Effects of Stress, Non-Stress Cyclicity on Hypothalamic Noradrenaline Release in Rats

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Summary: The effects of continuous stress and intermittent stress at short intervals on rat hypothalamic noradrenaline (NA) release were assessed by measuring the levels of a principal metabolite of NA, 3-methoxy-4-hydroxy-phenylethleneglycol sulfate (MHPG-SO$_4$) in male Wistar rats. The rats were exposed to one of five restraint stress conditions, unstressed (control), six 15 min intermittent stress periods (interspersed with 18 min non-stress periods), three 30 min intermittent stress periods (interspersed with 45 min non-stress periods), 90 min continuous stress period or 180 min continuous stress period. The 15 min intermittently stressed rats had significantly larger increases in hypothalamic MHPG-SO$_4$ than the single 90 min and 180 min continuously stressed rats, while the 30 min intermittently stressed rats were significantly different from only the 180 min continuously stressed rats. In a comparison of the 15 min and 30 min intermittently stressed rats, which had the same total duration of stress exposure; the 15 min group had larger increases in MHPG-SO$_4$ levels than the 30 min group. This study provides supporting evidence for the role of stress cyclicity in determining the extent of stress-induced NA release from the hypothalamus.

Key words: stress — nonstress cyclicity — intermittent stress continuous stress — restraint stress noradrenaline — MHPG-SO$_4$ — hypothalamus

Introduction

Temporal patterns of stress have been shown to influence the extent of stress-induced pathologies such as the development of gastrointestinal ulcers (see Pare & Glavin, 1986 for a review). Brady (1958) first reported that monkeys developed duodenal ulcers only when the animals were subjected to a series of six hour shock stress periods interspersed with six hour of non-stress periods. On the other hand, Murison et al. (1989) recently found that a single 180 minute period of restraint produced more extensive ulceration than did a series of six 30 minute restraint periods interspersed with 45 minute non-stress periods in the home cage. In addition to this contradictory evidence, there is very little evidence concerning the relative influence of the forms of continuous or intermittent stress on the neurochemical activity in the brain.

The objects of this project was to determine whether a cyclic stress paradigm could induce different changes in hypothalamic noradrenaline (NA) release than the continuous stress. The NA release was
studied by measuring the content of the major metabolite of NA (3-methoxy-4-hydroxyphenylethylene glycol sulfate, MHPG-SO₄) in the rat brain. It is well established that the hypothalamus is the site of the noradrenergic neurons which respond most markedly to various forms of stress (Tanaka et al. 1983; Ida et al. 1985; Tsuda et al. 1986).

Materials and Methods

Subjects

Forty male Wistar rats, weighing 180-200 g, were used in this project. They were kept in groups of 4 per plastic cage containing wood shavings at a constant temperature (24±1°C) and humidity (50±10%) under a 12 hour light-dark cycle (light from 0700 to 1900 hour). Food and water were provided to the animals ad libitum.

Stress procedure

For the restraint stress, each animal 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 (Tanaka et al. 1983).

Experimental Procedure

After balancing for body weight, rats were randomly assigned to one of five groups (n=8 for each group). As illustrated in Fig. 1, the rats in the control group (Group 1) were not subjected to restraint stress. The animals in Group 2 were intermittently subjected to a series of six 15 minute sessions of restraint stress, interrupted by 18 minute non-stress periods. The animals in Group 3 were intermittently subjected to three 30 minute sessions of restraint stress, interrupted with two 45 minute non-stress periods. During the non-stress periods, animals were placed in their home cages without food and water. The total duration of stress was 90 minutes (i.e., a total time of 180 minutes of stress and non-stress). Animals in Groups 4 and 5 were restrained for a single 90 minute and 180 minute (continuous) period, respectively.

Tissue preparation and biochemical detection

Immediately after the period of stress, the animals were decapitated and the hypothalamus was removed according to the method of Gispen et al. (1972). Hypothalamic MHPG-SO₄ levels were determined by a fluorometric method (Kohno et al. 1979). Statistical evaluation of the data was performed using a Student’s t-test.

Results

As shown in Fig. 2, hypothalamic MHPG-SO₄ levels were significantly higher in the four groups of restraint stressed rats, relative to the non-stressed controls (Group 1). The 15-min intermittently stressed rats (Group 2) had the largest and most significant elevations of MHPG-SO₄ levels in the hypothalamus among the stressed groups. While the 30 min intermittently stressed rats had significantly higher levels of MHPG-SO₄ than

Fig. 1. Temporal patterns of stress and non-stress employed in these experiments.
Fig. 2. MHPG-SO₄ levels (ng/g) in the hypothalamus of five rat treatment groups. Each value indicates the mean±SEM for 7-8 rats. The horizontal bar indicates the statistical significance between the two groups, compared by the Student’s t-test (two-tailed). (*p<0.05, **p<0.01, ***p<0.001).

Discussion

This experiment clearly demonstrated that restraint stress, either continuous or intermittent, altered the activity of the noradrenergic neuronal system in the hypothalamus. Stressed rats exhibited significant elevations of MHPG-SO₄ in the hypothalamus, as compared to the non-stressed controls. However, the increases in the 15 min intermittently stressed group were significantly higher than those in the 30 min intermittently stressed and 90 min continuously stressed groups, even though the total duration of stress exposure was the same for each group. Also, regardless of the shorter total stress duration, the 15 min intermittently stressed rats had significantly higher levels of hypothalamic MHPG-SO₄ than the 180 min continuously stressed rats. The 90 min continuously stressed rats had larger increases in MHPG-SO₄ than the 180 min continuously stressed rats.

The results from the present study indicate that the exposure of rats to intermittent stress produced more significant enhancement of hypothalamic NA release than did a single continuous stress exposure. Moreover, even the particular temporal pattern of the stress paradigm (i.e., six 15 min stress periods interspersed with 18 min non-stress periods) was of considerable significance in determining the extent of the resulting NA release. These data are in agreement with previous work on the effects of intermittent stress in enhancing the severity of gastric lesions (Brady, 1958; Rice, 1963). These findings indicate that cyclic patterns of stress not only enhance peripheral physiological activation, but also alter brain noradrenergic neuronal activity. In addition, 90 min of continuous stress yielded a more significant enhancement of NA release than did 180 min of continuous stress, in spite of the shorter total duration of stress exposure. It has already been found that hypothalamic NA release occurs mainly within the first 60 min of restraint stress consecutive 180 min restraint stress exposures (Tanaka et al. 1983).

The 15 min intermittently stressed rats exhibited considerably larger increases in NA release in the hypothalamus, relative to the remaining stressed groups. This difference seems to reflect a more marked distress due to the repeated episodes of stress, separated by stress-free periods.
Previous regional brain analyses have shown that the enhancement of NA release in the hypothalamus is related to many different aspects of stress such as arousal, negative emotions, pain, and previous history including changes of the autonomic and endocrine systems (Tanaka et al. 1983; Ida et al. 1985; Tsuda et al. 1986).

What is it about the particular temporal pattern of stress cyclicity which produces accentuating effects on the stress-induced alteration in NA release in the hypothalamus? At a glance, the degree of hypothalamic NA release was graded as a function of the number of exposures to restraint stress. Although the experimental design did not include a handled control group, it is unlikely that the repeated handling itself was a significant factor in producing the observed enhancement of NA release (Anisman and Zacharko, 1986). Instead, there are other interpretations which help to explain these results.

Firstly, the non-stress (or rest) periods in the home cages between exposure to restraint 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 increases in NA release in extended brain regions including the hypothalamus during the period following the stress (Gondoh et al. 1989). It was suggested that the increased NA release continued for some time after termination of the stress. In other words, the brain NA activity may automatically continue toward the completion of an organized sequence, even though cessation of the stress has occurred. Therefore, NA release in intermittently stressed rats would be augmented during repeated restraint episodes, because serial stress periods had occurred before the after-effects of the preceding stress were eliminated during by the non-stress period in the home cages.

More accurately, the present data demonstrated that the six 15 min intermittent stress periods reliably enhanced NA release more than three 30 min stress periods. The latter group did not differ from the 180 min continuous stress group. The important factors would appear to be the length of the non-stress time between each stress periods, as well as the duration of the stress period (Murison et al. 1989). Thus stress cyclicity may determine the magnitude of the hypothalamic NA release. Desiderato et al. (1974) have demonstrated that the temporal combination of sympathetic arousal during the stress period followed by parasympathetic activation during the rest period is critical to the ulcerogenic process.

Secondly, repeated exposure to intermittent stress interspersed with rest periods gradually sensitizes the animal to the stress and, over time, there is a marked enhancement of NA release. Anisman and Zacharko (1986) noted that hypothalamic NA release in mice could be sensitized to shock stress after several exposures. Thus it is possible to speculate that the temporal patterns of stress cyclicity are critical factors in determining the noradrenergic neuronal activity in the hypothalamus. This study seems to provide a possible neurochemical basis for the sensitization of the stress response.

Lastly, it should be mentioned that there is some evidence showing that a single stress exposure produced more extensive ulceration than a series of intermittent stress exposures (Murison et al. 1989). The bases of these divergent results are not immediately understood. However, the different outcomes are not particularly surprising, considering the large number of experimental variables that influence the pathophysiology of stress (e.g., stress schedules). Indeed, Pare and Glavin (1986) have explained the
differential effects of the cyclic stress paradigm on the general biochemical adaptive response. Future work should explore the mechanisms of these differential changes under specified stress cyclicity.

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Reference


