

Effects of ginseng root saponins on brain monoamines and serum corticosterone in heat-stressed mice¹

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ABSTRACT The rectal temperature and serum corticosterone increased in mice exposed to 45°C for 15 min; at the same time, the contents of brain 5-HT and NE reduced, brain DA unchanged. Ginseng root saponins (GRS) ip 200 mg/kg inhibited the increase of serum corticosterone and the decrease of brain 5-HT and NE in heat-stressed mice, but did not affect brain DA. GRS lowered mice body temperature at room temperature and inhibited the rise of body temperature under heat environmental conditions in mice. Reserpine eliminated the hypothermia of GRS at room temperature and its inhibitory effect on hyperthermia under heat-stress conditions. PCPA eliminated only the inhibition of GRS on hyperthermia under heat-stress, but had no significant effect on hypothermia at room temperature.

KEY WORDS ginseng; saponins; heating; stress; body temperature; serotonin; norepinephrine; dopamine; corticosterone; reserpine; *p*-chlorophenylalanine

The extracts of ginseng showed anti-heat-stress effects⁽¹⁾. Ginseng saponins also exhibited such effects and the effects were thought to be related to the changes of brain ACh⁽²⁾. In this paper the effects of ginseng root saponins (GRS) on brain monoamines and serum corticosterone level in heat-stressed mice were investigated further.

MATERIALS AND METHODS

Animal treatment Albino Kunming

mice, ♂ and ♀, body weight $21 \pm SD$ 1 g, were housed 10 per cage and handled regularly. Food and water were available *ad lib*. The experiments were performed at 8-10 AM in order to reduce the circadian changes. Naive, unstressed mice were used as the nonstress control group. Mice undergoing 15 min of 45°C exposure were the stress control group. Mice pretreated with GRS (ip) for 15 min, with reserpine (iv or ip) for 2 h, and with PCPA (ip) for 3 d before undergoing heat stress, were the GRS, reserpine, and PCPA groups respectively.

Drugs and reagents GRS, a yellowish brown powder, was extracted⁽³⁾ from ginseng cultivated 6 yr in Tonghua area of Jilin province. 11 spots of saponins were shown on the silica gel thin-layer plate. GRS was kindly provided by the Department of Phytochemistry of the Shenyang College of Pharmacy. The dosage of GRS used in the following experiments was calculated according to the saponin content. 5-Hydroxytryptamine creatinine sulphate (5-HT, Switzerland), norepinephrine (NE) and corticosterone (Sigma), dopamine hydrochloride (DA, National Institute for the Control of Pharmaceutical and Biological Products), reserpine (RP, the Shenyang First Pharmaceutical Factory), *p*-chlorophenylalanine (PCPA, Serva). Deionized water was used throughout the experiments.

Stress Mice were exposed to an environmental temperature of 45°C for 15 min except the nonstressed controls which were kept in the laboratory at 20°C.

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Measurement of body temperature

Each temperature measurement took approximately 20 s. Rectal temperature readings were taken for 20 s before and 15 min after GRS injection, and immediately after heat stress, respectively.

Determination of 5-HT, NE, DA, and corticosterone All mice were killed by decapitation immediately after the heat stress. The heads were placed into liquid nitrogen rapidly for at least 2 min, then the brains were removed, homogenized, and measured the contents of 5-HT, NE, and DA in brain by spectrofluorometric methods^(4,5). Blood was collected from the trunks of decapitated mice into centrifuge tubes for determination of serum corticosterone^(6,7).

RESULTS

Effect of GRS on rectal temperature

Body temperature increased when the mice were exposed to 45°C for 15 min. GRS ip 100 and 200 mg/kg lowered body temperature in dose-dependent manner in non-stressed mice ($P < 0.01$), and decreased body temperature in heat-stressed mice only after larger dose (200 mg/kg) was given ($P < 0.01$, Tab 1 and 2).

Effect of GRS on brain 5-HT

The contents of brain 5-HT in mice was reduced significantly ($P < 0.01$) after exposure

to heat stress (45°C, 15 min), compared with the nonstressed control. The reduction of brain 5-HT content by heat stress could be almost eliminated by ip GRS 200 mg/kg 15 min before stress ($P < 0.05$, Tab 1).

Effect of GRS on brain NE and DA

Heat stress caused a significant decrease in brain NE content ($P < 0.01$) and a non-significant reduction of the brain DA ($P > 0.05$). 30 min after ip GRS 200 mg/kg, the brain NE content significantly increased ($P < 0.01$) and the brain DA had an apparent but non-significant rise ($P > 0.05$, Tab 1).

Effect of GRS on serum corticosterone

Serum corticosterone concentration increased in mice exposed to 45°C for 10 min ($P < 0.05$) or 15 min ($P < 0.01$). Under heat stress conditions for 10 min, corticosterone of the stress control group ($2.03 \pm 0.67 \mu\text{mol/L}$, $n = 9$) was higher than that of nonstress control group ($1.44 \pm 0.21 \mu\text{mol/L}$, $n = 9$, $P < 0.05$); corticosterone of GRS (200 mg/kg) group ($1.84 \pm 0.38 \mu\text{mol/L}$, $n = 11$) had a non-significant decrease ($P > 0.05$) compared with stress control group, and it was higher than that of nonstress control group ($P < 0.05$). Under heat stress conditions for 15 min, the results were obtained as shown in Tab 1. Corticosterone of stress control groups

Tab 1. Effects of GRS on rectal temperature, brain 5-hydroxytryptamine (5-HT), norepinephrine (NE), dopamine (DA), and serum corticosterone in heat-stressed mice. Number of mice in parentheses. $\bar{x} \pm \text{SD}$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs non-stress. † $P > 0.05$, †† $P < 0.05$, ††† $P < 0.01$ vs heat-stress NS.

	Non-stress (20°C)		Heat-stress (45°C) 15 min	
	NS	NS	GRS (mg/kg)	
			100	200
Rectal temperature (°C)	$37.7 \pm 0.5(10)$	$40.1 \pm 0.7(10)^{***}$	$40.3 \pm 0.5(10)^{***\dagger}$	$39.4 \pm 0.5(10)^{***\dagger\dagger}$
5-HT (ng/g)	$610 \pm 60(7)$	$440 \pm 10(7)^{***}$	$480 \pm 24(7)^{***\dagger}$	$590 \pm 50(7)^{*\dagger\dagger}$
NE (ng/g)	$661 \pm 74(12)$	$520 \pm 48(12)^{***}$	—	$606 \pm 70(12)^{*\dagger\dagger}$
DA (ng/g)	$710 \pm 113(8)$	$680 \pm 157(8)^*$	—	$787 \pm 143(8)^*\dagger$
Corticosterone ($\mu\text{mol/L}$)	$1.24 \pm 0.37(15)$	$2.13 \pm 0.40(18)^{***}$	—	$1.80 \pm 0.25(23)^{***\dagger\dagger}$
	$1.44 \pm 0.21(9)$	$2.51 \pm 0.45(11)^{***}$	$1.90 \pm 0.29(12)^{***\dagger\dagger}$	—

increased significantly ($P < 0.01$). GRS ip 100 and 200 mg/kg reduced significantly the serum corticosterone in heat-stressed mice ($P < 0.01$). However, the corticosterone levels of GRS groups were all higher than that of the nonstress control groups ($P < 0.01$, Tab 1).

Effect of reserpine on body temperature lowered by GRS Reserpine 1.0 mg/kg iv had no significant effect on body temperature at room temperature or under heat stress conditions in mice ($P > 0.05$), and it did not alter the effect of GRS on lowering the body temperature under either conditions. Reserpine 2.5 mg/kg ip had no effect on body temperature in mice both under room temperature and heat environmental conditions ($P > 0.05$). Nevertheless it did eliminate the hypothermic effect of GRS in mice at room temperature and the inhibitory effect of GRS on hyperthermia in mice under heat stress conditions ($P > 0.05$, Tab 2).

Effect of PCPA on body temperature lowered by GRS PCPA ip 300 mg/kg had no significant effect on body temperature in mice at room temperature ($P > 0.05$),

but it decreased body temperature in mice under heat stress ($P < 0.05$); and it did not affect the body temperature lowered by GRS in both conditions. PCPA ip 300 mg/kg qd \times 3 d had no effect on body temperature in either condition as mentioned above ($P > 0.05$), or on body temperature lowered by GRS in mice at room temperature; however, it did eliminate the inhibitory effect of GRS on hyperthermia in mice in heat stress ($P > 0.05$, Tab 2).

DISCUSSION

The present study has demonstrated that in heat-stress environment (45°C, 15 min), the rectal temperature was raised, the level of serum corticosterone increased, and the contents of brain 5-HT and NE decreased simultaneously in mice. Earlier reports have shown that under 40°C for 1 h brain NE decreased in mice⁽⁸⁾, at 40°C for 3 h brain 5-HT was reduced in rats pretreated with inhibitors of monoamine biosynthesis⁽⁹⁾; the reduction or depletion of brain NE or 5-HT induced by administering drugs increased the levels of serum corticosterone^(10,11). Thus it can be seen that

Tab 2. Effects of reserpine (RP) and *p*-chlorophenylalanine (PCPA) pretreatment on the action of GRS on rectal temperature in heat-stressed mice. $n = 10$, $\bar{x} \pm SD$. * $P > 0.05$, ** $P < 0.05$, *** $P < 0.01$ vs NS. † $P > 0.05$, †† $P < 0.05$, ††† $P < 0.01$ vs RP or PCPA alone.

Group	Dose (mg/kg)	Rectal temperature (°C)		
		Before GRS (20°C)	after 15 min (20°C)	after 30 min (45°C)
NS	—	37.5 ± 1.0	37.2 ± 0.8	40.8 ± 0.7
GRS	100	37.9 ± 0.5	35.7 ± 1.0***	40.4 ± 0.5*
NS	—	37.6 ± 0.6	37.6 ± 0.7	40.2 ± 0.8
GRS	200	37.7 ± 0.8	34.1 ± 1.3***	39.2 ± 0.7***
RP	1.0	37.7 ± 0.3	37.3 ± 0.9*	40.0 ± 0.7*
RP + GRS	1.0 + 200	37.4 ± 0.9	34.5 ± 0.5***†††	39.4 ± 0.3***††
RP	2.5	37.6 ± 0.4	37.1 ± 1.8*	40.5 ± 1.0*
RP + GRS	2.5 + 200	37.7 ± 0.3	36.8 ± 1.0*†	40.7 ± 0.7*†
PCPA	300	37.4 ± 1.0	37.3 ± 0.6*	39.6 ± 0.4**
PCPA + GRS	300 + 200	37.8 ± 0.9	34.3 ± 1.1***†††	39.2 ± 1.0***†
PCPA	300 \times 3	37.1 ± 1.1	37.8 ± 1.0*	40.0 ± 0.9*
PCPA + GRS	300 \times 3 + 200	37.0 ± 1.1	36.4 ± 1.1***†††	40.3 ± 0.8*†

the disturbance of central thermoregulatory functions is related to the changes in brain 5-HT and NE, and that alteration of the function of hypothalamo-pituitary-adrenal axis is regulated by brain 5-HT and NE in heat-stressed mice.

GRS exhibited inhibitory effects on the raised temperature increased serum corticosterone and decreased brain 5-HT and NE in heat-stressed mice: while reserpine and PCPA both eliminated the inhibitory effect of GRS on the raised temperature under heat stress. These data suggest that this inhibitory effect and on the increased serum corticosterone in heat-stressed mice may be related to its regulatory action on brain 5-HT and NE.

Munck⁽¹²⁾ suggests that the increase of plasma glucocorticoid in stress is to suppress the primary defense function of the body, so as to make the defense reaction not so strong that it interferes with the steadiness of homeostasis. Under the experimental conditions of the present study, not that GRS causes further increase of serum corticosterone in heat-stressed mice, but that it inhibits the increase of serum corticosterone. Such an action of GRS is a protective effect on the function of hypothalamic-adrenal system^(13,14). So GRS may regulate the defense function of body by inhibiting the increase of serum corticosterone in heat-stressed mice, and maintain the defense function at a level which is advantageous to the body against stressor. Thus the progress of heat stress process will be delayed. This is probably one of the possible mechanisms of antiheat-stress effect of GRS in mice.

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人参根总皂甙对热应激鼠脑单胺递质和血清皮质酮的影响

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提要 小鼠在 45°C 15 min, 直肠温度升高, 血清皮质酮升高, 脑 5-HT, NE 减少, DA 无变化。人参根总皂甙(GRS) ip 200 mg/kg 使热应激鼠体温下降, 皮质酮降低, 5-HT, NE 增加, 不影响 DA。利血平 ip 2.5 mg/kg 消除 GRS 在室温(20°C)下降低体温和在热应激下抑制体温升高; 对氯苯丙氨酸(PCPA) ip 300

mg/kg qd × 3 d 仅消除 GRS 在热应激下对体温升高的抑制。

关键词 人参; 皂甙; 采暖; 应激; 体温; 血清素; 去甲肾上腺素; 多巴胺; 皮质酮; 利血平; 对氯苯丙氨酸

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石杉碱甲对兔脑电图及其功率谱的影响^{1,2}

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Effects of huperzine A on electroencephalography power spectrum in rabbits^{1,2}

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ABSTRACT Huperzine A (Hup-A) is a new alkaloid extracted from *Huperzia serrata* in China. The effects of Hup-A on learning and memory are superior to those of physostigmine (Phys). The purpose of this paper is to observe the effects of Hup-A on EEG and EEG power spectrum in rabbits with micro-computer analysis.

Hup-A 0.1 mg/kg iv in conscious rabbits produced, after 0.5 min, an alert EEG pattern, which showed decreases of lower frequency components and the total

EEG power in cortical area, and the dominant frequency transferred from delta rhythm to theta rhythm in hippocampus. The same effects were seen with Phys 0.1 mg/kg. Scopolamine (Scop, 0.2 mg/kg iv) reversed significantly these effects of Hup-A (10 µg/rabbit, icv), but Scop butylbromide (0.4 mg/kg, iv) which can not pass the blood-brain barrier did not. Hup-A 0.2 mg/kg iv or Phys 0.3 mg/kg iv antagonized the EEG effects of Scop 0.3 mg/kg iv. The results indicate that the effects of Hup-A are closely related to the action on the central cholinergic system.

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KEY WORDS huperzine A; physostigmine; scopolamine; electroencephalography; psychopharmacology