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Effects of Conditioned Fear Stress on 5-HT Release in the Rat Prefrontal Cortex

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YOSHIOKA, M., M. MATSUMOTO, H. TOGASHI AND H. SAITO. *Effects of conditioned fear stress on 5-HT release in the rat prefrontal cortex.* PHARMACOL BIOCHEM BEHAV **51**(2/3) 515-519, 1995.—The effects of conditioned fear stress (CFS) on 5-HT release in the medial prefrontal cortex were studied by in vivo microdialysis. CFS (exposure to an environment in which foot-shock had been delivered previously) induced a marked suppression of motility-that is, freezing behavior. The extracellular concentration of 5-HT in the medial prefrontal cortex increased during this freezing behavior, but no significant changes were observed in the concentration of its metabolite, 5-HIAA. The increased 5-HT concentration returned to pretreatment levels when the animals were returned to their home cages. Diazepam (0.5 mg/kg, intraperitoneally) reduced the CFS-induced freezing behavior and prevented the increases in extracellular 5-HT levels. A 5-HT_3 receptor antagonist, tropisetron (10 and 100 μg/kg), also inhibited both the CFS-induced increase in 5-HT release and the freezing behavior. These findings suggest that there is a relationship between anxiety and 5-HT release in the prefrontal cortex and that the 5-HT_3 receptor antagonist tropisetron might have anxiolytic properties.

Serotonin Conditioned fear stress Foot-shock Freezing behavior Medial prefrontal cortex In vivo microdialysis

A NUMBER of studies have shown that stress increases monoamine metabolism and turnover in rats (1,2,10,11,17,23). However, the stressors used in these studies were restraint and foot-shock, treatments that had direct physiologic effects upon the rats used in these experiments. Fanselow (12) reported that 24 h after the delivery of an electric foot-shock to rats, these animals exhibited freezing behavior when placed again into the same test location. This observation suggests the postshock freezing is a conditioned fear response elicited by cues associated originally with the shock. Diazepam, an established anxiolytic drug, has been reported to reduce the freezing response (7,13). This response, known as conditioned fear stress (CFS), is now regarded as a form of psychological stress in the absence of physiologic stimuli, and has been proposed to be a simple animal model of anxiety or fear.

The cerebral cortex is thought to have an important role in the emotional behavior elicited by stressors (14,17,19,30). A recent study found that a conditioned stressor induces *c-fos* mRNA in the rat cingulate cortex (28). Furthermore, Inoue et al. (19) reported that CFS increases serotonin metabolism in the rat medial prefrontal cortex, an observation that suggests that the 5-HT system in the prefrontal cortex is involved in emotional behavior related to anxiety or fear. There are, however, few studies of the effects of CFS on dynamic 5-HT release in the brain. One objective of the present study was to determine directly whether CFS changes neuronal serotonin release in the rat prefrontal cortex. In vivo microdialysis was used to examine the effects of CFS on 5-HT release. We also examined a 5-HT_3 receptor antagonist as a putative anxiolytic drug.

Various types of serotonergic agonists and antagonists modify behavioral responses to stress. For example, buspirone (7), a 5-HT_{1A} agonist, ritanserin (29), a 5-HT_2 receptor antagonist, and ondansetron (32), a 5-HT_3 receptor antagonist, have been shown to be pharmacologically effective in some behavioral models of anxiety. In the present study the effects of tropisetron, another 5-HT_3 receptor antagonist, were compared to those of diazepam upon CFS-induced freezing behavior and upon CFS-induced changes in the neuronal release of serotonin in rat prefrontal cortex.

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METHOD

Animals

Thirty-six male Sprague-Dawley rats (290-350 g, 9-12 weeks old) were used (Shizuoka Laboratory Animal Center, Hamamatsu, Japan). They were housed individually in cages and maintained on a 12 L : 12 D cycle, with free access to food and water. Five groups of rats were studied, namely control rats that received no shock and saline, CFS rats that were treated with saline, CFS rats that were treated with diazepam [0.5 mg/kg, intraperitoneally (IP)], and CFS rats that were treated with either tropisetron, 10 μg/kg, IP, or tropisetron, 100 μg/kg, IP. Each rat was used in only one of these experimental procedures.

Experimental Protocols

In CFS experiments, rats were subjected to inescapable electric foot-shock (1-mA scrambled shock, variable-interval schedule, mean intershock interval 30 s and shock duration 30 s) for 30 min in a chamber with a grid floor. In control experiments, rats were placed in the shock chamber, but no current was applied to the floor of the chamber. These procedures were performed once daily for 2 days. On the 3rd day, both CFS and control rats were placed in the same chamber but received no electric foot-shock. Behavior was observed over a 30-min period, using a time-sampling procedure (12). Every 10 s, the behavior in which the animal was currently engaged was classified as either freezing or active. Freezing was defined as the lack of all observable movement of any parts of the body and vibrissae, except for those movements related to respiration. All other behavior was scored as active.

Brain Microdialysis

Two days before the first foot-shock session, rats were anesthetized with ketamine (100 mg/kg, IP) and a 3-mm concentric guide cannula with an inserted dialysis probe was stereotaxically implanted into the medial prefrontal cortex. The stereotaxic coordinates with respect to the bregma and the dural surface were as follows: rostral, 3.2 mm; lateral, 0.7 mm; and ventral, 4.0 mm (24). Twenty-one hours after the second foot-shock session, the probe was perfused with Ringer's solution for 2 h while the rat was in the home cage, and then every 30 min (10-min intervals during exposure to CFS) during which the perfusates were collected and injected into a high performance liquid chromatography (HPLC) electrochemical detector (ECD) for the measurement of 5-HT and its major metabolite, 5-HIAA. One hour later, the rats were again placed in the same chamber, but no electric foot-shock was delivered; the perfusate was collected for 30 min. After this 30-min exposure to CFS, the rats were returned to their home cages. The HPLC-ECD system consisted of an EP-10 liquid chromatograph pump (Eicom, Kyoto, Japan), a reversed-phase ODS column (Eicom), and an ECD-100 (Eicom). This procedure has been described previously (33). Mean in vitro recoveries (the concentrations of the 5-HT or 5-HIAA in the dialysate expressed as a percentage of the concentrations of these compounds outside of the dialysis probe) ± SEM of eight determinations were 14.9 ± 2.7% for 5-HT and 11.7 ± 1.9% for 5-HIAA. Once an experimental procedure was completed with a rat, the animal was sacrificed and its brain examined histologically to determine the precise insertion site of the dialysis probe.

Drugs

Diazepam was obtained from Sigma (St. Louis, MO) and tropisetron from Sandoz (Basle, Switzerland). Diazepam was suspended in Tween 80, and tropisetron was dissolved in saline. Drugs or saline were administered 30 min before the third (CFS) session in the experimental chamber.

Data Analysis

All values are expressed as a percent of basal release and are presented as means ± SEM. The statistical analysis of data was performed using the two-tailed Student's *t*-test.

RESULTS

Effects of CFS on Behavior

CFS induced a marked suppression of motility (i.e., freezing behavior) during the initial 10-min period of the third session in the experimental chamber (Fig. 1A).

Effects of CFS on Concentrations of 5-HT and Its Metabolite in Perfusate From the Prefrontal Cortex

The extracellular concentration of serotonin in the medial prefrontal cortex increased during CFS-induced freezing behavior and returned to the pretreatment level when rats were returned to their home cages (Fig. 1B). Basal levels serotonin were 0.06 ± 0.01 pg/μl. During CFS-induced freezing serotonin levels increased more than 15-fold ($p < 0.05$, $n = 9$). In contrast, the major 5-HT metabolite 5-HIAA was not significantly changed by CFS. Basal levels of 5-HIAA were 20.2 ± 2.1 pg/μl.

Effects of Anxiolytic Drugs on CFS-Induced Changes in Behavior and 5-HT Release

Diazepam (0.5 mg/kg, IP) reduced the freezing behavior (Fig. 2A) and inhibited the increase in extracellular 5-HT levels in the prefrontal cortex during the time that the rats were placed in the same chamber in which they had received the shock (Fig. 2B). In the diazepam experiments basal levels of serotonin and of 5-HIAA were 0.16 ± 0.04 pg/μl and 10.0

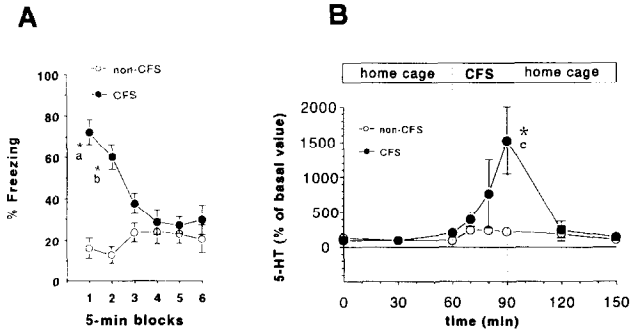

FIG. 1. (A) Effects of conditioned fear stress (CFS) on behavior. Mean percentage ± SEM of freezing scored for each 5-min testing block. Behavior was sampled at 10-s intervals. (B) Effects of conditioned fear stress on extracellular 5-HT. Dialysates were collected beginning 180 min after insertion of the dialysis probe. Values represent the means ± SEM of six to nine rats per group. *Significant difference from the corresponding nonstress control ($p < 0.05$; a, t 6.29 and $df = 16$; b, $t = 5.81$ and $df = 16$; c, $t = 2.65$ and $df = 14$.

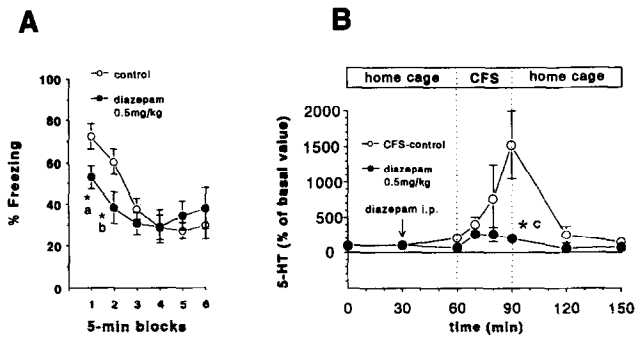

FIG. 2. (A) Effects of diazepam (0.5 mg/kg, IP) on conditioned fear stress-induced freezing behavior. Mean percentage ± SEM of freezing scored for each 5-min testing block. Behavior was sampled at 10-s intervals. (B) Effects of diazepam (0.5 mg/kg, IP) on conditioned fear stress-induced 5-HT release in the prefrontal cortex. Values represent the means ± SEM of six to 10 rats per group. *Significant difference from corresponding control ($p < 0.05$; a, $t = 2.12$ and $df = 16$; b, $t = 2.21$ and $df = 16$; c, $t = 2.34$ and $df = 12$.

FIG. 4. A photomicrograph showing sagittal section of typical implant site of a dialysis probe. Bar = 1 mm.

± 1.0 pg/μl, respectively. Tropisetron (10 and 100 μg/kg) inhibited both the CFS-induced freezing behavior (Fig. 3A) and 5-HT release in the prefrontal cortex (Fig. 3B). In the tropisetron experiments basal levels of serotonin and of 5-HIAA were 0.09 ± 0.02 pg/μl and 14.7 ± 1.2 pg/μl, respectively.

Figure 4shows a typical histologic section that demonstrates the implantation site of the dialysis probe in the rat prefrontal cortex.

DISCUSSION

To clarify the functionally dynamic changes in the serotonergic system induced by conditioned fear stress, we used the in vivo microdialysis method. Freezing behavior can occur when an animal is returned to the same environment in which it had previously received an electric foot-shock. The freezing behavior in such a situation is induced by a conditioned stressor, and not by a physical stressor, such as foot-shock, immobilization, or forced swimming.

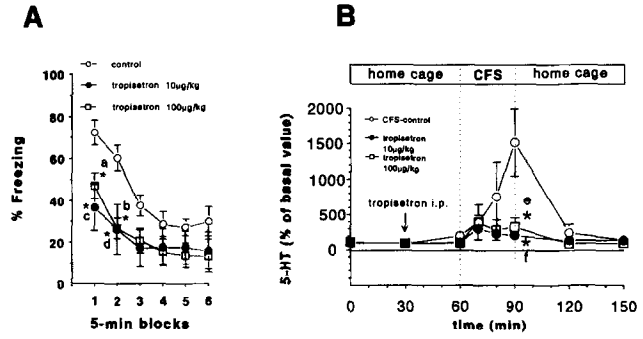

FIG. 3. (A) Effects of tropisetron (10 and 100 μg/kg, IP) on conditioned fear stress-induced freezing behavior. Mean percentage ± SEM of freezing scored for each 5-min testing block. Behavior was sampled at 10-s intervals. (B) Effects of tropisetron (10 and 100 μg/kg, IP) on conditioned fear stress-induced 5-HT release in the prefrontal cortex. Values represent the means ± SEM of six to 10 rats per group. *Significant difference from corresponding control ($p < 0.05$; a, $t = 2.85$ and $df = 16$; b, $t = 2.53$ and $df = 16$; c, $t = 2.66$ and $df = 16$; d, $t = 3.80$ and $df = 16$; e, $t = 2.51$ and $df = 13$; f, $t = 2.06$ and $df = 12$.

In this study, we observed an increase in extracellular 5-HT levels in the prefrontal cortex during CFS-induced freezing behavior. Pretreatment with diazepam, an established anxiolytic drug, attenuated this behavioral response as well as the increase in 5-HT release. These findings are in agreement with those reported by Rex and coworkers (26) in the guinea-pig. In their study exposure to an elevated plus-maze resulted in an increase in cortical extracellular 5-HT levels. These observations suggest that changes in 5-HT release in the prefrontal cortex may be implicated in emotional behavior related to anxiety or fear.

Unconditioned foot-shock stress has been shown to increase monoamine metabolism in such areas of the rat brain as the nucleus accumbens, caudate-putamen, amygdala, and hippocampus (1,2,6,11,17). In contrast to unconditioned stress, conditioned stress (CFS) was reported to increase 5-HT metabolism only in the rat prefrontal cortex (19). Thus, the content of the major metabolite of 5-HT, 5-HIAA, was increased by CFS in the prefrontal cortex, but there were no changes in the other brain regions examined. The lack of significant alterations in extracellular 5-HIAA during CFS observed in the present study contrasts with the findings of increased monoamine metabolites in these previous reports. One explanation for the differences between our study and these previous reports might be that the extracellular concentration of monoamine metabolites throughout the brain does not reflect their concentration at nerve terminals. Thus, extracellular 5-HIAA levels could reflect the turnover of the total 5-HT pool in the brain rather than the stimulated release of neuronal 5-HT (16,21).

Drugs that affect central serotonergic systems have been attracting interest recently as putative anxiolytic compounds. Some clinical studies have found that 5-HT_{1A} receptor agonists are effective in the treatment of anxiety (15), and other clinical studies have reported that the 5-HT_2 receptor antagonist ritanserin is effective in the treatment of generalized anxiety disorder (29). The 5-HT_3 receptor antagonists also seem to be effective in some behavioral models of anxiety (32). The 5-HT innervation of the frontal cortex mostly originates from the dorsal raphe nucleus (3), with the highest concentrations of

5-HT nerve terminals being found in the prefrontal cortex (25). The administration of benzodiazepines, either locally into the dorsal raphe nucleus or systemically, suppresses the firing rate of 5-HT neurons (31). Furthermore, benzodiazepines and 5-HT_{1A} agonists exhibit anxiolytic-like effects when injected directly into the dorsal raphe nucleus (8,18,27).

Microinjection of 5-HT_3 receptor antagonists into the dorsal raphe nucleus has been claimed to produce anxiolytic-like effects in rodents subjected to aversive conditions (8,9). With respect to the distribution of the 5-HT_3 receptors in the CNS (4,20), the highest density of 5-HT_3 binding sites occurs in the area postrema; other areas of the brain also contain 5-HT_3 sites, with the order of density being: entorhinal cortex > retrosplenic cortex > frontal cortex > amygdala > hippocampus > accumbens > septum > thalamus. Thus, it is conceivable that the dorsal raphe may not be a target for 5-HT_3 receptor antagonists. A 5-HT_3 receptor-mediated positive feedback mechanism is proposed in the serotonergic nerve terminals of the guinea-pig cortex (5) and the rat hippocampus (22). In this context, it is possible that 5-HT_3 receptor antagonists act directly in the prefrontal cortex so as to inhibit 5-HT release. Therefore, the neurobiologic mechanisms responsible for the anxiolytic effects of the distinct classes of drugs noted earlier are probably different.

In the present study, we observed that tropisetron, a 5-HT_3 receptor antagonist, blocked both CFS-induced freezing behavior and CFS-induced changes in extracellular serotonin levels, actions that were apparently maximal at the lower dose studied (10 μg/kg, IP).

In conclusion, the increase in extracellular 5-HT in the prefrontal cortex of rats placed in the foot-shock chamber, with the recovery to basal 5-HT levels on their return to a familiar environment, could indicate a relationship between a state of anxiety and 5-HT release. This view is supported by the findings that diazepam had an anxiolytic effect and inhibited 5-HT release during exposure to CFS. The 5-HT_3 receptor antagonist tropisetron may also have an anxiolytic-like effect.

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