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Daily Increase in Noradrenaline Turnover in Brain Regions of Activity-Stressed Rats

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TSUDA, A., M. TANAKA, Y. KOHNO, Y. IDA, Y. HOAKI, K. IIMORI, R. NAKAGAWA, T. NISHIKAWA AND N. NAGASAKI. *Daily increase in noradrenaline turnover in brain regions of activity-stressed rats.* PHARMACOL BIOCHEM BEHAV **19**(3) 393–396, 1983.—Changes in contents of noradrenaline (NA) and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in brain regions (the hypothalamus, amygdala, thalamus, hippocampus, midbrain, cerebral cortex, pons plus medulla oblongata and basal ganglia) of male Wistar rats were evaluated after 1, 3 or 5 days of exposure to the activity-stress paradigm, wherein rats were housed in a cage with a running-wheel and restricted to 1-hr of feeding per day. When compared to the non-stressed control rats, contents of MHPG-SO_4 in all the brain regions except of the basal ganglia in the stressed rats increased as rapidly as 1 day and continued to increase throughout the 5-day activity-stress period. Contents of NA did not change significantly in most of these brain regions. The daily increase in regional NA turnover by continuous exposure to the activity-stress paradigm was related to the large increases in running activity and gastric ulcers, and to body weight loss at the 3-day and 5-day testing periods. These data suggest that pathological states produced by a 5-day activity-stress paradigm may reflect concomitant disturbances of noradrenergic function in various brain regions. The activity-stress paradigm is regarded as an intense and progressive stress, because it induces an increase in NA response in extended brain regions.

Activity-stress paradigm Noradrenaline turnover Rat brain regions Gastric Ulcers Running activity

THE activity-stress paradigm consists of housing rats in running-wheel activity cages while at the same time, restricting their food intake to 1 hr per day over the course of 4 to 10 days [12]. It has been demonstrated that running activity of activity-stressed rats progressively increases and their food intake and body weight decrease. They eventually die and manifest extensive ulcers in the glandular portion of the stomach [2,22].

We have recently found that a 5-day activity-stress exposure causes marked increases in levels of the major metabolite of noradrenaline (NA), 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) [14], in the half brain [20] or in various brain regions [21] without significant reductions of NA levels. The marked increase in NA turnover in extensive brain regions results from the interaction of a 1-hr restricted feeding regimen and an increase in running-wheel activity, since no significant change in brain NA turnover is caused by housing rats in running-wheel activity cages with ad lib feeding or by housing rats in individual home cages with either restricted or ad lib feeding.

The present study was undertaken to investigate time-related and regional variations in NA metabolism by measuring contents of NA and MHPG-SO_4 in discrete brain regions of the rat at various phases (1, 3 or 5 days) during the activity-stress paradigm. We also examined how such neurochemical changes are related to the time-course of the behavioral and physiological changes seen in activity-stress paradigm, such as increase in running activity, development of gastric ulceration and body weight loss.

METHOD

Animals and Apparatus

Thirty-two male Wistar rats weighing 150–200 g were used. They were housed 4 per standard plastic cage containing wood shavings in an air-conditioned room (24±1°C, relative humidity 50±10%) kept on a 12:12 light-dark cycle (lights on 7 a.m. to 7 p.m.) and given food and water ad lib until the experiment.

The activity-stress paradigm employed in the present experiment was described elsewhere [19]. Briefly, a running-wheel activity cage consisted of a wire drum activity wheel and an adjoining chamber. Wheel revolutions for each cage were recorded by an electromagnetic counter.

Procedure

All rats were individually housed in the running-wheel activity cages with ad lib food and water for 48 hr prior to the activity-stress period. On Day 0 of the beginning of the

activity-stress period, food was removed from rats at 10 a.m. From Day 1 to Day 4 (the second last day of the experiment), rats were fed 1 hr per day between 10 a.m. and 11 a.m. Body weight and the number of wheel revolutions were recorded daily for all rats. Rats were divided into 4 groups of 8 rats each: a non-stressed control (0-day) and three (1-day, 3-day and 5-day) activity-stressed groups. Rats in the control group were sacrificed by decapitation at 10 a.m. on Day 0, and rats in the three stressed groups were sacrificed by decapitation at 10 a.m. on Day 1, 3 and 5, respectively. The stomachs and brains were removed immediately. The stomach was examined for the number of ulcers.

Tissue Preparation and Biochemical Determination

The brain was dissected into eight parts (the hypothalamus, amygdala, thalamus, hippocampus, midbrain, cerebral cortex, pons plus medulla oblongata and basal ganglia) by the method of Gispen *et al.* [3]. The contents of NA and MHPG-SO_4 in the brain tissues were determined simultaneously according to our fluorometric method [8].

All statistical comparisons were made using analysis of variance and subsequent Tukey pair wise comparisons, unless otherwise noted. They were considered statistical significant when p or α value was equal or less than 0.05.

RESULTS

Seven rats in the 5-day group developed gastric ulcers, whereas only two rats in the 3-day group and none of the 1-day and control rats exhibited ulcers (Fig. 1). Rats in the 5-day group had more ulcers than did each of the remaining groups. Figure 1 also shows that the activity-stress paradigm produced a progressive loss of the body weight. Although running activity (i.e., the number of wheel revolutions) was low for the first phase of the stress period, the 3-day and 5-day activity-stressed groups showed sharp increases in the activity.

An analysis of variance on the MHPG-SO_4 data revealed that the activity-stress paradigm produced significant increases in MHPG-SO_4 contents in all brain regions with the exception of the basal ganglia (Fig. 2). The most rapid and marked elevation of MHPG-SO_4 level occurred in the thalamus, where the peak elevation of MHPG-SO_4 appeared as early as 1 day after the activity-stress paradigm began. Similar changes were observed in the amygdala and hippocampus, with a significant increase within 1 day of stress and sustained elevations during the remaining period of the experiment. MHPG-SO_4 contents also significantly increased in the midbrain, cerebral cortex and pons plus medulla oblongata 1 day after the activity-stress began and geometrically increased 5 days after the stress. In the hypothalamus, MHPG-SO_4 contents tended to elevate across the course of stress.

As shown in Fig. 2, significant alterations in NA levels were found in three of the eight discrete brain regions. The midbrain and cerebral cortex showed significant increases in NA levels at the 3-day and at the 1-day and 3-day testing periods, respectively. In the basal ganglia, NA contents were elevated progressively throughout the activity-stress. NA levels in the hypothalamus, hippocampus and thalamus tended to decrease at the 5-day testing period. No marked alteration in NA contents was observed in the amygdala and pons plus medulla oblongata.

DISCUSSION

The present experiment confirmed the previous report

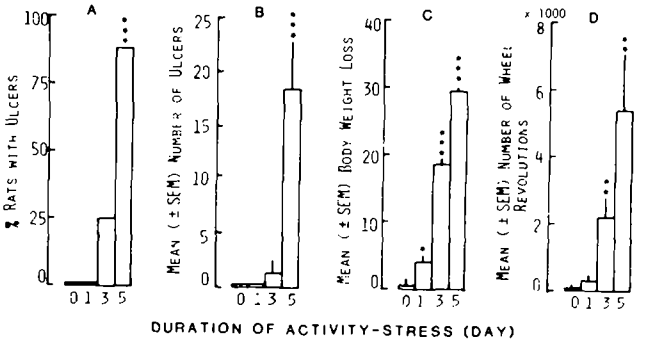

FIG. 1. Daily changes in (A) % rats with gastric ulcers, (B) number of gastric ulcers per rat, (C) % body weight loss of habituation period values and (D) number of wheel revolutions for 24 hr prior to sacrifice. The asterisk above the bar indicates the statistical significance as compared with 0-day control rat ($^*p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

[21] that the activity-stressed rats not only developed gastric ulcers, but also showed marked increase in NA turnover in extensive brain regions. Furthermore, the present study revealed a similarity in the time-course of changes induced by the activity-stress in terms of pathological symptoms and brain NA metabolism. Physiological changes such as the incidence and number of gastric ulcers, body weight loss and increased running activity appeared as a function of number of days of activity-stress. The activity-stress paradigm also progressively increased MHPG-SO_4 levels in most brain regions studied. NA levels in these regions displayed a variable pattern over time. The progressive increase in MHPG-SO_4 levels which occurred in most brain regions, seemed to be related to the increase in gastric ulcers after 5-days, and to the large increase in running activity after 3-days and 5-days exposed to the activity-stress. These patterns suggest that a marked increase in NA release in many brain regions resulting from long-term exposure to the activity-stress might be one of the neurochemical mechanisms underlying the physiological and behavioral changes caused by this paradigm.

In spite of the fact that MHPG-SO_4 levels increased progressively in most brain regions examined, there is a time-related, regional characteristic response of brain NA neurons to the activity-stress procedure. In the thalamus, MHPG-SO_4 levels markedly elevated after 1-day and remained at high levels after both 3-days and 5-days. On the other hand, MHPG-SO_4 levels in the hypothalamus and hippocampus gradually increased according to the time-course of the activity-stress. Similar time-related changes were observed in the amygdala. The cerebral cortex, midbrain and pons plus medulla oblongata displayed different regional characteristics of MHPG-SO_4 changes. As observed in other regions, levels of the metabolite in these three regions increased within 1-day and gradually increased after 3-days, however, MHPG-SO_4 levels after 5-days showed markedly higher values as compared to those seen after 1-day or after 3-days. In contrast to these seven regions, MHPG-SO_4 levels in the basal ganglia unchanged, while NA levels increased significantly over the stress period. This finding is consistent with previous reports where other stresses were employed [9,16] and suggests that the activity-stress dose not produce marked increase in NA release in this particular region.

The fact that the activity-stress elicited a progressively

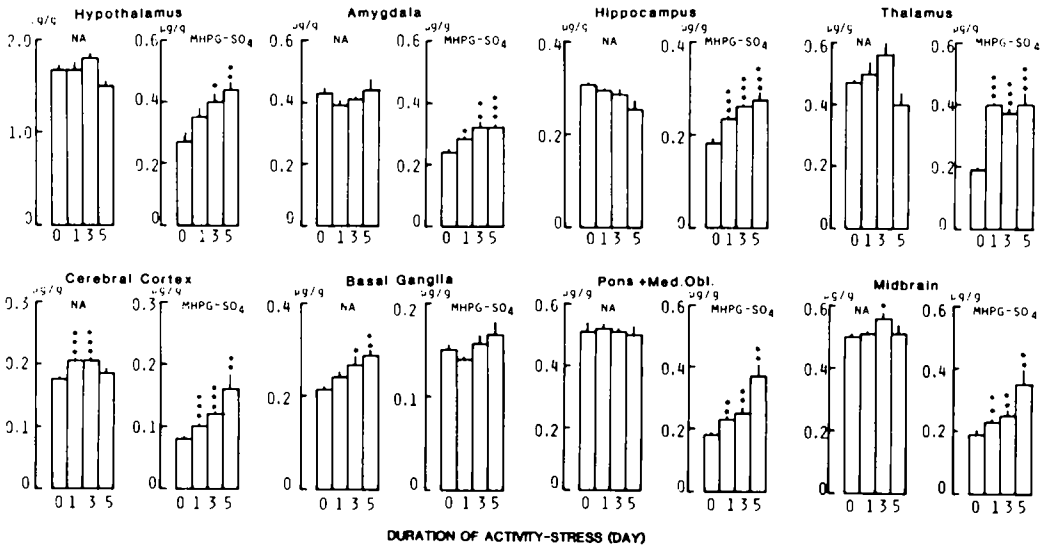

FIG. 2. Changes in contents of NA and MHPG-SO_4 in eight brain regions of rat at various times after exposure to the activity-stress paradigm. Each value represents a mean ± S.E.M. of 6–8 rats. The asterisk above the bar indicates the statistical significance as compared with 0-day control rat ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

more intense response of NA neurons in extended brain regions, especially after 5-days, is consistent with the previous finding that the activity-stress influences not only activity on the motor system and the autonomic nervous system, but also cortical activation as well as endocrine and immunological responses [4, 11, 13]. The finding that NA utilization in extensive brain regions was significantly increased from 1 day through 5 days after initiation of the activity-stress is not in agreement with previous reports showing that increases in brain NA release and metabolism caused by restraint [5] or footshock stress [15] diminished within a few days after repetition of these treatments. This finding indicates that adaptation to the stressful situation, in terms of NA metabolism, might not occur in the activity-stress paradigm. The progressive and continuous increase in NA release is considered to be one of the characteristics of the neurochemical basis of the activity-stress paradigm. It is further suggested that activity-stress continuously is stressful to the rat when they are exposed to this situation for 5 days, relative to shock [1, 7, 23] or restraint stress situations [6, 17, 18] which have well-defined "on-off" periods.

In summary, physiological and neurochemical changes seen 5 days after the activity-stress suggest that activity-stressed rats are close to death and exhibit obvious behavioral signs which Paré [10] termed the "disease state." It appears that daily increase in NA release in extensive brain regions might be related to the cause of death, and may parallel the development of severe gastric ulcers in the activity-stressed rats at a time prior to their death.

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