Behavioural Brain Research 226 (2012) 235–241

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

The effect of age and tongue exercise on BDNF and TrkB in the hypoglossal nucleus of rats

Allison J. Schaser[a,b,*], Kyle Stang[c], Nadine P. Connor[a,b], Mary Behan[c]

[a] Department of Surgery, University of Wisconsin-Madison, 53706, WI, USA
[b] Department of Communicative Disorders, University of Wisconsin-Madison, 53706, WI, USA
[c] Department of Comparative Biosciences, University of Wisconsin-Madison, 53706, WI, USA

ARTICLE INFO

Article history:
Received 2 September 2011
Accepted 14 September 2011
Available online 21 September 2011

Keywords:
Exercise
Aging
Neurotrophic factors
Hypoglossal nucleus
Tongue muscle
Swallowing

ABSTRACT

Age-associated changes in tongue musculature may contribute to dysphagia. One possible treatment is tongue exercise. Exercise induces synaptic plasticity by increasing neurotrophic factors in spinal cord and limb musculature. However, effects of exercise on neurotrophic factors in the cranial sensorimotor system are unknown. Our purpose was to examine the effects of age and exercise on brain-derived neurotrophic factor (BDNF) and its receptor TrkB in the rat hypoglossal nucleus.

Young, middle-aged, and old rats were assigned to exercise or no-exercise control conditions. Exercise animals were trained to perform a tongue press task for 8 weeks. Samples from the hypoglossal nucleus were analyzed for BDNF and TrkB immunoreactivity.

Baseline maximum tongue forces were similar in all age groups and increased significantly following exercise. BDNF immunoreactivity did not show a significant decrease with age in control group. However, in the exercise group, BDNF was significantly increased in young animals. TrkB immunoreactivity decreased significantly with age in control group, but did not change with exercise. BDNF and TrkB immunoreactivity levels were positively correlated with exercise in young and middle aged animals, but were negatively or weakly correlated with exercise in old animals and with a lack of exercise in no-exercise controls.

Tongue exercise was associated with increased tongue forces in rats at all ages. While increases in BDNF and TrkB levels associated with exercise may play a role in mechanisms contributing to increased tongue forces in young and middle-aged rats, other mechanisms may be involved in increased tongue forces observed in old rats.

1. Introduction

Aging is associated with muscle weakness and fatigue, a condition that has been termed sarcopenia [1–3]. The cause of sarcopenia is likely multifactorial and includes a variety of potential mechanisms including neuromuscular changes, decreased nutrition, hormonal changes, and inactivity [4–6]. Sarcopenia affects all elderly individuals to some extent because it is a consequence of normal aging [4]. In addition, sarcopenia has clinical relevance because the loss of muscle mass and strength in the aging musculature has functional consequences. Functional deficits have been well characterized in the limb musculature and include changes in balance and gait, increased risk of falls, and decreased independence [7,8]. Recent data suggest that similar age-related changes occur in the cranial musculature involved in swallowing, and may be associated with the age-related changes seen in the swallow of elderly individuals [9–11]. Elderly individuals swallow more slowly and have increased residue in the pharyngeal cavity following the swallow, both of which may result from reduced strength in tongue and pharyngeal musculature [12,13].

Due to the complex nature of sarcopenia, a variety of treatments have been suggested to decrease the functional consequences of this condition. Of the possible treatments proposed for limb musculature, resistance exercise appears to be the most promising [14–17]. In addition, resistance exercise training of the tongue musculature has been shown to have beneficial effects on tongue muscle strength and swallowing function in elderly individuals [18,19]. Therefore, tongue exercise protocols are currently being used in clinical practice to strengthen the lingual musculature and to improve age-related declines in swallowing function [20,21].

While evidence suggests that resistance exercise is a beneficial treatment for sarcopenia, the underlying mechanisms responsible for the increases in muscle strength and function associated with exercise are unknown. In addition to the changes in

* Corresponding author at: Department of Surgery/Communicative Disorders, University of Wisconsin, 483 Medical Sciences Center, 1300 University Ave, Madison, WI 53706-1102, USA. Tel.: +1 608 265 9130.
E-mail address: schaser@surgery.wisc.edu (A.J. Schaser).

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doi:10.1016/j.bbr.2011.09.027

musculature with exercise, there is evidence to suggest that exercise has a neuroprotective component [22–24] mediated by neurotrophins in both the central and peripheral nervous systems [25]. Neurotrophins are a family of proteins that, when activated through binding with tropomyosin-related kinase (Trk) receptors, initiate signaling cascades that promote the development, survival, and function of neurons [26–28]. Given that the neurotrophin receptors TrkB and TrkC are decreased in the spinal motoneurons of aged rats [29], it appears that decreases in neurotrophin levels may also have a role in the limb deficits seen with aging. Results of previous studies in the brain and spinal cord support the hypothesis that neurotrophins act as a therapeutic agent in cases of neurodegenerative disease and nerve injury [30–32]. In addition, neurotrophins appear to be regulated in an exercise dependent manner. Vaynman et al. found that mRNA of brain-derived neurotrophic factor (BDNF) and its receptor TrkB was up regulated in the hippocampus of rats after 3 and 7 days of wheel running [33]. The same group also found that both mRNA and protein levels of BDNF were increased in the spinal cord following 5 days of wheel running exercise [34]. Other work has shown that BDNF is important for plasticity in respiratory regions of the spinal cord after intermittent hypoxia, which is used as a method of inducing long term facilitation in phrenic and hypoglossal motor outputs [35,36]. Therefore, previous work has demonstrated a link between neurotrophins, aging, and activity-dependent neuroplasticity in the limb sensorimotor system.

No studies, however, have examined changes in neurotrophin levels in the cranial sensorimotor system with either age or exercise. Currently, our laboratory is using an animal model to study the underlying changes to the tongue musculature associated with progressive resistance exercise of the tongue [9,10]. We have shown that tongue exercise induces changes in muscle fiber size and variability in the genioglossus muscle of aged rats that are associated with increased protrusive tongue forces. We found that there was a trend toward an increase in muscle fiber size with tongue exercise and a significant increase in muscle fiber size variability with tongue exercise [9]. In addition, we have shown that neuromuscular stimulation results in a reduction in age-related changes to the morphology of the neuromuscular junction in aged rats [10]. In this study we used our previously described animal model to examine changes in neurotrophin levels in the hypoglossal nucleus of rats with both age and exercise. We hypothesized that levels of BDNF and TrkB immunoreactivity would be decreased with age and increase with exercise. Therefore, the purpose of our study was to examine the levels of BDNF and TrkB in the hypoglossal nucleus of rats at different ages, in both control and exercise conditions, to determine the effect of age and exercise on BDNF and TrkB in the cranial nucleus that controls the tongue musculature involved in swallowing.

2. Methods

All procedures were performed in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of Wisconsin. A total of 48 (16 young, 16 middle-aged, 16 old) male Fischer 344/Brown Norway (F344/BN) rats were obtained from the National Institute of Aging colony. At study completion, the animals were in one of three age groups: young (9–10 months), middle-aged (24–25 months), or old (32–33 months). The median life expectancy of F344/BN rats is 36 months [37]. Every effort was made to minimize the number of animals used and their suffering. Thus, tissues from these animals were assigned to more than one experiment [38]

2.1. Exercise

Animals were housed in pairs in standard polycarbonate cages on a 12:12 h light–dark reversed light cycle. Rats were obtained 8 weeks prior to the start of the experiment to allow acclimation to the animal care facility, reversal of light cycle, water restriction, and familiarization to the tongue force operandum. Food was given ad libitum. Water was restricted to 3 h per day to encourage the animals to press a disk for a water reward. Experimental methods for tongue press measurements in rats have been detailed previously [9,39] but are discussed briefly below.

Throughout the experiment, animals were placed individually into a polycarbonate cage resembling the home cage, but equipped with a 1×1 centimeter (cm) aperture and force operandum that delivered aliquots of water based on tongue press behaviors.

After familiarization with the task, baseline tongue force measurements were obtained for the rats in the exercise group allowing for a measurement of baseline maximum force (g). Following baseline testing the animals in the exercise group underwent an 8-week training paradigm. Throughout the 8 weeks of training the force required for a water reward was increased to mimic a progressive resistance training program. For the first 2 weeks of training the animals were required to press at 50% of their estimated maximum press (EMP) force, which was established during baseline testing. During the second 2 weeks the force was increased to 60% of their EMP force, then 70%, and finally 80%. After the 8 weeks of training, post-exercise maximum force (g) values were obtained.

The control rats were placed on a water restriction protocol and light/dark cycle reversal identical to the exercise-treatment rats. However, they were not given access to the operandum enclosure and did not receive any exercise treatment. Instead, they were placed in an enclosure that resembled the operandum enclosure and were permitted to drink water ad libitum from a water dish for 3 h.

2.2. Perfusion

Rats were anesthetized with isoflurane followed by sodium pentobarbital (120 mg/kg i.p.). Anesthetized rats were transcardially perfused with 200 mL of heparinized saline (10,000 units/L) followed by 400 mL of 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M sodium phosphate buffer (PB) (pH 7.4). Brains were removed and postfixed for 1 h at 4 °C, then cryoprotected for 24–36 h at 4 °C with 20% sucrose and 5% glycerol in 0.1 M phosphate buffer. Sections were cut coronally (40 μm) and stored in 0.1 M phosphate buffer containing 0.02% sodium azide at 4 °C.

2.3. Immunocytochemistry

Two sections through the hypoglossal nucleus from each animal were immunochemically reacted for the presence of BDNF. A separate pair of adjacent sections were reacted for the presence of TrkB. Specifically, sections were selected from the junction between middle-caudal (to be known as caudal sections) and middle-rostral (to be known as rostral sections) of the hypoglossal nucleus in the medulla. A dilution series was conducted to identify the optimal dilution for each antibody. Sections were washed in Tris-buffered saline (TBS), then in 0.1% Triton X-100 in TBS (TBST). After 2 h in blocking solution (5% normal donkey serum in TBST), primary antibodies were applied for 24 h at room temperature in blocking solution. Primary antibodies were used at 1:50: anti-BDNF (Santa Cruz, sc-546, Santa Cruz, CA), and 1:200 anti-TrkB (Santa Cruz, sc-8316, Santa Cruz, CA). An Alexa-Fluor conjugated secondary antibody (594 donkey anti-rabbit IgG. Invitrogen, Eugene, OR) was used for both BDNF and TrkB staining at 1:500 in 5% normal donkey serum in TBST. All sections were reacted at the same time. Negative controls were reacted simultaneously with the omission of either the primary or secondary antibody. Sections were mounted and coverslipped with Vectashield Hard Set mounting medium for fluorescence (Vector Laboratories, Burlingame, CA). There were no labeled cells in negative control sections from all behavioral states.

2.4. Analysis

All images were acquired during the same session using SPOT (Advanced version) computer software and SPOT RT Slider camera (Diagnostic Instruments, Sterling Heights, MI, USA) attached to a Nikon Eclipse E600 microscope. In each section, one image was taken from each side of the ventral middle hypoglossal nucleus (Fig. 1A) for both BDNF and TrkB immunoreactivity using the 40× objective, resulting in 8 images from each animal (4 of BDNF, 4 of TrkB). Images were analyzed using ImageJ [40]. To separate signal from background an Otsu thresholding algorithm was applied to each image. The average fluorescent intensity of each image, captured in relative fluorescent units (RFU), was then measured and used for statistical analysis.

We used an analysis of variance (ANOVA) to compare tongue forces between age groups. The pre-to-post exercise change in force was assessed within each age group using a paired *t*-test. The impact of age, exercise, and region (caudal vs. rostral) on average fluorescent intensity within the specified area of the hypoglossal nucleus was examined using a mixed model analysis for both BDNF and TrkB independently. Age, exercise, and region were included as fixed variables, and the rat itself was included as a random variable to account for the multiple measures taken from each animal. Post-hoc testing was completed on all significant interactions found during the mixed model analysis using a Fisher's LSD analysis to examine individual group differences. In addition, Spearman Correlation was used to examine the relationship between BDNF and TrkB immunoreactivity in the different age, exercise, and region groups. All analyses were performed using SAS statistical software version 9.1 (SAS Institute Inc., Cary, NC). *P*-values less than 0.05 were considered as significant.