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EFFECT OF *PANAX GINSENG* AND DIAZEPAM ON BRAIN 5-HYDROXYTRYPTAMINE AND ITS MODIFICATION BY DICLOFENAC IN RAT

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Abstract : Wistar male rats pretreated with anti-stress agents like, *Panax ginseng* (Pg) and diazepam (Diaz) were stressed by restraining for 1 h and 5-HT content of brain and hypothalamus as well as plasma corticosterone were measured spectrophotoflurometrically. Diclofenac (DICLO), a prostaglandin (PG) synthesis inhibitor was used to confirm the role of prostaglandin in restraint stress-induced elevation of central 5-HT correspondingly confirmed by elevation of plasma corticosterone and modification of the above anti-stress agents. Pg, Diaz and DICLO *per se* did not modify brain and hypothalamic 5-HT in control rats. But they attenuated stress-induced elevation of brain and hypothalamic 5-HT. Anti-stress action of both Pg and Diaz reflected by inhibition of stress-induced elevation of brain and hypothalamic content of 5-HT as also stress-induced concurrent elevation of plasma corticosterone were further diminished by DICLO. The mediatory action of 5-HT in anti-stress effects of Pg and Diaz may be modulated through prostaglandins.

Key words : restraint stress, 5-HT, corticosterone, diclofenac, *Panax ginseng*, prostaglandin

INTRODUCTION

The prime reaction in stress is the activation of the hypothalamo-hypophyseal-adrenocortical axis (H-H-A axis). 5-HT plays an important role in the regulation of stress-induced activation of H-H-A axis (1–3). Stress also enchances the activity of central prostaglandins (4–5). The role of PGs in stimulation of the H-H-A axis has also been proposed (5–7). It has been reported that PGs enhance 5-HT level as well as 5-HT turnover (8–9).

Stress attenuating actions of diazepam and *Panax ginseng* are well documented (10–12). Earlier studies indicated that *Panax ginseng* (Pg) and diazepam (Diaz) in a dose dependent manner attenuated restraint stress-induced elevation of brain and hypothalamic 5-HT and simultaneously diminished restraint stress-induced

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enhancement of plasma corticosterone (13–15). But their definite effect on the modification of 5-HT during stress could not be confirmed.

The purpose of this study was to examine the central action of DICLO, PG synthesis inhibitor (16) on the modifying effects of *Panax ginseng* and diazepam on 5-HTergic neurotransmission during restraint stress, measured by brain and hypothalamic 5-HT estimation with simultaneous estimation of plasma corticosterone as an index of H-H-A axis stimulation.

METHODS

Wistar strain male albino rats (120–150 g) were used. They were either segregated into groups of 10 for estimation of 5-HT or into groups of 12 for hypothalamic 5-HT, while plasma corticosterone was done in all animals. As the hypothalamii of 3 rats were pooled together to obtain the 5-HT level in hypothalamus, 12 rats were taken instead of 10 animals in groups for estimation of 5-HT in hypothalamus.

The animals were subjected to restraint stress for 1 h (RSl) as described earlier by completely immobilizing them (13–15). The anti-stress agents, Diaz (0.2 mg/kg) and Pg (50 mg/kg) were given intraperitoneally 30 min before the animals were subjected to stress. Animals were pretreated with DICLO (15 mg/kg) intraperitoneally 6 h prior to the experiment. The doses and duration of each drug were chosen based on earlier studies (14–15).

Estimation of 5-HT on whole brain and hypothalamii (pooled from 3 rats) was done spectrophotofluorometrically following the method of Amar et al (17). The measurement of 5-HT was done by reading the fluorescence at 495 nm, with activation at 385 nm (SPEKOL 11, Carl Zeis, GDR). Plasma 11-hydroxycorticosteroid was estimated fluorimetrically by the method of Mattingly (18). The readings were taken at 530 nm with activation at 470 nm.

All values were presented as mean ± SEM. Statistical comparisons were made by using Student "t" test. Statistical significance was considered as $P<0.05$.

RESULTS

Table I shows that in response to restraint stress (1h) there were elevations of 5-HT in brain (2.75 ± 0.12) and hypothalamus (3.28 ± 0.12) along with plasma corticosterone (71.28 ± 2.45), where as pretreatments with Pg, Diaz and DICLO and DICLO *per se* did not change these parameters in comparison to their respective control group.

Pg significantly reduced elevations of 5-HT in brain (1.98 ± 0.05), hypothalamus (2.24 ± 0.18) as also plasma corticosterone (58.18 ± 3.94) by RS1 are shown in Table II. Similarly, Diaz reduced RS1-induced increases of 5-HT in brain (2.02 ± 0.06), hypothalamus (2.52 ± 0.22) as also plasma corticosterone (61.85 ± 1.52) which are shown in Table II. DICLO significantly diminished RS1-induced enhancement of 5-HT in brain (1.87 ± 0.08), hypothalamus (2.42 ± 0.24) as also plasma corticosterone (49.32 ± 3.85), shown again in Table II.

TABLE I : Effect of restraint stress, *Panax ginseng*, diazepam and diclofenac on brain and hypothalamic 5-HT and plasma corticosterone.

Group	Treatment	*Whole brain 5-HT*[a] *(n=10)*	*Hypothalamic 5-HT*[a] *(n=12)*	*Plasma corticosterone*[b] (n=22)
1.	Control	0.85±0.03	1.74±0.16	32.12±2.12
2.	RS1	2.75±0.12**	3.28±0.12**	71.28±2.45**
3.	Pg	0.84±0.06	1.82±0.11	30.15±3.42
4.	Diaz	0.83±0.12	1.94±0.18	29.24±2.85
5.	DICLO	0.72±0.15	1.53±0.10	24.78±3.92

Values are expressed as Mean ± SEM
[a] = µg/g of wet tissue
[b] = µg/100 ml of blood
**P<0.001 as compared with control

TABLE II : Effect of *Panax ginseng*, diazepam on restraint stress-induced enhancement of brain and hypothalamic 5-HT and plasma corticosterone modification by diclofenac.

Group	Treatment	*Whole brain 5-HT*[a] *(n=10)*	*Hypothalamic 5-HT*[a] *(n=12)*	*Plasma corticosterone*[b] (n=22)
1.	RS1	2.75 ± 0.12	3.28 ± 0.12	71.28 ± 2.45
2.	RS1+Pg	1.98 ± 0.05**	2.24 ± 0.18**	58.18 ± 3.94**
3.	RS1+Diaz	2.02 ± 0.06**	2.52 ± 0.22*	61.85 ± 1.52*
4.	RS1+DICLO	1.87 ± 0.08**	2.42 ± 0.24*	49.32 ± 3.85**
5.	RS1+Pg+DICLO	1.54 ± 0.14*	1.57 ± 0.08*	39.68 ± 3.76**
6.	RS1+Diaz+DICLO	1.54±0.07**	1.74 ± 0.04*	45.25 ± 2.78**

Values are expressed as Mean ± SEM
[a] = µg/g of wet tissue
[b] = µg/100 ml of blood
*P<0.01; **P<0.001
Group 2, 3 & 4 are compared with Group 1; Group 5 is compared with Group 2; Group 6 is compared with Group 3

Table II also shows the effects of DICLO on the negating effect of Pg and Diaz on RS1-induced elevation of the same parameters. Significant accentuating effects of DICLO were thereby Pg antagonism of RS1-induced increases of 5-HT in brain (1.54 ± 0.14) and hypothalamus (1.57 ± 0.08)) as also plasma corticosterone (39.68 ± 3.76). Similar accentuations were seen on antagonism by Diaz on RS1-induced elevation of brain (1.54 ± 0.07) and hypothalamic (1.74 ± 0.04) 5-HT as also elevation of plasma corticosterone (45.25 ± 2.78).

DISCUSSION

5-HT has been implicated in a variety of central neuronal system-mediated processes including anxiety and stress.

Under the influence of any stressor, producing non-specific responses, changes are brought about in the concentration, synthesis, metabolism and turnover rate of 5-HT in the brain (19–20). It has been known that PGs markedly enhance rat brain 5-HT levels as well as 5-HT turnover (5, 8–9).

Restraint stress enhanced brain and hypothalamic content of 5-HT and plasma corticosterone in rat upto 1 h. Prologation of stress (more than 1 h) could not induce further enhancement in this parameter, probably due to adaptive changes (13–15). All subsequent experimentations were done with animals restrained for 1 h only, as before.

In our earlier studies, it was noted that Pg and Diaz significantly reduced RS1-induced elevation of brain and hypothalamic content of 5-HT and plasma corticosterone (index of H-H-A axis activation) dose dependently, which justifies their anti-stress action (14–15).

Pg, Diaz and DICLO *per se* did not modify the control levels of brain and hypothalamic 5-HT and plasma corticosterone, showing negligible action on basal H-H-A axis function. DICLO, the PG synthesis inhibitor significantly diminished RS1-induced enhancement of brain and hypothalamic 5-HT as also plasma corticosterone. These observations point to the involvement of PGs in stress-induced enhancement of 5-HTergic neurotransmission supporting the hypothesis that PGs modulate 5-HTerigc neurotransmission (6, 8, 13, 15) even during restraint stress. In this study significant accentuation of the inhibitory effects of Pg and Diaz on RS1-induced elevation of brain and hypothalamic 5-HT and plasma corticosterone by DICLO were noted which further justifies the above statement.

It is suggested that PGs may be involved in H-H-A axis activation during stress through the positive modulation of 5-HTergic system and PGs may also be involved in stress attenuating effect of *Panax ginseng* and diazepam.

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