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BRIEF COMMUNICATION

Stressor Predictability and Rat Brain Noradrenaline Metabolism[1]

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TSUDA, A., Y. IDA, H. SATOH, S. TSUJIMARU AND M. TANAKA. *Stressor predictability and rat brain noradrenaline metabolism.* PHARMACOL BIOCHEM BEHAV 32(2) 569–572, 1989.—This study examined the effects of stressor predictability on regional rat brain noradrenaline (NA) turnover, by measuring levels of a principal metabolite of NA (3-methoxy-4-hydroxyphenylethyleneglycol sulfate, MHPG-SO_4). Male Wistar rats were exposed to one of three shock conditions for 19 hr: nonshock, signalled, and unsignalled shocks. Rats in the shock conditions received shock (1.2 mA intensity, 2 sec duration) on a 2.5 min variable time (VT) either preceded by a 12-sec, 10-W light signal (signal-shock interval of 10 sec) or not preceded by this signal. The tail electrodes for these rats were in series, so that the shock received by all rats was of exactly the same number and duration. After 19 hr in a VT-2.5 min shock session, the rats exposed to unsignalled shock (unpredictable group) showed significantly greater increases in MHPG-SO_4 levels in the hypothalamus, amygdala, midbrain, cerebral cortex, thalamus and locus coeruleus, as well as in plasma corticosterone levels. Rats exposed to signalled shock (predictable group) showed significant increases in MHPG-SO_4 levels in the first four of these regions, as compared to the nonshocked rats. Moreover, the unpredictably shocked rats exhibited greater elevations in MHPG-SO_4 levels in the hypothalamus, amygdala, and thalamus, as well as in plasma corticosterone levels, when compared to the predictably shocked rats. These results are consistent with previous reports showing that unsignalled shock induced extensive somatic effects in comparison to signalled shock. The present study suggests that the presence of a signal attenuates the extent of NA release in some brain regions resulting from irregular inescapable shock stress.

Shock predictability Psychological stress Rat brain regions Noradrenaline metabolism MHPG-SO_4
Fear and/or anxiety

OVER the past 20 years, the concept of stressor predictability has been a main theme in understanding the psychopathology of fear, anxiety, and stress (21). Do animals suffer more stress when an inescapable stressor (e.g., electric shock) is predicted by a signal (e.g., light), or do they suffer more stress when the occurrence of the stressor is not predicted by a signal?

It has been well-documented that the predictable-unpredictable nature of a stressor can modulate the behavioral and physiological impact of that stressor [see (1) for a review]. Some studies show that unpredictable stressors are more stressful, arousing, or fear and/or anxiety-producing than predictable ones (10, 16, 17, 29, 31). For example, Weiss (31) reported that rats exposed to shock intermittently and which received a signal prior to shock, developed less severe gastric lesions than did the rats which received shock paired randomly with a signal. In addition, given a choice, rats showed a strong preference for a signal correlated with the presentation of shock (7).

However, only very few studies have thus far dealt with the effects of unpredictability versus predictability of shock affecting noradrenergic neuronal activity in the brain. Much interest has recently focused on the relationship between psychological factors in stressful situations and noradrenergic neuronal activity in the brain [see (9) for a review]. Moreover, our recent studies revealed that enhanced noradrenaline (NA) turnover in certain brain areas, such as the hypothalamus and limbic structures, was related to the appearance of fear and/or anxiety in rats exposed to stressful conditions (12, 23, 26, 28).

Accordingly, the present study examined differential changes in regional brain NA turnover in rats that could accurately predict when shock would occur in comparison to rats that received the same shock but could not predict its

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occurrence, by measuring the levels of a major metabolite of NA (15), 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in the rat brain.

METHOD

Subjects

The subjects were 24 male Wistar rats (8 triplets of three subjects each) about 9 weeks of age (weighing 200–230 g) at the beginning of the experiment, obtained from Kyudo, K. K. (Kumamoto). Rats were housed three per cage in a colony room maintained at a constant temperature (24±1°C) and humidity (50±10%) under a 12-hr light-dark cycle (light on at 0700 hr and off at 1900 hr). All rats were permitted ad lib access to water and standard chow.

Apparatus

The apparatus consisted of three Plexiglas chambers (9 cm wide, 19.5 cm long, and 15 cm high) placed in soundproof boxes which were filled with a masking noise generated by the chamber ventilators. The end walls of each apparatus were at an angle, and the top fitted across the rat's back to prevent the rat from backing out during the session. The floors were made of 0.2 cm diameter stainless steel rods spaced 0.5 cm apart (center to center). The rat's tail extended through a hole in the rear of each chamber and was held in place by a small piece of tubing taped to the tail. Unscrambled electric shock was produced by a solid state shocker (Lehigh Valley Electronics, No. 113–33) to the rat's tail through fixed tail bipolar electrode (0.5 cm diameter stainless steel bolts embeded in rubber tubing). A visual signal was delivered by a 10-W white bulb attached to the ceiling of each box. The chamber was illuminated by a 10-V tungsten lamp attached to the rear wall of each box. Presentation of stimulus events was controlled by a programmable controller (Sysmac S6; Omron, Tateishi Electronics Co., Osaka).

Procedure

Stress procedure. The three rats in each triplet were randomly assigned to one of three treatments. One rat was selected as the nonshock control group, and was given 12-sec, 10-W light presented on a variable time (VT) 2.5-min schedule (interval range, 20–215 sec) without any shock. The other two rats received the same lights as the nonshock group but also received shock (1.2 mA, 2-sec duration) on a 2.5-min VT. The tail electrodes for the two shocked rats were wired in series, so that the shock received by those rats was of exactly the same current, intensity and duration. One shock rat (unpredictable group) received uncorrelated stressor shock: the light and shock schedules were independent of each other. The other shock rat (predictable group) received the same shocks as the unpredictably shocked rat, but the shocks were always preceded by a 12-sec light: the light and shock terminated together. The shock intensity was initially set at 1.2 mA and increased to a maximum of 2 mA. The shock stress session started at 1700 hr and lasted for 19 hr, during which time the animals were deprived of food and water.

Tissue preparation and biochemical determination. At the end of the stress session, animals were decapitated and the brain was rapidly removed. Six brain regions (the hypothalamus, amygdala, thalamus, midbrain, hippocampus and cerebral cortex) were dissected out by the method of Gispen *et al.* (8). The locus coeruleus (LC) region was also dissected out by the method of Reis and Ross (19). Blood from the cervical wound was collected into heparinized tubes and centrifuged. Brain tissues and separated plasma were stored at −45°C until assayed. Levels of MHPG-SO_4 in the brain were determined fluorometrically by our method (15). Plasma corticosterone levels were measured fluorometrically by a slight modification of the method of van der Vies (30).

Data analysis. Data were analyzed by analysis of variance (ANOVA) and subsequent Tukey's HSD comparisons.

TABLE 1

MEAN (±SEM) LEVELS OF PLASMA CORTICOSTERONE IN THE NONSHOCK, PREDICTABLE, AND UNPREDICTABLE SHOCK GROUPS AFTER 19-HR STRESS SESSION

Group	Number of Subjects	Plasma Corticosterone (in μg/dl)
Nonshock	8	20.5 ± 1.5
Predictable Shock	8	22.6 ± 1.4
Unpredictable Shock	8	27.4 ± 1.9*†

*$p<0.05$, compared with nonshock group.
†$p<0.05$, compared with predictable shock group.

RESULTS

Table 1 illustrates plasma corticosterone levels for three groups after the shock stress. ANOVA of plasma corticosterone levels revealed a significant group effect, $F(2,21)=4.2$, $p<0.05$. Tukey's HSD test indicated that the unpredictable group showed significantly higher levels of corticosterone as compared with the nonshock and predictable groups. The latter groups did not significantly differ from each other. Two-way ANOVA of body weight changes revealed only a marginally significant stress effect, $F(1,42)=3.8$, $p<0.1$. Tukey's HSD test indicated that the nonshock group (mean±SEM=19.9±2.0 g) showed significantly less loss of body weight as compared with the unpredictable group (28.6±2.3 g), but not the predictable groups (25.8±2.3 g).

Figure 1 depicts regional brain MHPG-SO_4 levels for three groups after the shock stress. ANOVAs revealed significant group effects in the hypothalamus, $F(2,21)=35.0$, $p<0.01$, amygdala, $F(2,21)=16.6$, $p<0.01$, thalamus, $F(2,21)=7.0$, $p<0.01$, midbrain, $F(2,21)=11.5$, $p<0.01$, and cerebral cortex, $F(2,21)=12.4$, $p<0.01$. There was a marginally significant group effect in the LC region, $F(2,21)=3.4$, $p<0.1$, but there was no reliable group effect in the hippocampus. Tukey's HSD test indicated that, as compared to the nonshock group, the predictable and unpredictable shock groups significantly increased MHPG-SO_4 levels in all brain regions examined with the exception of the hippocampus. Among these brain regions, MHPG-SO_4 levels for the unpredictable shock group were significantly increased in the hypothalamus, amygdala, and thalamus, as compared with those in the predictable shock group.

DISCUSSION

This study clearly demonstrated that a purely psychological factor, whether or not rats could predict the occurrence of a shock stressor by a light signal, resulted in altered activ-

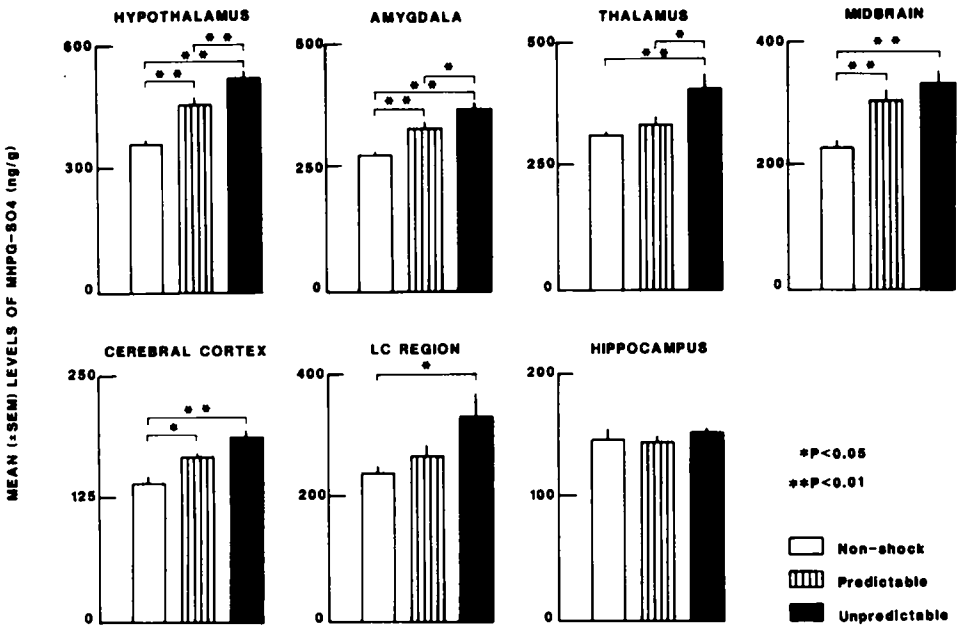

FIG. 1. Mean (±SEM) levels of MHPG-SO_4 (ng/g) in various brain regions for the nonshock, predictable shock and unpredictable shock groups.

ity of the brain noradrenergic neuronal system. Both shocked groups exhibited greater elevations of MHPG-SO_4 levels in many brain regions, as compared to the nonshock controls. However, the increases in the unpredictable shock group were significantly higher than those in the predictable shock group in the hypothalamus, amygdala, thalamus, and LC region. The former group also exhibited significantly higher levels of plasma corticosterone than those seen in the latter group.

These results replicate previous findings that signalled shocks were less aversive and stressful than unsignalled shocks, with respect to behavioral and physiological measures of aversiveness and stress, including preference behavior (7), behavioral suppression (11), pain sensitivity (10), plasma corticosterone (16,17), and gastric lesions (29,31). The data from this experiment suggest that the presence of a warning signal not only reduces extent of brain noradrenergic neuronal activity, but also decreases peripheral endocrine activation. Indeed, it has been assumed that brain NA and plasma corticosterone variations are affected by aversive stimuli (13).

The unpredictable shock group exhibited considerably higher increases in NA turnover, relative to the matched predictable shock group. This difference was particularly striking in the hypothalamus, amygdala, thalamus and LC region, and probably reflects the more marked distress and hyperemotionality due to the psychological dimension of the lack of ability to anticipate the occurrence of the shock. It has been reported that enhancement of NA turnover in these brain structures was associated with different attributes of stress (e.g., arousal, negative emotions, pain, previous stress history, etc.) (2, 22, 26). In earlier studies dealing with another important psychological factor (i.e., stressor controllability), we found that uncontrollable shock caused greater NA turnover preferentially in the hypothalamus and limbic system than that seen in conditions of controllable shock (25,27). Previous regional brain analyses also demonstrated that the attenuating effects of anxiolytics and opioid peptides on stress-enhanced NA turnover in these specific brain regions are related to the relief of fear or anxiety of rats exposed to restraint stress (12,24).

Taken together with these findings, it appears that enhancement of NA turnover induced by stress varies appreciably with the brain regions examined. Moreover, changes in NA turnover in the hypothalamus and limbic system seem to be indicators of psychological dimensions which reflect the unpleasant emotional component of the inability to cope with the stressor, such as uncontrollability and/or unpredictability of a stressor. In accordance with this view, Redmond (18) and Weiss and Simson (32) have hypothesized that the LC system acts as an "alarm system" in provoking anxiety or fear in animals, and distal regions innervated by the LC are also involved in the elaboration of adaptive responses to stress challenge. The LC is thought to make its projection to various terminal sites including the hypothalamus, amygdala, thalamus, hippocampus, and cerebral cortex (5).

What is it about the ability to predict a shock which produces attenuating effects against shock-induced increase in NA turnover in the brain, as well as increased endocrine activity in the adrenal cortex? It is reasonable to point out, on the basis of the safety-signal hypothesis (20), that unpredictable shock leads to the marked activation for neurochemical processes because animals that have no safety signal (i.e., no signal that predicts time out from shock) continue to be subjected to chronic fear.

Lastly, it should be noticed that despite much data and arguments concerning the stressfulness of unpredictable stressors (1, 11, 16, 17, 29, 31), there is some evidence showing that predictable conditions are physiologically more

stressful than unpredictable conditions, as measured by corticosterone response (3, 6, 14). The source for the divergent results is not immediately evident, however, the different outcomes are not particularly surprising in considering the large number of experiential variables that influence the pathophysiology of stress (e.g., the phasic versus chronic nature of predictable versus unpredictable stressor, the degree of behavioral control that an organism exerts to control the predicted event, or parameters of stressor including shock delivery, shock intensity, and stress schedules). Indeed, Bassett *et al.* (4) have explained the differential effects of warning signal and shock predictability on glucocorticoid activity. Future works should explore the mechanisms of these differential changes under specified conditions of predictable and unpredictable shocks.

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