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THE ANTIDEPRESSIVE EFFECT OF THE PHYSICAL EXERCISE CORRELATES WITH INCREASED LEVELS OF MATURE BDNF, AND proBDNF PROTEOLYTIC CLEAVAGE-RELATED GENES, p11 AND tPA

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Abstract—Clinical studies show an evident antidepressive effect of physical exercise and animal research corroborate such evidence. However, the neurobiological mechanisms underlying the antidepressive effect of exercise are not completely understood. Notwithstanding, it is known that exercise increases brain-derived neurotrophic factor (BDNF) expression in the hippocampus similarly to antidepressant drugs. BDNF is synthesized as a precursor molecule that undergoes a proteolytic cleavage to generate either a mature or a truncated isoform. Precursor and mature BDNF are assumed to elicit opposing biological effects in neuroplasticity. In the present study we investigated the effect of voluntary physical activity on precursor and mature brain-derived neurotrophic factor levels and on proBDNF cleavage related genes, p11 and tissue plasminogen activator (tPA), as well as the antidepressive and cognitive effects of voluntary physical activity. Mice had access to mobile or locked running wheels for 28 days and were submitted to forced-swim, tail suspension and water maze tests. Their hippocampi were dissected and analyzed by Western blot and real time RT-PCR. Voluntary physical activity, but not locked wheel exposure, induced a robust increase in hippocampal mature BDNF protein levels, as well as in p11 and tPA mRNA expression; and also promoted antidepressive effects and improved learning, when compared with sedentary mice. On the other hand, there were no significant differences between any groups in the expression of precursor or truncated isoforms of BDNF. Our data suggest that the antidepressive effect of the physical exercise may depend, at least in part, on changes in BDNF post-translational processing.

Key words: physical exercise, running wheel, brain-derived neurotrophic factor, hippocampus, clinical depression, memory.

Depression is a highly invalidating neuropsychiatric disease with increasing incidence worldwide (Kessler et al., 2005; Blumenthal et al., 2007). Although several antidepressant drugs are currently available, not all individuals with depression respond to treatment and only 50–70% of treated patients have complete remission of symptoms. Moreover, long term treatment is frequently needed with several side effects (Berton and Nestler, 2006; Racagni and Popoli, 2008). These drawbacks justify a great effort for the development of more effective treatments (Berton and Nestler, 2006; Hunsberger et al., 2007; Agid et al., 2007; Krishnan and Nestler, 2008; Mathew et al., 2008; Pittenger and Duman, 2008). In this scenario, it is relevant the investigation of alternative methods for the treatment of depression.

Extensive research with humans suggests that physical exercise has general beneficial effects on the central nervous system (Cotman and Berchtold, 2002). Many authors report the ability of physical exercise to reduce both incidence and severity of human depression (Dunn and Dishman, 1991; Fox, 1999; Paluska and Schwenk, 2000; Lawlor and Hopker, 2001; Brosse et al., 2002). Long-term studies with patients indicate that physical exercise is more efficient in preventing depression relapse than antidepressant medication (Babyak et al., 2000; Strawbridge et al., 2002). Physical exercise reduces depressive symptoms either as the sole therapy, or when combined with other treatments with reduction of side effects (Trivedi et al., 2006). Moreover, physical exercise promotes better cognitive performance in humans (Hillman et al., 2008) and rodents (van Praag et al., 1999a; Molteni et al., 2004; Vaynman et al., 2004, 2007; Ding et al., 2006; Creer et al., 2010). For these reasons, physical exercise is becoming an accepted intervention to reduce stress-related dysfunctions. However, the neurobiological underpinnings of physical exercise-mediated antidepressive effects are not well understood.

Studies performed in animal models of depression demonstrate that physical activity performed in a running wheel elicits physiological effects similar to those induced by antidepressant drugs, including increased neurogenesis in the dentate gyrus of the hippocampus and also increased expression of the neurotrophin brain derived neurotrophic factor (BDNF) and of the transcription modulator cAMP response element binding protein (CREB) (Neeper et al., 1996; Oliff et al., 1998; Russo-Neustadt et al., 1999, 2000; van Praag et al., 1999a,b; Vaynman et al., 2003, 2004; Adlard et al., 2004; Bjornebekk et al., 2005). Of note, most antidepressants drugs require modulation of BDNF expression in the hippocampus to exert their behav-

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Abbreviations: AIIt, annexin II tetramer; BDNF, brain-derived neurotrophic factor; CREB, cAMP response element binding protein; FST, forced swim test; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; matBDNF, mature BDNF; proBDNF, BNDF precursor; RT-PCR, real time reverse transcription polymerase chain reaction; tPA, tissue plasminogen activator; TrkB, tyrosine kinase B; TST, tail suspension test; WB, Western blot; WM, Morris water maze test.

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ioral effect (Duman and Monteggia, 2006) and BDNF has a fundamental role in neuronal plasticity molecular processes which are involved in learning and memory (Duman et al., 2000; Tyler et al., 2002; Calabrese et al., 2009). However, BDNF is also known to promote different effects in different brain areas involved in mood disorders (Krishnan and Nestler, 2008).

BDNF is synthesized as 32 kDa precursor isoform (proBDNF) that generates by proteolytic cleavage a 14 kDa mature isoform (matBDNF) or a truncated isoform of 28 kDa (Mowla et al., 2001; Seidah et al., 1999). According to the "yin-yang neurotrophin hypothesis" matBDNF preferentially binds to tyrosine kinase B (TrkB) receptor triggering an intracellular signaling cascade that promotes cell survival and neuronal plasticity, while proBDNF has high affinity for the p75 neurotrophin receptor, triggering pro-apoptotic and anti-plasticity effects (Lu et al., 2005). Extracellular cleavage of proBDNF is catalyzed by the protease plasmin that is expressed as an inactive zymogen called plasminogen. The plasminogen activation involves another protease, tissue plasminogen activator (tPA). Therefore, tPA converts plasminogen to plasmin which, in turn, cleaves proBDNF, generating matBDNF (Pang et al., 2004; Lu et al., 2005). In addition, the annexin II tetramer (AIIt) interacts on the extracellular surface with both plasminogen and tPA, enhancing the plasmin activation (Kim and Hajjar, 2002). AIIt is a heterotetramer complex that is composed of two p36 subunits, also referred to as annexin II, and two p11 subunits (Kassam et al., 1998a). p11, also called S100A10, is a member of the S100 family of proteins and is found in the cytosol or at the inner surface of the plasma membrane. It is also present on the extracellular surface, where it binds tPA (Svenningsson and Greengard, 2007). It was shown that the p11 subunit is critical for AIIt molecular interaction with plasminogen and stimulates a 300 fold increase of tPA-mediated plasminogen activation (Kassam et al., 1998b).

The role of matBDNF and proBDNF in the expression of depressive behavior and on the response to antidepressant drugs is still unclear (Martinowich et al., 2007) and limited studies addressed the effects of physical exercise on the regulation of BDNF isoforms (Griesbach et al., 2009; Sartori et al., 2009). Moreover, there are no studies investigating the expression of the p11 and tPA, molecules involved in the post-translational processing of BDNF, in exercising animals. In the present study we investigated the effect of voluntary physical activity on precursor and mature BDNF isoforms levels and on proBDNF cleavage related genes, p11 and tPA, as well as the antidepressive and cognitive effects of voluntary physical activity in mice.

EXPERIMENTAL PROCEDURES

Male C57BL/6J mice (20–25 g, 8–9 weeks old) obtained from the Multidisciplinary Center for Biological Investigation (CEMIB) at State University of Campinas (UNICAMP) were housed at 21 °C in a 12 h light/dark cycle (lights on at 7 AM), with access to food and water *ad libitum*. The experimental procedures were approved by the institutional Committee for Ethics in Animal Experimentation at UNICAMP (CEUA/IB-UNICAMP, 2070-1).

Experimental groups and physical activity

Mice were randomly allocated to one of three groups: (1) Sedentary, with no access to a running wheel; (2) Locked, in which mice were exposed to a locked running wheel; and (3) Exercise, with free access to a mobile running wheel. Mice from the Exercise and Locked groups were housed in individual cages ($40\times32\times16 cm^3$) with free access to a mobile or locked running wheel (12 cm diameter; 60 g), respectively. The activity performed by exercising mice on the mobile running wheel was recorded daily for 24 h by an electronic counter connected to a computer for data storage. Mice had a period of 28 consecutive days of voluntary access to the running wheel. Mice from the sedentary group were individually housed in standard cages ($28\times18\times12 cm^3$) for the same experimental period (28 days). The greater dimensions of cages where Exercise and Locked mice were housed were necessary for an adequate setup of running wheels. Each experimental group described above was subdivided in four cohorts and designated for the analysis of: 1) FST ($n=8$) forced swim test; 2) TST ($n=8$) tail suspension test; 3) WM ($n=8$) Morris water maze test; 4) WB/PCR ($n=5$) Western blot analysis of BDNF protein levels and real time RT-PCR for p11 and tPA mRNA. This design was used to avoid interference of one behavioral test into another; moreover, mice exposed to behavioral tests would not allow the distinction of changes in BDNF expression levels mediated by behavioral experiments or voluntary physical activity.

Forced swim test

In order to evaluate depressive behavior, mice from all three groups were submitted to the FST (Porsolt et al., 1977, with modifications) after the experimental period of 28 days above described. In this test, mice were placed in a glass cylinder (25 cm diameter ×65 cm height) filled to a depth of 50 cm with water (25 °C), in a setup that did not allow mice to touch the floor of the glass container and had no escape possibility. A 6-min swim test session was videotaped and the total immobility time during the last 4 min was recorded by a blind observer. Immobility time consisted in the total time mice stood still, not swimming, with minimal movements necessary only to keep the head above water surface, without escape attempts.

Tail suspension test

In order to evaluate depressive behavior, mice from another cohort of all three groups were submitted to the TST (Steru et al., 1985, with modifications) after the experimental period of 28 days above described. Mice were suspended by the tail for 6 min using a tape with 15 cm of length, placed approximately 2 mm from tail tip and attached to a metal support (50 cm height). With this setup mice were kept suspended approximately 20 cm from table top. This protocol, employing a long tape attached in a short distance from mice tail tip, was used to avoid that mice could climb their tail, a common behavior in C57BL/6J mice (Mayorga and Lucki, 2001; Gould et al., 2008a,b). Immobility time was defined as the absence of any movement, with exception of breathing and whisker movement. Tests were video recorded, and the resulting videos were used for immobility time analysis by a blind observer.

Water maze test

Morris water maze test, (Morris, 1984, with modifications) was used to evaluate spatial learning and memory of mice. A third cohort of all three groups was submitted to the water maze test after the experimental period of 28 days above described. For these tests, the mice were allowed to swim freely in a circular pool (120 cm in diameter, 50 cm high) filled with water (26±1 °C) that was made opaque with nontoxic, white paint. A movable, transparent circular plastic platform 9 cm in diameter, mounted on a