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BRIEF COMMUNICATION

Acute White Noise Exposure Affects the Concentration of Benzodiazepine Receptors in the Brain of the Rat

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LAI, H. AND M. A. CARINO. *Acute white noise exposure affects the concentration of benzodiazepine receptors in the brain of the rat.* PHARMACOL BIOCHEM BEHAV 36(4) 985–987, 1990.—Rats were acutely (45 min) exposed to 100-dB white noise, and benzodiazepine receptors in the cerebral cortex, hippocampus, and cerebellum were studied immediately after exposure by the receptor-binding assay using ^{3}H-flunitrazepam as the ligand. An increase in the concentration of receptors was observed in the cerebral cortex, whereas no significant change in receptor concentration was seen in the hippocampus and cerebellum. No significant effect of noise on receptor binding affinity was detected in the three brain regions studied. Experimental handling also did not significantly affect the benzodiazepine receptor properties. These data confirm previous reports that acute exposure to stressor can cause rapid changes in benzodiazepine receptors in the brain.

Noise Acute stress Benzodiazepine receptors Cerebral cortex Hippocampus Cerebellum

VARIOUS studies have shown that loud noise is a stressor. Acute and chronic exposure to noise cause changes in neuroendocrine, neurochemical, and behavioral functions (1, 5–9, 17). In the present study, we investigated the effect of acute exposure to loud noise (100 dB) on benzodiazepine receptors in different regions of the brain of the rat. Benzodiazepine receptors in the brain have been suggested to play a role in an animal's response and adaptation to a stressful situation (15). Rapid changes in benzodiazepine receptors have been reported in the brains of animals acutely exposed to various forms of stressor (10, 11, 13, 16, 18). Since experimental handling during the noise exposure procedure could constitute a stressful experience to the rats, a group of unhandled animals also was included as a control in this study.

METHOD

Animals

Male Sprague-Dawley rats (250–300 g) purchased from Tyler Laboratory, Bellevue, WA were used. They were housed three to a cage in a temperature-controlled (23°C) room adjacent to the room where the animals would be exposed to noise and were maintained on a 12-hr light-dark cycle (lights on 7:00–19:00 hr). The rats were housed in the laboratory for at least two days before an experiment and were provided with food and water ad lib.

Experimental Procedures

Noise exposure was done between 8:00–10:00 hr. Rats were exposed to noise in Plexiglas cylindrical cages (15 cm in diameter, 24 cm in length). Eleven rows of holes (1 cm in diameter, 6 holes per row) were equally spaced longitudinally on the wall of the cylinder. One end of the cage was sealed closed and the other end was a removable door. The cage was put on its side. A platform made of plastic rods was placed longitudinally in the cylinder (3 cm from the lower side) allowed waste to fall through. The rat had sufficient room to move freely inside the cage. A loudspeaker (Speakerlab, WA; Model KR 4580) was mounted at 30 cm above the cage and activated by a white-noise generator (Lehigh Valley Electronics, Model 581-02) powered by an amplifier system (Hewlett-Packard 467A power amplifier and 6215A power supply). The frequency range of the noise generator was up to 40 KHz. Noise level was set at 100 dB and found to be uniform inside the cage as monitored with a sound meter (B & K, Inc.). The background noise inside the cage was 60 dB, which came mostly from the ventilation system in the room. By putting two cages side by side, two animals were exposed at a time.

Experimental rats were exposed to the white noise for 45 min. Two control groups were studied: 1) sham-exposed controls were placed in the exposure cage and exposed to the ambient noise for 45 min before benzodiazepine receptors in their brains were assayed; and 2) unhandled controls consisted of animals taken directly from their home cage for immediate assay of benzodiazepine receptors in various regions of the brain. In an exposure experiment, the experimenter would put an animal in the cage,

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turn on the noise or leave the noise off in the case of sham-exposure rats, and leave the room. At the end of the 45-min exposure period, he would remove and sacrifice the rat. The cerebral cortex, hippocampus, and cerebellum were dissected out from the brain on ice for benzodiazepine receptor assay. The dissection took approximately 2 min for each animal. Animals were sacrificed by decapitation on a work-bench in the room adjacent to the exposure room. The two rooms are separated by a door, so that the animals still being exposed to noise or sham-exposed were not exposed to the sight and noise of the sacrifice procedure. Furthermore, we found no consistent effect of the order of sacrifice on the binding properties of the benzodiazepine receptors in the brain.

Benzodiazepine receptor binding assay was done according to the method described by Medina *et al.* (14) using ^{3}H-flunitrazepam as ligand. Brain tissues were homogenized in 10 vol. of 0.32 M sucrose solution using a glass homogenizer and centrifuged at 1,000 × g for 10 min. The supernatant was centrifuged at 30,000 × g for 25 min. The pellet was resuspended in 0.025 M Tris-HCl buffer (pH 7.4) using a polytron (10 sec at setting 5) and recentrifuged at 30,000 × g for 25 min. This washing procedure was repeated twice and the final pellet was resuspended in Tris-HCl buffer. Of this preparation, 100 μl (containing 0.1–0.15 mg of protein) was added to each of a set of tubes containing the Tris-HCl buffer with different concentrations of ^{3}H-flunitrazepam (New England Nuclear, specific activity 80–85 Ci/mmol). The total volume was 1 ml. Nonspecific binding was determined by addition of 3 μM of diazepam to each of a similar set of tubes. The tubes with their contents were incubated at 4°C for 25 min. Incubation was terminated by addition of 4 ml of cold Tris-HCl buffer and the solution was filtered with suction with a Brandel cell-harvester through Whatman GF/B filter paper. The filters were washed three times with 5 ml each of cold buffer and then dried overnight in scintillation vials. Nine milliliters of Biosafe NA (RPI Corp.) were then added and radioactivity was counted with a scintillation counter at 63% efficiency. Protein concentration of the tissue homogenate preparation was determined by the method of Lowry *et al.* (12) using bovine serum albumin as external standards. Concentration (B_{max}, in fmol/mg protein) and affinity (K_d, in nM) of the receptor sites were determined by Scatchard analysis using a computer program. Data points for the analysis were fitted by linear regression.

Data Analysis

Data were analyzed by the one-way analysis of variance and the difference between two treatment groups was compared by the Newman-Keuls test. A difference at $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Data on the effects of noise exposure on the concentrations of benzodiazepine receptors in the three brain regions are presented in Fig. 1. One-way analysis of variance of the data showed a significant treatment effect in the cerebral cortex, $F(2,18)=7.667$, $p<0.005$. Newman-Keuls test showed that the concentration of receptors in the cerebral cortex of the noise-exposed animals is significantly higher than that of the sham-exposed animals, whereas no significant difference was found between the data of sham-exposed and unhandled rats. No significant treatment effect on receptor concentrations was found in the hippocampus, $F(2,18)=3.329$, nonsignificant, and cerebellum, $F(2,17)=0.672$, nonsignificant. Furthermore, no significant treatment effect on receptor binding affinity was found in the three brain regions studied (Table 1).

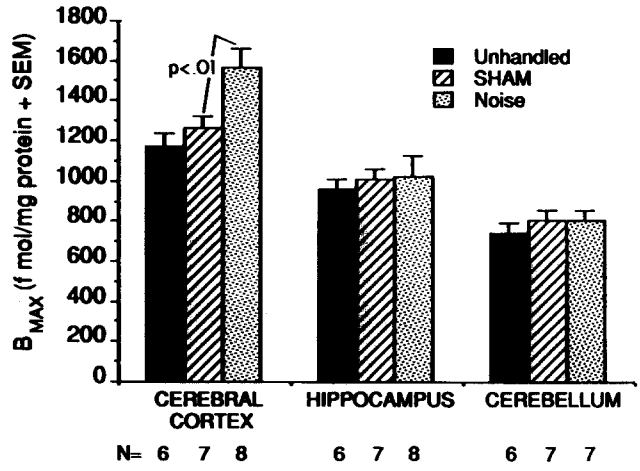

FIG. 1. Concentrations (B_{max}) of ^{3}H-flunitrazepam binding sites in different brain regions of the unhandled, sham-exposed, and noise-exposed rats.

Thus, our data show a brain-region selective change in the concentration of benzodiazepine receptors after acute exposure to loud noise. These data support previous reports that rapid change in benzodiazepine receptors occurs in the brain after acute exposure to stressor. The biological significance of a rapid change in the properties of the benzodiazepine receptors in the brain after stressor exposure is not clear. It may be related to an animal's response or adaptation to the stress situation.

However, a review of the literature shows that the direction of change in receptors after acute stress is not entirely consistent. Lippa *et al.* (11) reported a decrease in binding concentration of ^{3}H-diazepam in the frontal cortex of rats after a conflict-test or foot-shock session. Braestrup *et al.* (3) reported a decrease in benzodiazepine binding sites in the cerebral cortex and hippocampus of the rat after repeated electric foot-shock and postnatal isolation, but an increase in the frontal cortex after repeated

TABLE 1

BINDING AFFINITY (K_d) (nM ± SEM) OF ^{3}H-FLUNITRAZEPAM BINDING SITES IN DIFFERENT REGIONS OF THE BRAINS OF THE UNHANDLED, SHAM-EXPOSED, AND NOISE-EXPOSED RATS

Brain Regions	Unhandled	Sham-Exposed	Noise-Exposed
Cerebral cortex	1.625 ± 0.073 (n=6)	1.971 ± 0.157 (n=7)	1.946 ± 0.154 (n=8)
	$F(2,18)=1.689$, nonsignificant		
Hippocampus	1.427 ± 0.096 (n=6)	1.663 ± 0.118 (n=7)	1.788 ± 0.122 (n=8)
	$F(2,18)=2.396$, nonsignificant		
Cerebellum	1.404 ± 0.138 (n=6)	1.658 ± 0.110 (n=7)	1.772 ± 0.129 (n=7)
	$F(2,17)=2.17$, nonsignificant		

Data were analyzed by the one-way analysis of variance.

immobilization stress. Isolation, forced-swimming, and chronic amphetamine intoxication had no significant effect on the receptors. However, Soubrie *et al.* (18) demonstrated an increase in ^{3}H-flunitrazepam binding sites in the cortex of the rat after cold-water swimming (3 min in 6°C). Rago *et al.* (14) also showed an increase in cerebral cortical benzodiazepine binding sites in rats subjected to 5 min of forced-swimming at 20°C. Havoundjian *et al.* (4) showed an increase in the efficacy of chloride ions to enhance ^{3}H-flunitrazepam binding in the cerebral cortex of rats subjected to 10 min of forced-swimming in water at 25°C. On the contrary, Medina *et al.* (13) reported a decrease in ^{3}H-flunitrazepam binding sites in the cerebral cortex and hippocampus after 15 min of swimming in 18°C water. In addition, the hippocampus showed a rebound increase in binding sites at 1 hr after the stress episode. More recently, Weizman *et al.* (19) showed a decrease in in vivo binding of ^{3}H-RO 15-1788 to the cerebral cortex, hippocampus, and hypothalamus of mice subjected to repeated swim-stress. Bowers and Wehner (2) demonstrated an increase in GABA-stimulated ^{3}H-flunitrazepam binding in the cerebral cortex of an ethanol-selective strain of mice subjected to confinement in a maze. Interestingly, Lane *et al.* (10) showed a decrease in ^{3}H-diazepam binding sites in the cerebral cortex of rats exposed to a psychological stressor, a conditioned stimulus that had been associated with foot-shock.

These diverse findings suggest that the change in central benzodiazepine receptors may depend on the parameters of the stressor and possibly the experimental conditions. For example, increase (4, 16, 18), decrease (19), and biphasic changes (13,14) in benzodiazepine binding sites in the brain have been reported after swim-stress. However, the experimental conditions of these experiments are not exactly the same. An increase in benzodiazepine binding was observed after short period of acute swimming (3–10 min), whereas in the experiment in which a decrease in binding was reported, animals were subjected to repeated swim-stress (2 or 10 min per day for 7 days). Biphasic response (i.e., an initial decrease followed by an increase in binding sites) was observed in rats subjected to longer period (15 min) of swim-stress when the animals showed signs of exhaustion. Thus, the duration and frequency of stress exposure may determine the direction of response of the benzodiazepine receptors in the brain.

On the other hand, the response may be selective for a stressor and depends on the nature of the homeostatic disturbance triggered by the stressor. This may explain why some stressors [e.g., short period swimming, noise, immobilization, confinement in a maze (2, 3, 16)] cause an increase, whereas other stressors [e.g., foot-shock and psychological stress (3, 10, 11)] cause a decrease in central benzodiazepine receptor binding sites.

An important approach to further understand the stress-induced responses of the benzodiazepine receptors is to evaluate the effects of the different parameters of the stressors. Noise can be used the stressor for such a study. Unlike the other stressors commonly used in stress research, the intensity and duration of noise exposure can be precisely controlled. Thus, dose-response relationship and interaction effects of the different parameters of the stressor can be determined.

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