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BRIEF COMMUNICATION

Fighting During Shock Exposure Attenuates the Reduction in the Number of Low-Affinity $GABA_A$ Sites in the Cerebral Cortex

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CUADRA, G. R. AND V. A. MOLINA. *Fighting during shock exposures attenuates the reduction in the number of low-affinity $GABA_A$ sites in the cerebral cortex.* PHARMACOL BIOCHEM BEHAV **44**(1) 237–239, 1993. – Rats were submitted either singly or in pairs to a series of foot-shocks. Immediately following the stress event, animals were sacrificed and the binding of low-affinity $GABA_A$ sites assayed in the cerebral cortex. A reduced number of sites was observed in rats singly shocked. However, rats shocked in pairs and thus afforded the opportunity to fight during the shock did not present changes in the density of $GABA_A$ receptors as compared to unstressed animals. Our data may suggest that fighting during an aversive event could decrease the deleterious consequence of a highly stressful experience.

$GABA_A$ receptors Stress Aggressive behavior Cerebral cortex

SEVERAL reports have documented that rats shocked in pairs and thus being able to fight showed less enhancement of plasma corticotropin, and fewer gastric mucosal lesions as compared to single rats exposed to shock experience (8,9). Regarding the central noradrenergic system, it was shown that the increase in noradrenaline (NA) turnover, normally observed following stress exposure (6), was clearly attenuated when rats were able to perform aggressive behavior in response to the aversive situation (5,7).

Alterations in the activity of the GABAergic system have been also reported to occur following foot-shock experience (1). Thus, it was reported that exposure to foot-shocks lead to a rapid reduction of the number of cortical low-affinity $GABA_A$ sites in previously handled rats (1). Moreover, the same type of stressor also provokes changes in the basal Cl^- flux in rats with prior handling (1). Therefore, the goal of the present work was to study if the possibility to fight in response to shock could influence the effect of foot-shock experience on the binding of cortical low-affinity $GABA_A$ receptors.

METHOD

Animals

Male, adult Wistar rats from our own colony, weighing 200–250 g at the start of the experiments, were used. They were maintained at 22 ± 2°C in a 12 L : 12 D cycle (light on 7:00 a.m.) with food and water freely available.

Procedure

Rats were previously habituated to the manipulations that precede killing, that is, they were picked up from their cages, held on a platform with their head forced through the hold left open by the blades of the guillotine, and returned to their cages. These maneuvers were repeated four times daily for 7 days. After this period, animals were divided into three groups: a) control (placed in the shock chamber and received no shock). b) SI (received shocks individually), and c) SIF (received shocks in pair). The stress session consisted of delivering 50 foot-shocks (2 mA) of 0.5-s duration at intervals of

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TABLE 1

EFFECT OF EXPOSURE TO A FOOT-SHOCK STRESS SESSION IN RATS WITH OR WITHOUT OPTION TO FIGHT ON LOW-AFFINITY [^{3}H]GABA BINDING IN THE RAT CEREBRAL CORTEX

	Low-Affinity ^{3}H-GABA binding (B_{max})		
	pM mg/Protein	% Changes	K_d (nM)
Control	2.81 ± 0.31	100 ± 11	59.5 ± 21
SIF	2.66 ± 0.15	95 ± 5	39.7 ± 12
SI	1.78 ± 0.28*	63 ± 10*	41.8 ± 13

Low-affinity [^{3}H]GABA binding was measured in frozen-thawed cortical membrane preparations in the presence of 10–600 nM [^{3}H]GABA. Each value represent the mean ± SE of five separate experiments (n = 5 animals per group).

*$p < 0.05$ compared to control or SIF group.

15 s in a 25 × 25 × 22-cm chamber. The front viewing wall was made of Plexiglas, and the floor was fitted with a stainless steel grid from which scrambled shock was delivered. Animals were killed immediately after foot-shock or after context exposure (control), the brain rapidly removed, and the cerebral cortex dissected for binding assay.

Low-affinity [^{3}H]GABA Binding

[^{3}H]GABA binding was determined according to Enna and Snyder (2) with minor modifications. The fresh tissue was homogenized in 10 vol of 0.32 mol/l sucrose and centrifuged at 0°C, 900× g for 10 min; the supernatant was centrifuged at 11,000× g for 20 min. The resultant pellet was submitted to hypotonic shock in 10 vol of water and centrifuged at 20,000× g for 30 min. The final crude membrane fraction was stored at −20°C for at least 24 h before assay. Frozen membranes were resuspended in 50 mmol/l Tris-HCl buffer (pH 7.1) containing 0.5 g/l Triton X-100 incubated at 37°C for 30 min and centrifuged at 0°C, 100,000× g for 30 min. The resulting pellet was washed twice by resuspension in Tris-HCl buffer. Specific [^{3}H]GABA (92.5 Ci/mmol, New England Nuclear, Boston, MA) binding was determined in aliquots of crude membrane fraction (0.2–0.4 mg protein) incubated for 5 min at 0°C with 0.6 ml buffer containing [^{3}H]GABA. The concentration of [^{3}H]GABA varied between 10–600 nmol/l (low-affinity receptor); [^{3}H]GABA was maintained constant (10 nmol/l), whereas the concentration of nonradioactive GABA varied. The reaction was stopped by centrifugation of the vials at 17,000× g at 4°C. The supernatant was discarded and pellets rapidly rinsed with 1 ml ice-cold buffer. The pellets were transferred to vials to count the radioactivity in 3 ml of a solution containing toluene–Triton X-100–2,5 diphenyloxazole (PPO, Sigma Chemical Co., St. Louis, MO). The nonspecific binding, assayed in the presence of 1 mmol/l GABA, was subtracted from the total radioactivity, yielding values of specific binding. Protein was determined by the method of Lowry et al. (3). Scatchard analysis of saturation data were analyzed using the EDBA-LIGAND program (4).

Statistics

Differences between groups were analyzed by one-way analysis of variance (ANOVA), and subsequent posthoc comparisons were performed using the Newman-Keuls test. A p value of 0.05 or less was considered to represent a significant difference between groups.

RESULTS

Effect of Exposure to a Foot-Shock Session in Rats With or Without Option to Fight on Low-Affinity [^{3}H]GABA Binding Sites

As observed in Table 1, rats individually submitted to a foot-shock session show a decreased density of $GABA_A$ receptors. However, the same foot-shock session applied to pair of rats (given the option to fight) did not modify the total number of low-affinity [^{3}H]GABA binding sites as compared with control. On the other hand, this stress session failed to change the apparent affinity of [^{3}H]GABA for their binding sites. Individual comparisons were confirmed by Newman-Keuls posthoc test ($p < 0.05$). A one-way ANOVA revealed a significant shock effect, with or without option to fight, $F(2, 14) = 4.56, p < 0.05$.

DISCUSSION

In accordance with previous findings (1), handled rats that were individually shocked presented a reduced number of low-affinity $GABA_A$ sites in the brain cortex without changes in receptor affinity. This reduction was not evident when rats were shocked in pairs and therefore able to fight during the foot-shock event.

Aggression performed during aversive situations reduced the peripheral physiological–endocrine changes (8,9) and attenuated the enhanced activity of the central noradrenergic system (5,7), normally observed following stress. These data, including that reported in the present work, have been observed following the expression of different types of aggressive behaviors in response to stressful experience, so, regardless of the type of aggression involved, the possibility to aggress during stressful situations may reduce the deleterious consequences induced by stress exposure.

It is well known that benzodiazepines, as well as $GABA_A$ agonists, exert their pharmacological action as anxiolytics by a selective facilitation of the GABA receptor complex. In this line, it was proposed that anxiety may result from a reduction in the functioning of the central GABAergic system (1). Hence, exposure to stress experience such as foot-shock decreases the activity of the $GABA_A$ receptor complex and produces a preconflict effect, similar to those effects provoked

by administration of anxiogenic β-carbolines and opposite those induced by anxiolytic agents (1).

The present data show that fighting in response to shock attenuates the decrease in $GABA_A$ receptor binding elicited by the aversive experience, so it seems reasonable to suggest that aggression as a behavioral response to stress could act as a protective mechanism, leading to decreased anxiety following exposure to highly aversive situations.

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