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Expression of Aggression Attenuates Both Stress-Induced Gastric Ulcer Formation and Increases in Noradrenaline Release in the Rat Amygdala Assessed by Intracerebral Microdialysis

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TANAKA, T., M. YOSHIDA, H. YOKOO, M. TOMITA AND M. TANAKA. *Expression of aggression attenuates both stress-induced gastric ulcer formation and increases in noradrenaline release in the rat amygdala assessed by intracerebral microdialysis.* PHARMACOL BIOCHEM BEHAV **59**(1) 27–31, 1998.—The effects of an aggressive biting response on stress-induced noradrenaline (NA) release in the rat amygdala and gastric ulcer formation were studied with an intracerebral microdialysis technique. Rats were exposed to a 60-min period of cold restraint stress with or without being allowed to bite a wooden stick. They were sacrificed 100 min after release from stress to investigate gastric ulcer formation. Cold-restraint stress increased NA release to 304 ± 22.3 and 206 ± 23.8% of basal levels (mean ± SEM) in the nonbiting and biting groups, respectively. The stress-induced increases in NA release in the nonbiting group were significantly higher than those in the biting group. In the nonbiting group, significant increases in NA release continued for 80 min after release from stress; however, NA levels in the biting group recovered to basal levels immediately after the cessation of stress. Although many severe gastric lesions with bleeding were found in the nonbiting group, fewer gastric lesions without bleeding were found in the biting group. The cumulative length of gastric lesions in the nonbiting group and in the biting group was 26.2 ± 7.4 and 6.8 ± 3.9 mm (mean ± SEM), respectively. The mean number of ulcers in the nonbiting group and the biting group was 11.8 ± 1.3 and 1.8 ± 0.7 (mean ± SEM), respectively. Both the cumulative length of ulcers and the number of ulcers were significantly lower than those seen in the nonbiting group. These findings strongly suggest that expression of aggression during stress exposure attenuates not only stress-induced increases in NA release in the rat amygdala but also gastric ulcer formation consequent to stress. © 1998 Elsevier Science Inc.

Cold restraint stress Noradrenaline release Basolateral nucleus of the amygdala Expression of aggression Gastric lesions

IT has been reported that rats exposed to restraint stress while being allowed to bite a brush or wood block developed less acute gastric mucosal lesions than did rats that were unable to display aggressive biting or gnawing responses (5,18). Tsuda et al. (17) reported that giving rats an opportunity to express aggression during stress exposure results in a significant attenuation of stress-induced increases in noradrenaline (NA) turnover in the hypothalamus, amygdala, thalamus, and basal ganglia. These studies have suggested that expression of aggression is closely related not only to reduction of brain noradrenergic neuronal activity, but also to attenuation of peripheral pathological changes such as gastric ulcer formation produced by stress.

It is well established that noradrenergic neurons in many brain regions in rats are activated by a variety of stressful stimuli (13,14). Psychological stress increased NA turnover

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selectively in the hypothalamus, amygdala, and locus coeruleus region (9). The amygdala is particularly closely related to negative emotional changes (fear and anxiety) observed in animals exposed to stressful stimuli (8,14). We have also reported that immobilization and tail-pinch stress increase NA release in the rat amygdala (15). Using the in vivo microdialysis technique, we can more accurately define changes in the time course of NA release induced by immobilization stress with or without being allowed to bite a wooden stick.

The purpose of the present study was to determine whether or not an aggressive biting response could attenuate both increases in NA release in the amygdala and gastric ulcer formation produced by cold restraint stress.

METHOD

All animal procedures were performed in accordance with the Guiding Principles for The Care and Use of Animals in The Field of Physiological Sciences (The Physiological Society of Japan) and were approved by the Committee of Animal Experimentation, Kurume University School of Medicine.

In Vivo Microdialysis

Male Wistar rats (body weight 200–220 g) were used. Animals were kept in a temperature-controlled room (24 ± 1°C) under a 12-h (0700–1900 h) light–dark cycle and anesthetized with sodium pentobarbital at 50 mg/kg (IP) and stereotaxically implanted with a U-shaped microdialysis probe into the right basolateral nucleus of the amygdala. The coordinates for placement of the tip of the probes were: A −3.0 mm, L 5.2 mm, V 9.0 mm from bregma and the dural surface, according to the atlas of Paxinos and Watson (10). The active region of the cellulose hollow dialysis tube (0.25 mm diameter molecular weight cut off: 50,000) was 4 mm in length. All experiments were carried out one day after the implantation of the dialysis probe, on freely moving, conscious rats during the light phase of the light–dark cycle.

The rat was connected directly to the HPLC equipment for on-line analysis of NA (EICOM Co., Kyoto, Japan). The dialysis tube was perfused with the following solution (mM): (NaC1 140, KC1 3.35, $MgC1_2$ 1.15, $CaC1_2$ 1.26, Na_2HPO_4 1.20, and NaH_2PO_4 0.3, pH 7.4) at a flow rate of 3.0 ml/min using a microperfusion pump. The dialysis sample (60 ml) was injected every 20 min via an autoinjector (EICOM AS-10). The mobile phase consisted of 0.1 M sodium acetate, 0.03 mM EDTA, 2.0 mM octanesulfonic acid, and 12% methanol at pH 4.3. NA was separated on an Eicompack MA-ODS column (4.6, 3.6 × 150 mm, EICOM Co.) at 25°C. The graphite working electrode was set at +600 mV vs. an Ag/AgCl reference electrode (EICOM ECD-100 electrochemical detector) and the flow rate (EICOM EP-10 pump) was 0.9 ml/min. An integrator (Chromatocorder 12, SIC, Hachioji, Japan) was used to record the signal.

The average of three baseline samples immediately preceding stress was defined as 100% and all subsequent measures were related to these values (% changes).

Procedure

Dialysis experiments were carried out one day after the implantation of the probe. Food and water were provided ad lib before the surgical implantation of the dialysis probe. All food was removed for 24 h before the dialysis experiment was begun. Rats were never deprived of water after the implantation of the probe. After a stable baseline was obtained, rats were exposed to a 60-min period of cold restraint stress by immobilizing the rat on a 13 × 22 cm wooden board (3) with (biting group) or without (nonbiting group) biting a piece of wooden stick. Each leg was extended at a 45° angle from the body midline and secured with a cotton thread. During the stress, the animals in both groups were moved into an expanded polystyrene foam box (46 × 21 × 16 cm) that was filled with shaved ice. After the rats were moved into the box, it was covered with a lid to keep the temperature cold. The temperature in the box was taken during the cold restraint stress procedure and was maintained at 6 ± 1°C. The rats in the biting group were given visual and tactile stimulation by a wooden stick (18 cm of length and 0.5 cm of diameter), which was extended toward the mouth of the rat by the experimenter. The rats were then given an opportunity to bite the wooden stick. The rats in the nonbiting group were exposed to the same cold restraint stress for 60 min in the same expanded polystyrene foam box but were not confronted with the wooden stick. After release from stress, the animals were moved into a Plexiglas cage (28 × 28 × 30 cm), which was used to obtain a stable baseline, and were kept therein for 100 min. They were then sacrificed by administration of sodium pentobarbital (more than 250 mg/kg, IP) and the stomachs were removed, opened along the greater curvature, pinned on a cork board, fixed in 10% (v/v) formalin solution, and examined immediately under a microscope by an observer naive to experimental conditions. The number and cumulative length of lesions were recorded. The total number and length of lesions were used as a measure of gastric pathology. At the end of each experiment, the position of the microdialysis probe was verified histologically by a method that we previously reported (15).

Statistical Analysis

Differences between the baseline dialysate concentrations and the samples during or after the stress exposure were analyzed by one-way analysis of variance followed by the Newman–Keuls test. Comparison between the effects of cold restraint stress with biting and without biting on NA levels was analyzed by two-way analysis of variance followed by the Newman–Keuls test. Comparison between the effects of cold restraint stress with biting and without biting on the number and length of gastric lesions was analyzed by Student's *t*-test.

RESULTS

Effect of Cold Restraint Stress With or Without Biting on Extracellular NA Concentrations

The average basal extracellular NA levels detected in all animals used in these experiments ($n = 8$) were 3.42 ± 0.34 pg/60 ml/20 min. In the nonbiting group, cold restraint stress caused significant increases in NA release, $F(7,24) = 15.086$, $p < 0.001$. The maximum increase was observed in the first sample following application of stress (304%, $n = 4$) and then increases in NA concentrations decreased gradually. However, significant increases in NA release continued for 80 min following cessation of stress. The same stress also caused significant increases in NA release in the biting group, $F(7,24) = 10.470$, $p < 0.001$. The elevation of NA levels in the biting group examined both during and after cessation of stress were significantly lower as compared with those in the nonbiting group, $F(13,42) = 14.591$, $p < 0.001$. NA levels in the biting group returned to basal levels immediately following cessation of stress, whereas those in the nonbiting group never re-

turned to basal levels even 80 min after the release from stress (Fig. 1).

Stomach Lesions

Analysis of the gastric ulcer data indicated that the biting rats had significantly fewer and smaller lesions as compared to the non-biting rats (Fig. 2).

DISCUSSION

It is clear from this study that giving rats an opportunity to engage in an aggressive biting response subsequently results in a significant attenuation of both the enhancement of NA release from the amygdala and gastric ulcer formation induced by cold supine restraint stress. These results demonstrate that expression of aggression during stress exposure has a protective effect not only in terms of central noradrenergic response but also in terms of peripheral physiological-endocrine activation (ulcerogenic procedure) induced by stress.

Tsuda et al. (17) reported that giving rats an opportunity to express aggression (bite a wooden stick) during stress exposure resulted in a significant attenuation of stress-induced increases in NA turnover in the hypothalamus, thalamus, midbrain, and basal ganglia compared to rats that were not given an opportunity to bite. They measured levels of NA and 3-methoxy-4-hydroxyphenylenethylenglycol sulfate (MHPG-SO_4), a major metabolite of NA, in discrete brain regions at 0 min and 50 min after release from a brief period of supine restraint stress. In the amygdala, they found significant attenuation of stress-induced increases in MHPG-SO_4 levels in the rats allowed to bite a wooden stick at 50 min, but not at 0 min after release from stress. The present study, using the in vivo microdialysis technique, clarified the different changes in NA release related to the time course during and after release from stress. The present data clearly show that biting rats exhibited less elevation of NA release during stress and after release from stress as compared to nonbiting rats which were exposed to the same stress situation without the opportunity to bite. It should be noted that NA levels in the biting rats returned to basal levels immediately after release from stress. This result is different from the previous study (17) in which they found significant attenuation of stress-induced increases in MHPG-SO_4 levels in the amygdala of biting rats at 50 min, but not at 0 min after release from stress. They used a brief (10-min) period of supine restraint stress and measured tissue NA and MHPG-SO_4 levels using a fluorometric method, while we used 60 min of cold plus restraint stress and measured extracellular NA levels using a microdialysis technique. These different methods may be the main reason for the different results.

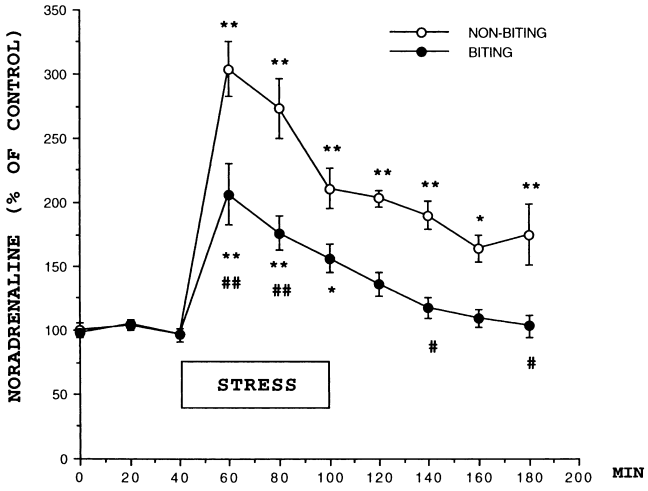

FIG. 1. Effect of cold resistant stress for 1 h on amygdalar noradrenaline release with and without being allowed to bite a wooden stick. Each value indicates the mean ± SEM of four rats. BITING: biting group rats were exposed to cold restraint stress for 1 h and allowed to bite a wooden stick. NONBITING: nonbiting group rats were given the same stress without being allowed to bite. $^{*}p < 0.05$ (vs. basal values); $^{**}p < 0.01$ (vs. basal values); $^{\#}p < 0.05$ (vs. NONBITING); $^{\#\#}p < 0.01$ (vs. NONBITING).

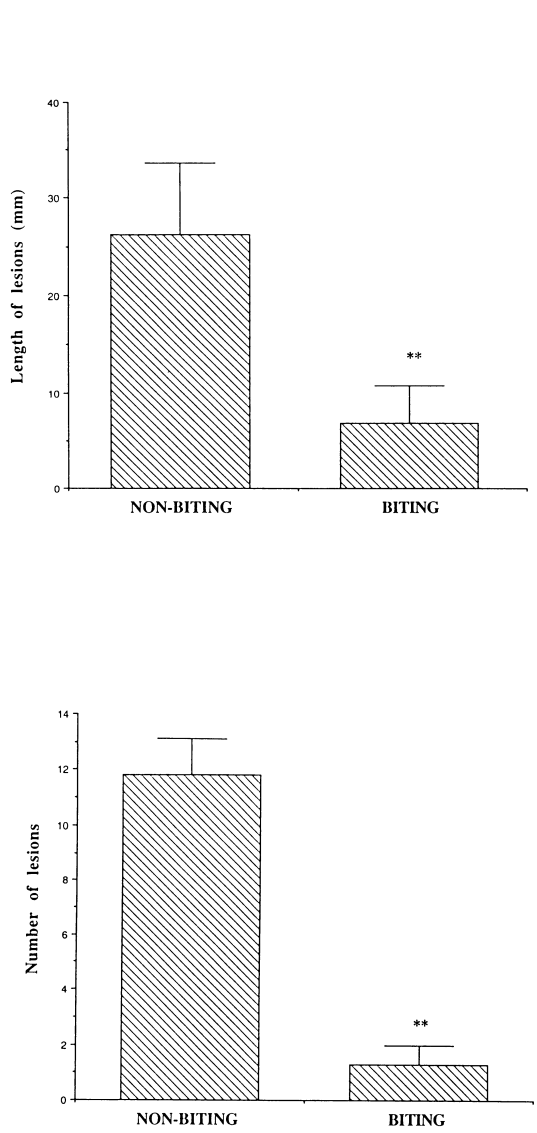

FIG. 2. Effect of cold resistant stress for 1 h on the number (A) and length (B) of gastric lesions with and without being allowed to bite a wooden stick. Each value indicates the mean ± SEM of four rats. BITING: biting group. NONBITING: nonbiting group. See the legend for Fig. 1 for details. $^{**}p < 0.01$ (vs. NONBITING group).

On the contrary, a significant elevation of NA release in the nonbiting rats was maintained until 80 min after release from stress. This result is consistent with the previous report (17).

We have reported that stress-induced increases in NA turnover in the amygdala are closely related to the provocation of negative emotion such as anxiety and/or fear observed in animals during stress exposure (14,15). Changes in NA turnover in the amygdala were regarded as indicators of psychological dimensions including the ability to cope with the stressor (9,16). Therefore, the present data suggest the possibility that expression of aggression during stress exposure might attenuate stress-induced fear and/or anxiety. It might be speculated that aggressive biting behavior distracts attention from the negative emotion (anxiety and/or fear) of the cold restraint stress situation and thereby reduces the adverse effects of that stressor (15). If this hypothesis holds true for humans, then expression of aggression may have a therapeutic benefit in the form of a coping behavior for the psychosomatic diseases such as stress ulcer (1) or neurosis.

Much evidence suggests that the central noradrenergic system is activated when animals are subjected to ulcerogenic stress procedures (4). The amygdala is regarded as an important brain site for modulating the development of stress-induced gastric erosions (4). For example, stimulation (7) or lesions (6) of the central nucleus of the amygdala produced or reduced gastric ulcers, respectively. These findings suggest that the central nucleus of the amygdala is an important site in which stressful experiences influence gastric functions (4). In the present study, we found that both stress-induced NA release in the basolateral nucleus of the amygdala and gastric lesions in the biting group were significantly smaller than those in the nonbiting group. Taken together, these data indicate that not only the central nucleus of the amygdala but also the basolateral nucleus of the amygdala are closely related to stress-induced gastric ulcer formation.

The present study indicates that the number and length of gastric lesions resulting from cold restraint stress were markedly fewer and smaller in rats that had access to an aggressive biting response as compared to rats which could not. Thus, it seems that expression of aggression has a protective effect both in a situation that causes negative emotion (anxiety and/or fear) and gastric ulcers. There are several hypotheses to explain these results. One possibility is that biting an object during stress exposure may decrease anxiety and/or fear in the biting group rats, thus decreasing stress-induced NA release, which, in turn, produces less elevation of plasma corticosterone and adrenocorticotropin (ACTH) (20). As a result, biting rats develop less severe ulceration than animals not allowed biting behavior. Brain CRF exerts a protective role against the damaging effects of cold stress (11); therefore, our hypothesis is not consistent with this report. Second, it is possible that biting behavior represents a manifestation of stress-induced negative emotion, which helps to terminate sympathetic arousal and restore autonomic balance (2). Thus, animals with biting exhibited less ulceration. A third possibility is that biting behavior may distract the animal's attention from the discomfort of the cold stress; thus, stress-induced ulceration decreased in the biting group (17,19). Another possibility is that the biting response may keep the rat warmer as a result of the considerable movements such as struggling and head moving compared to the nonbiting rats. Consequently, aggressive biting reduces the ulcerogenic effect of restraint stress, because stress-induced hypothermia is related to gastric ulcer formation (12,18).

Because we did not take body temperature in both groups, we could not confirm this hypothesis in the present study. The last possibility is that aggressive behavior may reduce input of stressors that produce increases in NA release in the amygdala. Thus, attenuation of stress-induced NA release reduces gastric lesions in the biting group. Although we cannot clarify why biting behavior reduces ulcer formation in this study, we can speculate that attenuation of stress-induced increases in NA release in the amygdala may be closely related to the reduction in stress-induced gastric ulcer formation.

In conclusion, the present study demonstrates that expression of aggression during stress exposure attenuates both stress-induced increases in NA release in the amygdala and gastric ulcer formation, compared to rats that were not allowed to express aggression by biting. These results strongly support the hypothesis that suppression of aggression during stress exposure may lead to psychosomatic diseases such as peptic ulcer and/or neuroses in humans.

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