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Influence of kidney tonic formula on the hippocampal neurotrophic factors and proliferation and differentiation of neural stem cells in natural aging rats

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The aim of this research was to explore the influence of the quantity of NGF and GDNF in hippocampus on the proliferation and differentiation of hippocampal neural stem cells (NSCs) in natural aging rats and the function of Zuogui pill and Yougui pill. Methods: SD rats were divided into Old group, Zuogui group and Yougui group. Young group (5 months) was set additionally. All rats were fed with common food and free drinking water. Old group was fed to 24 months old. From 20 months old, Zuogui group and Yougui group were fed with food mixing two formulas (0.675g/100g/d) accordingly for 4 months. Western Blot method was utilized to assay the protein expression of NGF, GDNF, nestin and ki-67 in rat hippocampal tissue. Results: compared with Young group, the protein expressions of NGF, GDNF, nestin and ki-67 were all down-regulated in rat hippocampus in Old group, $P<0.05$; compared with Old group, the protein expression of NGF, GDNF, nestin and ki-67 were all up-regulated in both Zuogui group and Yougui group, $P<0.05$. Conclusion: the change of protein expression of NGF and GDNF can influence the proliferation and differentiation of NSCs in hippocampus in aged rats, and Zuogui and Yougui pills can ameliorate these trends.

Keywords: Natural aging rats, Hippocampus, NGF and GDNF, Proliferation and differentiation of NSCs, Kidney tonic formula

INTRODUCTION

New neurons continue to be produced in adult mammals. This update focuses on the emerging concept that adult central nervous system (CNS) neurogenesis can be regulated by targeting neurotransmitter receptors, which, in turn, drive expression of crucial neurotrophic and growth factors. Such an approach might enable the development of pharmacological treatments that harness the endogenous potential of the CNS to replace lost cells in neurological disorders such as stroke, Alzheimer's and Huntington's diseases [1]. As the nerve growth factor was found, the neuro scientists believed, there were some kind of nutritions that survival of neurons are depended on. These nutritions come from the target tissue that neurons dominate. With the development of the studies on these nutritions, they have been named as neurotrophic factors (NTFs), which include nerve growth factor (NGF), brain derived growth factor (BDNF), glial derived growth factor (GDNF), etc. NTFs can prevent cell death in degenerative processes, and at the same time NTFs are important for the regulation of proliferation and differentiation of neural stem cells (NSCs) [2], and can induce differentiation of NSCs into different neurons and self proliferation [3]. There is a significant amount of evidence from animal and human studies that links neurodegenerative related cognitive deficits with changes on brain and peripheral trophic factor levels [4]. NTFs can promote the proliferation and differentiation of NSCs. Here, we aim to explore the influence of the quantity of NGF and GDNF in hippocampus on proliferation and differentiation of hippocampal NSCs in natural aging rats and the function of Zuogui pill and Yougui pill.

EXPERIMENTAL

Materials and analytical methods

Animals: Male Sprague Dawley (SD) rats, SPF grade. Licence number: SCXK(Yu)2010-0002, provided by the Centre of Henan province for experimental animals. Reagents: NGF antibody, GDNF antibody, nestin antibody, ki-67 antibody, Santa Cruz; goat anti-mouse IgG-HRP, Santa Cruz; mouse anti-beta actin, Zhongshan jin qiao. Formula: Zuogui pill and Yougui pill, sixth "Chinese medicine formula" teaching materal. Instruments: electrophoresis and electrotransfer instrument, Bio-Rad; refrigerated centrifuge, Thermo; automatic glass homogenate machine, Qilinbeier; electronic analytical balance, Denver Instruments. Method: thirty SD male rats were divided into Old group, Zuogui group and Yougui group. Young group (5 months) was set additionally. All rats were fed with common food and free drinking water. Old group was fed to 24 months old. From 20 months old, Zuogui group and Yougui group were fed with food mixed with two formulas (Zuogui pill and Yougui pill, both 0.675g/100g/d), respectively, for 4 months.

When rats were 24 months old, all rats were anaesthetized from abdomen (urethan, 0.75mg/kg). Bilateral hippocampus were separated from the brain

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and kept in a refrigerator at -80°C.

Western Blot method was utilized to assay the protein expressions of NGF, GDNF, nestin and ki-67 in rat hippocampal tissue.

Data were presented as $\bar{x} \pm s$. Statistical software SPSS17.0 was used to analyse all data (one-way ANOVA), a=0.05. There was a significant difference at P<0.05.

RESULTS AND DISCUSSION

Influence of Zuogui pill and Yougui pill on protein expressions of hippocampal NGF and GDNF in natural aging rats (Table 1)

NGF is a neuro regulating factor, it can nourish neurons and promote synapse development. It was proved that the combined use of NGF/BDNF/bFGF significantly improved the ability of NSCs proliferation and differentiation and the percentage in the multi-factor groups was significantly higher than that in the single-factor groups [5]. Ontogenetic life and stress can have different effects on the NGF in the structures of the limbic system. A significant age-related decrease in NGF and TrkA in the PVN of stressed rats was noted with immunofluorescence staining. However, in the hippocampus, an age-related decrease in NGF-ir or TrkA-ir cells was observed in all rats except in acute forced swim stressed rats. The changes are possibly associated with involutional aging processes caused by insufficient control of hypothalamic-pituitary-adrenal (HPA) axis functioning in P720 rats and may contribute to disturbances in NGF signaling [6], while the hyperfunction of HPA axis is founded in the process of aging.

GDNF have shown potent neuroprotective effects on different neuronal populations and they are also useful in maintaining the integrity of the corticostriatal pathway. These neurotrophic factors may be suitable for the development of a neuroprotective therapy for neurodegenerative disorders of the basal ganglia [7]. GDNF promotes the survival, growth, and regeneration of dopamine neurons and its tissue distribution is more important than dose for trophic stimulation of dopamine neurons [8]. Clinically relevant and long-lasting regeneration of the dopaminergic system in rhesus macaques lesioned with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine 3-6 months before GDNF gene delivery (AAV2-GDNF). The observed progressive amelioration of functional deficits, recovery of dopamine, and regrowth of fibers to the striatal neuropil demonstrate that high GDNF expression in the putamen promotes restoration of the dopaminergic system in a primate model of advanced PD [9]. The aged primate brain challenged by MPTP administration has the potential to respond to trophic factor delivery and that the degree of neuroprotection depends on GDNF levels [10]. GDNF protects dopamine (DA) neurons from 6-hydroxydopamine (6-OHDA) toxicity [11]. GDNF is essential for regulating dopamine (DA) release in the basal ganglia and for the survival of dopaminergic neurons; GDNF-deficient mice are considered an animal model for aging-related Parkinsonism [12].

It was found in our experiment that compared with Young group, the protein expression of NGF and GDNF were both down-regulated in rat hippocampus in Old group, P＜0.05. Compared with Old group, the protein expressions of NGF and GDNF were both up-regulated in the Zuogui group and Yougui group, P＜0.05.

Table 1. Level of protein expression of hippocampal NGF and GDNF in rats of all groups

Groups	*n*	NGF(*OD*)	GDNF(*OD*)
Young Group	8	0.657±0.061▲	0.482±0.015▲
Old Group	8	0.403±0.013	0.275±0.014
Zuogui Group	8	0.624±0.015▲	0.433±0.010▲
Youogui Group	8	0.605±0.009▲	0.395±0.011▲

▲Compared with Old Group, P<0.05

Influence of Zuogui pill and Youogui pill on protein expressions of hippocampal nestin and ki-67 in natural aging rats (Table 2)

NSCs exist in mammalians in life process. But their overall neurogenic potential declines considerably in the early postnatal period. The deficiency of NSCs is related to aging or aging-related diseases.

Hippocampus is an important brain area related to aging. It has been proved that there are NSCs in hippocampal dentate gyrus in aging. Multipotent NSCs also persist in both CA1 and CA3 subfields of the hippocampus in the postnatal period. Such NSCs also retain their ability to give rise to both GABA-ergic and non-GABA-ergic neurons [13]. With aging, Hippocampal neurogenesis diminished and this phenomenon is related to deficiency of NSCs. Nestin and ki-67 are markers of proliferation and differentiation of NSCs.

It was found in our experiment that compared with Young group, the protein expressions of nestin and ki-67 were both down-regulated in rat hippocampus in Old group, P＜0.05. Compared with Old group, the protein expressions of nestin and ki-67 were both up-regulated in the Zuogui group and the Yougui group, P＜0.05.

Table 2. Levels of protein expression of hippocampal nestin and ki-67 in rats of all groups

Groups	*n*	Nestin(*OD*)	Ki-67(*OD*)
Young Group	9	0.744±0.101▲	1.017±0.256▲
Old Group	9	0.339±0.097	0.697±0.178
Zuogui Group	9	0.666±0.886▲	0.968±0.303▲
Youogui Group	9	0.568±0.089▲	0.989±0.304▲

▲Compared with Old Group, P<0.05

CONCLUSION

The change in protein expression of NGF and GDNF can influence the proliferation and differentiation of NSCs in hippocampus in aged rats, and Zuogui pill and Yougui pill can ameliorate these trends.

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