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Stress-Induced Changes in Histaminergic System: Effects of Diazepam and Amitriptyline

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GHI, P., C. FERRETTI, M. BLENGIO AND P. PORTALEONE. *Stress-induced changes in histaminergic system: Effects of diazepam and amitriptyline.* PHARMACOL BIOCHEM BEHAV 51(1) 65-68, 1995.—The involvement of the histaminergic system in the regulation of weak stress was studied in rats. The parameters examined were the brain receptors and corticosterone (CS) plasma levels. The benzodiazepine diazepam [(2 mg/kg intraperitoneally (IP)] influenced neither foot-shock-induced changes in CS levels nor [^{3}H]-histamine [(^{3}H)-HA] binding site constants, whereas the tricyclic antidepressive amitriptyline (10 mg/kg IP) partially counteracted a plasma CS increase and prevented changes in [^{3}H]-HA binding in the stressed rat brain. These observations are in agreement with the known activities of amitriptyline on monoaminergic metabolism and receptors. Moreover, these data provide further experimental evidence of the functional role of the central histaminergic system in organized stress response.

Stress Histamine receptor Corticosterone Amitriptyline Diazepam Rat brain

SEVERAL reports have substantiated the role of histamine (HA) as a neurotransmitter in the CNS (27). Although various findings indicate a central involvement of HA in thermoregulation (19), water balance (18), nociception (14), neuroendocrine control (17), and behavior (8), the exact role played by HA within the CNS is largely subject to speculation (29).

All of these observations support the hypothesis of a possible involvement of brain HA in the organization of response to stress. Complex neurochemical mechanisms have been shown to regulate organized stress response, and the involvement of several neurotransmitters has been suggested (15,16). Previous research showed that HA is released during stress (22,26,35), but conflicting data were reported when different stress stimuli widely ranging in intensity were applied to various mammalian species (7,14,23,28). Currently, the type of histaminergic receptors in some stress responses involved, H_1 and/or H_2 has not been completely clarified. Whereas H_1 receptors appear to be relevant to both visceral and endocrine responses, H_2 receptors seem to be more crucial in the latter, as H_2 receptor antagonists inhibit the stress-induced release of neurohormones (3,4).

In our former studies on rats, we reported that weak electric foot-shock stress induced a significant decrease in brain [^{3}H]-HA site density and a rise in plasma corticosterone (CS) levels (10).

This work was carried out to investigate further the mechanisms of stress-induced effects. Because anxiety and depression are generally associated with adverse stressful conditions, it is interesting to investigate whether stress-induced variations could be involved in these behavioural desease. For this purpose, the capacity of the widely used anxiolitic diazepam and the antidepressant amitriptyline to affect both a receptor density decrease and a rise in plasma CS has been examined.

METHODS

Animals

Male Sprague-Dawley rats (180-200 g) were housed in Plexiglas cages (four rats/cage), with controlled temperature (24 ± 2°C) and humidity (60 ± 5 %). They were exposed to a 10 L : 14 D cycle. The stress stimulus used was an inescapable scrambled electric foot-shock given by a Campdel shock

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TABLE 1

[^{3}H]-HA BINDING IN CORTEX MEMBRANES OF MALE RATS FOLLOWING FOOT-SHOCK STRESS AND DIAZEPAM TREATMENT

Treatment Group	B_{max} (pmol/g prot)	K_d (nM)
Nontreated	38.18 ± 1.86	3.35 ± 0.52
Nontreated stress	21.07 ± 1.65*	4.05 ± 0.73
Vehicle	39.74 ± 3.05	6.09 ± 1.02
Vehicle stress	23.23 ± 2.09*	6.69 ± 1.58
Diazepam	31.47 ± 2.57	5.20 ± 1.23
Diazepam stress	22.70 ± 1.99*	4.91 ± 1.00

Data are the mean ± SEM of five repeated experiments. Each experimental group was composed of 6 animals. *$p < 0.05$ ANOVA and Newman-Keuls test versus nonstress groups. Rats were injected with diazepam (2 mg/kg IP) or vehicle 60 min before the stress procedure, and all animals were sacrificed 30 min later.

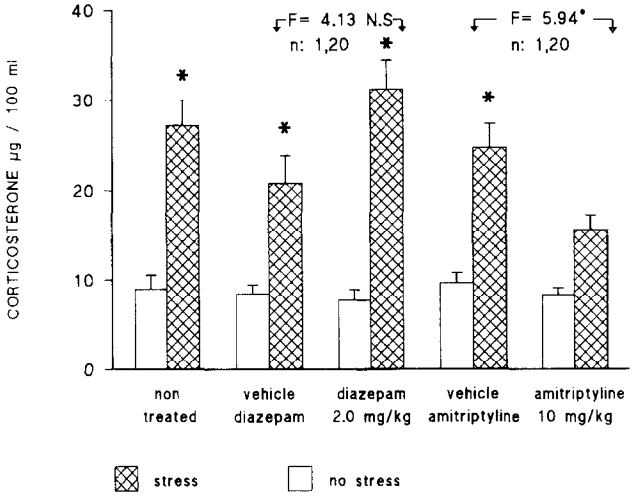

FIG. 1. Effect of footshock stress (0.5 mA × 1 sec × 5 times), diazepam 2.0 mg/kg IP and amitriptyline 10 mg/kg IP treatments on plasma CS in male rats. Each column represents the mean (± SEM) of 6 animals.
*$p < 0.05$ ANOVA and Newman-Keuls test versus nonstress group
•$p < 0.05$ ANOVA 2 × 2 test.

generator (London, UK). The rats to be stressed were individually placed in a shock box [rat chamber (Grason-Stadler Co., West Concord, MA) 32 × 30 × 30 cm] and received five electric foot-shocks (0.5 mA/1 s) with 1-min intervals.

In the first experiment, three groups of 12 rats each were used. The rats in the first group received diazepam [2 mg/kg intraperitoneally (IP)]; 1 h after drug treatment, six rats were subjected to the stress procedure while the other six were left alone. The 12 shock-stressed animals and controls were killed 30 min later. A second group of 12 animals received the vehicle [ethanol-benzylalcohol-phosphate-buffered saline (PBS)-distilled water] alone by the IP route. Six of them were then subjected to the same shock-stress procedure as was used with the first group. The third group consisted of 12 rats that received no injection; again, six rats were subjected to shock while six remained untreated.

In the second experiment, which also used three groups of 12 rats, the procedure described before was followed, except that amitriptyline (10 mg/kg IP in distillated water) was used instead of diazepam. All rats were decapitated 90 min after experiments were started. Therefore, the experimental design consisted of the following groups of six rats: a) diazepam; b) diazepam + stress; c) vehicle; d) vehicle + stress; e) nontreated; and f) nontreated + stress. The same groups were maintained for amitriptyline treatment. Both of the experiments were performed independently five times.

After the rats were decapitated, blood was collected from their severed necks into tubes for plasma CS determination. The brains were rapidly removed and dissected on a glass surface at 0°C according to the method of Glowinski and Iversen (12). To avoid circadian fluctuation of plasma CS, the procedure was carried out between 0900 and 1300 h.

The animal experiments were approved and financed by the MURST (Ministero dell'Universita' per la Ricerca Scientifica e Tecnologica) with 60% funds for the year 1991.

TABLE 2

[^{3}H]-HA BINDING IN CORTEX MEMBRANES OF MALE RATS FOLLOWING FOOT-SHOCK STRESS AND AMITRIPTYLINE TREATMENT

Treatment Group	B_{max} (pmol/g prot)	K_d (nM)
Nontreated	31.94 ± 1.29	4.96 ± 0.84
Nontreated stress	21.51 ± 4.40*	5.01 ± 0.49
Vehicle	34.07 ± 3.23	6.72 ± 0.63
Vehicle stress	17.29 ± 2.20*	3.91 ± 0.88
Amitriptyline	30.42 ± 1.25	5.99 ± 0.38
Amitriptyline stress	40.82 ± 2.75†	7.26 ± 0.53

Data are the mean ± SEM of five repeated experiments. Each experimental group was composed of 6 animals. *$p <$ 0.05 ANOVA and Newman-Keuls test vs. non-stress group. †$p < 0.01$ ANOVA two-way test. $F(1,8) = 13.55$ vs. vehicle group. Rats were injected with amitriptyline (10 mg/kg *ip*) or vehicle 60 min before the stress procedure, and all animals were sacrificed 30 min later.

[^{3}H]-HA Binding

[^{3}H]-HA binding was determined according to the procedure of Barbin et al. (2) with minor modifications. Tissues were homogenized with a Teflon-glass Potter-type homogenizer in 30 vol (vol./wt.) of cold 50 mM Tris-HCl buffer, ph 7.4. Homogenates were centrifuged at 1000 × *g* for 10 min, and supernatants were spun at 15,000 × *g* for 25 min. The pellet of the second centrifugation was resuspended in cold Tris-HCl containing 50 mM NaCl. A 300-µl aliquot of the particulate fraction (containing 500 µg protein) was preincubated for 15 min at 30°C. Incubation was started by the addition of 150 µl of the same buffer containing 1–10 nM [^{3}H]-HA (spec. act. 50 Ci/mmol; Amersham, Buckinghamshire, UK) and 5 µM unlabeled HA. Incubation was ended after 15 min at 30°C by the addition of 3 ml cold Tris-HCl buffer and rapid filtration under vacuum on AAWP Millipore filters (0.8-µm pore size; Millipore, Bedford, MA). Tubes were rinsed with 5 ml cold buffer and filters were washed twice with 10 ml of the same buffer. Radioactivity retained on the filters was measured by liquid scintillation counter at 44% efficiency. Saturable binding of the [^{3}H]-HA was calculated as the difference between total and nonspecific binding obtained in the presence of 5 µM unlabeled HA.

Plasma Corticosterone Assay

Corticosterone was extracted from the plasma with diethyl-ether (1 ml/100 μl plasma). After vigorous shaking, the diethyl-ether was dried under a stream of nitrogen. The dry residue was taken up with 100 μl 50 mM phosphate buffer, NaN_3 0.5%, EDTA 4 mM, BSA 1%, pH 7.4. CS levels were calculated by radioimmunoassay using: 100 μl CS standards (0.025–2.5 ng/tube), 50 μl [^{3}H]-CS, corresponding to 8000 dpm (spec. act. 84 Ci/mmol; Amersham, UK), and 50 μl anti-CS antiserum 3 CMO-BSA, diluted 1 : 1000 (UCB bioproduct; S.A. Brain, Alleud, Belgium). After overnight incubation at 4°C, a mixture of 200 μl dextran (0.05%) and activated charcoal (0.5%) was added to each tube. The contents were mixed for 15 s on a vortex, allowed to stand for 15 min, and centrifuged at 1000 × *g* for 15 min at 4°C. Finally, the supernatant was decanted to a counting vial and dissolved in 5 ml Picofluor (Packard, Meriden, USA). The sensitivity of the assay was 10 pg/ml. The inter- and intra-assay coefficients of variation were 11.4 and 2.2%, respectively.

Statistical Analysis

The effect of stress treatments was evaluated with a one-way analysis of variance (ANOVA). When overall significance was evident, [^{3}H]-HA binding and CS levels observed after each treatment were compared to the corresponding results of nontreated rats by Newman–Keuls test (30). ANOVA 2 × 2 factorial analysis was used to evaluate the influence of the treatments either alone or in association with stress.

RESULTS

The [^{3}H]-HA binding constants from cortical membrane preparations of diazepam treatment are summarized in Table 1. No significant differences in [^{3}H]-HA binding site density and affinity were observed in the vehicle-treated and diazepam groups in comparison to the nontreated one. However, stress administration induced a significant decrease (compared to corresponding nonstressed animals) in [^{3}H]-HA binding site density in nontreated + stress, vehicle + stress, and diazepam + stress groups. On the contrary, amitriptyline treatment prevented [^{3}H]-HA binding site variations in cortex membranes of rats exposed to stress (Table 2). No changes in K_d values were observed in any treatment.

A dramatic increase in CS plasma levels was observed after electric foot-shock stress of untreated rats and in rats treated with either diazepam or only vehicle. On the other hand, amitriptyline treatment significantly counteracted the foot-shock-induced plasma CS increase (Fig.1).

DISCUSSION

The physiologic role of the brain histaminergic system in stress-induced responses has not yet been defined. Differing degrees of involvement of the histaminergic system, according to the severity of emotional engagement and stress applied, have been demonstrated (11,29). Several investigations showed that electric foot-shock applications induce analgesia in mice and rats, and that endogenous opioids are involved in this antinociceptive response (5,17,20). On the other hand, two distinct antinociceptive responses to foot-shock, different in both duration and intensity, have been recognized: an opioid-dependent antalgic response (foot-shock intensity: 2.0 mA for 3 min) and an HA-mediated analgesic response (3.5 mA for 3 min) (13,14).

To verify whether the histaminergic system is involved in the stress response to stimuli below the antalgic and analgesic threshold, we applied a foot-shock of weaker intensity and length (0.5 mA for 1 s).

In agreement with our previous observations (10), this study shows that a weak foot-shock induced a significant decrease in cortical H_2 receptor density, identified by [^{3}H]-HA binding (9,31–33). The rise in plasma CS confirmed that this kind of stress stimulation was also effective in inducing a neurohormonal response.

It is possible that a histaminergic response could be involved in anxiety control, even though conflicting observations have been reported on diazepam activity in stress-induced effects (21).

Our data indicate that changes in H_2 receptor density and the CS increase were not antagonized by diazepam, suggesting that HA response to stress cannot be suppressed by an anxiolitic drug. On the other hand, it has been suggested that depression may sometimes be precipitated by environmental factors such as stress (1). Acute amitriptyline treatment was found to be able to reverse both H_2 receptors reduction and a CS increase induced by foot-shock. This finding suggests that the histaminergic systems may be implicated in the depression syndrome, probably acting through neurohormonal feedback. Amitriptyline could interact directly on the H_2 receptor, because an antagonistic effect has also been shown on these receptors (34). Furthermore, antihistaminic drugs have been demonstrated to elicit the same behavioural patterns as the classic tricyclic antidepressant on forced swimming and feeding tests (24,25). The capacity of amitriptyline to reverse stress-induced H_2 down-regulation could be explained by its antagonistic action on these receptors. Because a stress-induced increase in HA release has been reported (35), the HA receptor density reduction may be considered an adaptative response to increased receptor stimulation. Amitriptyline could therefore prevent a receptor decrease by binding the H_2 receptors and inhibiting their up-stimulation. However, amitriptyline could also produce its antistress effect by acting on a different receptor type. It is well established that HA release is inhibited by an α_2 eteroreceptor stimulation (6); thus, by inhibiting noradrenaline re-uptake, amitriptyline could in turn reduce HA release.

Finally, our data confirm the existence of a functional link between stress-HA receptors and CS secretion. However, further experiments are required to establish whether the central histaminergic system modulates CS secretion or vice versa.

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