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Ginsenosides Rg1 and Rb1 enhance glutamate release through activation of protein kinase A in rat cerebrocortical nerve terminals (synaptosomes)

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Abstract

We examined the effect of ginsenoside Rg1 or Rb1, the active ingredients of ginseng, on the release of endogenous glutamate from glutamatergic nerve terminals purified from rat cerebral cortex. Result showed that the Ca^{2+}-dependent release of glutamate evoked by 4-aminopyridine was facilitated by ginsenoside Rg1 or Rb1 in a concentration-dependent manner. Sequential experiments reveal that ginsenoside Rg1 or Rb1-mediated facilitation of glutamate release (i) results from an enhancement of vesicular exocytosis; (ii) is not due to an alternation of synaptosomal excitability; (iii) is associated with an increase in Ca^{2+} influx through presynaptic N- and P/Q-type voltage-dependent Ca^{2+} channels; (iv) appears to involve a protein kinase A pathway. These results conclude that ginsenoside Rg1 or Rb1 exerts their presynaptic facilitatory effect, likely through the activation of protein kinase A, which subsequently enhances Ca^{2+} entry to cause an increase in evoked glutamate release from rat cortical synaptosomes. This finding might provide important information regarding the action of ginseng in the central nervous system.

Keywords: Ginsenoside; Glutamate exocytosis; Voltage-dependent Ca^{2+} channel; Protein kinase A; Cerebrocortical synaptosomes

1. Introduction

Natural products and/or their synthetically developed active components have been used in medicine to prevent and treat a variety of disorders. Ginseng is one of the most commonly used natural products with a number of pharmacological effects (Gillis, 1997). Many pharmacological actions of ginseng are attributed to its active ingredient ginsenoside. Within more than 30 different ginsenosides, Rg1 and Rb1 have been shown to have beneficial effects on the central nervous system, especially cognitive function like learning and memory (Attele et al., 1999; Radad et al., 2004). It has been demonstrated, for example, that ginsenoside Rg1 or Rb1 administration is able to increase the performance in different animal models of learning/memory, such as passive avoidance and Morris water maze tasks (Benishin et al., 1991; Yamaguchi et al., 1995; Mook-Jung et al., 2001).

Although the cellular and molecular mechanisms that underlie the effect of ginsenosides on memory are not understood fully, studies in the literature suggest that the improving effect of ginsenosides on cognitive function could be related to an enhancement of central cholinergic function (Zhang et al., 1990; Benishin, 1992). Apart from facilitating central cholinergic neurotransmission, however, the increase of other neurotransmitter systems relating to cognitive function may be possibly involved in the beneficial effect of ginsenosides on learning and memory. For instance, glutamate, an excitatory neurotransmitter that exists in very high concentration in the mammalian brain, plays a crucial role in cognitive processing such as maintaining learning and memory functions (Izquierdo and Medina, 1997). There is evidence that the central glutamatergic transmission declines with age and that this decrease is associated with cognitive disturbances related to senility beyond its physiological role (Lipton and Rosenberg, 1994; Farber et al., 1998). Based on the above-studies, the memory improving effect of ginsenosides might be correlated with the regulation of brain glutamatergic neurotransmission. Indeed, data from electrophysiological experiments have shown that ginsenoside Rg1 can

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induce long-term potentiation (LTP) (Wang and Zhang, 2001), a physiological correlation of synaptic plasticity that is thought to underlie learning and memory (Bliss and Collingridge, 1993).

In the central nervous system, the regulation of glutamate release from the presynaptic neurons is one of the main mechanisms for altering synaptic efficiency that are considered to be necessary for learning and memory. Indeed, an increase of presynaptic glutamate release has been coupled with formation of several certain forms of cognitive function (Bekkers and Stevens, 1990; Reid et al., 2004). That is, there is a possibility that the presynaptic enhancement of central glutamate function is involved in the enhancing effects of ginsenoside on cognition and memory. To elucidate this possibility, we used isolated nerve terminals (synaptosomes) prepared from the rat cerebral cortex to investigate the effect of ginsenoside Rg1 or Rb1 on the release of glutamate. The synaptosome preparation provides a useful system for analysing the presynaptic action of ginsenosides on glutamatergic transmission given that synaptosomes, which are capable of accumulating, storing, and releasing neurotransmitters, do not contain intact neuronal circuits and thus are free of indirect effects mediated by functional glial or postsynaptic neurons, as what occur in more intact preparations, such as brain slices (Nicholls, 1993). Using an established method for looking at endogenous glutamate release, we found that ginsenoside Rg1 or Rb1 markedly facilitates the depolarization-evoked glutamate release from cerebrocortical synaptosomes. Also, this release facilitation seems do be mediated by positively modulating voltage-dependent Ca^{2+} channel activation through a signaling cascade involving protein kinase A.

2. Materials and methods

2.1. Materials

3′,3′,3′-dipropylthiadicarbocyanine iodide ($DiSC_3(5)$) and Fura-2-acetoxymethyl ester (Fura-2-AM) were obtained from Molecular Probes (Eugene, or, U.S.A.). Bafilomycin A1, and ω-conotoxin MVIIC (ω-CgTX MVIIC) were obtained from Tocris Cookson (Bristol, U.S.A.). Cyclic 3, 5-(hydrogenphosphorothioate) triethylammonium (Sp-cAMPS), *N*-[2-(p-bromocinnamylamino)-ethyl]-5-isoquinolinesulfonamide dihydrochloride (H89), and all other reagents were obtained from Sigma (Poole, U.K.) or Merck (Poole, U.K.).

2.2. Isolation of synaptosomes from rat cerebral cortex

All experiments were carried out in accordance with the guidelines established by the Fu Jen Institutional Animal Care and Utilization Committee. Synaptosomes were purified by discontinuous Percoll gradients as described previously (Nicholls et al., 1987). The cerebral cortex from male Sprague–Dawley rats (2–3 months) was isolated and homogenized in a medium containing 0.32 M sucrose, pH 7.4. The homogenate was centrifuged at 3000 g for 2 min at 4 °C. The supernatant fraction was collected and centrifuged at 14,500 g for 12 min. The resulting pellet was resuspended in 8 ml of 0.32 M sucrose, pH 7.4. 2 ml of this synaptosomal suspension was loaded onto discontinuous gradients consisting of three 2 ml layers of filtered Percoll density gradient medium (23%, 10%, and 3%) in 0.32 M sucrose containing 0.25 mM dithiothreitol and 1 mM ethylenediaminetetraacetic acid, pH 7.4. The gradients were centrifuged at 32,500 g for 7 min at 4 °C. The synaptosomal fraction was collected from the 23%/10% Percoll interface and diluted in a volume of 30 ml of HEPES buffer medium consisting of 140 mM NaCl, 5 mM KCl, 5 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 1 mM $MgCl_2$, 10 mM glucose and 10 mM HEPES (pH 7.4). The pellets were centrifuged at 27,000 g for 10 min and resuspended in the appropriate HEPES buffer medium to remove Percoll. The protein concentration of the synaptosomal preparation was determined by the method of Bradford using bovine serum albumin as a standard. 0.5 mg of the synaptosomal suspension was diluted in 10 ml of HEPES buffer medium and centrifuged at 3000 g for 10 min. The supernatants were discarded and the pellets containing the synaptosomes were stored on ice and used within 4–6 h.

2.3. Measurement of glutamate release

Glutamate release was assayed by on-line fluorimetry as described previously (Nicholls et al., 1987). Synaptosomal pellets (0.5 mg/ml) were resuspended in 2 ml of HEPES buffer medium containing 16 μM bovine serum albumin and incubated in a stirred and thermostated cuvette maintained at 37 °C in a Perkin-Elmer LS-50B spectrofluorimeter (Beaconsfield, U.K.). $NADP^+$ (2 mM), glutamate dehydrogenase (50 units/ml) and $CaCl_2$ (1 mM) were added after 3 min. After a further 5 min of incubation, 4AP (1 mM), or KCl (15 mM) was added to stimulate glutamate release. The oxidative decarboxylation of released glutamate, leading to the reduction of $NADP^+$, was monitored by measuring NADPH fluorescence at excitation and emission wavelengths of 340 nm and 460 nm, respectively. Data points were obtained at 2.2-s intervals. A standard of exogenous glutamate (5 nmol) was added at the end of each experiment and the fluorescence change produced by the standard administration was used to calculate the released glutamate as nmol glutamate/mg synaptosomal protein. Release traces are shifted vertically to align the point of depolarization as zero release. Unless otherwise indicated, release values quoted in the text are levels attained at steady-state after 5 min of depolarization (nmol/mg/5 min).

2.4. Cytosolic Ca^{2+} measurement

Synaptosomes (0.5 mg/ml) were preincubated in HEPES buffer medium with 16 μM bovine serum albumin (BSA) in the presence of 5 μM Fura-2-acetoxymethyl ester and 0.1 mM $CaCl_2$ for 30 min at 37 °C in a stirred test tube. After Fura-2-AM loading, synaptosomes were centrifuged in a microcentrifuge for 30 s at 3000 g. The synaptosomal pellets were resuspended in HEPES buffer medium with bovine serum albumin and the synaptosomal suspension stirred in a thermostatted cuvette in a Perkin-Elmer LS-50B spectrofluorimeter. $CaCl_2$ (1 mM) was added after 3 min and further additions were made after an additional 5 min, as described in the legends of the figures. Fluorescence data were accumulated at excitation wavelengths of 340 nm and 380 nm (emission wavelength 505 nm) at data