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Stress and Brain Histaminergic System: Effects of Weak Electric Foot-Shock

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GHI, P., M. BLENGIO, C. FERRETTI AND P. PORTALEONE. *Stress and brain histaminergic system: Effects of weak electric foot-shock.* PHARMACOL BIOCHEM BEHAV **41**(2) 317–320, 1992.—A weak electric foot-shock stressful stimulus (0.5 mA × 1 s × 5 times) significantly increases plasma corticosterone (CS) levels and modifies [^{3}H]-histamine ([^{3}H]-HA) binding site constants related to H_2 receptors in rat cortical membranes. Progressive and total recovery of basal binding characteristics occurs 90 min later. A double-foot-shock stress procedure delays [^{3}H]-HA binding characteristic recovery instead of strengthening stress effects. This finding is further evidence of involvement of the brain's histamine receptors in the response to mild stress.

Histamine-receptor Corticosterone Foot-shock Stress Cortex Rat

THE involvement of several central neurotransmitters in stress response has been widely described and various types of stress stimuli have been used as tools to investigate the physiological role of neurotransmitters. Although strong evidence supports histamine (HA) as putative central neurotransmitter, involved in some types of responses to stress, its role played under stressful conditions is far from clear because of conflicting results reported (6,13,19).

A wide range of different stress stimuli that induce significant variations in cerebral HA synthesis, release, and concentrations in discrete rat brain areas (18,19,28) is known to support the HA involvement in stress-induced reactions.

Stress induced by an electric foot-shock was shown to increase HA levels in the rat cortex and hypothalamus (4). Furthermore, the intracerebroventricular (ICV) administration of HA in the rat induced a dose-dependent increase in blood serum corticosterone (CS) levels (2), which is considered to directly indicate ACTH activation following a mild stress (12).

On the other hand, both H_1 and H_2 brain HA receptors have been shown to modulate the secretion of many stress-related neuropeptides (29), and in rats stress-induced CS increase is mediated by both central H_1 and H_2 receptors (3).

The aim of our research was to investigate the effect of low-intensity foot-shock stress on brain HA binding sites. This binding can be defined as H_2 receptors—mediated on the basis of biochemical and pharmacological evidences that these sites possess characteristics like H_2 receptors (9,23,25–27). Some of these indicated that: 1) [^{3}H]-HA binding sites interact with a G/F protein in the way characteristic of cyclase-linked receptors; 2) H_2 antagonists inhibit [^{3}H]-histamine ([^{3}H]-HA) binding with constants characteristic of an action at H_2 receptors, but the inhibition appears to be competitive; 3) the biphasic inhibition of [^{3}H]-HA binding by H_2 agonist is characteristic of compounds with H_2 agonist activity, and it may thus be suggested that such inhibition is both allosteric and cooperative (25–27).

METHOD

Animals

Male Sprague-Dawley rats (200–220 g) were housed in Plexiglas cages (four rats per cage) with controlled temperature and humidity (24°C; 60%). They were exposed to a 14 L: 10 D cycle. Stress was induced by consecutive administrations of inescapable electric foot-shock. Rats were individually placed in a shock-box and received an electrical foot-shock (0.5 mA/1 s) five times consecutively with 1-min intervals. Rats not subjected to stress (no-stress) were used as control and rats placed in the shock-box but not stressed were used as sham-stress. Each experimental group was composed of 12 animals. The animals were killed by decapitation 15, 30, 60, and 120 min after the last foot-shock administration. In another set of experiments, rats (12 animals per group) were exposed twice to the same stress procedure but with 30-min intervals between the first and second stress exposures.

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Rats were then killed by decapitation 30, 60, 120, and 180 min after the second stress administration. Trunk blood was collected into tubes for plasma CS determination. Brains were rapidly removed and dissected on a glass surface at 0°C according to Glowinski and Iversen (10). To avoid circadian fluctuations of plasma CS, all experiments were carried out between 9 a.m. and 1 p.m.

[^{3}H]-Histamine Binding

[^{3}H]-HA binding was determined according to Barbin et al. (1) with minor modifications. Tissues were homogenized with a Teflon-glass potter homogenizer in 30 vol (w/v) of cold 50 mM Tris-HCl buffer, pH 7.4. Homogenates were centrifuged at 1,000 g × 10 min and the supernatants spun at 15,000 g × 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 (at 500-μl protein concentration) was preincubated 15 min at 30°C. Incubation was started by addition of 150 μl of the same buffer containing 1-10 nM [^{3}H]-HA (spec. act. 50 Ci/mmol., Amersham, UK) and 5 μM 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). 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 a 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

CS 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 RIA using: 100 μl CS standards (0.025-2.5 ng/tube), 50 μl [^{3}H]-CS, corresponding to 8,000 dpm (spec. act. 84 Ci/mmol., Amersham, UK), and 50 μl anti-CS antiserum 3-CMO-BSA, diluted 1 : 1,000. (UCB bioproduct, S.A. Brain, Alleud, Belgium). After overnight incubation at 4°C, 200 μl dextran (0.05%) and activated charcoal (0.5%) mixture were added to each tube. The contents were mixed for 15 sec on a vortex, allowed to stand for 15 min, and centrifuged at 1,000 g for 15 min at 4°C. Finally, the supernatant was decanted to a counting vial and dissolved in 5 ml Picofluor (Packard, USA). The sensitivity of the assay was 10 pg/ml. The inter- and intraassay 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). If overall significance was evident, then [^{3}H]-HA binding and CS levels at each treatment were compared to control results by Newman-Keuls test (24).

RESULTS

[^{3}H]-HA binding patterns in membrane preparations obtained from cerebral cortex of control and stressed rats are represented in Fig. 1. No significant modifications of [^{3}H]-HA binding characteristics were observed in sham-stress rats (B_{max}: 36.55 ± 4.61 pmol/g prot; K_d: 7.70 ± 0.96 nM). A statistically significant reduction of binding site density (B_{max} −47.5% of controls) occurred instead in animals killed 15 and 30 min after stress administration (Table 1). Likewise, slight but significant variation in receptor density was still evident in rats killed 60 min after the electric foot-shock procedure (−14% of controls), whereas after 120 min a complete recovery of the [^{3}H]-HA binding characteristics was observed (Table 1).

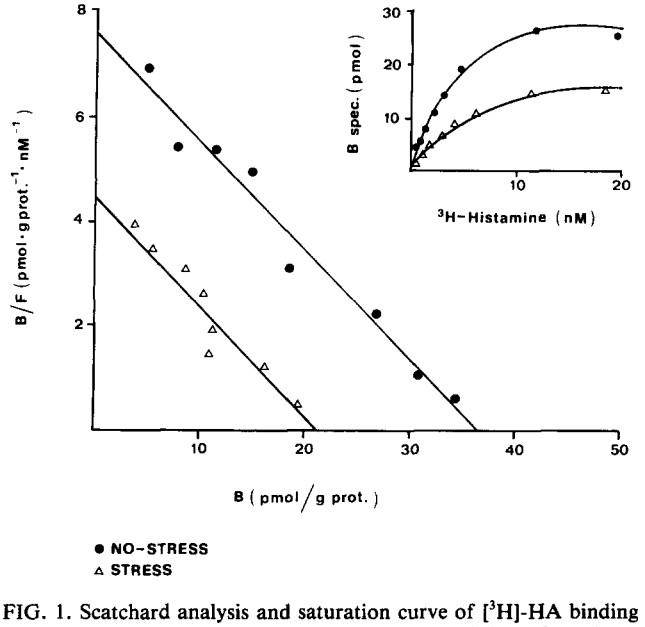

FIG. 1. Scatchard analysis and saturation curve of [^{3}H]-HA binding to cortex membranes of male rats no-stress (●) and single foot-shock stress (△) sacrificed after 30 min. Values are the mean (± SEM) of B_{max} (pmol/g prot) and K_d (nM) obtained from three repeated experiments (four rats per group). ●, B_{max} 36.52 ± 2.51 pmol/g protein; △, B_{max} 20.83 ± 1.87 pmol/g protein; ● K_d, 4.79 ± 0.09 nM; △, K_d 4.44 ± 0.08 nM; $p < 0.01$ (Newman-Keuls test).

The foot-shock stress-induced changes in plasma CS levels are shown in Fig. 2. A significant increase of CS was observed 15 min (+310% of controls) and 30 min (+187% of controls) after stress application. On the contrary, a slight reduction of plasma CS levels was observed in animals killed 60 and 120 min after the stress procedure (−19% and −42% of controls). In sham-stress rats, a nonsignificant increase of plasma CS (+26% of controls) was obtained (Fig. 2). When the single-foot-shock procedure was compared to double exposure to stress (five foot-shocks, twice, with 30-min interval), results

TABLE 1

RECOVERY OF [^{3}H]-HA BINDING IN CORTEX MEMBRANES OF MALE RATS AFTER A SINGLE FOOT-SHOCK STRESS

Time (min after stress)	B_{max} (pmol/g prot)	K_d (nM)
–	38.76 ± 2.84	5.33 ± 2.30
15	31.89 ± 2.09*	6.16 ± 0.46
30	20.81 ± 2.34**	6.40 ± 0.42
60	33.36 ± 2.25*	5.63 ± 2.07
120	33.71 ± 3.05	6.86 ± 0.65

Data are the mean ± SEM of three repeated experiments. Each experimental group was composed of four animals.
*$p < 0.05$; **$p < 0.01$ by Newman-Keuls test.

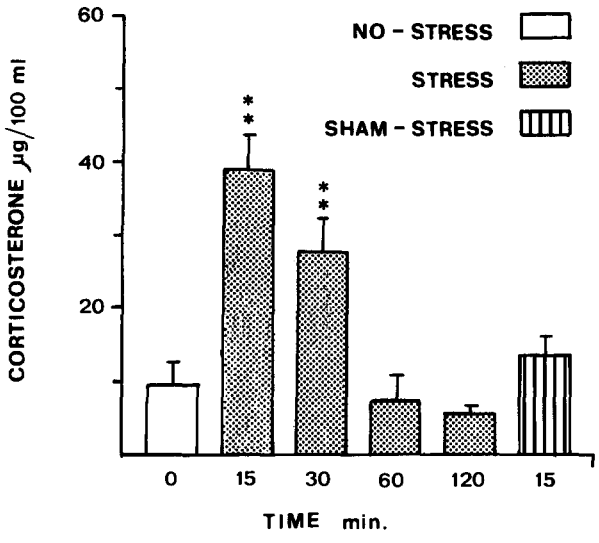

FIG. 2. Effect of single foot-shock stress (0.5 mA × 1 s × 5 times) on plasma CS in male rats. Each column represents the mean (± SEM) of 10–12 animals; $^{**}p < 0.01$ (Newman-Keuls test) significantly different from no-stress control group.

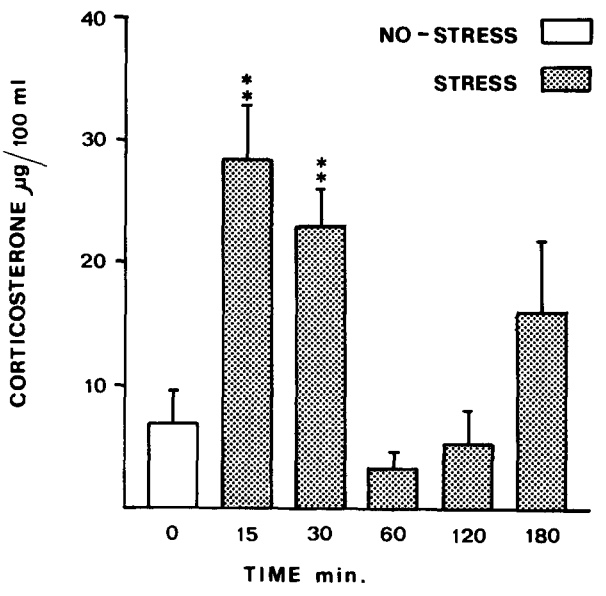

FIG. 3. Effect of double foot-shock stress (0.5 mA × 1 sec × 5 times repeated after 30 min) on plasma CS in male rats, after second stress. Each column represents the mean (± SEM) of 8–10 animals; $^{**}p < 0.02$ (Newman-Keuls test) significantly different from no-stress control group.

did not reveal significant variations in the [^{3}H]-HA binding characteristics (Table 2) except for significant binding pattern variation found within 30 min, whereas a slow recovery of basal values began 60 min after the last exposure to stress. Therefore, the recovery of [^{3}H]-HA binding appeared to occur 180 min after the double-stress treatment (Table 2).

Repeated exposure to stress modified plasma CS levels (Fig. 3) in a manner similar to that observed in the single-stress administration (15 min: +311% of controls; 30 min: +232% of controls) and the decrease observed at 60 and 120 min disappeared within 180 min (Fig. 3).

It seems of interest to point out that the increased plasma CS levels and cortical [^{3}H]-HA binding site variations exhibit similar patterns. Plasma CS peaked at 15 min and [^{3}H]-HA binding site density at 30 min after both single and double exposure to stress.

DISCUSSION

Brain HA seems to be involved in various physiological processes such as thermoregulation (11), water intake (16), analgesia (14), and neuroendocrine regulation (13,15). Furthermore, there is also evidence of brain histaminergic receptor site involvement, in particular of the H_2 type, in several behavioral models (8). It has been shown in mice that the H_2 receptor population, identified by ICV administration of selective H_2 receptor agonists and/or antagonists may be responsible for swimming despair-induced depression and for excitatory effects in aggressive behavior (21,22).

The aim of our work was to investigate possible changes in the functional state of brain histamine receptors induced by stress. In our binding assays, [^{3}H]-HA has been used as specific H_2 receptor site ligand instead of [^{3}H]-cimetidine or [^{3}H]-tiotidine since Gajtkowski et al. (7) reported that these latter ligands failed to give reliable results in rat brain. Furthermore, considering the [^{3}H]-HA high-affinity binding and that the binding sites recognized by [^{3}H]-HA display biochemical and pharmacological characteristics related to H_2 receptors (9, 23,25–27), the use of [^{3}H]-HA appeared reliable.

Electric foot-shock proved to be a reliable stimulus to produce an experimental stress model; in fact, it may be quantified and repeatedly applied without tissue damage and its effects rapidly disappear after the stimulus is discontinued. Our procedure showed to be an effective stimulus for inducing stress as indicated by the fast increase of plasma CS levels in foot-shock-stressed rats.

In agreement with other observations (19,28), our results support the involvement of HA receptors of H_2-related type in electric foot shock-induced mild stress response. The significant decrease in [^{3}H]-HA binding site density, observed 30 min after foot-shock, may represent a receptor down-regulation, probably induced by the raised HA synthesis and release from terminals during stress (18,20) and the subsequent HA receptor occupation. This effect gradually disappeared and [^{3}H]-HA binding showed no variations in the cortex membranes of rats killed 2 h after the stress procedure. In rats in which foot-shock adminis-

TABLE 2
[^{3}H]-HA BINDING IN CORTEX MEMBRANE OF MALE RATS FOLLOWING DOUBLE FOOT-SHOCK STRESS

Treatment Group	Time (min after second stress)	B_{max} (pmol/g prot)	K_d (nM)
No stress	–	37.81 ± 2.33	3.35 ± 0.39
Stress	15	26.16 ± 3.48	3.91 ± 0.45
Stress	30	18.01 ± 1.72*	5.92 ± 0.71
Stress	60	32.93 ± 4.45	8.97 ± 1.46
Stress	120	34.38 ± 3.86	7.51 ± 1.16
Stress	180	37.32 ± 2.89	4.71 ± 0.70

Data are the mean ± SEM of three repeated experiments. Each experimental group was composed of four animals.
*$p < 0.02$ by Newman-Keuls test.

tration was repeated, the total recovery of [^{3}H]-HA binding characteristics was found extended by 3 h. These data suggest that stress response was not strengthened; however, the possibility that habituation to thisweak stress takes place cannot be ruled out. Taken as a whole, therefore, the variations in [^{3}H]-HA binding observed in our study point to involvement of the brain's histaminergic system in stress.

Several investigations showed that electric foot-shock applications induce analgesia in mice and rats and that endogenous opioids are involved in this antinociceptive response (5,15,17). 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 × 3 min) and an HA-mediated analgesic response (3.5 mA × 3 min) (13,14). Our foot-shock procedure is remarkably different (0.5 mA × 1 s × 5 times), but the peak of CS plasma level proved foot-shock efficacy in inducing stress. Central histamine receptor involvement in stress-induced responses of serum CS was shown, though the mechanism is not completely understood (2,20).

A central adaptative histaminergic mechanism with short-term [^{3}H]-HA binding changes can be hypothesized after a weak stress stimulus, whereas following a stress of higher intensity the histaminergic system may activate protective (antalgic-opioid response) or inhibitory (histaminergic-controlled response) mechanism. Therefore, a different electric foot-shock intensity may lead to different histaminergic mechanisms and then to varied stress responses.

Investigations are in progress to define the functional role of central HA receptors in the stress response by integrating the data on HA interaction with other neurotransmitters related to the neuroendocrine control of the hypothalamus–pituitary–adrenal axis under stress.

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