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Blunted response of hippocampal AMPK associated with reduced neurogenesis in older versus younger mice

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ABSTRACT

The rate of hippocampal neurogenesis declines with aging. This is partly explained by decreased neural responsiveness to various cues stimulating metabolism. AMP-activated protein kinase (AMPK), a pivotal enzyme regulating energy homeostasis in response to metabolic demands, showed the diminished sensitivity in peripheral tissues during aging. AMPK is also known to be involved in neurogenesis. We aimed to see whether AMPK reactivity is also blunted in the aged hippocampus, and thus is associated with aging-related change in neurogenesis. Following subchronic (7 days) intraperitoneal and acute intracerebroventricular (i.c.v.) administration of either 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR; AMPK activator) or saline (sham) to young (16-week-old) and old (72-week-old) mice, we measured changes in AMPK activity, brain-derived neurotrophic factor (BDNF) expression or neurogenesis in the hippocampus. AICAR-induced changes in AMPK activity were observed in the hippocampus of young mice after acute i.c.v. injection. However, neither subchronic nor acute treatment induced significant changes in AMPK activity in old mice. Intriguingly, directions of AICAR-induced changes in AMPK activity were opposite between the hippocampus (decrease) and skeletal muscle (increase). ATP levels were inversely correlated with hippocampal AMPK activity, suggesting that the higher energy levels achieved by AICAR treatment might deactivate neuronal AMPK in young mice. The blunted response of AMPK to AICAR in old age was also indicated by the observations that the levels of neurogenesis and BDNF expression were significantly changed only in young mice upon AICAR treatment. Our findings suggest that the blunted response of neuronal AMPK in old age might be responsible for aging-associated decline in neurogenesis. Therefore, in addition to activation of AMPK, recovering its sensitivity may be necessary to enhance hippocampal neurogenesis in old age.

1. Introduction

Hippocampal neurogenesis occurs throughout the life span. However, its rate declines with increasing age (Kuhn et al., 1996). The aging-associated reduction in adult neurogenesis is partly explained by decline in responsiveness of neural stem cells to various environmental cues such as metabolic stimulation by several growth factors (Adlard et al., 2005; Hayashi et al., 2001; Kuhn et al., 1996; Lichtenwalner et al., 2001; Rafalski and Brunet, 2011). Recently, dysfunctional energy metabolism has emerged as a key factor for reduced adult neurogenesis (Rafalski and Brunet, 2011). For example, studies have shown that several conditions causing resistance to metabolic signaling, such as high-fat diet, obesity or type 2 diabetes mellitus, lead to impaired neurogenesis (Beauquis et al., 2006; Lindqvist et al., 2006; Lopresti

Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, adenosine monophosphate activated protein kinase; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo-2-deoxyuridine; BSA, bovine serum albumin; DAPI, 4′,6-diamidino-2-phenylindole; DCX, doublecortin; DG, dentate gyrus; i.c.v., intracerebroventricular or intracerebroventricularly; i.p., intraperitoneal or intraperitoneally; MOM, mouse on mouse; PBS, phosphate-buffered saline; PFA, paraformaldehyde; RT, room temperature; SE, standard error.

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and Drummond, 2013; Tozuka et al., 2009), while improving energy metabolism by exercise or calorie restriction contributes to enhanced neurogenesis (Lee et al., 2002; Van Praag et al., 2005). In this regard, aging-related decline in neural responsiveness to metabolic stimuli might be associated with diminished neurogenesis in old age (Kapogiannis and Mattson, 2011; Kuhn et al., 1996).

AMP-activated protein kinase (AMPK) is a pivotal enzyme regulating energy metabolism (Kahn et al., 2005; Schimmack et al., 2006). This enzyme senses the AMP:ATP ratio in cells to maintain energy homeostasis. When the AMP:ATP ratio increases (i.e., there is an energy deficiency), AMPK is activated to enhance cellular catabolism, which results in a net increase in ATP levels (Hardie et al., 2012). Conversely, when AMP:ATP ratio decreases (ATP abundance), AMPK is deactivated, which implies sufficient cellular energy. As expected, altered functionality of AMPK has been associated with multiple metabolic disorders, such as obesity, type 2 diabetes, and cardiovascular disorders (Lage et al., 2008; Steinberg and Kemp, 2009; Winder and Hardie, 1999).

Consistent with such a pivotal role in energy homeostasis, neuronal AMPK is significantly involved in neurogenesis (Dagon et al., 2005; Spasić et al., 2009). Previous studies have shown that activation of neuronal AMPK by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR; an AMPK activator) enhances neurogenesis as well as cognitive function in rodents (Dagon et al., 2005; Kobilo et al., 2011; Yau et al., 2014). Also, neuronal AMPK is among key signaling pathways to expression of brain-derived neurotrophic factor (BDNF), an essential mediator for neurogenesis (Kim and Leem, 2016; Liu et al., 2014).

Interestingly, it has been found that, in peripheral tissues, responsiveness of AMPK to various stimuli is attenuated in old age (Li et al., 2012; Mulligan et al., 2005; Reznick et al., 2007). For example, activation of muscular AMPK in response to AICAR or exercise was blunted in old mice in contrast with its successful activation leading to increased mitochondrial biogenesis in young mice (Reznick et al., 2007). Consistently, AMPKα2 activity measured upon exercise training was lower in old than young individuals (Li et al., 2012).

The brain is the most vulnerable organ to energy stress. If responsiveness of neuronal AMPK to various cognitive and metabolic demands is also reduced by aging, such alteration would be manifested as reduced neurogenesis, as a result of bioenergetic failure in the brain tissue. A previous study showed that a genetic polymorphism of AMPK was associated with cognitive impairment in the community-dwelling elderly (Kim et al., 2012). However, to our knowledge, few experimental studies have directly shown whether neuronal AMPK reactivity is indeed attenuated in old age and is associated with levels of neurogenesis.

Therefore, we aimed to examine the effect of aging on the sensitivity of neuronal AMPK in responding to its activator, AICAR, and its association with neurogenesis in younger versus older mice.

2. Materials and methods

2.1. Animals and drug treatment

Young (16-week-old) and old (72-week-old) male C57BL/6 mice (Animal Facility of Aging Science, Kwangju) were used in this study and housed under a 12-h light/dark cycle with food and water given ad libitum. All procedures for animal experiments were approved by the Committee for the Care and Use of Laboratory Animals at Yonsei University Health System and performed according to the National Institute of Health guidelines for the Care and Use of Laboratory Animals.

AICAR (sc-200659, Santa Cruz Biotechnology, Santa Cruz, CA) was dissolved in saline. For subchronic experiments, mice were injected intraperitoneally (i.p.) with a dose of 125 mg/kg/day AICAR or saline vehicle, for 7 days. Injections were performed from 10:00 am to 12:00 pm. Mice were sacrificed on the 7th day, about 5 h after last drug treatment. The number of mice in the first subchronic experiment was 6 per group, and additional 4 mice per group were used in the second experiment for analysis of muscle. For the results of neuronal tissues, data from a total of 10 mice per group were pooled to be analyzed since the direction of changes in neuronal AMPK activity upon AICAR treatment in young mice were the same in both the first (n = 6 per group) and second (n = 4 per group) cohorts. For acute experiments, AICAR (25 nM) or saline was administered intracerebroventricularly (i.c.v.) at a rate of 0.2 μL/min by stereotaxic injection using a Hamilton syringe at the coordinates of 0.2 mm posterior, 1.0 mm lateral, and 3.1 mm deep from bregma. Surgery was performed under isoflurane anesthesia (2.0% isoflurane in 30% oxygen and 70% nitrous oxide using a facemask). Brains were extracted about 3 h after i.c.v. treatment. The number of mice in acute experiment was 3 per group.

2.2. Western blot analysis

For Western blot analysis, hippocampi and right anterior quadriceps muscle were immediately dissected and prepared after sacrifice of animals. Hippocampal and muscle tissues were homogenized in lysis buffer consisting of: 20 mM Hepes (pH 7.0), 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 10 mM KCl, 1.5 mM $MgCl_2$, 250 mM sucrose, and cocktails of phosphatase inhibitors and protease inhibitors. Homogenates were centrifuged at 8000 ×*g* for 30 min. Equal amounts of each protein sample were resolved on 8% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Millipore, Bedford, MA). After blocking, membranes were probed overnight at 4 °C with the following antibodies: anti-Thr^{172}-phosphorylated AMPKα (p-AMPK; 1:1000, 2531s, Cell Signaling Technology, Beverly, MA), anti-AMPKα (1:1000, 2603s, Cell Signaling Technology). Membranes were washed and incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:5000, 7074s, Cell Signaling Technology) for 1 h at room temperature (RT), followed by enhanced chemiluminescence detection (ECL plus; Amersham Biosciences, Piscataway, NJ). Measurement of signal intensity was done by Multi Gauge 3.0 analysis software (FUJIFILM, Tokyo, Japan). Signal intensity of p-AMPK was normalized by that of total AMPK, and the absolute value of p-AMPK/AMPK ratio was converted to relative values by the reference value (p-AMPK/AMPK set as 1 from a randomly selected, young control mouse).

2.3. ATP assay

ATP assays were performed once with hippocampi, which were dissected 3 h after i.c.v. drug treatment. Fresh hippocampal tissues were homogenized with sterilized ice-cold phosphate-buffered saline (PBS). Homogenates were centrifuged for 30 s and supernatants were transferred into new tubes. The supernatant (10 μL) was analyzed for ATP levels using the colorimetric ATP assay kit (BioVision, Mountain View, CA). The remaining supernatant was quantified for protein analysis. Concentration of ATP was presented as amount of ATP per milligram of protein.

2.4. Assessment of neurogenesis

For analysis of neurogenesis in vivo, 5-bromo-2-deoxyuridine (BrdU; B5002, Sigma-Aldrich Co., St. Louis, MO) was injected i.p. at a dose of 100 mg/kg/day for 7 consecutive days. Mice were anesthetized and transcardially perfused with 4% paraformaldehyde (PFA). The brains were removed and post-fixed overnight in 4% PFA followed by equilibration in 15% and 30% sucrose for 24 h each. Tissues were then quick-frozen in optimal cutting temperature compound (Sakura Finetek, Zoeterwoude, the Netherlands) and cut coronally into 30 μm sections through the anteroposterior extension of the hippocampi using a cryostat. Every sixth section from each brain was mounted onto gelatin-coated slides and processed for immunohistochemistry. We pretreated sections for permeabilization (with 0.3% Triton-X-100 in PBS at RT for 1 h), and quenching (with 3% H_2O_2 in methanol at RT for 40 min). For BrdU staining, we performed