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Role of Histaminergic Mechanisms in the Regulation of Some Stress Responses in Rats

S. PURI, A. RAY, A. K. CHAKRAVARTY AND P. SEN[1]

Department of Pharmacology, University College of Medical Sciences and G. T. B. Hospital, Shahdara, Delhi 110 095, India

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PURI, S., A. RAY, A. K. CHAKRAVARTY AND P. SEN. *Role of histaminergic mechanisms in the regulation of some stress responses in rats*. PHARMACOL BIOCHEM BEHAV **39**(4) 847–850, 1991.—The involvement of histaminergic mechanisms in the regulation of some stress responses was studied in rats. The brain neuronal histamine (HA) depletor, α-fluoromethyl histidine (α-FMH), at doses (50 or 100 mg/kg) which markedly lower brain HA, significantly attenuated the gastric ulcer formation and the elevation in plasma corticosterone in response to cold restraint stress (CRS). α-FMH also appreciably reduced gastric mucosal HA content. The H_1-antagonist, pheniramine (25 mg/kg), attenuated both the gastric mucosal and endocrine response to CRS, while the effects of the H_2-antagonist, cimetidine (200 mg/kg), were on the plasma corticosterone levels. These results are discussed in light of complex HA-ergic mechanisms in the maintenance of physiological homeostasis during stress.

Brain histamine Restraint stress Gastric ulcer Plasma corticosterone H_1 and H_2 receptor

COMPLEX neurochemical mechanisms regulate the organism's response to stress, and the role of several neurotransmitters is suggested (2,26). In recent years, histamine (HA) has been identified as a chemical messenger in the brain (20, 24, 26), and several physiological and pharmacological actions are shown. In the brain, the hypothalamus is densely populated with HA-ergic neuronal elements (31), and this area is particularly crucial for the organism's perception of and reaction to aversive stimuli like stress. Though preliminary reports indicate that HA is released during stress (15, 17, 18, 26, 31), the nature of HA-ergic involvement in some stress responses is not clearly defined. Physical restraint is one of the commonly used experimental methods to study stress pathology, and gastric ulcer formation and plasma corticosterone are recognized markers of stress. The present study thus investigated the role of HA-ergic mechanisms on cold restraint stress (CRS)-induced changes in gastric ulcerogenesis and plasma corticosterone in rats.

EXPERIMENT 1

Histamine is known to exist in the brain in neuronal (nerve terminals) as well as extraneuronal (mast cells) sites (13, 19, 30). α-Fluoromethyl histidine (α-FMH), a histidine decarboxylase inhibitor, specifically prevents HA biosynthesis in brain HA neurons, thereby depleting neuronal (endogenous) HA (3, 12, 14). We evaluated the effects of α-FMH on stress (CRS)-induced gastric ulcer formation and plasma corticosterone in rats.

Method

Inbred male Wistar rats (200–250 g) were used. They were housed individually in temperature (22 ± 2°C) and light (12 h dark-12 h light cycle) controlled conditions with free access to food and water. All animals were food (but not water) deprived 18 h prior to the experiment. The experiments were performed between 0900 to 1600 h. Cold restraint stress (CRS) was applied by immobilizing 18 h food-deprived rats in specially designed Plexiglas restrainers (INCO, Ambala) for 3 h at 4°C. α-FMH (50 or 100 mg/kg) was dissolved in distilled water and injected IP in a volume of 2 ml/kg, 3 h prior to the CRS procedure. Immediately after termination of the CRS procedure, blood samples were collected after light ether anesthesia from the retro-orbital plexus using the microcapillary technique. The animals were then sacrificed with an overdose of ether. The stomachs were dissected out, cut open along the greater curvature, washed in cold water and examined microscopically (× 10) under a dissecting microscope (Zeiss, GDR). The number of gastric mucosal lesions and their cumulative length (in mm, to the nearest 0.1 mm) per rat were measured. Plasma samples were assayed fluorimetrically for corticosterone using the method of Glick et al. with modifications (6,16).

A group of rats received drug or vehicle treatment as before and were then returned to their home cages (no CRS). After 6 h (i.e., 3 h pretreatment + 3 h home cage stay) each of these nonstressed rats were sacrificed with ether and their brains and stomachs removed. The brains (minus cerebella) were immediately frozen, and the mucosal surface of the stomachs was scraped off and suspended in 5% trichloroacetic acid. The homogenates of brain and gastric scrapings were assayed for their HA content by the method of Hakanson (7,8).

The results were analysed using the Mann-Whitney U-test (two-tailed). A p value of at least 0.05 was used as the level of significance in all statistical tests.

[1]Requests for reprints should be addressed to Prof. P. Sen.

TABLE 1

EFFECTS OF α-FLUOROMETHYL HISTIDINE (α-FMH) AND COLD RESTRAINT STRESS (CRS) ON GASTRIC ULCER FORMATION AND PLASMA CORTICOSTERONE LEVELS IN RATS

Treatment (mg/kg, IP)	n	Stress Parameters (Mean ± SE) Gastric Pathology Ulcer Number	Cumulative Length (mm)	Plasma Corticosterone (μg/dl)
Controls*	9	1.5 ± 0.8	0.2 ± 0.1	19.0 ± 1.3
α-FMH (50)	6	1.1 ± 0.7	0.1 ± 0.06	17.9 ± 2.0
α- FMH (100)	6	0.2 ± 0.1	0.02 ± 0.01	22.6 ± 1.9
CRS	14	8.9 ± 0.8†	2.1 ± 0.3†	35.7 ± 4.2†
α-FMH (50) + CRS	10	4.3 ± 0.5§	0.7 ± 0.2§	28.8 ± 2.4‡
α-FMH (100) + CRS	7	2.5 ± 0.3#	0.4 ± 0.09#	24.7 ± 2.0§

*No CRS (only 18 h food deprived).
†$p<0.002$ (compared to control group).
‡$p<0.05$; §$p<0.02$; #$p<0.002$ (compared to CRS group).

Results and Discussion

Cold restraint stress (CRS) consistently induced gastric mucosal lesions and elevations in plasma corticosterone levels in 18 h food-deprived rats, when compared to nonstressed controls (Table 1). α-FMH (50 or 100 mg/kg) attenuated the CRS-induced gastric ulcerogenesis. Both the number and severity of ulcers were lower as compared to vehicle-treated stressed animals. Similarly, α-FMH showed clearcut inhibitory effects on CRS-induced elevations of plasma corticosterone, when compared to the respective controls. In the nonstressed rats, α-FMH was without influence on the formation of gastric lesions or on basal plasma corticosterone levels ($p>0.05$). α-FMH is a known neuronal HA depleter in the brain. Our results with this drug clearly shows that rats with lowered brain neuronal HA are less reactive to the effects of CRS with respect to gastric mucosal damage and corticosterone response than vehicle- treated CRS controls.

Biochemical analysis showed that brain HA was significantly lower in α-FMH-treated rats when compared to vehicle-treated controls. As shown in Table 2, both doses of the drug had similar effects (reductions by 33% and 36% respectively). The gastric mucosal HA content was also lower after α-FMH pretreatment. These data indicate that both brain and gut mucosal HA content were actually reduced after α-FMH treatment. Taken together, it is suggested that: (a) HA is one of the facilitatory mediators in the brain released from neurons during stressful situations; and (b) the HA-ergic brain-gut axis is crucial in stress ulcer formation.

TABLE 2

EFFECTS OF α-FLUOROMETHYL HISTIDINE (α-FMH) ON BRAIN AND GASTRIC MUCOSAL HISTAMINE CONTENT IN RATS

Treatment (mg/kg, IP)	n	Mean Histamine Content (± SE) Brain (ng/g)	Gastric Mucosa (μg/g)
Controls	8	63.5 ± 2.6	37.0 ± 3.5
α-FMH (50)	8	42.6 ± 2.8†	25.6 ± 1.4†
α-FMH (100)	7	40.8 ± 5.0†	28.6 ± 3.0*

*$p<0.05$; †$p<0.002$ (compared to controls).
n = 6, for gastric mucosa.

EXPERIMENT 2

Earlier data have shown that H_1 or H_2 receptors are involved in the mediation of several central as well as peripheral effects of HA (29). The purpose of the present experiment was to determine the probable receptor mechanisms involved in the HA-ergic regulation of stress effects like gastric ulcerogenesis and plasma corticosterone response. Thus the effects of HA antagonists, pheniramine (H_1) or cimetidine (H_2) were assessed on these stress markers.

Method

Inbred male Wistar rats were housed under similar conditions as described for Experiment 1. The method for stress (CRS), evaluation of gastric pathology and assay of plasma corticosterone were essentially similar to those done for the earlier study. The drugs used were pheniramine maleate (Hoecht, India) and cimetidine (Smith Kline & French, India). Pheniramine maleate was dissolved in distilled water, while cimetidine was dissolved in 0.1 N HCl, neutralised to pH 5.5–6.0 with 0.1 N NaOH and volume made up with distilled water. All drugs were injected IP in a volume of 2 ml/kg, 30 min prior to the CRS procedure.

Results and Discussion

As seen in Experiment 1, CRS effects on gastric mucosal integrity and adrenocortical corticosterone release were more pronounced as compared to "no stress" controls (Table 3). The H_1-antagonist, pheniramine, significantly reduced the number and severity (mm) of gastric lesions in stressed rats. The plasma corticosterone response was also less when compared to CRS controls. On the other hand, the H_2-blocker, cimetidine, was not as effective as pheniramine in attenuating the gastric mucosal resonse to CRS, and though the ulcer number and severity was reduced by 25% and 20% respectively, this was not statistically significant ($p>0.05$). The levels of plasma corticosterone, however, were lower in the cimetidine-treated group when compared to controls ($p<0.002$). These data suggest that while both H_1 and H_2 receptors mediate the corticosterone response to CRS,

TABLE 3

EFFECTS OF HISTAMINE ANTAGONISTS ON COLD RESTRAINT STRESS (CRS)-INDUCED GASTRIC ULCER FORMATION AND PLASMA CORTICOSTERONE LEVELS IN RATS

Treatment (mg/kg, IP)	n	Stress Parameters (Mean ± SE) Gastric Pathology Ulcer Number	Cumulative Length (mm)	Plasma Corticosterone (μg/dl)
Controls*	14	1.1 ± 0.6	0.2 ± 0.1	15.2 ± 1.0§
CRS	12	6.6 ± 0.4	1.4 ± 0.3	36.6 ± 2.1
Pheniramine (25) + CRS	9	3.2 ± 0.9‡	0.6 ± 0.2‡	32.6 ± 3.3
Cimetidine (200) +CRS	9	4.9 ± 1.0	1.1 ± 0.4	30.8 ± 1.8§

*No CRS (only 18 h food deprived).
†$p<0.05$; ‡$p<0.02$; §$p<0.002$ (compared to CRS group).

the gastric mucosal response is probably H_1 receptor mediated.

In fact, the lack of involvement of H_2 receptors in this brain-gut axis concept of stress ulceration has been suggested in an earlier study (10). Cimetidine in high doses is reported to cross the blood-brain barrier, and it can be assumed that in the doses used in this study (200 mg/kg), it would have achieved adequate levels in the CNS to interfere with HA effects on central H_2 receptors (1,29). On the other hand, the effects of pheniramine on stress ulcer formation may well have been due to its ancillary anticholinergic property. Several reports have shown that atropine-like drugs protect the gastric mucosa from the disruptive effects of experimental stressors (10, 21, 22).

GENERAL DISCUSSION

The experimental data presented indicate that brain neuronal HA plays a crucial role in the regulation of one visceral and one endocrine response during stress. The involvement of hypothalamo-pituitary adrenal axis during aversive stimuli like stress is well documented, and HA is found in high concentrations in the hypothalamus. Further, the brain (hypothalamus)-gut axis is important in stress-induced gastric ulcer formation (9, 20, 25, 30). The neuronal HA depletor, α-FMH, is reported to inhibit brain HA synthesis (3, 12, 14, 23), and this is reaffirmed by our biochemical data (Table 2). Further, α-FMH-pretreated rats showed decreased gastric mucosal damage and less elevated plasma corticosterone (Table 1). It is thus apparent that brain neuronal HA is probably one of the "robust" forces during the organism's reaction to the stressful experience. Alternatively, neuronal HA, at the level of the hypothalamus, could act as a neuromodulator, which in turn could influence the complex neurochemical events that ultimately regulate visceral (gastric ulcerogenesis) and endocrine (plasma corticosterone) stress responses. Interestingly, α-FMH pretreatment also lowered gastric mucosal HA content, in a manner closely parallelling HA level changes in the brain. The finding strongly supports the contention that the HA-ergic brain-gut axis might be important for stress ulcer formation.

Complex H_1 and H_2 receptor mechanisms are seemingly involved in the regulation of the stress responses studied. While the H_1 receptor is important for both visceral (gastric mucosal) and endocrine (corticosterone) responses, the H_2 receptor is probably more crucial for the latter. Both HA (H_1 and H_2) receptors have been shown to mediate several neuroendocrine responses like changes in levels of catecholamines, β-endorphin, ACTH and renin. A generalized inhibitory effect for H_2 receptor antagonists on stress-induced release of neurohumors is also suggested (1, 11, 20). Our present data with cimetidine on corticosterone levels is in agreement with these views. Further, involvement of H_2 receptors in the gastric mucosal response to stress seems unlikely, and this also has been reported in an earlier study (10).

Taken together, the present findings show that: (a) brain HA is an important mediator in the regulation of physiological homeostasis during aversive inputs like stress; (b) changes in brain HA can influence stress effects on gastric mucosal integrity and plasma corticosterone in a manner which is consistent with an important role for the transmitter; and (c) differential H_1 and H_2 receptor mechanisms are seemingly involved in the HA-ergic regulation of these stress effects. However, the physiological role of extraneuronal brain HA in mast cells cannot be totally discounted (13). The significance of these findings is further highlighted by the fact that HA-ergic neurons and receptors have been identified in those areas of the CNS (like the hypothalamus and the amygdala) which are seemingly critical for triggering the stress indices we observed (9,28).

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