BRAIN RESEARCH 1357 (2010) 19–25

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Ginsenosides Rb1 and Rg1 promote proliferation and expression of neurotrophic factors in primary Schwann cell cultures

Wei Liang[a,1], *Shuhua Ge*[b,1], *Lihong Yang*[c], *Min Yang*[a], *Zhengxu Ye*[a], *Ming Yan*[a], *Junjie Du*[a], *Zhuojing Luo*[a,*]

[a]*Institute of Orthopaedics, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032, China*
[b]*Department of Respiratory Medicine, Xijing Hospital, The Fourth Military Medical University, Xi'an 710032, China*
[c]*Headquarters of the Military District in Lanzhou, Lanzhou 730032, China*

ARTICLE INFO

Article history:
Accepted 27 July 2010
Available online 2 August 2010

Keywords:
Ginsenoside Rb1
Schwann cells
Proliferation
Nerve growth factor
Brain-derived neurotrophic factor
Protein kinase A

ABSTRACT

Ginsenoside Rb1 (GRb1) and ginsenoside Rg1 (GRg1), two major ingredients in ginseng root, have gained extensive attention because of its neuroprotective properties. Thus far, most of the studies on GRb1 and GRg1 have been focused on their neuroprotective effects on neurons. The potential beneficial effects of GRb1 and GRg1 on Schwann cells have not been investigated comprehensively. The present study was designed to examine the possible beneficial effect of GRb1 and GRg1 on proliferation and expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in Schwann cells. Schwann cells were incubated without or with GRb1 and GRg1 at different doses. The proliferation of Schwann cells was examined by cell counting. The expression and secretion of NGF and BDNF were examined by western blotting and ELISA. We found that both GRb1 and GRg1 were capable of increasing the proliferation of, and the expression and secretion of NGF and BDNF in Schwann cells. Further studies showed that both GRb1 and GRg1 were able to increase intracellular cyclic AMP (cAMP) level and protein kinase A (PKA) activity. Preincubation with 10 μM H89 (a PKA inhibitor) significantly inhibited the beneficial effects of GRb1 and GRg1 on Schwann cells. These findings indicate that the beneficial effects of GRb1 and GRg1 on proliferation and expression of NGF and BDNF occurs mainly through the PKA pathway in cultured Schwann cells.

Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.

1. Introduction

Schwann cells are glial cells in the peripheral nervous system. They are essential for nerve regeneration as they provide a permissive environment for nerve regrowth (Frostick et al., 1998; Pearse et al., 2004). After peripheral nerve injuries, Schwann cells proliferate and synthesize neurotrophic factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3, to provide trophic support for regenerating axons (Frostick et al., 1998;

* *Corresponding author*. Fax: +86 29 84775285.
E-mail address: zjluo@fmmu.edu.cn (Z. Luo).

Abbreviations: GRb1, ginsenoside Rb1; GRg1, ginsenoside Rg1; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; cAMP, cyclic AMP; PKA, protein kinase A; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; DMSO, dimethyl sulfoxide; PBS, phosphate buffered saline; MPP+, 1-methyl-4-phenylpyridinium-iodide; PKA, protein kinase A

[1] Wei Liang, and Shuhua Ge contribute equally to this work.

0006-8993/$ – see front matter. Crown Copyright © 2010 Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2010.07.091

Pearse et al., 2004). The unique characteristics of Schwann cell make it an attractive addition to artificial nerve. In these cases, a large amount of Schwann cells with good biological properties would be required. However, the biological properties of Schwann cells declined over time under conventional culture condition. Therefore, searching for bioactive substances which are capable of improving the biological properties of Schwann cells is very important.

Ginseng (Panax ginseng C.A. Meyer) is a well known Chinese herbal medicine. It has been widely used for thousands of years in Asia for the prevention and treatment of aging-associated disorders (Jia et al., 2009). The bioactive components responsible for the pharmacological actions of ginseng are ginsenosides. Thus far, more than 30 different ginsenosides have been identified from ginseng (Attele et al., 1999). They can be divided into two classes, namely protopanaxadial (Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3) and protopanaxatriol (Rg1, Rg2, Re, Rf, and Rh1). Among the 30 ginsenosides, ginsenside Rb1 (GRb1) and ginsenside Rg1 (GRg1) are the main active ingredients of ginseng. Recently, GRb1 and GRg1 have gained extensive attention because they have been shown to possess neuroprotective properties in many studies.

The neuroprotective effects of GRb1 and GRg1 have been identified in many *in vitro* and *in vivo* studies. GRb1 and GRg1 are capable of promoting neurite outgrowth and neurite branching in neurons which are exposed to MPP+ (Radad et al., 2004a) and glutamate (Radad et al., 2004b). GRb1 and GRg1 have also been shown to increase neurotransmission in PC12 cell culture, and the effect of GRb1 was mediated through a cAMP-dependent protein kinase pathway (Xue et al., 2006). In *in vivo* studies, GRb1 (Chen et al., 2002) and GRg1 (Ma et al., 2010) have been found to enhance peripheral nerve regeneration in rat models of nerve injuries. In addition, GRb1 and GRg1 have been reported to reduce neuronal death following transient cerebral ischemia (Jiang et al., 2000) and promote survival and outgrowth of spinal cord neurons (Liao et al., 2002). All those evidences reveal the neuroprotective effect of GRb1 and GRg1. Thus far, most of the studies concerning GRb1 and GRg1 have focused on their impact on neurons. The potential beneficial effects of GRb1 and GRg1 on the biological properties of Schwann cells were not investigated comprehensively. Therefore, the present study was designed to investigate the effect of GRb1 and GRg1 on proliferation and expression of NGF and BDNF in Schwann cells.

2. Results

2.1. *GRb1 and GRg1 promoted proliferation of Schwann cells*

To investigate the effects of GRb1 and GRg1 on proliferation of Schwann cells, we counted the number of Schwann cells after various drug treatments (Fig. 1A–H). Compared with control cells (without GRb1 or GRg1), both GRb1 and GRg1 significantly promoted proliferation of Schwann cells (Fig. 1A, B, C, D, G, H). The concentration at which GRb1 and GRg1 elicited 50% of the maximal proliferation was 31.2 μM and 14.6 μM, respectively. To investigate the possible involvement of PKA pathway in the beneficial effects of GRb1 and GRg1 on proliferation of Schwann cells, Schwann cells incubated with GRb1 (45 μM) or GRg1 (45 μM) were pre-incubated with or without 10 μM H89 (a PKA inhibitor). We found that pre-incubation with H89 almost abolish the promotive effects of GRb1 and GRg1 on proliferation of Schwann cells (Fig. 1E, F, G, H). These results indicate that PKA pathway was involved in the increased proliferation of Schwann cells by GRb1 and GRg1.

2.2. *GRb1 and GRg1 increased cAMP level and PKA activity*

To further investigate the possible activation of PKA pathway by GRb1 and GRg1, the intracellular cAMP level and PKA activity were determined in each group in the present study. We found that both GRb1 and GRg1 were capable of increasing intracellular cAMP level and PKA activity (Fig. 2). After preincubation with 10 μM H89, the intracellular cAMP level and PKA activity in control cells were in the similar range to that without H89 treatment. The intracellular cAMP level and PKA activity in cells treated with GRb1 (45 μM) and GRg1 (45 μM) significantly decreased to baseline level after incubation with H89, confirming activation of PKA pathway by GRb1 and GRg1.

2.3. *GRb1 and GRg1 increased the protein levels of β-NGF and BDNF*

The effects of GRb1 and GRg1 on expression of β-NGF and BDNF were investigated in the present study. We found that GRb1 and GRg1 significantly increased the protein levels of β–NGF and BDNF in Schwann cells (GRb1, Fig. 3; GRg1, Fig. 4). The concentration at which GRb1 and GRg1 elicited 50% of the maximal expression of β-NGF was 19.4 μM and 16.7 μM, respectively. The concentration at which GRb1 and GRg1 elicited 50% of the maximal expression of BDNF was 28.2 μM and 23.5 μM, respectively. Pretreatment with 10 μM H89 showed little effect on the expression of NGF and BDNF in control cells. The protein levels of β-NGF and BDNF in cells treated with GRb1 (45 μM) and GRg1 (45 μM) were significantly decreased by preincubation with H89 (GRb1, Fig. 3; GRg1, Fig. 4), suggesting that upregulation of β–NGF and BDNF by GRb1 and GRg1 occurs mainly through the PKA pathway.

2.4. *GRb1 increased secretion of NGF and BDNF*

The effects of GRb1 and GRg1 on secretion of NGF and BDNF were investigated by an ELISA method. We found that GRb1 and GRg1 significantly increased the release of NGF and BDNF from cultured Schwann cells (Fig. 5). The concentration at which GRb1 and GRg1 elicited 50% of the maximal release of NGF was 18.4 μM and 22.3 μM, respectively. The concentration at which GRb1 and GRg1 elicited 50% of the maximal release of BDNF was 13.5 μM and 26.2 μM, respectively. Pretreatment with 10 μM H89 showed little effect on the release of NGF and BDNF in control cells (Fig. 5). The increased release of NGF and BDNF induced by GRb1 and GRg1 was significantly inhibited by preincubation with H89 (Fig. 5).

3. Discussion

The present study revealed a positive effect of GRb1 and GRg1 on cultured Schwann cells. GRb1 and GRg1 were able to

Download English Version:

https://daneshyari.com/en/article/4326405

Download Persian Version:

https://daneshyari.com/article/4326405

Daneshyari.com