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Research report

Aging process alters hippocampal and cortical secretase activities of Wistar rats

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HIGHLIGHTS

- Aged brains have an imbalance between amyloidogenic and non-amyloidogenic pathways.
- Lower cortical TACE activity was linked to aging-induced aversive memory impairment.
- Treadmill exercise was unable to alter hippocampal and cortical secretase activities.

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ABSTRACT

A growing body of evidence has demonstrated amyloid plaques in aged brain; however, little attention has been given to amyloid precursor protein (APP) processing machinery during the healthy aging process. The amyloidogenic and non-amyloidogenic pathways, represented respectively by β- and α-secretases (BACE and TACE), are responsible for APP cleavage. Our working hypothesis is that the normal aging process could imbalance amyloidogenic and non-amyloidogenic pathways specifically BACE and TACE activities. Besides, although it has been showed that exercise can modulate secretase activities in Alzheimer Disease models the relationship between exercise effects and APP processing during healthy aging process is rarely studied. Our aim was to investigate the aging process and the exercise effects on cortical and hippocampal BACE and TACE activities and aversive memory performance. Young adult and aged Wistar rats were subjected to an exercise protocol (20 min/day for 2 weeks) and to inhibitory avoidance task. Biochemical parameters were evaluated 1 h and 18 h after the last exercise session in order to verify transitory and delayed exercise effects. Aged rats exhibited impaired aversive memory and diminished cortical TACE activity. Moreover, an imbalance between TACE and BACE activities in favor of BACE activity was observed in aged brain. Moderate treadmill exercise was unable to alter secretase activities in any brain areas or time points evaluated. Our results suggest that aging-related aversive memory decline is partly linked to decreased cortical TACE activity. Additionally, an imbalance between secretase activities can be related to the higher vulnerability to neurodegenerative diseases induced by aging.

1. Introduction

Aging process and age-related diseases are usually accompanied by cognitive decline and structural alterations in brain areas such as cortex and hippocampus [1–3]. Substantial data have shown that β-amyloid (Aβ) brain deposition, a 39 to 43 amino acid peptides derived from amyloid precursor protein (APP) is implicated in both Alzheimer disease (AD) and normal aging process [4–6]. Although

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amyloid plaques have been found in aged human brain [7,8] little attention has been given to APP processing machinery during the healthy aging process.

APP can be proteolytically cleaved by amyloidogenic or non-amyloidogenic pathways. The amyloidogenic pathway comprises the sequential APP cleavages by β-secretase and γ-secretase leading to Aβ production [9,10]; β-secretase activity is represented by BACE (β-site APP-cleaving enzyme-1) [11]. There are paradoxical findings about the relationship between Aβ and cognitive performance since Aβ has demonstrated a dual neuromodulatory effect, which can improve or impair memory performance [12–15]. Moreover, recent in vivo imaging studies have failed to find any correlations between cognitive performance and amyloid deposition [16]. Taken together, we can suggest that in fact APP processing machinery rather than the Aβ levels is related to cognitive performance during the aging process.

The non-amyloidogenic APP processing pathway is represented by α–secretases. The members of a Disintegrin and Metalloprotease (ADAM) family, such as tumor necrosis factor (TNF)-α converting enzyme (TACE or ADAM17) and ADAM10 have α–secretase activity and generate α-APPs that play a role in synaptic plasticity, neuroprotection and synaptogenesis [17,18]. Although sAPPα levels are reduced in cerebrospinal fluid (CSF) of 23-month-old rats [19], to the best of our knowledge, there are no studies evaluating the non-amyloidogenic pathway role such as TACE activity during normal brain aging process. However, increased TACE activity and consequently higher sAPPα levels may be related to neurotrophic effects as synaptic formation and cognitive improvement [17,20].

A significant body of clinical and experimental evidences has indicated that regular physical exercise improves cognitive functions during normal aging and age-associated disorders [21–24]. The effects of forced and voluntary exercise protocols on Aβ-associated neuropathology and APP pathways processing have been investigated [25–29]. Recently, Zhao et al. [30] demonstrated that forced treadmill exercise ameliorates the spatial memory impairment accompanied by alterations in hippocampal plaques in APP/PS1 mice. In accordance, this transgenic model subjected to a voluntary exercise had improved memory, lower Aβ aggregation and BACE activity in hippocampus and cortex [31]. Despite these findings demonstrating that exercise can modulate secretase activities in AD models little is known regarding the relationship between APP processing and exercise effects during the healthy aging process.

Previously, we demonstrated that the treadmill exercise protocol used here (20 min/day during 2 weeks) was able to reduce in vitro ischemic damage in hippocampal slices of Wistar rats [32]. Besides, this forced exercise protocol transiently improved the inhibitory avoidance aversive memory performance in aged and young rats [33,34]. Recently, it has been described the impact of this neuroprotective protocol on inflammatory and epigenetic marks in hippocampus and frontal cortex of rats [33–37].

Our working hypothesis is that the normal aging process could imbalance amyloidogenic and non-amyloidogenic pathways, specifically BACE and TACE activities. Moreover, we expect that our exercise protocol would be able to alter aging-induced impairments. Taken together, the aim of this study was to evaluate the effects of aging process and a daily running exercise protocol on hippocampal and cortical BACE and TACE activities in Wistar rats.

2. Methods

2.1. Animals

Male Wistar rats of different ages, 3-month-old (n = 38), 21-month-old (n = 27) and 26-month-old (n = 24) were used. The animals were provided by the Centro de Reprodução Animal de Laboratório (CREAL) and maintained under standard conditions (12-h light/dark, 22 ± 2 °C) with food and water *ad libitum*. All animal procedures and experimental conditions were approved by the Local Ethics Committee (CEUA – Comissão de Ética no Uso de Animais – UFRGS; nr.23464). Their ages were chosen based on previous studies by Takahashi et al. [10] describing increased Aβ1-42 levels in brains of 26-month-old healthy rats. Additionally, work performed by our group demonstrated that this exercise protocol improves the cognitive performance of young adult and 20–21-month-old rats [33,34].

2.2. Treadmill exercise protocol

The animals were divided in sedentary (SED) or exercised groups (EXE). The aged groups, 21 and 26-month-old animals, had 12–14 rats per group; since we had control animals for each set of experiments and because the youngest animals (3-month-old) were taken as a control group an increased number per group was examined (n = 18–20). Exercise training consisted of running sessions on a motorized rodent treadmill (AVS Projetos, São Paulo, Brazil) using a moderate daily treadmill protocol (20 min running session each day for 2 weeks). All animals ran at 60% of their maximal oxygen uptake (VO_{2max}), which was measured indirectly prior to training [34,36]. Animals in the SED group were daily placed on the treadmill for 5 min without any stimulus to run. Gentle tapping on their back encouraged rats that initially refused to run. Neither electric shock nor physical prodding was used in this study, and the treadmill exercise was performed between 2:00 and 5:00 PM

2.3. Inhibitory avoidance test

Single-trial step-down inhibitory avoidance conditioning was used as an established model of fear-motivated memory where the animals learned to associate a location in the training apparatus (a grid floor) with an aversive stimulus (foot shock). In the training session, the rats received a 0.6 mA foot shock for 3.0 s. The test session was performed 24 h after a single training session to evaluate long-term memory; no foot shock was delivered, and the step-down latency (maximum 180 s) was used as memory retention [38]. The behavioral test was conducted 30 min after the last exercise training and 30 min prior to euthanasia. Previously, we described a transitory treadmill exercise effects on aversive memory [33,34]. After behavioral test rats were randomly subdivided into 1 and 18 h groups in order to verify transitory and delayed exercise effects on biochemical outcomes (Fig. 1).

2.4. Sample preparation

Rats were decapitated 1 and 18 h after the last exercise training session, consequently 30 min or 17.5 h respectively, after behavioral measurement (Fig. 1). It is important to cite that 1 h after exercise was performed in the afternoon while 18 h was taken early in the morning. Each exercised group had its sedentary control group. The hippocampi and prefrontal cortices were quickly dissected, immediately snap-frozen in liquid nitrogen and stored at −80 °C.

2.5. β-secretase (BACE) activity

BACE activity was evaluated using a commercially available kit (Abcam, catalog number ab65357) according to the manufacturer's instructions. The hippocampus and prefrontal cortices (n = 5–9) were homogenized, incubated on ice for 10 min and centrifuged 10.000 × *g* for 5 min at 4 °C; thereafter, the supernatant was collected. In a black 96-wells plate, 30 μL of the sample (total protein