Vol. 3/1 (2003) 90 - 94

JOURNAL OF NATURAL REMEDIES

Panax ginseng on brain and hypothalamic 5-hydroxytrytamine during immobilization stress: and its modification by parachlorophenylalanine

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Received 20 November 2002; Accepted 25 November 2002

Abstract

Objective : The involvement of central 5-hydroxytryptamine (5-HT) in the anti-stress effects of *Panax ginseng* was studied in immobilized rats after blockade of 5-HT synthesis with parachlorophenylanine (PCPA). Materials and methods : Adult male Wistar rats were pretreated with PCPA (100 mg/kg, i.p.) once daily for 3 days. *Panax ginseng* (50 mg/kg, i.p.) was administered 30 min before the rats were subjected to immobilization stress (IS) for 1 h. The levels of 5-HT in brain and hypothalamus, and plasma corticosterone, were measured spectrofluorometrically. Results : IS for 1 h resulted in enhanced 5-HT levels in brain and hypothalamus and increase in plasma corticosterone. Administration of PCPA reduced all the above parameters. *Panax ginseng* significantly attenuated IS-induced elevations in the above parameters when either given alone or when co-administered with PCPA. Conclusions : These data suggest that anti-stress effect of *Panax ginseng* may be modulated by central 5-HT-ergic system.

Key words: Immobilization, stress, brain, 5-HT, *Panax ginseng*, corticosterone.

1. Introduction

Panax ginseng (PG) is a well-known traditional medicine both in the western and the eastern world, including India. Medicinal properties have been attributed to the plant in several systems of medicine. The active constituents of ginseng are known as ginsenosides. Twenty eight ginsenosides have been identified [1, 2]. Investigations on PG indicate that the plant has significant anxiolytic [3], immunostimulant [4], anti-ulcerogenic [5], anabolic [6], anti-carcinogenic [7] and anti-diabetic [8] activities. It has been suggested that the basic effect in ginseng action is its capacity to increase non-specific resistance of the organism to various untoward influences [1].

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In view of wide range of pharmacological actions exhibited by PG, search for a likely common modality led to the investigation of its anti-stress action. But literature about its specific effect on central monoamines particularly during stress is scanty.

One of the major phenomena related to stress response is the activation of the hypothalamo-hypophyseal-adrenocortical (HHA) axis. A variety of stimuli produce an overall increase in adrenocorticotrophic hormone (ACTH) and corticosterone secretion [9]. The central 5-HT neuronal system may influence the activity of the HHA axis via direct action upon CRH synthesizing neurons during stress [10].

Immobilization stress (IS) produces typical non-specific manifestations in animals, and it affects the steady state concentrations and turnovers of all established as well as putative neuro-transmitters, along with hormonal changes [11].

Earlier it has been reported that, IS elevated 5-HT and norepinephrine in brain and hypothalamus in rats. It was also reported that IS induced accentuation of these parameters have been negatively modified by PG [12,13]. These findings are consistent with anti-stress effect of PG. But its actual mechanism of action as an anti-stress agent remains unclear. The present investigation was conducted to study the involvement of central 5-HT-ergic neuronal system during stress and also its negation by PG.

2. Materials and methods

2.1 Animals

Male Wistar rats, 150±10 g, kept in colony cages under identical housing conditions were used for this study. Animals were divided into groups of 9 for estimation of whole brain 5-HT or estimation of hypothalamic 5-HT. Plasma corticosterone was measured in all animals.

2.2 Drugs and reagents

5-hydroxytryptamine (5-HT), parachloro-phenylalanine (PCPA) and corticosterone were purchased from Sigma Chemical Co. (St. Louis, MO. USA). *Panax ginseng* (PG) was a gift from Biological E Limited (India). All other reagents were of analytical grade.

2.3 Experimental procedures

Rats were divided into several groups. 5-HT synthesis inhibitor, PCPA was intraperitoneally administered as pretreatment at the dose of 100 mg/kg once daily for 3 days. The water extract of the root of PG (50 mg/kg) was given intraperitoneally 30 min before the animals were subjected to IS. Stress was induced by immobilizing the animals as described elsewhere [12,14] for 1 h under identical ambient temperature (25°C) at a fixed time (12-13 h) of the day.

2.4 Analysis of 5-HT in brain and hypothalamus

Whole brain and hypothalamic content of 5-HT were estimated by the modified spectrofluorometric method [15]. The reading of 5-HT was taken at 495 nm using activation at 385 nm in SPEKOL 11 with fluorometric attachment (Carl Zeiss, Germany). The 5-HT content was calculated by comparison with internal standards, and expressed as μg/g of wet tissue. Hypothalamii of three animals were pooled to estimate 5-HT in hypothalmus.

2.5 Analysis of plasma corticosterone

The spectrophotofluorometric method of Mattingly (1962) was followed for estimation of plasma corticosterone [16]. The readings were taken at 530 nm with activation at 470 nm. The concentration was expressed as μg/100 ml of blood.

2.6 Statistical analysis

Results were expressed as mean ± SEM and analysed by Student's unpaired *t* - test.

A probability value of $P < 0.05$ was accepted as being statistically significant.

3. Results

3.1 Whole brain and hypothalamic 5-HT

IS significantly elevated both whole brain and hypothalamic 5-HT in rats, whereas pretreatment with PG *per se* did not change this in comparison to control group. However, pretreatment with PCPA *per se* reduced the concentration of brain and hypothalamic content of 5-HT (Table 1).

PG significantly reduced elevations of 5-HT in brain and hypothalamus by IS (Table 2). Similarly, PCPA diminished IS-induced enhancement of 5-HT. Pretreatment with PCPA significantly further accentuated PG-induced antagonism of IS-induced elevation of 5-HT in both rat brain and hypothalamus (Table 2).

3.2 Plasma corticosterone

Plasma corticosterone level was enhanced by IS which corroborates stress induced HHA axis activation. PG *per se* did not alter, but PCPA pretreatment reduced the concentration of plasma corticosterone when compared to control (Table 1). Both PG and PCPA individually attenuated elevations of plasma corticosterone by IS. PCPA pretreatment negated plasma corticosterone when compared to PG treated IS group (Table 2).

4. Discussion

Forced immobilization stress (IS) is one of the best explored models of stress in rats. Painful stimuli are not involved in IS and it is more akin to physiological stress as it combines emotional stress (escape reaction) and physical stress (muscle work) resulting in both restricted motility and aggression [11,14]. IS for 1 h enhanced central 5-HT and increased the level of corticosterone in plasma. It has been reported that ascending 5-HT neurons from raphe nuclei innervate hypothalamic and limbic sites and have an overall role in the secretion of CRH/ACTH during stress [17].

In the present study, PG attenuated stress-induced elevation of brain and hypothalamic 5-HT and diminished plasma corticosterone level, which are consistent with its anti-stress properties. With the purpose of determining the central regulation of 5-HT secretion following IS, we have used PCPA, the most frequently used 5-HT synthesis inhibiting drug. PCPA inhibits tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of 5-HT [18].

Table 1.
Effects of immobilization stress (IS), *Panax ginseng* (PG), and parachlorophenylalanine (PCPA) on whole brain and hypothalamic 5-HT and plasma corticosterone in rats. (Data represents mean ± SEM)

Groups	Whole brain 5-HT (μg/g of wet tissue) n=9	Hypothalamic 5-HT (μg/g of wet tissue) n=9	Plasma corticosterone (μg/100 ml of blood) n=18
Control	0.85 ± 0.04	1.73 ± 0.18	32.10 ± 2.22
IS	2.75 ± 0.10[a]	3.30 ± 0.15[a]	71.32 ± 2.40[a]
PG	0.84 ± 0.05[b]	1.82 ± 0.12[b]	30.18 ± 3.46[b]
PCPA	0.56 ± 0.02[b]	1.16 ± 0.15[b]	25.14 ± 1.76[b]

[a] $P<0.01$ as compared with control group; [b] $P<0.01$ as compared with IS group

Table 2.
Effects of *Panax ginseng* (PG) on Immobilization stress (IS) induced enhancement of brain and hypothalamic 5-HT and plasma corticosterone and accentuated of this effect by parachlorophenylalanine (PCPA). (Data represents mean ± SEM)

Groups	Whole brain 5-HT (μg/g of wet tissue) n=9	Hypothalamic 5-HT (μg/g of wet tissue) n=9	Plasma corticosterone (μg/100 ml of blood) n=18
IS	2.75 ± 0.10[a]	3.30 ± 0.15[a]	71.32 ± 2.40[a]
PG + IS	1.96 ± 0.07[a]	2.20 ± 0.16[a]	57.18 ± 1.94[a]
PCPA + IS	0.88 ± 0.04[a]	1.58 ± 0.04[a]	45.16 ± 3.86[a]
PCPA + PG + IS	0.76 ± 0.08[a,b]	0.89 ± 0.12[a,b]	37.52 ± 2.16[a,b]

[a] P<0.01 as compared with IS group; [b] P<0.01 as compared with PG + IS group

Prior treatment with PCPA caused a significant depletion in brain and hypothalamic 5-HT. It also significantly reduced the IS-induced elevation of brain and hypothalamic 5-HT, and the reduction was so severe that reduction of 5-HT concentration was below the normal levels. The rise in plasma corticosterone after IS was also inhibited concurrently by PCPA. PG induced inhibition of elevation of brain and hypothalamic 5-HT after IS, was further inhibited on pretreatment of the rats with PCPA.

The present experiment clearly indicates that 5-HT may play a role as mediator in the activation of HHA axis during IS, and anti-stress activities of *Panax ginseng* may be modulated by central 5-HT-ergic system.

5. Acknowledgement

This work was supported by grant from Indian Council of Medical Research (ICMR), Government of India, New Delhi.

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