots were stripped and reprobed with an antibody against this protein (1:2000; Sigma-Aldrich, St Louis, MO, USA). Band intensities were quantified using the ImageQuant 5.0 software.

2.4. STATISTICAL ANALYSIS

Statistics were performed using a Mann-Whitney U-test to detect differences among groups. Results are expressed as mean ± SEM, and the level of $p<0.05$ was accepted as statistically significant.

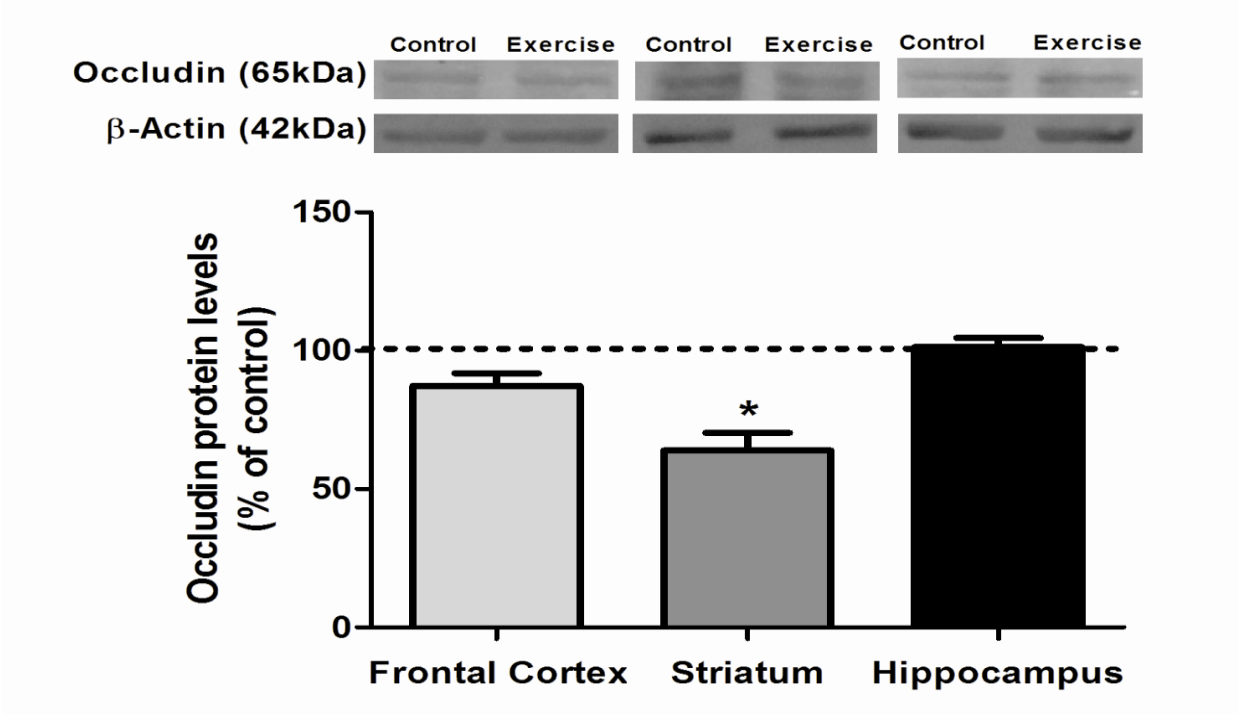
3. RESULTS

The BBB endothelial cells are linked by a junctional complex formed by the tight junctions and adherens junctions; however, it is primarily the TJs that are responsible for the low paracellular permeability and high electrical resistance of the BBB (Silva et al., 2010). In fact, the alteration in the content of TJ proteins, like ZO-1, claudins and occludin, is associated with an impairment of the BBB and to the increase in its permeability (Martins et al., 2011).

The impact of acute physical exercise on BBB function is yet unknown, so we analyzed the protein levels of claudin-5 and occludin in the frontal cortex, striatum and hippocampus. In this study, we observed a significant decrease of the occludin protein levels in the striatum when compared with the control (63.9±6.4% of the control, n=9, p=0.034; Fig. 1B).

Figure 1

A.



B.

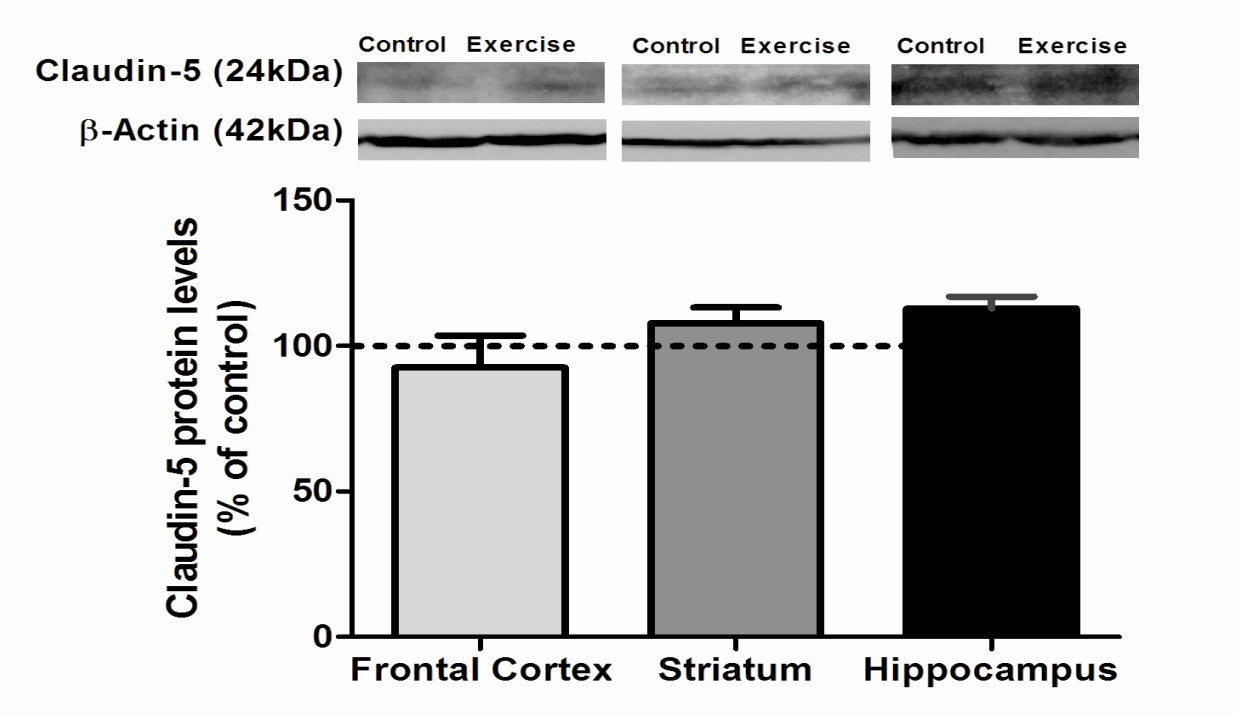


Fig. 1 – Acute physical exercise decreases protein levels of occludin in the rat striatum. Quantification of (A) occludin (65 kDa) and (B) claudin-5 (24 kDa) protein levels in the hippocampus, frontal cortex and striatum. Representative western blots for occludin, claudin-5 and β-actin (42 kDa) are shown above the bars. The results are expressed as mean ± SEM, and the level of $p<0.05$ was accepted as statistically significant, when compared with control.

There were no significant alterations in occludin protein levels in the frontal cortex and hippocampus in the exercise group in comparison with controls (87.37±4.4% of control, n=14, p=0.49; and 101.3±3.3% of the control, n=14, p=0.87; respectively Fig. 1A).

There were also no significant alterations in claudin-5 protein levels as follows: frontal cortex 92.54±11.1% of control, n=12, p=0.76; striatum 107.8±5.4% of control, n=13, p=0.46; hippocampus 112.8±4.1% of control, n=5, p=0.071 (Fig.1B).

4. DISCUSSION

Increase in the BBB permeability, by using the S100β protein, has been observed during exercise (Watson et al, 2006; Nierwinska et al, 2008; Netto et al, 2006. However, S100β protein has now been found to be also released by extracerebral sources that contribute to the increased levels of the protein during physical activity. It might also be released by activated adipocytes (Netto et al., 2006) and by damaged muscle cells (Stocchero et al., 2010). Other studies using other techniques, such as Evans Blue show increased BBB permeability after forced swimming (Sharma et al., 1996).

To investigate if exercise can increase the BBB permeability, and for a better understanding of the mechanisms underlying the increased BBB permeability, we need to investigate potential alterations of TJs protein levels, assuming that the increase of the permeability is caused by BBB disruption, which could be caused by alterations in the structural BBB proteins.

Tight Junctions (TJs), together with the Adherens Junctions (AJ) are the main responsible to keep the BBB integrity (Ballabh P et al., 2004). TJs are constituted by the transmembrane, claudin and occludin proteins, and by the intracellular, zonula occludens (ZO) proteins. Claudin, the smallest protein (24 kDa), is responsible for the primary seal of the TJ (Furuse et al., 1999); occludin (64 kDa), a major transmembrane protein localized in the TJ and present in high levels, is distributed continuously at cell-cell contacts in brain endothelial cells. Occludin appears to be a regulatory protein that can alter paracellular permeability in different tissues (Hirase et al., 1997). Linked to the intracellular actin by ZO-1, -2 and -3 intracellular proteins, it is also responsible for an additional support. Decrease in claudin-5 and occludin protein levels could mean an increase of the BBB permeability and its impairment (Martins et al., 2011). Another

factor which can affect the paracellular barrier formed by TJs in endothelial cells is the integrity of adherens junctions by the removal of extracellular Ca^{2+} (Hirase et al., 1997).

Our results demonstrate no alterations in claudin-5 protein levels in the three cerebral regions measured and no alterations in occludin protein levels of the hippocampus and frontal cortex, suggesting no alterations of the BBB integrity. However, we demonstrated a significant decrease of occludin protein levels of the striatum, suggesting a an increase in BBB permeability in the striatum during acute physical activity.

Striatum is a cerebral region responsible for the control of motor activity. Its activity increases with exercise (McCloskey et al., 2001), and it has also a high dopamine content as the frontal cortex. Deleterious effects can be observed on the striatal dopaminergic system in response to intense exercise (Teixeira et al., 2009). Thus strenuous exercise can lead to enhanced dopamine brain synthesis that may form reactive oxygen species (ROS) by either dopamine metabolism by monoamine oxidase or autoxidation. Oxidative stress can cause DNA and protein damage (Acikgoz et al., 2006). Exercise can also lead to increased serum glucocorticoid levels, which increase the toxicity of oxygen radical generators and may increase the basal level of ROS and alter antioxidant enzyme activities in the brain. However, there are only a few reports showing that acute exercise result in protein oxidation and DNA damage in the brain, and the results are conflicting (Acikgoz et al., 2006). In fact, Liu et al., (2000) demonstrated that acute exhaustive exercise does not cause protein oxidation and DNA damage in the brain, and also showed that no significant changes are found in the activity of glutamine synthetase (GS) in the brain as a result of acute exercise. GS facilitates the clearance of glutamate. The excitatory neurotransmitter glutamate is quickly removed from glutamatergic synapse clefts by uptake into the astrocytes. This

glutamate is then converted by GS to glutamine. GS activity is sensitive to inactivation by oxidative stress and glutathione peroxidase helps to protect GS from inactivation by oxidative stress, (Acikgoz et al., 2006). In fact, in the literature there are some contradictory results. Some authors (Acikgoz et al., 2006; Aksu et al., 2009) showed that acute treadmill exercise performed at different strengths did not cause oxidative stress in prefrontal cortex, striatum and hippocampus cerebral regions. However, another experiment demonstrated that acute exercise significantly increases lipid peroxidation in striatum, but not in the cortex, cerebellum, medulla and hypothalamus (Acikgoz et al., 2006). An increased vulnerability to oxidative damage of the striatum induced by high-intensity exercise is associated with the disruption of implicit memory in mice and accompanied by an alteration of signaling proteins involved in the plasticity of this brain structure (Aguiar et al., 2010). Another experiment, that used chronic intense exercise, verified deleterious effects on the striatal dopaminergic system after intense exercise with the increase in the striatal catalase activity, which can be a compensatory mechanism involved in the detoxification of reactive oxygen species in the dopaminergic brain regions that can be related to a higher consumption of oxygen during chronic intense exercise (Teixeira et al., 2009). Another recent study suggested that intense exercise has the potential to increase BBB permeability without causing structural brain damage subsequent to a free radical-mediated impairment in the dynamic cerebral autoregulation (Bailey et al., 2011).

Other study showed that disruption of BBB results in delayed functional and structural impairments (Tomkins et al., 2007). Moreover, descriptions of spatial memory disruption and object recognition impairment after high levels of running exercise, suggest that antioxidant imbalance and cellular signaling impairment observed with high-intensity trained animals resulted in basal ganglia-dependent cognitive

impairment (Aguiar et al., 2010), namely in the striatum. This could be due to the impairment of the cAMP response element-binding (CREB) phosphorylation within the frontal cortex and striatum after high-intensity exercise. So, a possible interaction between these findings and BBB leakage should be explored.

All these processes can be correlated with our findings, but since there are some contradictory results in the literature, more studies are needed to clarify the possible involvement of the oxidative stress in the BBB leakage, and if this could be the reason for the decrease of occludin protein levels in striatum, increasing that way the BBB permeability.

We also suggest a possible role of cytokines in BBB impairment, due to the increase of cytokines during strenuous exercise. It increased the levels of several pro-inflammatory and anti-inflammatory cytokines, naturally occurring cytokine inhibitors and chemokines. Thus, increased plasma levels of tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1, IL-6, IL-1, TNF receptors, IL-10, IL-8 and macrophage inflammatory protein-1 are found after strenuous exercise (Perdersen, 2000). It is also known that cytokine signaling at the level of the BBB is a crucial feature of the dynamic regulation that can rapidly change BBB function and affect brain health and disease (Pan et al., 2011). Cytokines can cross the BBB and compromise the integrity of the BBB, potentially disrupting the compartmental model of brain calcium homeostasis (Yarlagadda et al., 2009).

So, more studies are needed to clarify the main reason for the dysregulation and decrease of occludin in the striatum. It is important to futher clarify if the decrease of occludin really means an increase of the BBB permeability, despite some reports (Martins et al., 2011) suggesting that alterations in TJs could lead to an increase of BBB permeability. Hirase et al., (1997) demonstrated the possible role of occludin in TJ

permeability and it is also well known that occludin is responsible for sealing of TJs (Wolburg et al., 2002).

The stress induced by forced exercise could be a problem to take into account, but we know that various stress modalities have no repercussions on the BBB permeability (Ovadia et al., 2001), except in diencephalon (specially in the hypothalamus and thalamus) and cerebellum, where acute stress increases BBB permeability through activation of brain mast cells (Esposito et al., 2001). Sharma et al., (1996) also suggested that the response of the BBB varies with age. It is known that the age of the animals, and duration or intensity of the exercise, are important factors in protocols of exercise. The oxidative damage increases in the brain of aged animals. Therefore, it is probable that acute exhaustive exercise may cause oxidative stress in aged rat brains (Acikgoz et al., 2006). So it would be interesting to seek for differences between old and young rats, since our study only included young rats.

These findings about exercise may be important in doping, because a recent study reports that drugs like amphetamine may induce an increase in the BBB permeability in the hippocampal proteins ZO-1, claudin and occludin. (Martins T et al., 2011). Since exercise may also induce a decreased level of occludin in striatum, its effect on BBB permeability may be addictive to amphetamines, or other drugs, making apparent a functional and structural impairment (Tomkins et al., 2007).

Another approach that we can take into account, since our results suggest an increase of the BBB permeability, is the future perspectives for brain pharmacotherapies, because many neurological disorders have been largely refractory to pharmacotherapy. BBB is at the same time the protector of the brain from harmful substances and a huge hurdle to drug pharmacotherapy development (Cecchelli et al., 2007). Some studies documented the implications of drug transport processes at the

BBB (Hermann, 2008), and how BBB can be the physical obstacle that denies most drugs from entering the brain from the blood, blocking therapeutic intervention in many neurological diseases (Karkan et al., 2008). Thus, we can assume that our findings could be a potential target for pharmacotherapy industries, on the way to overtake BBB interface and provide more effective strategies to treat neurological disorders.

In conclusion, in this study we show a decrease of occludin protein levels in the striatum which suggests an increase of the BBB permeability (Martins et al., 2011), this could be correlated with alterations in the ROS equilibrium, although the evidences in literature are not very clear. Therefore, more studies are needed to investigate the reason for this decrease of occludin protein levels in striatum and if this effect could really mean an increase of the BBB permeability. It would be very interesting in to investigate the interactions between these findings and drugs taken by competition athletes, and the impairments associated with the BBB leakage.

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