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Institute of Experimental Medicine and Intercosmos Council
Hungarian Academy of Sciences
H-1450 Budapest, P.O.B. 67
Hungary

NÁNDOR LUDVIG*
LÁSZLÓ G. HÁRSING
JÁNOS HIDEG
E. SYLVESTER VIZI

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* To whom all correspondence should be sent.

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Ginseng saponin treatment does not alter brain or pituitary levels of beta-endorphin and dynorphin

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The root of *Panax ginseng* has been used extensively in oriental communities as a vitalizing agent to counteract the deterioration of bodily functions accompanying old age. Scientific investigations on the pharmacology and biochemistry of ginseng have begun only in recent years. More than twelve ginseng saponins have been isolated [1–4], and these compounds have been shown to have a variety of metabolic actions ranging from stimulation of protein and RNA synthesis in the liver [5] to increase of cAMP level in the adrenal gland [6] and stimulation of ACTH release from the pituitary [7]. In addition to these biochemical changes, administration of ginseng to experimental animals can induce a number of physiological effects such as prevention of body temperature fluctuation after hot and cold exposure [8], improved exercise rates and prolonged swimming times [9], and reduction in radiation damage [10].

Although the experimental evidence gathered so far has provided some support for the pharmacological action of ginseng, the scientific establishment of how it can mediate all the actions claimed, no doubt, requires more detailed and careful studies. The opioid peptides have been shown to be involved in a number of neurological functions; the most noteworthy of which are pain and behaviors. Since an increase in stress tolerance is frequently claimed after intake of ginseng [9], an augmented release of the opioid peptides may be the underlying cause. In the experiment reported in this study, we examined the effect of ginseng administration on the levels of β-endorphin and dynorphin A in the hypothalamus and the pituitary gland.

Materials and methods

A ginsenoside (ginseng saponin) fraction, prepared according to the method of Sanada *et al.* [3] as modified by Yeung *et al.* [11] was used in this study. Briefly, pulverized lateral roots were extracted three times with hot methanol (2 ml/g) and after evaporation of the solvent under reduced pressure, the residue was suspended in water and washed with ether. The aqueous fraction was then extracted with *n*-butanol saturated with water. The *n*-butanol layer was concentrated *in vacuo* to give the ginsenoside fraction with a yield of 11%. The potency of this preparation was independently confirmed by its suppressive action on delayed-type of hypersensitivity [11]. Male Sprague–Dawley rats weighing between 160 and 200 g were used in this study. Animals were housed in a temperature-controlled room at 25° with 12 hr of artificial light starting from 0600 hr. Food and water were freely accessible. Treatment with ginseng was initiated after a stabilization period of three days. Rats were injected intraperitoneally in the morning with either a 1 ml saline solution or ginseng extract dissolved in the same volume of saline. The dose used was 3 mg/kg body weight per day for three consecutive days. No adverse reaction was noticed after ginseng administration. Rats were sacrificed by decapitation 24 hr after the last injection. Their brains and pituitary glands were quickly removed, chilled and dissected on ice. Extraction of opioid peptides was carried out immediately afterwards according to the procedure of Rossier *et al.* [12]. Briefly, the tissue was first incubated in 1 N acetic acid (10 ml for hypo-

thalamus and 5 ml for pituitary glands) at 95° for 15 min. After cooling in an ice-bath, the tissue was homogenized and the homogenate obtained was centrifuged at 12,000 rpm for 30 min at 4°. The supernatant was removed and subsequently lyophilized. The β-endorphin and dynorphin A contained in the samples were separated by HPLC in a Vydac C-18 reverse phase column (4 mm × 250 mm). Elution was carried out over a 60 min period using the following conditions: from zero to 10 min with 5 mM trifluoroacetic acid (TFA), from 10 to 50 min with a linear gradient of acetonitrile (0–50%) in 5 mM TFA, and from 50 to 60 min with 50% acetonitrile in 5 mM TFA. Flow rate was maintained at 1 ml/min and 2 ml fractions were collected. According to calibration with standard peptides, β-endorphin, dynorphin A and [Met]-enkephalin were eluted at 39%, 31% and 27% acetonitrile, respectively. Fractions corresponding to the positions where β-endorphin and dynorphin A came out were pooled and lyophilized. The β-endorphin and dynorphin A content in these fractions was then radioimmunoassayed by procedures essentially identical to those described by Guillemin *et al.* [13] and Ghazarossian *et al.* [14]. The β-endorphin antiserum (RB100, kindly provided by Dr. N. Ling, Salk Institute) used crossreacts completely with β-lipotropin but not with the enkephalins, ACTH, dynorphin A or dynorphin B. The dynorphin A antiserum (R3C, kindly provided by Dr. A. Goldstein, Addiction Research Foundation) shows no crossreactivity with β-endorphin, the enkephalins, ACTH, dynorphin-(1–8) and dynorphin B. However, it crossreacts completely with the 1–13 fragment of dynorphin A.

Results and discussions

Rats weighing 160–200 g were treated by intraperitoneal injection with a ginsenoside fraction of ginseng (3 mg/kg) for three days. Twenty-four hours after the last injection the rats were decapitated and the levels of β-endorphin and dynorphin A in the hypothalami and pituitary glands were measured. In contrast to our expectation, the level of β-endorphin and dynorphin A in the hypothalami and pituitary glands of animals treated with ginseng was not significantly different from those injected with saline (Table 1). Although the mean dynorphin level in the pituitary glands of animals treated with ginseng was twice that of the control, because of the large standard error associated with these determinations the level of significance was very low ($P > 0.2$). In view of these results, we conclude that the treatment of ginseng has no effect whatsoever on the level of these peptides in both the hypothalamus and the pituitary gland.

The antisera used in the present study have been well characterized and were used in a number of independent studies [12–15]. Although the anti-serum against β-endorphin crossreacts 100% with β-lipotropin, the immunological activities that we have measured were unlikely to be due to this peptide since all our samples were prepurified by HPLC for β-endorphin. In the case of dynorphin A, due to the crossreactivity of the antiserum, we were not able to differentiate it from its partially degraded fragments (e.g. dynorphin 1–13). Moreover, since our HPLC procedure was not refined enough to completely separate dynorphin A from dynorphin-(1–13) the present results should be interpreted with these limitations in mind. Compared with values published in the literature, the β-endorphin level of our control animals is within the same range as those reported. On the contrary, the level of dynorphin A that we obtained is only one tenth of the value reported by Goldstein and Ghazarossian [15]. This discrepancy may be accounted for by at least two reasons. First, dynorphin is known to be very "sticky" [16] and since we have used a number of steps to prepurify our samples before radioimmunoassay, a proportionally higher adsorptive loss may result. According to calibration with standard peptide this loss was in the region of 20–30%. Second, both Seizinger *et al.* [17] and Goldstein and Ghazarossian [15] have observed that immunoreactive dynorphin in the pituitary gland does not exclusively consist of dynorphin A. Thus, the value reported by Goldstein and Ghazarossian [15] may be higher than expected since their samples were total extracts of the pituitary gland. Irrespective of these arguments, since the determination of dynorphin activity in the ginseng- and saline-treated group was done in parallel, it is likely that if there was a significant difference between these groups it would have been noticed.

The role of ginseng in influencing mental activity, such as an increase in propensity to stress, has been documented [9]. Hiai *et al.* [7] have shown that after an acute administration of a large dose (70 mg/kg) of ginseng into rats, a significant elevation of plasma ACTH and corticosterone level was observed. Since the hypothalamic–pituitary–adrenal axis is known to play a crucial role in the stress response, it is not unreasonable to speculate that ginseng administration may be beneficial. Other than the ACTH/corticosterone system, the opioid peptides have been implicated in the regulation of pain, mood and behaviour [18]. Since administration of ginseng is known to counteract mental and physical stress [9], we had expected that it may also have some stimulatory action on the endorphin system. However, based on the results of the present study, we have to conclude that the established active ingredients of

Table 1. Effect of ginseng saponins on HPLC purified β-endorphin and dynorphin A immunoreactivities in the hypothalami and pituitaries of rats

Peptide	Brain area	
	Hypothalamus (fmoles/mg)	Pituitary (pmoles/mg)
β-Endorphin*		
Treated Group (N = 7)	219 ± 50	70.7 ± 16.4
Control Group (N = 7)	181 ± 34	92.4 ± 10.8
Dynorphin A*		
Treated Group (N = 7)	1.40 ± 0.38	0.029 ± 0.012
Control Group (N = 7)	1.11 ± 0.27	0.014 ± 0.005

* According to the *t*-test no significant difference between the treated and the control values was observed. Results are mean ± S.E.M. and are not adjusted for handling losses which have been estimated to be in the region of 20–30%. The concentrations of the peptides are expressed as amount per unit wet weight.

ginseng (i.e. the butanol-extractable ginsenosides) do not have any appreciable effect in changing the level of β-endorphin and dynorphin A in the hypothalamus and pituitary gland of the rat.

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Department of Biochemistry W. K. K. HO
The Chinese University of Hong Kong T. B. NG
H. W. YEUNG
Shatin, N.T.

Neurosurgical Unit H. L. WEN
Kwong Wah Hospital
Kowloon
Hong Kong

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