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Psychological Stress Enhances Noradrenaline Turnover in Specific Brain Regions in Rats

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IIMORI, K., M. TANAKA, Y. KOHNO, Y. IDA, R. NAKAGAWA, Y. HOAKI, A. TSUDA, AND N. NAGASAKI. *Psychological stress enhances noradrenaline turnover in specific brain regions in rats*. PHARMAC. BIOCHEM. BEHAV. **16**(4) 637–640, 1982.—Concentrations of noradrenaline (NA) and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in the hypothalamus, amygdala, cerebral cortex and pons+medulla oblongata were examined in male Wistar rats exposed to foot-shock or to psychological stress for 1 hour. Animals in the psychological stress group were prevented from receiving foot shock, but were exposed to responses of shocked rats. Foot shocked rats exhibited a significant reduction in NA content and a significant elevation in MHPG-SO_4 level in all brain regions when compared to control rats which were neither shocked nor exposed to shocked rats. Rats exposed to the psychological stress displayed a significant reduction of NA level in the amygdala, significant elevation of MHPG-SO_4 content in the hypothalamus and amygdala, and a moderate elevation of plasma corticosterone level. These results suggest that psychological stress produces mild enhancement of NA release preferentially in the hypothalamus and amygdala; while foot shock stress elicits a more intense response of noradrenergic neurons in more extended brain regions.

Noradrenaline 3-Methoxy-4-hydroxyphenylethyleneglycol sulfate Regional difference
Psychological stress Foot shock stress

ENHANCEMENT of noradrenaline (NA) turnover in the brain has been demonstrated by exposing animals to various stressful situations such as electric foot shock [2, 3, 20], cold environment [5,13], immobilization [3,6], fighting [3], immobilization combined with tail shock [14] and ether vapor [22]. In the literature, the marked acceleration of NA release has been reported to occur in the hypothalamus in animals exposed to foot shock [3], immobilization [3], cold environment [5] or ether vapor [22]. Data from our laboratory suggest that changes in NA turnover caused by immobilization [19] or its combination with tail shock [14] exhibit regional differences in terms of both the degree of responsiveness and in the time course of these changes. These data also suggest that stress-induced changes in brain NA metabolism occurred in animals subjected to forms of stress which were considered to be both physical and psychological.

Welch and Welch [23] found that mice which observed fighting in other animals displayed lowered NA levels in the pons+medulla oblongata brain region. In addition, Bliss and Ailion [1] observed that aggregation of familiar and non-familiar mice produced similar reductions in brain NA levels.

In order to further investigate the significance of psychological factors in the function of central noradrenergic neurons, we measured NA and MHPG-SO_4 levels in four brain regions of rats exposed to either psychological stress or to physical stress. Levels of MHPG-SO_4, the major metabolite of NA in the rat brain [15,16], have been shown to be a useful index of NA turnover in the brain as a whole [4, 12, 18] as well as in discrete brain regions [11].

METHOD

Animals

Male Wistar rats weighing 200–230 g were used. They were housed in groups of four in a temperature-controlled room (24±1°C) under a 12 hr light-dark cycle and provided food and water ad lib throughout the experimental period.

Apparatus and Experimental Groups

Stress treatments were produced using the apparatus shown in Fig. 1. This apparatus was originally employed as a communication chamber for mice [9], but was modified for use with rats in the present study. 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). This chamber was subdivided into 25 smaller compartments (18×19 cm) by the use of transparent plastic walls. A scrambled electric shock was delivered through the floor grid by a fixed impedance A.C. stimulator (60 Hz pulse wave). The shock consisted of 80 msec pulses separated by 420 msec intervals and was given for a 5 sec duration at intervals of 30 sec. The shock intensity was fixed at 70 V (about 3.5 mA). Plastic plates were placed on the grids of four compartments (shown as solid parts in Fig. 1) to prevent rats in these compartments from receiving foot shock. These four compartments and the other 21 compartments were designated as non-shock compartments and shock compartments, respectively.

Prior to the experiment, all animals were placed into the compartments without foot shock for 1 hr every day for 5

TABLE 1

NORADRENALINE (NA) AND 3-METHOXY-4-HYDROXYPHENYLETHYLENEGLYCOL SULFATE (MHPG-SO_4) LEVELS IN BRAIN REGIONS OF RATS PLACED IN SHOCK OR NON-SHOCK COMPARTMENTS OF THE COMMUNICATION CHAMBER

	NA (ng/g)			MHPG-SO_4 (ng/g)		
Regions	Control	Psychological stress	Foot-shock stress	Control	Psychological stress	Foot-shock stress
Hypothalamus	1653 ± 56	1587 ± 61	1160 ± 39‡	243 ± 8	286 ± 8†	423 ± 14‡
Amygdala	383 ± 10	348 ± 9*	289 ± 8‡	155 ± 9	182 ± 9*	261 ± 8‡
Pons+med. obl.	817 ± 23	794 ± 17	621 ± 20†	168 ± 8	167 ± 7	250 ± 13‡
Cerebral cortex	290 ± 11	261 ± 11	228 ± 12†	95 ± 2	98 ± 4	142 ± 6‡

Electric shock was delivered to animals in foot-shock group; animals in psychological stress group were not shocked but were exposed to the responses shown by shocked rats. Control rats merely were placed in non-shock compartments. Each experimental treatment was employed for 1 hr. Further explanations are in the text. Each value is shown as the mean ± S.E.M. of 8 animals. Statistical significance when compared to controls is $*p<0.05$, $\dagger p<0.01$, $\ddagger p<0.001$.

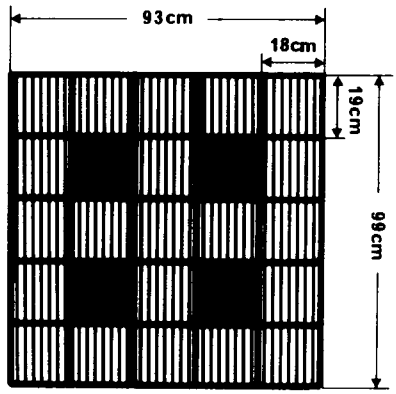

FIG. 1. A floor plan of the communication chamber. Foot-shocked rats were placed individually into the 21 shaded areas (shock compartments); rats in the psychological stress group, into solid areas (non-shock compartments). Electric shock was delivered through grids of the floor in shaded areas and was prevented by plastic plates in solid areas.

days. This was regarded as a habituation phase. Following the fifth habituation day, rats were selected by balancing body weights and were divided into three experimental groups as follows: a control group consisting of four rats placed into the non-shock compartments for 1 hr while the shock compartments remained empty; a foot shock group consisting of 21 rats placed into the shock compartments for 1 hr (four of these rats were randomly selected for biochemical determinations); and a psychological stress group consisting of four rats placed into the non-shock compartments while foot shock was delivered to rats in the shock compartments. Animals in this group were never shocked, but were exposed to the responses of shocked animals which included struggling, vocalizing, defecating, urinating and jumping. Each experimental group described above consisted of 8 rats. All treatments were administered between 0930 hours and 1330 hours.

Biochemical Determinations

Immediately after each experimental treatment, rats were sacrificed by decapitation and the hypothalamus, amygdala, pons+medulla oblongata and cerebral cortex were dissected out by the method of Gispen *et al.* [7] and frozen on dry ice. The blood from the cervical wound was collected in heparinized tubes and centrifuged. The plasma and brain tissues were kept at −45°C until assayed. NA and MHPG-SO_4 levels in brain regions from individual rats were estimated fluorometrically by a method developed in our laboratory and reported previously [10]. Plasma corticosterone level was determined according to the fluorometric method of van der Vies [21] with a slight modification. Statistical analyses were performed using non-directional Student's *t*-tests.

RESULTS

The foot shock group revealed significant decreases in NA and increases in MHPG-SO_4 concentrations in all brain regions when compared to control animals (Table 1). The alterations in NA metabolism occurred primarily in the hypothalamus (NA and MHPG-SO_4 levels in the foot shock group were 70% and 174% of control values, respectively) and less extensively in the amygdala (NA 76%; MHPG-SO_4 168%). The changes in the pons+medulla oblongata (76%; 148%) and cerebral cortex (79%; 149%) were moderate and similar in extent.

In the psychological stress group, regional NA level tended to be reduced, however a significant change was found only in the amygdala where the NA level was 90% of control value. MHPG-SO_4 levels were significantly increased in the hypothalamus (118%) and amygdala (117%), while the pons+medulla oblongata and cerebral cortex showed no significant change in the metabolite level.

A significant elevation in the plasma corticosterone level was observed in the foot shock group when compared to that in the control group (Table 2). The corticosterone level in the psychological stress group showed a marginally significant increase (152%; $p<0.1$).

DISCUSSION

The present study compared the regional changes in NA

TABLE 2

CHANGES IN PLASMA CORTICOSTERONE LEVELS
(μg/dl)

	Control	Psychological stress	Foot-shock stress
Corticosterone	13.9 ± 3.8	21.1 ± 2.4	33.9 ± 1.1‡

Each value is expressed as the mean ± S.E.M. of 8 animals. Statisical significance, when compared to controls is ‡$p<0.001$. (See the legend of Table 1 for details.)

metabolism in foot shocked rats and in rats exposed to only the responses of shocked animals. Foot shock for 1 hr resulted in a marked increase in MHPG-SO_4 levels, accompanied by a significant reduction of NA contents in all brain regions studied. These results show that the enhancement of functional activity of noradrenergic neurons caused by the foot shock stress leads to marked acceleration of NA release and consequently lower NA levels. The regional differences in responsiveness of noradrenergic neurons in the foot shock group were quite similar to our previous findings observed with immobilization stress [19] or the combined stress of immobilization and tail shock [14]. The increased response of noradrenergic neurons in the hypothalamus observed in the present experiment was also in agreement with that reported previously for foot shock stress [15], ether stress [22] and cold exposure [15]. An increase in NA turnover was shown by rats in the psychological stress group. Since all rats used in this study had been familiarized with the compartments of the communication box for 5 days prior to the experiment, the novelty of the compartments cannot account for the significant difference in the NA turnover between the psychological stress group and the control groups. In addition, the animals in these two groups were kept in the non-shock compartments for the same period of time during the course of the study. The only difference between the two groups was exposure to the responses of the rats receiving foot shock. The rats in the psychological stress group are considered to have been affected by visual, auditory and olfactory stimulation arising from the emotional behavior of the shocked rats which included jumping, struggling, vocalizing, defecating and urinating. This situation seems to have been mildly stressful since the plasma corticosterone level was elevated to 152% of control value in these animals. Hennessy and Levine [8] have reported that corticosterone level is a sensitive index which reflects different intensities of psychological stimulation to which animals are exposed. It may be that exposure of rats to stimulation such as that used in the present study could be characterized as psychologically stressful and thus, the difference in NA turnover between control rats and psychologically stressed rats may have been produced by this psychological stimulation. The present study showed that psychological stress enhanced NA turnover in limited brain regions including the hypothalamus and amygdala but not in the pons+medulla oblongata or cerebral cortex. Welch and Welch [23] originally suggested that the increased NA turnover caused by psychological stimulation was confined to NA reduction in the pons+medulla oblongata region in naive mice exposed to the sights, sounds and odors of vigorous fighting among other mice. Regional differences obtained in the present study, however, are in agreement with other data which suggest that the greatest decrease in NA occurred in the hypothalamus following aggregation of familiar and non-familiar mice [1].

The comparative evaluation of regional NA metabolism suggests at least one possible difference between psychological stress and physical stress. Psychological stress appears to produce mild enhancement of NA turnover preferentially in the hypothalamus and amygdala, while a more intense physical stress (foot shock) produces a greater increase in NA turnover in more extended brain regions. It is not clear from this study whether enhanced NA turnover during psychological stress depends upon the quality or quantity of the stress. The conclusion that psychological stimulation selectively enhances NA turnover in the hypothalamus and amygdala is further supported by recent data from this laboratory (unpublished observations) demonstrating a similar pattern of regional NA turnover following repeated treatments with psychological stress.

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