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Psychological Stress-Induced Increases in Noradrenaline Release in Rat Brain Regions Are Attenuated by Diazepam, But Not by Morphine

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TANAKA, M., A. TSUDA, H. YOKOO, M. YOSHIDA, K. MIZOGUCHI AND T. SHIMIZU. *Psychological stress-induced increases in noradrenaline release in rat brain regions are attenuated by diazepam, but not by morphine.* PHARMACOL BIOCHEM BEHAV **39**(1) 191–195, 1991.—By measuring levels of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in the hypothalamus, amygdala and locus coeruleus region, we investigated the effects of diazepam 5.0 mg/kg, morphine 6.0 mg/kg, or naloxone at 5.0 or 10 mg/kg injected SC immediately before stress exposure, on increases in NA release caused by psychological stress. Psychological stress, wherein rats were exposed to emotional responses which were displayed by other electrically shocked rats, significantly increased MHPG-SO_4 levels in the three brain regions examined and elevated plasma corticosterone levels. Both increases in brain MHPG-SO_4 levels and elevations of plasma corticosterone levels induced by stress were attenuated significantly by diazepam but neither by morphine nor by naloxone. MHPG-SO_4 levels in the hypothalamus and amygdala in the morphine-stress group were significantly higher than those in the saline-stress group. These findings suggest that psychological stress, in which an emotional factor is predominantly involved, causes increases in NA release in these brain regions examined and that these increases are attenuated only by diazepam, in contrast to the previous report, where increases in brain NA release caused by immobilization stress are attenuated not only by diazepam but also by morphine and are enhanced by naloxone.

Psychological stress Noradrenaline release Diazepam Morphine Naloxone Brain regions Plasma corticosterone

IT has been well documented that the marked increases in noradrenaline (NA) release in rat brain regions are caused by immobilization stress (3, 14–16). These increases in NA release induced by immobilization stress are attenuated by morphine (16), a potent opiate, and diazepam (6), a typical benzodiazepine anxiolytic, in a naloxone- (an antagonist of opioids) (16) and Ro 15-1788- (an antagonist of benzodiazepines) (6) reversible manner, respectively. In contrast, pretreatment with naloxone results in enhancement of increased NA release induced by immobilization stress in the hypothalamus, amygdala and thalamus (15). The enhancing effect of naloxone is considered to be due to its blockade of endogenous opioid peptides released during stress at the opioid receptor sites (15).

Emotional factors have been considered to be involved in immobilization stress, however, there is a possibility that physical factors also affect brain NA release. The psychological stress which was employed in the present study is characterized by no direct involvement of physical stimuli, i.e., the animals were merely placed in the compartment which was in full sight, sound and smell of other electrically shocked rats. The previous reports indicate that immobilization stress increases regional NA release markedly and extensively (3, 14–16), whereas psychological stress produces slight increases preferentially in the hypothalamus, amygdala and locus coeruleus (LC) region but not in the cerebral cortex, hippocampus, thalamus and midbrain (7,22). These findings raise the possibility that effects of these drugs on stress-induced NA release might be different depending on differences in the nature of the stressors given to the animals, i.e., immobilization and psychological stressors.

In order to clarify these problems, the present study was undertaken to investigate effects of diazepam, morphine and naloxone on increases in NA release in those brain regions known to be affected by psychological stress.

METHOD

Animals

In our previous studies, the male Wistar rats were used. In order to compare these data, male Wistar rats weighing 170–190 g were subjected in this study, however, another study should be needed on the female rats. They were housed in groups of

four in a temperature-controlled room (24 ± 1°C) under a 12-h light-dark cycle and provided with food and water ad lib throughout the experimental period.

Apparatus and Psychological Stress Procedure

Stress treatments were produced using the apparatus originally employed as a communication chamber for mice (12), which was modified for rats by us as previously reported (7). Briefly, the box measured 93 × 99 × 53 cm with a floor composed of 0.3 cm stainless steel rods placed 1.3 cm apart (center to center). The chamber was subdivided into 25 smaller compartments (18 × 19 cm) by transparent plastic walls. A scrambled electric shock was delivered through the floor grid by a fixed impedance AC stimulator (60 Hz pulse wave). The shock consisted of 80 ms pulses separated by 420 ms intervals and was given for a 5-s duration at intervals of 30 s. The shock intensity was fixed at 70 V (about 3.5 mA). Fifteen rats were individually placed in the shock compartment and electric shock was delivered. Ten rats were placed in the nonshock compartment where the rats did not receive any shock, since the plastic plates were placed on the grids of these compartments to prevent the rats from receiving foot shock. This situation, wherein the rats received no shock but were exposed to the emotional responses which were displayed by other electrically shocked rats, was designated as psychological stress. The electrically shocked rats showed struggling, vocalizing, jumping and defecating in the first 30 min but these behaviors were gradually reduced in the last 30 min.

Experimental Procedure

Prior to the experiment, all animals were placed into the compartments without foot shock for 1 h every day for 3 days for a habituation phase. One day before the experiment, the rats were divided into six experimental groups by balancing body weights. Control rats were injected with saline and placed in the compartment of the same box, wherein the electric shock was given to the shocked animals, for 1 h neither with electric shock nor together with electrically shocked rats. All rats in the remaining five groups were injected SC with either saline, diazepam at 5.0 mg/kg, morphine at 6.0 mg/kg, or naloxone at 5.0 mg/kg or at 10 mg/kg, and immediately exposed to psychological stress for 1 h. In order to compare to the previous results on immobilization stress (6,16), only one dose of diazepam and morphine was used and the time of drug administration was fixed. These animals received no shock but were exposed to the emotional responses of shocked rats, which included struggling, vocalizing, defecating, urinating and jumping.

Drugs

Morphine hydrochloride (Sankyo K.K.) and naloxone hydrochloride (a gift from Sankyo K.K.) were dissolved in physiological saline, and diazepam (a gift from Nippon Roche K.K.) was suspended in 0.3% carboxymethylcellulose sodium.

Tissue Preparation and Biochemical Determinations

Immediately after each treatment, the rats were sacrificed by decapitation. The brain was rapidly removed and the hypothalamus and amygdala were dissected out according to the method of Gispen et al. (2) and the LC region by the method of Reis and Ross (13), and frozen on solid CO_2. The blood from the cervical wound was collected in heparinized tubes and centrifuged. The plasma and brain tissues were stored at −45°C until assayed.

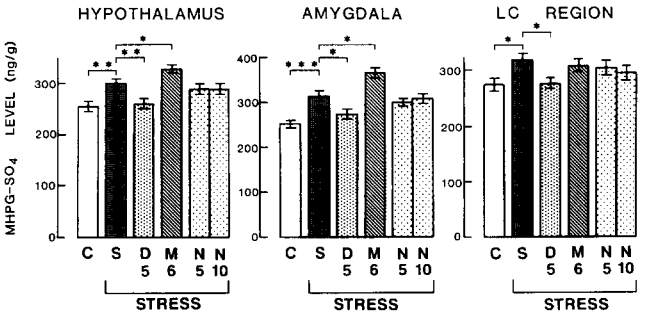

FIG. 1. Effects of psychological stress for 1 h on levels of 3-methoxy-4-hydroxyphenyletyleneglycol sulfate (MHPG-SO_4) in the three rat brain regions and their modifications by the drugs. Each value indicates the mean ± S.E.M. of 8 rats. Abbreviations: C: control, S: saline, D5: diazepam 5.0 mg/kg, M6: morphine 6.0 mg/kg, N5: naloxone 5.0 mg/kg, N10: naloxone 10 mg/kg. The horizontal bar indicates statistical significance between the respective two groups. Statistical significance: $*p<0.05$, $**p<0.01$, $***p<0.001$

Levels of NA and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), the major metabolite of rat brain NA and indicative of brain NA release, were measured in the hypothalamus and amygdala according to our fluorometric method (9). In the LC region, only MHPG-SO_4 levels, which were considered to reflect dendritic transmission of NA neurons, were determined according to the same method, because of the small amount of this tissue (around 15–20 mg of tissue weight). Plasma corticosterone levels were determined according to the method of van der Vies (23).

Statistical Analysis

Data were analyzed by a one-way (treatment) analysis of variance (ANOVA) and subsequent Tukey's honestly significant difference (HSD) pairwise comparisons.

RESULTS

MHPG-SO_4 levels in the hypothalamus, amygdala and LC region for all six groups are indicated in Fig. 1. One-way ANOVAs revealed that MHPG-SO_4 levels significantly differed among the six groups in the hypothalamus, $F(5,42)=8.03$, $p<0.01$, and the amygdala, $F(5,42)=10.99$, $p<0.01$, but did not in the LC region, $F(5,42)=1.95$, $p>0.10$. Tukey's HSD post hoc comparisons ($p<0.05$) indicated that psychological stress for 1 h caused significant increases in MHPG-SO_4 levels in the hypothalamus and amygdala. Also, these increases were significantly attenuated by pretreatment with diazepam at 5.0 mg/kg in these brain regions but not affected by naloxone at 5.0 mg/kg and 10 mg/kg. The metabolite levels in the rats, stressed and treated with morphine at 6.0 mg/kg, were significantly higher than those in saline-stressed rats.

Although an ANOVA revealed that MHPG-SO_4 in the LC region were not significantly influenced by treatments, Tukey's HSD post hoc comparisons ($p<0.05$) were conducted where a prior prediction had been made. These comparisons indicated that, when compared to the controls, psychological stress produced significant elevations of MHPG-SO_4 which were reliably attenuated by pretreatment with diazepam at 5.0 mg/kg. These data showed the tendency that psychological stress caused in-

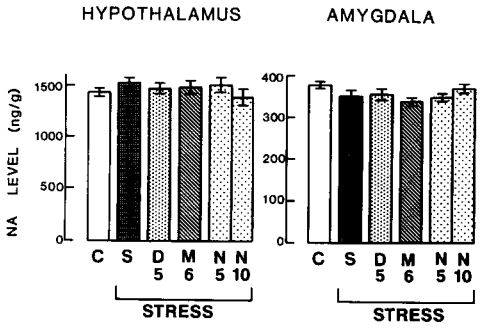

FIG. 2. Effects of psychological stress for 1 h on noradrenaline (NA) levels in the two rat brain regions and their modifications by the drugs. Each value indicates the mean ± S.E.M. of 8 rats. Abbreviations: C: control, S: saline, D5: diazepam 5.0 mg/kg, M6: morphine 6.0 mg/kg, N5: naloxone 5.0 mg/kg, N10: naloxone 10 mg/kg.

creases in MHPG-SO_4 levels and that these increases were attenuated by diazepam.

Levels of NA in the hypothalamus and amygdala for all six groups are shown in Fig. 2. One-way ANOVA revealed that none of psychological stress and drug effects significantly influenced NA levels in any of brain regions examined.

Plasma corticosterone levels for all six groups are indicated in Fig. 3. One-way ANOVA revealed that plasma corticosterone levels were reliably affected by these treatments, $F(5,42) = 39.01$, $p<0.01$. Tukey's HSD post hoc comparisons ($p<0.05$) indicated that plasma corticosterone levels were significantly elevated by psychological stress and these increases were significantly attenuated by diazepam. The corticosterone levels in the rats, stressed and treated with either morphine at 6.0 mg/kg or naloxone at 10 mg/kg, were significantly higher than those in saline-stressed rats.

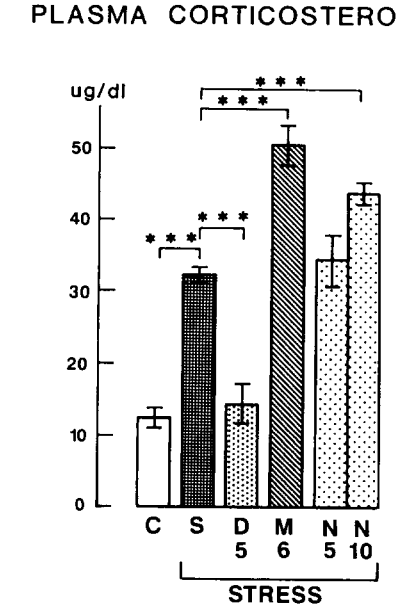

FIG. 3. Effects of psychological stress for 1 h on plasma corticosterone levels and their modifications by the drugs. Each value indicates the mean ± S.E.M. of 8 rats. Abbreviations: C: control, S: saline, D5: diazepam 5.0 mg/kg, M6: morphine 6.0 mg/kg, N5: naloxone 5.0 mg/kg, N10: naloxone 10 mg/kg. The horizontal bar indicate statistical significance between the respective two groups. Statistical significance: ***$p<0.001$

DISCUSSION

Psychological stress, employed in the present study, is characterized by involvement of no direct physical stimuli. The rats were merely placed in the compartment where they were exposed to emotional responses which were shown by other electrically shocked rats, such as defecation, urination, vocalization and struggling, by seeing, hearing and smelling them. In these respects, psychological stress is considered to consist of various components such as emotional, cognitive and social aspects, however, it seems to be difficult to define one special component.

In the present study, we selected three brain regions, i.e., the hypothalamus, amygdala and LC region, since we have found that psychological stress increases NA release preferentially in these three regions but not in other regions as the hippocampus, thalamus, cerebral cortex and midbrain (7,22). Psychological stress for 1 h significantly increased MHPG-SO_4 levels in the hypothalamus, amygdala and LC region without affecting NA levels. The degrees of psychological stress-induced increases in the metabolite levels in the hypothalamus, amygdala and LC region, i.e., 18%, 24% and 16% of the control values, respectively, were less than those induced by electric shock, i.e., 74% and 68% (7), respectively (the data in the LC by electric shock were not obtained). The reductions of NA levels were not accompanied by changes in MHPG-SO_4 as observed in the previous report, wherein immobilization stress caused both marked increases in MHPG-SO_4 levels and marked reductions in NA levels (14,16), which suggests that marked NA release exceeded amine synthesis. This finding indicates that psychological stress causes increases in NA release in these regions as previously reported (7,22), although there might be a possibility that increases in NA release might occur in other brain regions which have not been examined in the present study, i.e., the septal area and prefrontal cortex, etc.

The finding that psychological stress increases NA release in the hypothalamus was further supported by our recent observations using microdialysis, that NA levels in the dialysates in the anterior hypothalamus were significantly increased by stressors similar to psychological stress (conditioned fear), wherein the rats were replaced to the environment where the rats had received footshock previously (25).

Diazepam significantly attenuated psychological stress-induced increases in MHPG-SO_4 levels in the three brain regions examined. We have reported that diazepam at the present dose, when given to nonstressed rats, did not affect MHPG-SO_4 levels with the exception of slight increases in the metabolite levels in the hypothalamus (6). The same dose of diazepam also significantly attenuated increases in MHPG-SO_4 levels caused by immobilization stress in the same three regions (6). These findings suggest that diazepam attenuates increases in NA release in these regions induced not only by psychological but also by immobilization stresses.

In contrast, the effects of morphine are quite different depending on the nature of the stressor. In spite of the fact that morphine at 6.0 mg/kg, which is a common dose for inducing analgesia in animals (4,5), significantly attenuates increases in MHPG-SO_4 levels caused by immobilization stress (16), the same dose of the drug failed to attenuate increases in MHPG-SO_4 levels caused by psychological stress in any brain region. This finding indicates that the attenuating effects of morphine

on stress-induced increases in NA release are different depending on the nature of the stressor: morphine is likely to effectively attenuate stress-induced neurochemical changes only in stress situations wherein physical or nociceptive stimuli are involved. This suggestion agrees with the clinical findings that morphine has an action to relieve distress in patients suffering from severe pain (8).

Naloxone failed to affect increases in MHPG-SO_4 levels induced by psychological stress, although the drug enhanced the increases caused by immobilization stress (15) and virtually unaffected brain NA release in nonstressed rat (15). Together with our previous report that Met-enkephalin or β-endorphin injected ICV significantly attenuates increases in MHPG-SO_4 levels induced by immobilization stress (17, 19–21), it is suggested that the enhancing effects of naloxone in the case of immobilization stress might be due to the blockade of endogenous opioid peptides released during stress. We have also observed that analgesia appears in a naloxone-reversible manner under immobilization stress but not under psychological stress (18). It is suggested that release of opioid peptides is increased under immobilization stress but not under psychological stress, which might account for the lack of naloxone effects on changes in MHPG-SO_4 levels caused by psychological stress.

Plasma corticosterone levels were significantly elevated by psychological stress, consistent with previous reports (22) and these increases are significantly attenuated by diazepam but not by either morphine or naloxone, which enhanced secretion of this hormone. Morphine, given to nonstressed rats, increases plasma corticosterone levels in a dose-dependent manner (16) and the drug enhanced elevation of this hormone levels caused by immobilization stress (16). These effects might be due to the enhanced secretion of ACTH from the anterior pituitary gland produced by morphine (1, 10, 24), however, the reason why enhancement of stress-induced elevations of plasma corticosterone levels caused by naloxone is unknown.

In the present study, only one dose of diazepam and morphine was used and the injection time was fixed in order to compare the drug effects on increases in brain NA release caused by two different stresses, i.e., psychological and immobilization stresses, since the dose is effective in attenuation of brain NA release caused by immobilization stress (6,16). There might be a possibility that differences in pharmacokinetics of these drugs could lead to different actions on stress-induced increases in brain NA release. However, it is unlikely, since the doses used, the time of the drug administration and the duration of stresses were almost the same in the two studies where the two different stresses were employed (6, 15, 16).

The present study further suggests that increases in NA release in the hypothalamus, amygdala and LC region are, in part, closely related to the provocation of fear and/or anxiety in the animals (24). As a role of brain NA, tuning between signal in the central nervous system is pointed out (11), however, further studies should be needed to clarify the role of the brain NA system.

In conclusion, diazepam, but not morphine, shows attenuating effects on increases in NA release in the hypothalamus, amygdala and LC region as well as elevations of plasma corticosterone levels caused by psychological stress. The present study suggests that psychological stress possesses different neurochemical mechanisms from that of immobilization stress and, furthermore, that the drug effects are different depending on the nature of the stressor. Diazepam may be effective on changes induced by both psychological and immobilization stresses, however, morphine may affect only changes induced by immobilization stress.

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