Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 4, pp. 905–909, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00219-3

Differential Changes in Rat Brain Noradrenaline Turnover Produced by Continuous and Intermittent Restraint Stress

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Received 28 December 1992

SHIMIZU, T., M. TANAKA, H. YOKOO, Y. GONDOH, K. MIZOGUCHI, N. MATSUGUCHI AND A. TSUDA. *Differential changes in rat brain noradrenaline turnover produced by continuous and intermittent restraint stress.* PHARMACOL BIOCHEM BEHAV **49**(4) 905–909, 1994.—This experiment was performed to investigate differential effects of continuous and intermittent restraint stress on noradrenaline (NA) turnover in brain regions of male Wistar rats by measuring levels of a major metabolite of NA, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) levels, as well as by measuring levels of plasma corticosterone and organ weights of the thymus, spleen, and adrenal glands. Rats in the 15-min and 30-min intermittently stressed groups showed significantly larger increases in MHPG-SO_4 levels in most brain regions relative to those in the 90-min and 180-min continuously stressed groups, even though the total stress duration was equal or shorter. Body weight loss and loss of relative thymus weight in the 15-min intermittently stressed groups were the most marked among the five treatment groups. These findings suggest that stress-rest cyclicity is critical in determining the extent of stress-induced brain NA turnover and peripheral physiological responses.

Stress-rest cyclicity Intermittent stress Continuous stress Noradrenaline turnover Plasma corticosterone Brain regions Organ weights

THE DIFFERENTIAL impacts of intermittent-continuous stress on stress pathology have been a main theme in understanding the adaptation/sensitization of stress responses syndrome (14).

Despite a number of studies that have been concerned with this issue, there is yet no coherent understanding of how the continuous and intermittent nature of a stressor modulates the behavioral, physiological, and neurochemical impact of that stressor (3–5,7,10,12,17). For instance, Glavin (7) comared rats exposed to a single continuous restraint stress with rats given various durations of intermittent stress interspersed with nonstress period, and found maximum gastric pathology after a single stress period. Murison et al. (12) also found that a single continuous restraint produced more extensive ulceration than did a series of repetitive restraint periods interspersed with nonstress periods. On the other hand, Kahn et al. (10) reported that when rats were subjected to repeated restraint stress gradually, they exhibited less corticosterone release than did rats exposed to a single stress.

In addition to this contradictory evidence, only very few studies have thus far dealt with the relative influence of the form of continuous or intermittent stress on neurochemical changes in the brain, as an index of stress responses. Various stressful stimulations have been shown to induce excitation of brain noradrenergic neurons and, hence, enhance noradrenaline (NA) turnover (18–21,23). These findings are further supported by the reports wherein the in vivo microdialysis method was employed (22,25). We have preliminarily assessed the effects of continuous stress and intermittent stress on rat hypothalamic NA turnover by measuring the levels of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) (11), and found that intermittently stressed rats had larger increases in hypothalamic MHPG-SO_4 levels than single, continuously stressed rats (16).

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Therefore, this study was undertaken to confirm our previous findings (16) and to provide a more detailed examination of how continuous vs. intermittent stress affect NA activity in an extended number of brain regions, as well as assessing stressor effects on some peripheral stress responses such as body weight, organ weights, and plasma corticosterone levels.

METHOD

Animals

Forty male Wistar rats, that had been obtained from Kyudo K. K. (Kumamoto), 7 weeks old (weighing 180–200 g) at the beginning of the experiment, were used as subjects. Rats were housed, with free access to food and water, four per standard plastic cages containing wood shavings, which were situated in a 12 L : 12 D cycled room (lights on at 0700 h and off 1900 h) at constant temperature (24 ± 1°C) and humidity (50 ± 10%).

Procedure

For restraint stress, each rat was immobilized in a flexible wire mesh (3 × 3 mm), initially formed into a cone, which was bent to conform to the size of the individual animal (18). After balancing for body weight, rats were randomly assigned to one of five groups (n = 8 for each group). As illustrated in Fig. 1, rats in the nonstressed control group (group 1) were neither handled nor exposed to a restraint stress. The animals in group 2 were intermittently subjected to a series of six 15-min sessions of restraint stress, interrupted by 18-min nonstress periods. The animals in group 3 were intermittently subjected to three 30-min sessions of restraint stress, interrupted by two 45-min nonstress periods. During the nonstress periods, animals were placed in their home cages without food and water. The total duration of stress sessions was 0 min (i.e., total time of 180 min of stress and nonstress sessions). Animals in groups 4 and 5 were continuously restrained for a single 90-min and 180-min period, respectively.

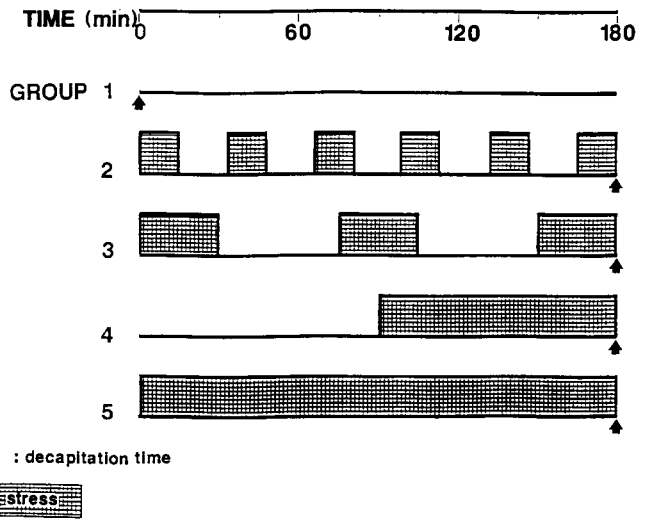

FIG. 1. Temporal patterns of stress and nonstress (rest) employed in this experiment.

Tissue Preparation and Biochemical Determination

The body weight was measured immediately after the stress session, and the rats were sacrificed by decapitation. Immediately after this, the brain, thymus, spleen, and adrenal gland were rapidly removed and wet weights were obtained. The brain was dissected into various discrete regions according to the method of Gispen et al. (6). The following regions were selected: hypothalamus, amygdala, hippocampus, cerebral cortex, and thalamus, because these are the terminal areas of the ventral and dorsal bundle pathways of the major brain NA systems. The locus coeruleus (LC) region was also dissected out by the method of Reis and Ross (15), because this area includes the brain stem incorporating the NA cell body sites. MHPG-SO_4 levels in the brain regions were fluorometrically determined by our method (11). Blood from the cervical wound was collected into heparinized tubes. Plasma corticosterone levels were determined fluorometrically by the method of van der Vies (23).

Statistical Evaluation

Statistical comparisons were made using Student's t-test (two tailed).

RESULTS

Regional Brain MHPG-SO_4 Levels

As illustrated in Fig. 2, four stressed groups (groups 2–5) exhibited significant elevations of MHPG-SO_4 levels, as compared to nonstressed controls (group 1) in all brain regions examined. Among the stressed groups, MHPG-SO_4 levels of the 15-min (group 2) and 30-min (group 3) intermittently stressed groups were significantly higher than those of the 90-min (group 4) and 180-min (group 5) continuously stressed groups in most brain regions. The 15-min intermittently stressed group (group 2) did not differ significantly from the 30-min intermittently stressed group (group 3), with the exception of the hippocampus and cerebral cortex. Although MHPG-SO_4 levels of the 90-min continuously stressed group (group 4) were generally similar to those of the 180-min (group 5) continuously stressed group, the former group had significantly larger increases in MHPG-SO_4 levels than the latter group in the hypothalamus and LC region.

Body Weight and Plasma Corticosterone Levels

As shown on the left side of Fig. 3, restraint stress caused significant decreases in body weight for four stressed groups (group 2–5), as compared to nonstressed controls (group 1). The body weight losses of the 15-min (group 2) and 30-min (group 3) intermittently stressed groups were significantly larger than those of the 90-min (group 4) and 180-min (group 5) continuously stressed groups. There were no statistical differences in body weight loss either between the intermittently stressed groups or between the continuously stressed groups. As illustrated on the right side of Fig. 3, the four stressed groups exhibited significant elevations of plasma corticosterone levels, as compared to nonstressed controls. There were no statistical differences in plasma corticosterone levels among the stressed groups, except for the difference between the 90-min (group 4) and 180-min (group 5) continuously stressed groups.

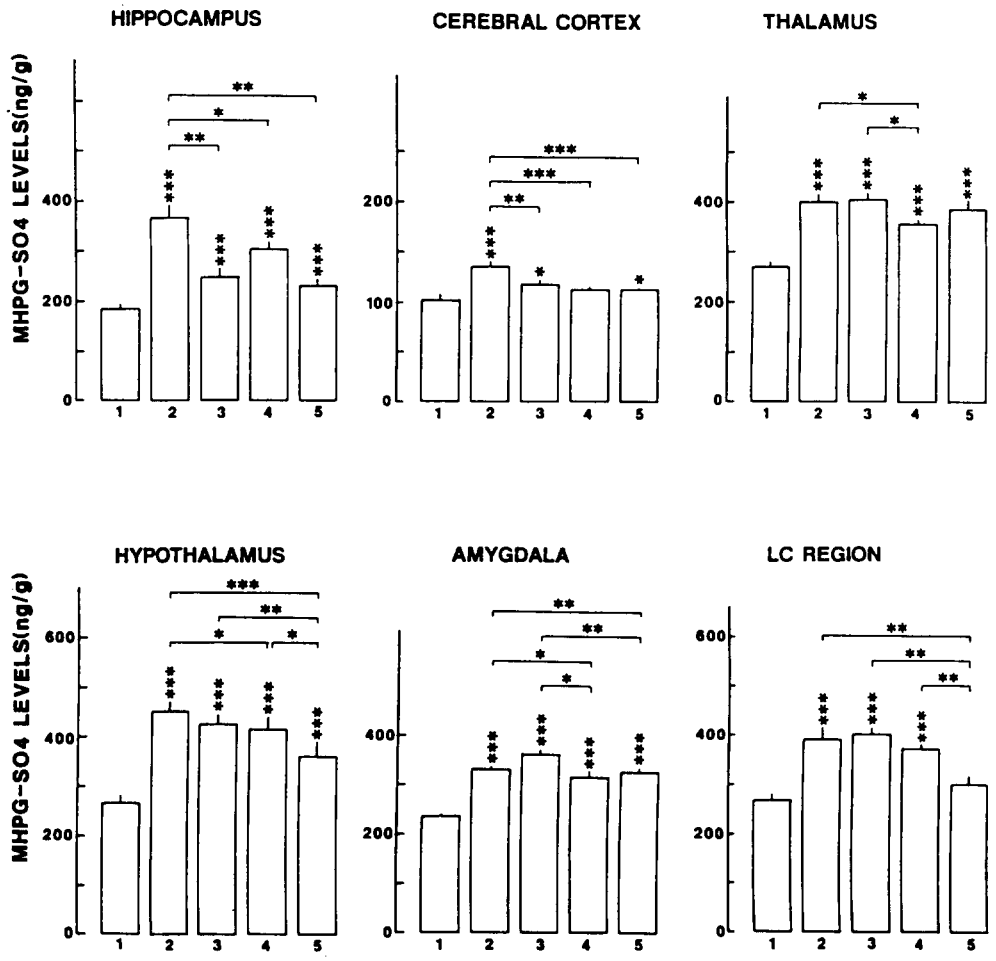

FIG. 2. MHPG-SO_4 levels (ng/g) in six brain regions for nonstressed control (group 1), 15-min (group 2), and 30-min (group 3) intermittently stressed, 90-min (group 4) and 180-min (group 5) continuously stressed groups. Each value indicates a mean ± SEM of seven to eight rats. The asterisk above the horizontal bar represents the statistical significance between the two groups compared. The asterisk above the column indicates the statistical significance as compared with nonstressed control (group 1) (two-tailed Student's *t*-test: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$).

Organ Weights

The relative wet weights of the thymus, spleen, and adrenal glands are outlined in Table 1, which were expressed as organ weight (mg) per body weight (g). When compared to nonstressed controls (group 1) and two continuously stressed groups (group 4 and 5), the 15-min intermittently stressed group (group 2) showed significant reductions in the thymus and spleen size. There were no significant group differences in adrenal weights.

DISCUSSION

A variety of stressful stimuli have been reported to cause the activation of central noradrenaline systems (18-21,23). We have reported that immobilization stress increases NA turnover in various brain regions. These changes have been considered to be related to the functions of the endocrine system and the autonomic nervous system. It is further suggested that increases in NA turnover in the hypothalamus, amygdala, and LC are closely related to the provocation of anxiety and/or fear (19). Physiologically, the locus coeruleus shows sustained responses to repeated presentations of noxious stimuli originating from these afferents even in anesthetized animals (5). We have preliminarily reported that the exposure of rats to intermittent stress produced more significant enhancement of hypothalamic NA turnover than did a single continuous stress exposure (16). The present study confirms and extends these findings to include altered activity of the noradrenergic neuronal system in many brain regions and peripheral physiological activation, such as body weight loss and reduced organ weights.

These data are in agreement with previous work on the effects of intermittent stress in enhancing the severity of gastric lesions (4,10). For example, Brady (4) found that monkeys developed duodenal ulcers only when the animals were subjected to a series of 6-h shock stress periods intererspersed with 6 h of nonstress periods. On the other hand, the present findings are inconsistent with the findings of Murison et al.

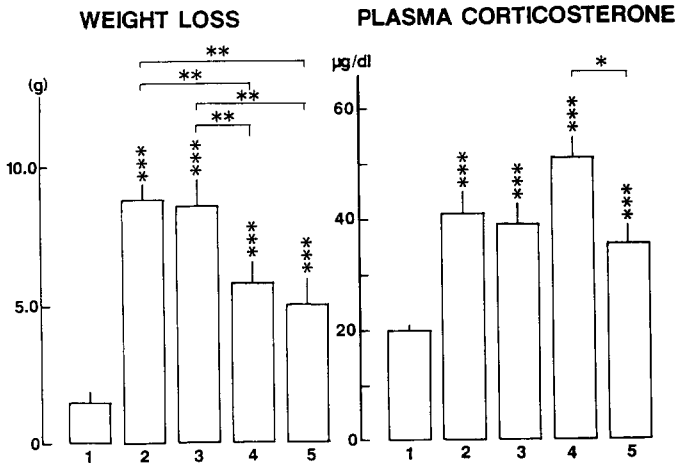

FIG. 3. The body weight loss (g) and plasma corticosterone levels (μg/dl) for nonstressed control (group 1), 15-min (group 2), and 30-min (group 3) intermittently stressed, 90-min (group 4) and 180-min (group 5) continuosly stressed groups. Each value indicates a mean ± SEM of seven to eight rats. The asterisk above the horizontal bar represents the statistical significance between the two groups compared. The asterisk above the column indicates the statistical significance as compared with nonstressed control (group 1) (two-tailed Student's t-test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

(12), who found that a single 180-min period of restraint produced more extensive ulceration than did a series of six 30-min restraint periods interspersed with 45-min nonstress periods. It is suggested that this disagreement is due to stress duration of one single exposure. Their data (12) were defined by the develoment of gastric pathology. It is suggested that at least 3 h of continuous stress exposure are required to produce gastric ulceration [see (8) for review], whereas our data were mainly concerned with the extent of brain NA turnover, and it is believed that changes in brain NA metabolism are observed within 10 min of exposure to stress (1). Therefore, it seems that in the study of Murison et al. (12), a series of six 30-min restraint periods were not enough to produce ulcers.

The results from the present study further indicate that even within intermittent stress exposure, the particular temporal pattern of the stress–rest cyclicity was of considerable significance in determinig the extent of the resulting NA turnover and peripheral physiological activation. The extent of NA turnover for the 15-min intermittently stressed rats (i.e., six 15-min stress periods interspersed with 18-min nonstress periods) was significantly higher than that for the 30-min intermittently stressed rats (three 30-min stress periods interspersed with 45-min nonstress periods), even though the total duration of stress exposure was the same for each group. It has been reported that the frequency of stressor presentation affects the adaptation pattern of neuroendocrine responses to intermittent stress (5). This result provides supporting evidence for the role of stress–rest cyclicity (e.g., the length of the nonstress time between each stress period, thus, interstressor interval) in determining the extent of stress-induced NA turnover in the brain. In addition, 90-min of continuous stress yielded more significant enhancement of NA turnover in the hypothalamus and LC region than did 180-min of continuous stress, in spite of the shorter total duration of stress exposure. It has already been found that NA turnover in these regions occurs mainly within the first 60-min of restraint stress in the course of a single consecutive 180-min stress exposure (18).

Although plasma corticosterone levels were significantly elevated by restraint stress, consistent with previous reports (18,24), there were no differences between intermittent stress and continuous stress. It seems that the restraint procedure employed in this experiment was sufficient to increase corticosterone levels to their maximum. It seems that changes of plasma corticosterone levels are very sensitive indice as compared to MHPG-SO_4, so the corticosterone levels increased and easily reached to the ceiling by various different stresses. To differentiate stress situations, MHPG-SO_4 levels seem to be more suitable indice. Moreover, the relative weights of the thymus, spleen, and adrenal glands, as well as body weight loss, were assessed to examine peripheral physiological symptoms of stress. As compared to the continuously stressed rats, 15-min intermittently stressed rats exhibited significant decreases of relative weights in the thymus and body weight. These differences seem to reflect a more marked distress due to the repeated episodes of stress, separated by stress-free periods.

What is it about the particular temporal pattern of stress–rest cyclicity that produces exacerbating effects on stress-induced alteration in brain NA turnover and peripheral physiological activation? First, the nonstress (rest) periods in the home cages between exposure to stress may act as a continuation of the stress. It was recently found that restraint stress for a short duration of 10 min could cause persistent increase in

TABLE 1

MEAN CHANGES OF RELATIVE WEIGHTS (mg/g) FOR THE THYMUS, SPLEEN, AND ADRENAL GLANDS IN TREATMENT GROUPS

Treatment	Thymus (mg/g)	Spleen (mg/g)	Adrenal Gland (mg/g)
Nonstressed control group	3.21 ± 0.10	3.45 ± 0.10	0.13 ± 0.01
15 min intermittent group	2.64 ± 0.11*	3.10 ± 0.14*	0.13 ± 0.01
30 min intermittent group	2.79 ± 0.12 *	3.12 ± 0.14	0.12 ± 0.02
90 min continuous group	2.95 ± 0.08 *	3.30 ± 0.11	0.13 ± 0.01
180 min continuous group	3.17 ± 0.21	3.15 ± 0.14	0.12 ± 0.02

Each value indicates a mean ± SEM of seven to eight rats. The asterisk above the horizontal bar represents the statistical significance between the two groups compared. The asterisk above the column indicates the statistical significance as compared with nonstressed control (group 1) (two-tailed Student's t-test: $^{*}p < 0.05$).

NA turnover in extended brain regions during the nonstress periods following the stress (9). It was suggested that even though cessation of the stress has occurred, increased NA turnover continued until the completion of an organized sequence of coping which persisted following stress offset (23). Therefore, NA turnover in intermittently stressed rats would be augmented during repeated restraint stress episodes, because serial stress periods had occurred before the aftereffects of the preceding stress were eliminated during the nonstress period. Second, it is possible to speculate that repeated exposure to intermittent stress would gradually sensitize the animal to the stress and, over time, there would be a significant enhancement of NA turnover. Anisman and Zacharko (2) reported previously that brain NA turnover could be sensitized to shock stress after several exposure. This suggests that enhancement of NA turnover is related to arousal and negative emotions, including changes in the autonomic and endocrine systems. In summary, although it is certainly conceivable from the present study that intermittent stress causes marked increases in brain NA turnover and peripheral physiological resposes as compared to continuous stress, we are well aware further study is needed to clarify the mechanisms of these differential changes induced by specific alteration in stress-rest cyclicity.

ACKNOWLEDGEMENTS

This research was supported, in part, a Research Grant from the Ministry of Education of Japan to M. Tanaka (#04670131 and #5680686). We express our gratitude to Dr. Gray B. Glavin of the Department of Pharmacology and Therapeutics, University of Manitoba, Winnipeg, Canada, for his kind help in preparing this English draft.

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