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Effects of ginsenoside Rg_1 on c-*fos* gene expression and cAMP levels in rat hippocampus[1]

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KEY WORDS ginseng; saponins; *fos* genes; proto-oncogene proteins c-*fos*; cyclic AMP; Northern blotting; Western blotting; hippocampus

AIM: To study the mechanisms of Rg_1 antiaging and nootropic function. **METHODS**: Using Northern and Western blot analyses, the levels of c-*fos* mRNA and *fos* protein were determined in the hippocampus of young and old rats treated with or without ginsenoside Rg_1. **RESULTS**: The expression of c-*fos* gene and protein was decreased in the hippocampus of aged rats, but dose-dependently increased in young and aged rats after the administration of Rg_1. Furthermore, Rg_1 increased the level of cAMP in the hippocampus of both young and old rats. **CONCLUSION**: The changes at the genomic and protein levels, arisen from the increase of cAMP, provide an explanation of the mechanisms of Rg_1 nootropic and antiaging function.

The c-*fos* proto-oncogene is the prototype of the early-response class of genes. It encodes for a nuclear phosphoprotein, which after forming a complex with the protein product of another oncogene c-*jun*, binds to the AP-1 sites of DNA, and acts as a regulatory factor for gene transcription[1]. The c-*fos* gene expression can be considered as a marker for neuronal activity[2]. Moreover, *fos* protein is believed to be involved in processes related to neuronal plasticity[3].

Ginsenoside Rg_1 is one of the important pharmacological principles of ginseng and shares many pharmacological effects of this plant, such as, facilitating learning and memory, and alleviating many ailments, especially those associated with aging. Since the c-*fos* gene expression might be of crucial importance for neuronal activity and cAMP is a factor regulating gene expression[4]. The present work was to study the effects of Rg_1 on c-*fos* mRNA and protein expression as well as cAMP and cGMP levels.

MATERIALS AND METHODS

PolyATtract mRNA isolation systems and Protoblot Western blot AP systems were purchased from Promega Co, USA. Human c-*fos* gene probe (Oncogene Sciences Co, USA) was a gift from Dr CHEN Jin-Dong (Institute for Cancer Research, CAMS, Beijing). Sheep monoclonal c-*fos* antibody (Oncogene Sciences Co, USA) was a gift from Prof WAN Xian-Cai (Institute of Basic Medical Research, CAMS, Beijing). Rg_1, isolated from notoginseng (*Panax pseudoginseng* Wall var *notoginseng* (Burk) Hoo *et* Tseng) with a purity of 96 %, was supplied by the Guangzhou Institute of Medicinal Industry. cAMP and cGMP assay kits were purchased from Chinese Academy of Nuclear Sciences, Beijing

Rats Young (5 months, 175 ± *s* 20 g) and old (24 months, 515 ± *s* 25 g) ♂ Wistar rats, obtained from the Animal Breeding Center, Beijing Medical University, were maintained under barrier conditions for a minimum of 2 wk before experiments. Food and water were available *ad lib*. Rg_1 10, 20, and 40 $mg \cdot kg^{-1}$ were injected ip for 5 d. On d 5, after ip Rg_1 for 1 h, the rats were decapitated, and the brains were dissected on ice.

Northern blot analysis The induction of c-*fos* RNA is known to be rapid and transient, therefore, an early time point of 1 h was used to characterize c-*fos* mRNA induction in response to Rg_1 injection. Total RNA was isolated by a single step extraction[5]. The mRNA was isolated from total RNA with Pormega's PolyATtract mRNA isolation systems using paramagnetic particles. Northern blot analysis was performed[6]. Five μg samples of denatured mRNA from hippocampus were electrophoresed on formaldehyde denatured 1 % agarose gel. mRNA was then transferred overnight onto 0.45 μm nitrocellulose (NC) filter by passive diffusion. The NC filter was baked at 80 ℃ for 2 h to fix the mRNA, which was hybridized to nick-translated [^{32}P] c-*fos* probe, and the hybridization was detected by autoradiography.

Western blot analysis The hippocampus was washed with ice-cold PBS and cut into small pieces. The tissue fragments were dispersed in 5 volume of ice-cold suspension buffer [NaCl 100 $mmol \cdot L^{-1}$, Tris·Cl 10 $mmol \cdot L^{-1}$ (pH 7.6),

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edetic acid 1 nmol·L^{-1} (pH 8.0), aprotinin 1 mg·L^{-1} and phenylmethylsulfonyl fluoride 100 mg·L^{-1}], and then added an equal volume of 2 × SDS gel-loading buffer [Tris·Cl 100 mmol·L^{-1} (pH 6.8), dithothreitol 200 nmol·L^{-1}, 4 % SDS, 0.2 % bromophenol blue, and 20 % glycerol]. The samples were placed in a boiling-water bath for 10 min, and centrifuged at 10 000 × g at 15 ℃ for 10 min.

The supernants, equivalent to 30 – 40 μg of protein (30 μL), were separated by SDS-polyacrylamide gels, and then transferred from the gel to a NC filter with an electrophoresis systems[7]. The NC filter was incubated with anti-*fos* antibody (1∶1 000 dilution) for 1 h, and then transferred to a solution of the secondary antibody (1∶1500 dilution) conjugated with alkaline phosphatase. The violet color developed by subsequent reaction with alkaline phosphates substrate solution provided a visual indication of *fos* protein.

Intracellular cAMP and cGMP determinations The intracellular cAMP and cGMP contents were measured[8]. The hippocampus was placed in ice-cold 10 % trichloroacetic acid (TCA). Homogenates (5 %) were centrifuged (800 × g, 10 min). The supernatants were extracted by diethyl ether saturated with water for 4 times to remove TCA. The medium was dried at 60 ℃ under a stream of air. The residue was dissolved in 0.5 mL buffer [Tris·Cl 50 mmol·L^{-1} (pH 7.5), containing edetic acid 4 nmol·L^{-1} for cAMP assay, and NaAC 50 nmol·L^{-1} (pH 6.2) for cGMP assay]. cAMP and cGMP concentrations were determined using assay kits. Proteins were determined[9].

Statistics Data were expressed as $\bar{x} \pm s$ and evaluated by t test.

RESULTS

c-*fos* gene expression The control rats had extremely low, or undetectable, levels of c-*fos* mRNA; treatment with Rg_1 elicited a dramatic increase of c-*fos* mRNA in the hippocampus of young and old rats. The expression of c-*fos* mRNA from old rats was lower than that from young rats. The maximal effect was obtained at 20 mg·kg^{-1} for young rats, and 40 mg·kg^{-1} for old ones. But at the higher dose over 20 mg·kg^{-1} in young rats, the effect promoting c-*fos* gene expression by Rg_1 decreased (Fig 1).

***fos* protein expression** Since the levels of *fos* protein in control rats were very low, staining was barely detectable on NC filter. After treatment with Rg_1 for 1 h, the intensities of staining on NC filter were notably greater, indicating that the levels of *fos* protein increased. Densitometric scanning showed that the effects of Rg_1 on *fos* protein expression of old rats was dose-dependent. In young rats, however, the *fos* protein levels did not rise in proportion to dose. The maximal dose was 20 mg·kg^{-1}. Thus, qualitatively, the level of *fos* protein mirrored that of c-*fos* mRNA in young and old rat hippocampus (Fig 2).

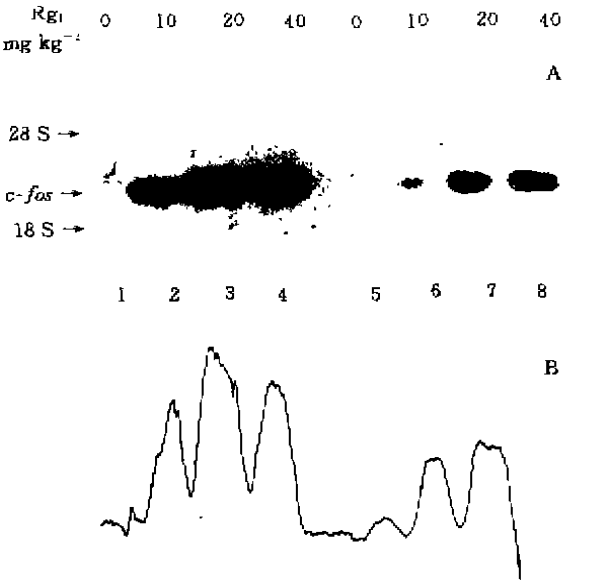

Fig 1. c-*fos* mRNA expression in hippocampus from young (lanes 1 – 4) and aged (lanes 5 – 8) rats treated with Rg_1. Arrows indicate the migration rRNA. A) Northern blot analysis; B) densitometric analysis.

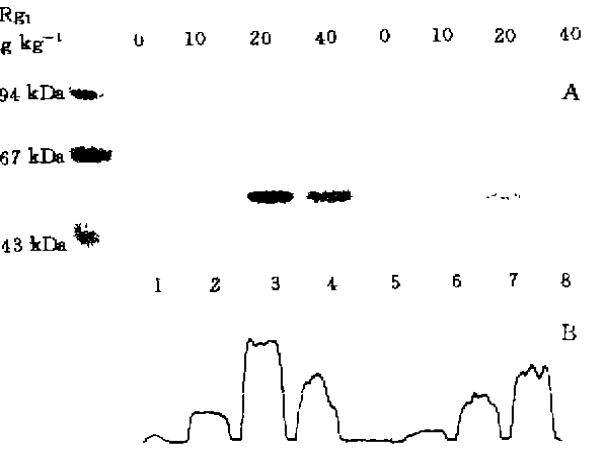

Fig 2. Western blot analysis of *fos* protein in hippocampus from young (lanes 1 – 4) and aged (lanes 5 – 8) rats treated with Rg_1. A) Western blot analysis; B) Densitometric analysis.

cAMP and cGMP level In comparison with young rats, decreases of the cAMP and cGMP levels

were found in the hippocampus of aged rats, which was in agreement with the report[10]. After treatment with Rg_1, the cAMP content obviously increased, but cGMP concentration showed no obvious changes, so the ratio of cAMP/cGMP increased. The optimal increase induced by Rg_1 in cAMP level occurred at 20 $mg \cdot kg^{-1}$ for young rats and 40 $mg \cdot kg^{-1}$ for old ones, suggesting that the changes of cAMP levels were consistent with those of c-*fos* mRNA and *fos* protein expression in Rg_1-treated rats (Tab 1).

Tab 1. Effects of Rg_1 on cAMP and cGMP levels in hippocampus of rats. $n = 6$ rats, $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* young rat control;
$^dP > 0.05$, $^eP < 0.05$, $^fP < 0.01$ *vs* aged rat control.

Rg_1/ $mg \cdot kg^{-1}$	nmol/g protein cAMP	cGMP	cAMP/ cGMP
Young rats			
0	5.5 ± 1.0	0.78 ± 0.12	6.987
10	7.1 ± 1.2^b	0.81 ± 0.99^a	8.790
20	12.8 ± 2.1^c	0.90 ± 0.13^a	14.167
40	9.3 ± 1.6^c	0.86 ± 0.12^a	10.802
Aged rats			
0	4.0 ± 0.8^b	0.62 ± 0.11^b	6.419
10	5.6 ± 1.0^e	0.69 ± 0.14^d	8.073
20	8.2 ± 1.3^f	0.72 ± 0.10^d	11.375
40	10.7 ± 2.4^f	0.73 ± 0.12^d	14.658

DISCUSSION

Recent rapid accumulation of papers on c-*fos* gene expression in the central nervous system (CNS) has raised interest in the physiological significance of those findings. This interest is spurred by an emerging consensus that elevated c-*fos* expression is a good marker of long-term cellular response. It is widely accepted that learning and memory are the feature of neuronal networks c-*fos* Proto-oncogene expression and *fos* protein biosynthesis are critical for the acquisition of learning and the establishment of a long-lasting memory trace[11]. In our previous study, Rg_1 was found to improve learning and memory in animals. In the present experiment, results showed that Rg_1 increased the c-*fos* mRNA and *fos* protein levels in the hippocampus of rats. These finding provided an explanation of the molecular mechanisms of Rg_1 nootropic action and other action on CNS.

Aging is accompanied by a number of biochemical, electrophysiolocal and morphological changes in the brain. Learning, memory and *in vitro* paradigms of neuronal plasticity decreased as a result of senescence. These changes are believed to be the end result of altered gene expression. The steady-state levels of the proto-oncogene c-*fos* mRNA and cAMP were decreased in aged rat brain[12]. Ginseng, a tonic in the Traditional Chinese Medicine, can be used for treatment and prevention of many diseases, especially those associated with aging or low physiological function. In this study, Rg_1, injected for 5 d, increased the levels of c-*fos* mRNA and *fos* protein as well as contents of cAMP in aged rats. Thus, Ginsenoside Rg_1 might become an effective agent for the treatment of age-related learning and memory deficits and other senile diseases.

The results of our investigation showed that the increase in cAMP content, caused by Rg_1 in the hippocampus of both young and old rat, was accompanied by a similar rise in c-*fos* mRNA and *fos* protein levels. cAMP is an important intracellular second messenger in many tissues. The elevation of intracellular cAMP levels induces c-*fos* expression[13]. Therefore, our results indicated that the action of Rg_1 on expression of c-*fos* mRNA and *fos* protein was mediated through a cAMP-dependent mechanism.

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人参皂苷 Rg_1 对大鼠海马 c-*fos* 基因表达和 cAMP 含量的影响

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关键词 人参；皂苷类；*fos* 基因；原癌基因蛋白 c-*fos*；腺苷环一磷酸；RNA 印迹；蛋白质印迹；海马 cAMP 大鼠

目的：探讨 Rg_1 对神经系统作用的机制。**方法**：采用 Northern 和 Western 印迹分析法，检测了 Rg_1 处理前后大鼠海马组织的 c-*fos* 基因和蛋白的表达。**结果**：老年鼠 c-*fos* 基因和蛋白的表达明显低于青年鼠，但给 Rg_1 后老年鼠和青年鼠均呈现显著性增强效应。此外，Rg_1 还明显增加青年鼠和老年鼠海马组织的 cAMP 含量。**结论**：Rg_1 升高 cAMP 水平及促进 c-*fos* 基因和蛋白的表达有助于阐明其促智和抗衰老作用的机制。

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Effects of *Coriolus versicolor* polysaccharides on superoxide dismutase activities in mice

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KEY WORDS *Coriolus versicolor*; polysaccharides; superoxide dismutase; delayed hypersensitivity; Ehrlich tumor carcinoma; radiation

AIM: To study if *Coriolus versicolor* polysaccharides (CVP) influence the superoxide dismutase (SOD) activities in mice. **METHODS**: Normal, tumor-bearing, and radiated ICR mice were injected ip with CVP daily for 3 - 15 d. The SOD activity was assayed by epinephrine autoxidation test. **RESULTS**: The SOD activities in lymphocytes and thymus were increased by CVP in both the normal mice with or without delayed hypersensitivity (DH). In tumor-bearing mice, CVP exerted not only inhibitory effects on tumor growth and SOD activity in tumor tissue but also complete or partial restorative effects on the suppressed DH and on the declined SOD activities in lymphocytes, spleen, and thymus. The total SOD and manganese-containing SOD (MnSOD) activities in lymphocytes and thymus were dose-dependently enhanced by CVP (5 - 20 mg · kg^{-1}) on d 3 after the tumor transplantation. In the mice exposed to ^{60}Co (3 or 6 Gy),

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