Neuroscience 271 (2014) 56–63

EFFECTS OF EXERCISE ON MITOCHONDRIAL FUNCTION, NEUROPLASTICITY AND ANXIO-DEPRESSIVE BEHAVIOR OF MICE

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Abstract—The present study was aimed at analyzing the effects of physical exercise on mitochondrial physiology, anxio-depressive-like behaviors and neuroplasticity in mice. Adult C57BL/6J male mice were isolated in home cages equipped or not with free-running wheels. After 6 weeks of exercise, mice were tested in various behavioral paradigms to evaluate anxiety- and depressive-like behaviors. The hippocampi were dissected for neurochemical assays, including mitochondrial activity, monoamines content and the expression of genes involved in energy metabolism and brain-derived neurotrophic factor (BDNF) regulation. Exercise decreased anxiety-like behaviors in the open field and elevated plus maze, and exerted antidepressant-like effects in the tail suspension test. Exercise stimulated brain mitochondrial activity and increased resistance against rotenone, an inhibitor of complex I activity. Furthermore, mRNA expression of *Bdnf*, *Gdnf*, *Tfam* (mitochondrial transcription factor A), and *Ndufa6* (mitochondrial I subunit) genes, as well as the phosphorylation of cAMP response element-binding protein were increased after exercise. In summary, exercise appears to engage mitochondrial pathways and to potentiate neuroplasticity and might be associated to mood improvement.

Key words: depression, epigenetic, hippocampus, mitochondria, mood, neurotrophin.

INTRODUCTION

Although studies on the pathophysiological mechanisms of depression and anxiety have given birth to several hypothesizes, the etiology of those diseases remains mostly unknown. New evidence suggests the impairment of mitochondrial plasticity as one potential mechanism (Moretti et al., 2003; Rezin et al., 2008; Scaini et al., 2010). Indeed, it has been demonstrated that brain cortical inhibition of mitochondrial complexes I, III and IV activities occurs in mice under chronic stress, and this can be recovered following antidepressant treatments (El Idrissi and Trenkner, 1999; Markham et al., 2004). In humans, post-mortem downregulation of mitochondrial complex I subunits was observed in the brain of patients with major depression (Ben-Shachar and Karry, 2008). These dysregulated mitochondrial pathways might result in less efficient mitochondrial activity and compromised ATP synthesis, which can increase neuronal vulnerability to neurobehavioral changes, including mood disorders.

Depression is characterized by a broad range of dysregulations including those impacting the monoamine systems, mostly serotonin (5-hydroxytryptamine, 5-HT) and noradrenaline (NA) neurotransmission, in addition to those impacting the activity of hypothalamus–pituitary adrenal (HPA) axis (Lanfumey et al., 2008). More recently, hypotheses pointing out the role of brain neuroplasticity and epigenetic regulations in depression have also been recently proposed (Krishnan and Nestler, 2008; Massart et al., 2012). The neurotrophin hypothesis of depression was supported by evidence showing decreased brain-derived neurotrophic factor (BDNF) mRNA levels, in the hippocampus and the frontal cortex in severely depressed patients (Shimizu et al., 2003; Knable et al., 2004). In turn, antidepressant treatments such as selective serotonin reuptake inhibitors (SSRI) and electroconvulsive therapy, as well as physical exercise (Duman, 1998; Laske et al., 2010), have consistently been reported to enhance the expression of neuroplasticity factors and to improve mood.

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Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CREB, cAMP response element-binding protein; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; DHPG, dihydroxyphenylglycol; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; EPM, elevated plus maze; HVA, homovanillic acid; MeCP2, methyl-CpG-binding protein 2; NA, noradrenaline; NADH, nicotinamide adenine dinucleotide; PCR, polymerase chain reaction; PMSF, phenylmethanesulfonylfluoride; SSRI, selective serotonin reuptake inhibitors; TBS, Tris-buffered saline; UCP2, uncoupling protein 2.

http://dx.doi.org/10.1016/j.neuroscience.2014.04.027

Attention has recently been drawn around the effects of exercise on neuroplasticity in the context of depression models (Sigwalt et al., 2011; Cunha et al., 2013). In particular, several reports have associated exercise-induced antidepressant effects to the activation of neuroplasticity signaling factors such as BNDF and VGF, which themselves display antidepressant-like effects in rodents (Hunsberger et al., 2007; Laske et al., 2010; Sigwalt et al., 2011). Furthermore, these neurotrophic changes were associated with increased brain mitochondrial activity in exercised animals (Vaynman et al., 2006; Aguiar et al., 2007). Exercise also appears to upregulate several mitochondrial genes in the mouse hippocampus, including mitochondrial ribosomal proteins and the electron-transferring flavoprotein (Stranahan et al., 2010), creatine kinase and the uncoupling protein 2 (UCP2) (Gomez-Pinilla et al., 2008). Synaptogenesis, a neuroplasticity effect also triggered by exercise, seems dependent on UCP2 (Dietrich et al., 2008), a molecular sensor and suppressor of mitochondrial reactive oxygen species. Moreover, the relationship between mitochondria and BDNF was strengthened by data showing that expression of BDNF is critical for exercise-induced neuroprotection against mitochondrial inhibition (Gerecke et al., 2012).

Exercise might engage antidepressant-like epigenetic mechanisms, which would upregulate BDNF gene expression. Antidepressant compounds have indeed been shown to regulate chromatin remodeling and the molecular machinery associated with this process (Newton and Duman, 2006). SSRIs could modulate transcription factors, such as the methyl-CpG-binding protein 2 (MeCP2) (Cassel et al., 2006; Wang et al., 2011), and reduce DNA methylation (Melas et al., 2012) in murine models of depression. Exercise could also reduce both histone deacetylase 5 (HDAC5; (Tsankova et al., 2006), and DNA methylation of the exon IV region of the BDNF promoter, and increase phosphorylation of MeCP2 (Gomez-Pinilla et al., 2011), leading to a transcriptional activation of the *Bdnf* gene within the hippocampus. To deepen our understanding about the relationship between exercise-induced mitochondrial changes and antidepressant-like effects, we investigated mitochondrial activity and expression of neurotrophins and epigenetic factors in the hippocampus of mice, in conjunction with anxiety- and depression-related behaviors.

EXPERIMENTAL PROCEDURES

Animals and exercise

C57BL/6 mice (male, 8–10 weeks, 20–25-g; Charles River facilities, France) were housed under controlled environment (12-h light–dark cycle, lights on at 7:00 h, humidity 60%, room temperature 21 ± 1 °C) with *ad libitum* access to food and water. Mice were housed in individual cages (27 × 18 × 13 cm) equipped with running wheels (RW, 4½″, Super Pet, Chicago, IL, USA) to stimulate voluntary exercise during 6 weeks ($N = 16$) (Aguiar et al., 2013). Control animals had access to a "locked" RW version (not spinning) ($N = 16$). All animal studies were approved by local ethics committee: Comissão de Ética no Uso de Animais (Universidade Federal de Santa Catarina – UFSC, Florianópolis, Brazil) and Comités d'Ethique appliquée à l'animal de laboratoire (Institut National de la Santé et de la Recherche Médicale – INSERM, Paris, France).

Behavioral tasks

Habituation to the experimental conditions and behavioral tasks were conducted in a sound attenuated and 12-lux dim lit room during the light phase of the mouse circadian cycle (10:00–17:00). All experiments were video recorded for an off-line rating using the ANY-maze video-tracking system (Stoelting Co., Wood Dale, IL, USA).

Elevated plus maze (EPM)

The EPM is a 50-cm high cross-shaped apparatus having two open arms and two closed arms (5-cm edge) connected by a central open square. At the beginning of experiments, animals were placed in the central square and allowed to explore the EPM for 5-min (Burghardt et al., 2004). Measures of anxiety include percentage $\left(\frac{\text{open}}{\text{open+closed}} \times 100\right)$ of time spent in the open arm, the number of entries, and the locomotor performance (number of closed arm entries and total distance traveled in the EPM).

Open field

The free exploration of a circular open field (160-cm diameter, 50-cm height) was analyzed for 15-min. The apparatus is further divided into three virtual concentric circles (30-, 60- and 160-cm in diameter). The behaviors examined included the distance (m) and the time (s) of exploration of the center and the periphery.

Tail suspension test (TST)

The tail of the mice was set in a high bar (1.2-cm diameter, 30-cm elevation) for 6-min to assess immobility (Steru et al., 1985). Immobility parameters examined included the latency for the first episode, number of episodes, and time spent in immobility.

Neurochemical assays

After behavioral experiments, mice were killed by cervical dislocation; the *quadriceps femoris* muscle and hippocampi were then dissected. Tissues were freshly processed according to the procedures described below.

Quantitative reverse transcription-polymerase chain reaction (RT-qPCR)

Total RNA was prepared from dissected, snap-frozen hippocampi and cDNA was generated by reverse transcription. Primers for the *Bdnf, Gdnf, Tfam, Ndufa6, Arc*, *MeCP2, His2Av, Neurog1, Neurog2* and *Neurod1* isoforms and for housekeeping *Gapdh* were designed using Primer-BLAST tool (2.2.27, National Institute of Health, Bethesda, MD, USA). Primers are described in