Basic Science

Chronic treadmill running does not enhance mitochondrial oxidative capacity in the cortex or striatum

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ABSTRACT

Objective. The aims of the present study were to determine in healthy animals if 1) acute exercise stimulated traditional exercise signaling pathways in the cortex and striatum, and 2) if chronic exercise training increased the oxidative capacity of these brain regions.

Methods. Male C57BL/6 mice were left sedentary, acutely exercised for 15 or 60 min to examine potential signaling cascades activated by exercise, or chronically exercise for 4 wk to examine the impact of prolonged training. The cortex and striatum were analyzed for changes in the phosphorylation of AMPK, CAMKII, ERK1/2, and P38 with acute exercise, or markers of mitochondrial protein content, mtDNA copy number, and mitochondrial respiration with chronic exercise.

Results. In mice, acute treadmill running did not alter the phosphorylation of AMPK, CAMKII, or P38 in either the cortex or the striatum, but decreased ERK1/2 phosphorylation in only the cortex for the duration of the exercise bout. Following chronic exercise training, mitochondrial respiration, mtDNA copy number, and protein content of various subunits of the electron transport chain were not altered in adult mice.

Conclusion. Combined, these data suggest that exercise does not result in increased phosphorylation of traditional signaling kinases or enhanced mitochondrial oxidative capacity in either the cortex or the striatum of healthy animals.

1. Introduction

Acute exercise is well known to cause metabolic perturbations to contracting skeletal muscle, which with repeated exercise-training enhances mitochondrial protein content, mitochondrial DNA (mtDNA) abundance, and oxidative phosphorylation [1–3].

Where chronic exercise is known to induce these adaptations in skeletal muscle, the effect of exercise training in the brain has received far less attention. Previous work has shown that exercise creates a metabolic perturbation in the brain as evidenced through reductions in brain glycogen concentrations following acute training [4], and increased mitochondrial activity

has been reported in neurogenically active regions of the brain [5]. However, adaptations in brain mitochondrial content as a result of chronic exercise are limited to observations of increased mtDNA and mRNA abundance [6], and therefore the extent to which mitochondrial content is enhanced with exercise remains largely unknown.

Within skeletal muscle, mitochondrial biogenesis is induced through various cellular stresses repeatedly activating one or several key signaling kinases such as AMPK (AMP-activated protein kinase), ERK1/2 (extracellular signal regulated kinase 1/2), CAMKII (calcium/calmodulin-dependent protein kinase 2), and stress-activated protein kinase P38 [7]. These events have been implicated in the stimulation of PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha), which coordinates the transcription of nuclear and mitochondrial genomes and stimulates the replication of mtDNA [8–10]. While roles for AMPK, ERK1/2, CAMKII, and P38 have been identified in the regulation of many processes in brain homeostasis [11–13], the ability of exercise to activate these signaling molecules in the brain has not been examined. Given the different biological roles of neuronal and contractile tissues, it is unlikely that signaling experiments conducted in exercising muscle can be directly transferred to the brain, particularly as these kinases appear to possess unique roles in neuronal tissue. For instance, AMPK has been shown to regulate axon formation during metabolic stress [14] and AMPK over-activation during key growth phases inhibits neuronal polarization [15]. Further, ERK1/2 demonstrates a nucleus-oriented migration away from brain mitochondria with maturity [16], suggesting that regulatory kinases may undergo functional remodeling with aging. Therefore, although it has been presumed that exercise activates signaling pathways in a similar manner in both the brain and skeletal muscle to result in enhanced mitochondrial content [17–19], investigations supporting these mechanisms have not yet been performed.

Therefore, the current study aimed to investigate the influence of exercise training in healthy animals on mitochondrial oxidative capacity in two key brain regions that regulate motor movement, the cortex and striatum. It was hypothesized that exercise would provoke similar responses in the brain as previously observed in skeletal muscle. Specifically, acute exercise was hypothesized to activate diverse signaling cascades (e.g. AMPK, ERK1/2, CAMKII and P38), such that chronic exercise training would increase the oxidative capacity of the cortex and striatum as a result of the accumulation of mitochondrial proteins (i.e. mitochondrial biogenesis). While typical responses were observed within the muscle, chronic exercise did not increase the oxidative capacity of the cortex or striatum, suggesting the absence of mitochondrial biogenesis within these brain regions.

2. Methods

2.1. Animals and exercise protocols

C57BL/6 mice were bred on site and housed on a 12 h:12 h light:dark cycle at the University of Guelph. All experimental protocols were approved by the University of Guelph Animal Care Committee. Initial experiments were performed in mice at 12 wk of age and follow-up experiments investigating age-related effects of chronic exercise were conducted in mice at 7 wk of age. For experiments with acute exercise, mice were left sedentary (n = 6), were exercised for 15 min (n = 6), or exercised for 60 min (15 m/min, 10% incline) (n = 6) before euthanasia to examine early and prolonged effects of acute exercise. When examining prolonged exercise training, mice were randomly allocated to either a sedentary group (n = 6) or a 4 wk exercise training group (n = 6). Exercising mice were familiarized on three occasions for 10 min (15 m/min, 10% incline) separated by a day each, and exercise training occurred five days per week for 60 min (wk 1: 20 m/min, 15% incline; wk 2: 22 m/min, 20% incline; wk 3: 23 m/min, 20% incline; wk 4: 25 m/min, 20% incline) [20]. Samples from the cortex, striatum, and vastus lateralis muscle were taken 48-hours following the final exercise bout. Isoflurane (3%) was used to rapidly anesthetize animals at all time points. Brain and muscle samples were removed, rapidly dissected, and flash-frozen in liquid nitrogen for further analysis, or used immediately for analysis of mitochondrial respiration.

2.2. High-resolution respirometry

Analysis of mitochondrial respiration was performed as previously described for skeletal muscle [21,22] and brain [23]. Following rapid removal from the animal, muscle samples were separated into bundles in ice-cold BIOPS (50 mmol/L MES, 7.23 mmol/L K_2EGTA, 2.77 mmol/L CaK$_2$-EGTA, 20 mmol/L imidazole, 0.5 mmol/L DTT, 20 mmol/L taurine, 5.77 mmol/L ATP, 15 mmol/L PCr, and 6.56 mmol/L MgCL$_2$.H$_2$O; pH 7.1), permeabilized with 40 μg/mL saponin for 30 min, and then washed for 15 min in MiR05 respiration buffer (0.5 mmol/L EGTA, 10 mmol/L HH$_2$PO$_4$, 110 mmol/L sucrose, and 1 mg/ml fatty acid free BSA; pH 7.1). Fiber bundles were then added to an Oxygraph-2K respirometer (Oroborus Instruments, Austria) in the presence of 25 μmol/L blebbistatin. Brain samples (~ 2 mg) were quickly weighed and placed in the respirometer and were given 5–10 min for equilibration before the addition of 50 μg/mL saponin. All samples were analyzed in MiR05 respiration medium at 37 °C with constant stirring at 750 rpm. When examining pyruvate-stimulated respiration in the brain, 10 mmol/L pyruvate and 5 mmol/L malate were added, followed by 5 mmol/L ADP, 10 mmol/L glutamate, and 10 mmol/L succinate, in succession.

2.3. Immunoblotting

Analysis of protein content by Western blotting was performed in the cortex, striatum, and vastus lateralis under conditions previously described [21]. Brain and muscle samples were loaded with 20 μg and 15 μg of protein, respectively, and antibodies for phosphorylated and total ERK1/2, AMPK, CAMKII, and P38 (Cell Signaling, diluted 1:1000), as well as complex I NDUFB8 and complex IV COXI using MitoProfile Total oxidative phosphorylation (OXPHOS) rodent antibody cocktail (Mitosciences, diluted 1:500), complex IV COXIV (Invitrogen, diluted 1:1000), PDH-E1α