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Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats

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Abstract

The impact of age on the effect of treadmill exercise on cell proliferation in the dentate gyrus of rats was investigated via 5-bromo-2′-deoxyuridine immunohistochemistry. Animals of different ages were used: 4-week-old, 8-week-old, and 62-week-old. Based upon the present study, the most prominent cell proliferation in the dentate gyrus was observed in the 4-week-old rats, and decreased in direct relation to the age of the animals. In addition, although treadmill exercise increased cell proliferation in the dentate gyrus of animals in all age groups, the most potent enhancing effect appeared in the 8-week-old rats. The present results demonstrate that age is an important factor in the regulation of cell proliferation in the dentate gyrus and that the enhancing effect of the treadmill exercise on cell proliferation also depends on age status.

Keywords: Treadmill exercise; Cell proliferation; Dentate gyrus; Immunohistochemistry; Age-dependence

It is well known that the hippocampus plays a pivotal role in learning and memory acquisition [13]. The mammalian brain continues to produce neuronal precursor cells throughout the developmental period [1]. It has been well established that new cells are produced continuously in the hippocampal dentate gyrus of adult mammals including humans [5]. Previous studies have shown that several factors such as learning, *N*-methyl-D-aspartate (NMDA) receptor antagonists, seizure, ischemia, and physical exercise enhance the proliferation of granular cell precursors and neurogenesis in the adult dentate gyrus [2,5,7,14]. In contrast, the rate of production of new cells in the dentate gyrus is known to be dramatically decreased in the aged animals [10]. Memory impairments in elderly populations are characterized by memory loss in self perception and decline in objective memory performance [11], similar to the symptoms shown by patients with mild Alzheimer's disease [15]. Hippocampal formation in aged persons shows structural and functional changes including loss of neurons in the CA regions and hilus [20].

The beneficial effects of physical exercise on the brain functions have been suggested in numerous studies. Voluntary physical activity is known to enhance the performance of spatial learning [4] and passive avoidance memory in experimental animals [16]. Voluntary wheel running in mice enhanced cell proliferation and survival in the dentate gyrus, and increased the magnitude of hippocampal long-term potentiation (LTP) and spatial learning [18]. Recent studies have shown that treadmill exercise in rats enhances cell proliferation and that this increase of cell birth is induced by insulin-like growth factor (IGF-I) [17]. A recent study also reported that treadmill exercise increases cell proliferation in the dentate gyrus without altering apoptosis [8].

Although previous studies have documented that treadmill exercise increases cell proliferation in the dentate gyrus, the age-dependence of the enhancing effect of exercise on cell proliferation in the dentate gyrus has not been reported to date. In the present study, the impact of age on the effect of treadmill exercise on cell proliferation in the dentate gyrus was investigated via 5-bromo-2′-deoxyuridine (BrdU) immunohistochemistry.

Male Sprague–Dawley rats were obtained from a

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commercial breeder (Daehan Biolink Co., Chungbuk, South Korea) for the experiment. The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under controlled temperature (20 ± 2 °C) and lighting (07:00–19:00 h) conditions with food and water made available ad libitum.

The animals were divided into six groups: the 4-week-old control group (90 ± 10 g); the 8-week-old control group (250 ± 10 g); the 62-week-old control group (580 ± 15 g); the 4-week-old exercise group; the 8-week-old exercise group; and the 62-week-old exercise group ($n = 5$ in each group). Animals of the exercise groups were made to run on a treadmill for 30 min once a day for 5 consecutive days, whereas those of the control groups were left on the treadmill for the same duration of time, but were not made to run. The exercise load consisted of 3 m/min for the first 5 min, 5 m/min for the next 5 min, and 8 m/min for the last 20 min. All animals received 50 mg/kg of BrdU intraperitoneally (Sigma, St. Louis, MO) once a day 1 h prior to the start of treadmill exercise for 5 consecutive days. The animals were sacrificed immediately after finishing the last session of exercise.

Animals were weighed and overdosed with Zoletil 50® (10 mg/kg, i.m.; Vibac Laboratories, Carros, France). After a complete lack of response was observed, the rats were transcardially perfused with 50 mM phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde (PFA) in 100 mM sodium phosphate buffer (PB) at pH 7.4. The brains were removed, post-fixed in the same fixative overnight, and transferred to a 30% sucrose solution for cryoprotection. Coronal sections of 40 μm thickness were made using a freezing microtome (Leica, Nussloch, Germany).

For detection of cell proliferation in the dentate gyrus, BrdU-specific immunohistochemistry was performed according to a previously described method [19]. In brief, sections were first pretreated by incubation in 50% formamide/2 × standard saline citrate (SSC) at 65 °C for 2 h, denaturing in 2 N HCl at 37 °C for 30 min, and rinsing twice in 100 mM sodium borate (pH 8.5). After the pretreatment, the sections were incubated overnight at room temperature with a BrdU-specific mouse monoclonal antibody (1:600; Boehringer Mannheim, Germany). The sections were then washed three times with PBS and incubated for 1 h with a biotinylated mouse secondary antibody (1:100; Vector Laboratories, Burlingame, CA). Next, the sections were incubated for another 1 h with an avidin–biotin–horseradish peroxidase complex (1:100; Vector Laboratories, Burlingame, CA). For staining, the sections were reacted with 0.02% 3,3′-diaminobenzidine (DAB) containing nickel chloride (40 mg/ml) and 0.03% H_2O_2 in 50 mM Tris–HCl (pH 7.6) for 5 min. Finally, the sections were mounted onto gelatinized glass slides.

The area of the granular layer of the dentate gyrus was measured using an image analyzer (Multiscan, Fullerton, CA). The number of BrdU-positive cells was counted hemilaterally and expressed as the number of cells per mm^2 of the granular layer of the dentate gyrus. Statistical analyses were performed with two-way ANOVA followed by Scheffé's post-hoc test using the SAS program. Differences were considered significant at $P < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

Photomicrographs of BrdU-positive cells in the dentate gyrus are presented in Fig. 1. In the control groups, the number of BrdU-positive cells in the dentate gyrus was 155.33 ± 7.98/mm^2 in the 4-week-old control group, 125.56 ± 8.70/mm^2 in the 8-week-old control group, and 26.22 ± 1.86/mm^2 in the 62-week-old control group. The most active cell proliferation in the dentate gyrus was observed in the 4-week-old rats, and was decreased in direct relation to the age of the animals.

In the exercise groups, the number of BrdU-positive cells in the dentate gyrus was 198.00 ± 16.02/mm^2 in the 4-week-old exercise group, 186.78 ± 10.98 in the 8-week-old exercise group, and 39.33 ± 10.16/mm^2 in the 62-week-old exercise group. Treadmill exercise increased cell proliferation in the dentate gyrus in all of the age groups, and the most potent enhancing effect of the treadmill exercise on cell proliferation in the dentate gyrus was observed in the 8-week-old rats (Fig. 2).

In the present study, cell proliferation in the dentate gyrus appeared in all of the groups: the most prominent cell proliferation in the dentate gyrus was observed in the 4-week-old rats, and the rate of cell proliferation in the dentate gyrus declined with increasing age. The present results reveal that new cell formation in the dentate gyrus is most prevalent in young rats. New cell proliferation in the dentate gyrus is known to play an important role in the hippocampus-dependent learning [12]. In contrast, it has

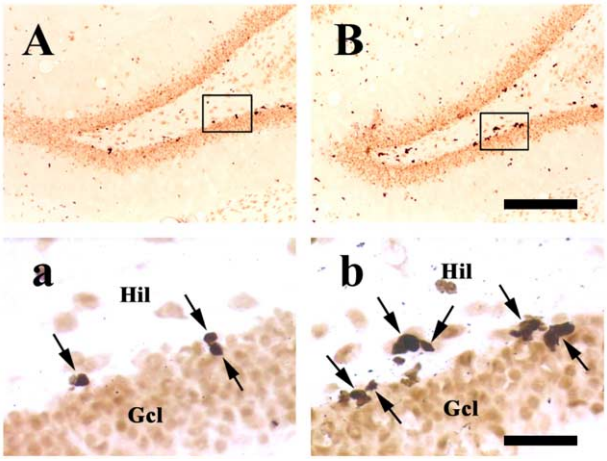

Fig. 1. Photomicrographs of BrdU-positive cells in the dentate gyrus. (A) Eight-week-old control group; (B) 8-week-old exercise group. The scale bar represents 100 μm in (A,B). The scale bar represents 25 μm in (a,b). Arrows indicate the location of BrdU-positive cells in the subgranular layer. Gcl, granule cell layer; Hil, hilus.

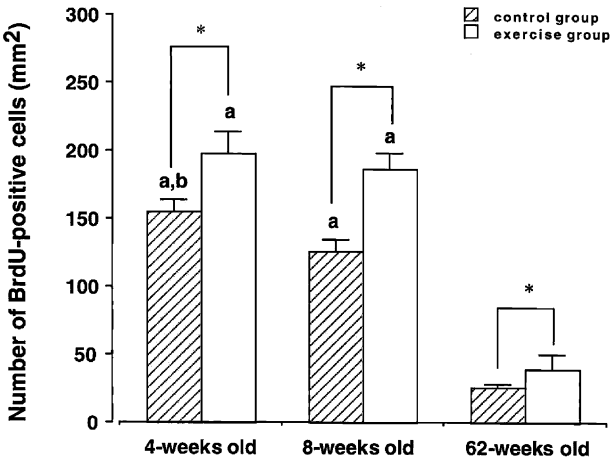

Fig. 2. Age-dependence of the effect of treadmill exercise on cell proliferation in the dentate gyrus. $^{*}P < 0.05$ between the control groups and the exercise groups. $^{a}P < 0.05$ compared to the 62-week-old groups. $^{b}P < 0.05$ compared to the 8-week-old groups.

been suggested that decreased neurogenesis may contribute to age-related memory impairment [11]. The present results showing the decrease of cell proliferation in the old aged rats are consistent with other reports [10]. Increased secretion of glucocorticoid, which suppresses neurogenesis in the dentate gyrus, is suggested as an underlying mechanism of age-related decrease in cell proliferation [6].

Treadmill exercise has been reported to increase cell proliferation in the dentate gyrus. In the present results, cell proliferation in the dentate gyrus was increased by treadmill exercise and the most potent enhancing effect of the treadmill exercise on cell proliferation in the dentate gyrus appeared in the rats of the 8-week-old exercise group. Exercise is known to increase the survival rate of newly formed cells and maturates cells to neurons [3]. Another study reported that the enhancing effect of the treadmill exercise on cell proliferation in the dentate gyrus is modulated by the intensity and duration of the exercise [9].

The results presented in this study demonstrate that age is an important factor in the regulation of cell proliferation in the dentate gyrus and that the enhancing effect of the treadmill exercise on cell proliferation also depends on age status.

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