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Effects of Preshock Experience on Enhancement of Rat Brain Noradrenaline Turnover Induced by Psychological Stress[1]

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TSUDA, A., M. TANAKA, Y. IDA, S. TSUJIMARU, I. USHIJIMA AND N. NAGASAKI. *Effects of preshock experience on enhancement of rat brain noradrenaline turnover induced by psychological stress.* PHARMACOL BIOCHEM BEHAV **24**(1) 115–119, 1986.—The present study examined alterations of brain noradrenaline (NA) turnover as a function of preshock and psychological stress treatments, by measuring contents of NA metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), in discrete brain regions of male Wistar rats. Psychological stress induced by exposing to the sight, sound and odor of other rats being shocked produced higher levels of MHPG-SO_4 in the hypothalamus, amygdala and locus coeruleus (LC) region, as well as higher levels of plasma corticosterone. Preshock experienced rats also showed marked increases of MHPG-SO_4 levels in the same regions described above and elevated plasma corticosterone levels when placed but not shocked in the same environment in which the rats had previously received shocks. The effects of psychological stress on brain NA turnover were affected by the animal's shock history preferentially in the hypothalamus and amygdala. These results suggest that: (1) a purely psychological stressor caused acutely enhanced NA turnover in specific brain regions; (2) regional NA activity appeared to be reinstated simply by reexposure to the environment previously associated with shock; (3) preshock experience further intensified the enhancement of amygdaloid NA turnover evoked by psychological stress. An additional experiment, studying the aftereffects of preshock experience, clearly showed that these findings result from sensitization or conditioning to the environment previously paired with shock, and not merely from the aftereffects of the shock *per se*.

Noradrenaline turnover MHPG-SO_4 Rat brain regions Psychological stress Preshock experience Sensitization

WITHIN the past several years, much interest has focused on the relationship between behavioral-psychological factors in stressful situations and noradrenergic neural activity in the brain including conditioned anxiety and/or fear [2,19], controllability of stressor [7, 23, 28], immobilization [6, 8, 9, 21], physical pain [27] and activity-stress [18, 24, 25]. One of the available ways by which to explore this relationship is to expose rats to the sound, sight and odor of other rats being shocked in the same location [3,29].

This psychological stress model has shown, thus far, that witnessing effects of footshock on other animals without the influence of direct physical stimuli produces increases in nociceptive threshold [11], development of gastric lesions [4], depression in food-motivated behaviors [3] and lowered noradrenaline (NA) levels in the pons plus medulla oblongata [29]. In addition, we [10] have recently found that rats which witnessed responses of other rats to footshock, show enhancement of NA turnover preferentially in the hypothalamus and amygdala, as indicated by measuring the major central metabolite of NA, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) [16]. It appears that the increased brain NA turnover produced by this type of psychological stress may be mediated by certain emotions, e.g., fear or anxiety. Tanaka *et al.* [22] have observed that enhanced NA activity in the brain is related to distress-evoked hyperemotionality, as evidenced by increased struggling, vocalization and defecation.

In order to further characterize the influence of witnessing effects of footshock on NA turnover in a more extended number of brain regions than those previously examined [10], the present study was carried out using a prior fear conditioning technique [17]. Rats were first given preshock and were then exposed to the sight, sound and smell of other rats receiving footshock in compartments wherein they had previously experienced footshock. It would be of interest to examine how preshock experience influences alterations of regional brain NA turnover induced by affective cues associated with inescapable shock, such as visual, auditory and olfactory stimuli arising from the distress behavior of the shocked rats (i.e., psychological stress).

Thus, the present study was undertaken to delineate the differential changes in regional brain NA turnover in

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preshock-experienced rats followed by psychological stress (preshock-stress group) and in nonshock-experienced rats followed by psychological stress (nonshock-stress group). The present experiment also attempted to investigate whether regional NA activity would be reinstated or sensitized by reexposure to the environment previously associated with preshock (preshock-control group). Recent studies have suggested that an initial stressor is able to sensitize or condition brain NA activity in animals exposed to environmental stimuli previously associated with stress [1,2].

METHOD

Animals

Male Wistar rats, weighing 170–190 g, were used as subjects. Rats were housed in groups of four in standard polypropylene cage (26.5×42.5×15.0 cm) containing wood shavings in an air-conditioned room (24±1°C, relative humidity 50±10%) kept on a 12 hr (light on 0700 to 1900 hr) light-dark cycle. Food and water were provided ad lib.

Apparatus

A rectangular clear plastic box (93 cm in width, 99 cm in length and 53 cm in height) was used for exposing rats to inescapable footshock in preshock sessions and to the affective cues of inescapable footshock in subsequent psychological stress sessions. The electrified floor was constructed of stainless rods of 0.3 cm in diameter and spaced 1.3 cm apart (center to center). The apparatus was subdivided into 25 smaller compartments (18×19×53 cm) by clear plastic walls. In subsequent stress session, eight of the compartment floors, located in center of the box, were covered with plastic plates to prevent rats assigned to these compartments from receiving footshock. The plastic plates were not placed on the remaining 17 compartment floors, so that animals in these compartments received footshock from the grid through an AC shock source.

In an additional experiment assessing the aftereffects of preshock *per se*, animals in the second session were placed in different wire mesh cages subdivided into 8 chambers (25×15×18 cm) from the plastic box in which they had received footshock in the preshock session.

Experimental Procedure

Rats were randomly assigned to one of four groups of 8 rats each. During the 1-hr preshock session, two preshock groups were placed in the compartments of the plastic box and exposed to 3-mA, 5-sec inescapable footshocks on a fixed-interval 30 sec schedule. The other nonshocked groups were placed into the compartments for the same period of time but received no shock. After the preshock session, all rats were returned to their home cages.

Forty-eight hours later, during the 1-hr stress session, one group of preshock (preshock-stress) and one group of nonshock (nonshock-stress) were again placed into their respective compartments (at this time, floors of their compartments were covered with plastic plates so that they were never shocked) while footshock was delivered to other rats in the shock compartments of box. Therefore, both preshock-stress and nonshock-stress rats were exposed to various responses exhibited by the shocked other rats, such as vocalizing, jumping, struggling, defecating and urinating, but did not receive any physical footshock. Other rats were used only in order to provide rats to send affective cues, such as visual, auditory and olfactory sensations described above during the stress sessions. Each of the 17 other rats were exposed to 5-sec, 3-mA inescapable footshocks on a fixed-interval 30-sec schedule for 1 hr. This procedure was conducted twice for the preshock-stress and nonshock-stress groups, respectively. The second group of preshock rats (preshock-control) and the second group of nonshock rats (nonshock-control) were again placed into their respective compartments for 1 hr while the shock compartments remained empty. Therefore, both preshock-control and nonshock-control rats were not exposed to any affective responses of other rats.

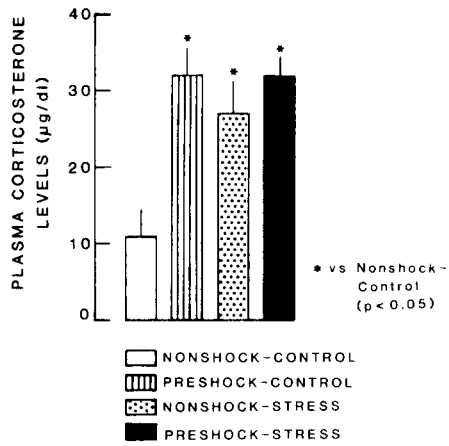

FIG. 1. Mean (±SEM) levels of plasma corticosterone as a function of preshock and psychological stress treatments.

In an additional experiment studying the aftereffects of preshock *per se*, two groups of 8 rats each were initially exposed to preshock or no shock in the shock compartments of the box described above. During the second session 48 hr after the preshock session, however, animals were merely placed into different wire mesh cages for 1 hr without any affective cues from the preshock compartments in which they had been previously shocked.

All experimental procedures were performed between 0900 and 1400 hr, since we found no diurnal variation of MHPG-SO_4 levels during this period [14].

Tissue Preparation and Biochemical Assay

Immediately after the stress session, all rats (except those rats used to produce shock-elicited affective cues) were decapitated. The brain was removed, dissected by the method of Gispen *et al.*, [5] and frozen on solid CO_2. The dissected brain regions were: the hypothalamus, amygdala, locus coeruleus (LC) region, thalamus, hippocampus, midbrain, cerebral cortex and basal ganglia. The LC region was dissected out according to the method of Reis and Ross [20]. Blood from the cervical wound was collected into heparinized tubes. Separated plasma and brains were stored at −45°C until assayed. Plasma corticosterone levels were determined fluorometrically by the method of van der Vies [26]. MHPG-SO_4 levels in the brain were determined by our fluorometric method [13].

Statistical Analysis

All statistical evaluations were performed using 2-way

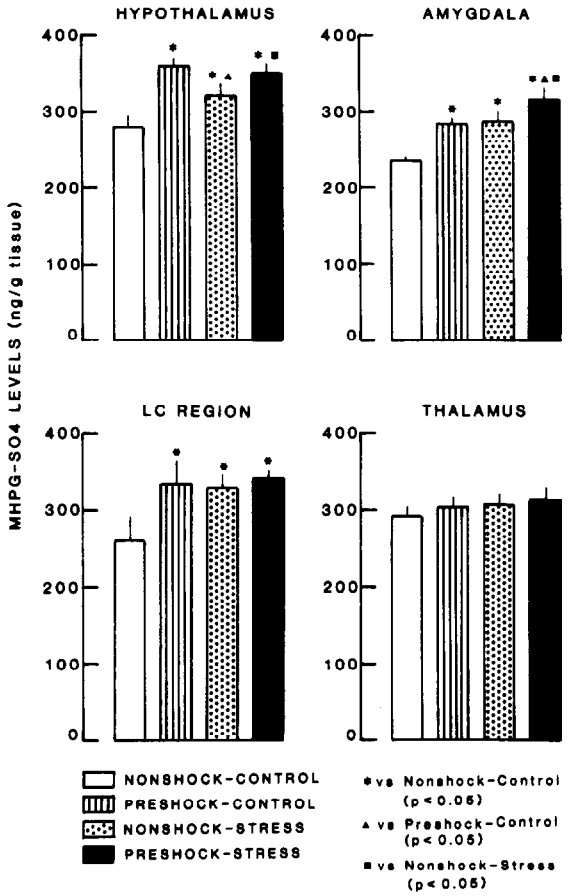

FIG. 2. Mean (±SEM) levels of MHPG-SO_4 in the hypothalamus, amygdala, LC region and thalamus as a function of preshock and psychological stress treatments.

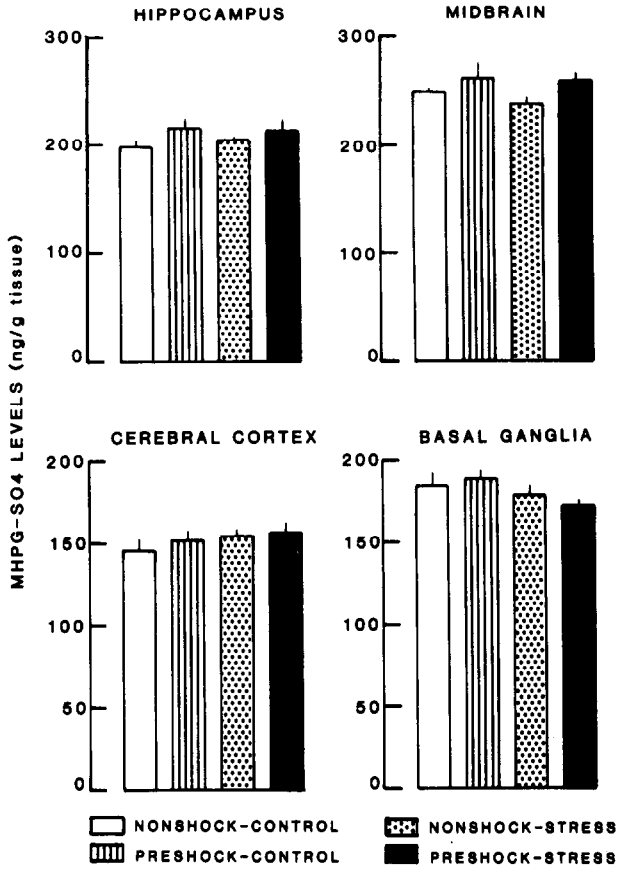

FIG. 3. Mean (±SEM) levels of MHPG-SO_4 in the hippocampus, midbrain, cerebral cortex and basal ganglia as a function of preshock and psychological stress treatments.

analysis of variance (ANOVA) with replication in which orthogonal decomposition for linear comparisons was conducted [30], unless otherwise noted.

RESULTS

A 2-way ANOVA revealed significant main effects of preshock, $F(1,28)=13.51$, $p<0.01$, and stress, $F(1,28)=4.63$, $p<0.05$, as well as a significant interaction between these factors, $F(1,28)=5.46$, $p<0.05$, on plasma corticosterone levels (Fig. 1). Tukey's HSD post hoc comparisons ($p<0.05$) indicated that the nonshock-control group exhibited significantly lower levels of plasma corticosterone than the remaining three groups. The nonshock-stress, preshock-control and preshock-stress groups did not differ reliably from one another.

Figure 2 depicts levels of MHPG-SO_4 in the hypothalamus, amygdala, LC region and thalamus for all four groups. Two-way ANOVAs revealed that preshock experience resulted in significant increases in MHPG-SO_4 in the hypothalamus, $F(1,28)=13.24$, $p<0.01$, amygdala, $F(1,28)=7.21$, $p<0.05$, and LC region, $F(1,28)=4.32$, $p<0.05$, respectively. Although the preshock and stress interaction did not reach statistical significance, Tukey's HSD post hoc comparisons ($p<0.05$) were conducted where a prior prediction had been made. With regard to the hypothalamus, these comparisons indicated that, when compared to the nonshock-control group, the remaining three groups produced significant elevations of MHPG-SO_4 levels. Among these three groups, MHPG-SO_4 levels in the preshock-stress group were significantly higher than those in the nonshock-stress groups, but did not differ from the preshock-control group. The preshock-control group showed reliable increases in MHPG-SO_4 contents when compared to the nonshock-stress group. In the case of the amygdala, preshock-control, nonshock-stress and preshock-stress groups produced significant increases in MHPG-SO_4 relative to the nonshock-control group. MHPG-SO_4 contents in the preshock-stress group were highest among the former three groups. In the LC region, MHPG-SO_4 levels in the nonshock-control group were reliably lower than those in the remaining three groups. The preshock-control, nonshock-stress and preshock-stress groups did not differ from one another with respect to MHPG-SO_4 levels in the LC region.

Figure 3 shows levels of MHPG-SO_4 in the hippocampus, midbrain, cerebral cortex and basal ganglia for all four groups. Two-way ANOVAs revealed that none of the preshock and stress main effects and none of interactions between these factors, significantly influenced MHPG-SO_4 levels in these brain regions.

Table 1 shows that regional MHPG-SO_4 levels were not affected by a 1 hr exposure to a different environment from

TABLE 1

MEAN (±SEM) LEVELS OF MHPG-SO_4 (ng/g TISSUE) IN VARIOUS BRAIN REGIONS OF PRESHOCKED AND NONSHOCKED RATS FOLLOWING A 1-HR EXPOSURE TO CONTROL CAGES

	Hypothalamus	Amygdala	LC Region	Thalamus
Nonshocked	287.8± 7.0	233.6± 5.8	251.8± 9.8	264.3±16.9
Preshocked	282.0±10.9	245.7±11.7	269.1±13.2	288.5±12.7
	Hippocampus	Midbrain	Cerebral Cortex	Basal Ganglia
Nonshocked	164.7± 8.4	204.8± 3.8	113.6± 3.6	187.8± 7.0
Preshocked	170.4± 9.5	207.1± 8.7	109.6± 3.3	182.0±10.9

that of the preshock situation in rats exposed to preshock and in rats not exposed to prior shock. No significant differences were observed between the preshocked and nonshocked groups in all regions examined ($p<0.05$, Student's t-test).

DISCUSSION

The psychological stressor employed in this experiment produced no physical insult administered to the animals. Psychological-stressed rats were placed into a plastic compartment which was in full sight, sound and smell of rats receiving electric footshock [3, 4, 29]. Psychological stress in the nonshock-stress group caused significant elevations of MHPG-SO_4 levels in the hypothalamus, amygdala and LC region, but did not affect the thalamus, hippocampus, midbrain, cerebral cortex and basal ganglia, relative to the nonshock-control group.

These results not only support our previous report [10], but also provide more detailed information concerning which brain regions are affected by psychological stress with regard to activity of noradrenergic neurons. The primary brain regions exhibiting enhancement of psychological stress-induced NA turnover were the hypothalamus, amygdala and LC region. Previous regional brain analyses concerning alterations of NA turnover suggested that changes in these brain regions had a strong relationship to emotional disturbances [22,23]. Therefore, the present study suggests that the increased NA turnover in the hypothalamus, amygdala and LC region elicited by psychological stress is probably mediated by certain emotional reactions to distress signals [4,29].

The effects of psychological stress on brain NA turnover were affected by the animal's shock history preferentially in the hypothalamus and amygdala. Preshock experience for the preshock-stress group further potentiated the enhancement of hypothalamic and amygdaloid NA neural activity evoked by psychological stress, relative to the nonshock-stress group. Church [3] observed that rats which had been shocked showed greater fear in responses to the pain reactions of others than did rats which had not previously experienced a painful stimulus. According to Church's conditioned-response interpretation of stressful "sympathy" to other shocked rats [3], the present findings suggest that when rats are subsequently exposed to the pain responses of others after they were themselves shocked, they exhibit conditioned fear to the pain responses of others.

It seems that the enhanced amygdaloid turnover seen in the preshock-stressed rats may be elicited by synergism between conditioned and unconditioned fear to the pain reactions of others. Anisman and Sklar [1] reported that exposure to footshock elicited more pronounced hypothalamic NA reductions upon stress reexposure. It is possible to speculate from the present study that prior to exposure to inescapable footshock results in sensitization or conditioning of the mechanisms responsible for NA release in the amygdala.

Preshock experience for the preshock-control group elicited increases of NA turnover in the hypothalamus, amygdala and LC region when placed in the same environment in which the rats had previously received shock. These changes are not unconditioned reactions to shock itself, but are reactions to situational stimuli which have been paired with shock. The additional shock-control experiment indicated that regional MHPG-SO_4 contents were not affected by exposure to a different environment from that in which preshock had been given. Kameyama and Nagasaka [12] have shown that when simply placed into an environment in which they had received shock, rats exhibited a marked reduction of motor activity, which was interpreted as an index of conditioned emotional response. With respect to direct biochemical measurements, Cassens *et al.* [2] have reported that conditioned stimuli associated with shock increased the use of NA in the whole brain upon subsequent presentation of these stimuli. The present investigation support Cassens' findings and adds more precise evidence that noradrenergic neural activity in the hypothalamus, amygdala and LC region can be conditioned or sensitized to the cues of an environment associated with shock.

The results are interpreted as indicating that these NA functions may be an index of fear or anxiety. Redmond [19] has hypothesized that the LC system is considered as an "alarm system" in producing anxiety or fear. The LC is thought to have highest density of NA containing neurons in the brain and to make its projection (ventral and dorsal bundles) to various terminal sites, especially limbic areas [15]. Tanaka *et al.* [22] also have found that changes in MHPG-SO_4 in the hypothalamus and amygdala, relative to other regions studied are most highly correlated with stress-related responses, of which fear behavior is an integral part. In addition, Tsuda and Tanaka [23] reported that NA function in these regions is essential for the psychological dimension of coping with stress versus helplessness.

Regarding the peripheral response of plasma corticosterone levels, both psychological and preshock treatments activated the release of corticosterone, respectively. However, a combination of these treatments failed to further potentiate the increased secretion of corticosterone.

The results of this research support our hypothesis that: (1) exposure to the sight, sound and odor of other rats exposed to footshock (i.e., psychological stress) activated a limited number of brain regions subserving emotional responses; (2) after one exposure to preshock, a second exposure to the same shock situation can readily evoke increased NA turnover; (3) increased NA turnover induced either by psychological stress or by preshock experience is potentiated by a combination of these treatments. In conclusion, we suggest that noradrenergic neural activity in the hypothalamus, amygdala and LC region can be sensitized or conditioned to certain emotional responses (possibly fear or anxiety) established through the principals of Pavlovian conditioning [17].

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