Pharmacology Biochemistry & Behavior, Vol. 19, pp. 287–290, 1983. © Ankho International Inc. Printed in the U.S.A.

Regional Rat Brain Noradrenaline Turnover in Response to Restraint Stress[1]

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Received 3 February 1983

GLAVIN, G. B., M. TANAKA, A. TSUDA, Y. KOHNO, Y. HOAKI AND N. NAGASAKI. *Regional rat brain noradrenaline turnover in response to restraint stress.* PHARMACOL BIOCHEM BEHAV **19**(2) 287–290, 1983.—Male Wistar rats were starved for 12 hr and then subjected to either 2 hr of wire mesh "envelope" restraint at room temperature; 2 hr of supine restraint in a specially constructed harness at room temperature or were not restrained. Eight brain regions were examined for NA level and the level of its major metabolite, MHPG-SO_4. Plasma corticosterone and gastric ulcer incidence were also measured. All restrained rats displayed marked elevations in MHPG-SO_4 levels in most brain regions. In addition, several brain regions in restrained animals showed a reduction in NA level. All restrained rats showed elevated plasma corticosterone levels and evidence of gastric lesions. In general, supine restraint produced greater alterations in regional brain NA turnover, greater evidence of ulcer disease, and higher plasma corticosterone levels than did wire mesh restraint. These data suggest that acute but intense stress in the form of restraint causes markedly altered brain NA activity—a possible neurochemical mechanism underlying the phenomenon of stress-induced disease.

Noradrenaline Stress Restraint MHPG-SO_4

INCREASING attention is being given to the relationship between stress and central brain neurochemical changes [1, 15, 18]. Much of this research effort has been directed toward an understanding of the complex relationship between stress, brain catecholamine changes and pathological behavioral consequences, especially depression. For the last several years, we have been examining the relationship between stress and gastrointestinal pathology [4, 5, 6, 7, 8]. Recently, these two lines of research have merged in the form of an investigation of the neurochemical antecedants and or parallels of such stress-induced gastric disease. Tsuda *et al.* [15] measured noradrenaline (NA) levels and levels of its major central metabolite, 3-methoxy- 4-hydroxy phenyl ethylene glycol sulfate (MHPG-SO_4) [12] in various brain regions of rats exposed to the severe and chronic activity-stress ulcer paradigm. The cerebral cortex, midbrain, thalamus, hippocampus, and pons plus medulla oblongata showed the most marked changes in response to activity stress. It would be of interest to examine the regional brain NA turnover response in animals exposed to an acute stressor (as opposed to the more chronic procedure used by Tsuda *et al.*) in order to identify the neurochemical-biochemical processes which may mediate the responses of organisms to these acute stressors. Accordingly, in the present study, we examined regional brain NA and MHPG-SO_4 responses in rats exposed to the more short-term, acute stressor of restraint, which does not involve extensive periods of starvation, electric shock, or muscular exertion. Two popular forms of restraint were used: short-term (2 hr) restraint in the supine position, which has been shown to induce gastric glandular ulcers, thymico-lymphatic involution, and adrenal hypertrophy—all indices of stress in rats [6,17]; and wire mesh "envelope" restraint, a popular pharmacological research tool and one which is also associated with stress pathology in rats [2, 11, 13].

METHOD

Subjects and Procedure

Thirty male Wistar rats (200±10 g at the start of the study) were used. Animals were kept in a temperature-controlled room (24±1°C) under a 12:12 (light on 0700 to 1900 hr) light-dark cycle. Rats were randomly assigned to three groups of equal size: wire mesh "envelope" restraint [13]; restraint in the supine position on a specially constructed harness [17]; or a non-restrained control group. All rats were starved for 12 hr prior to the restraint treatment. Rats in the restraint groups remained in these treatments for 2 hr at room temperature. Control rats remained housed in individual cages at room temperature until sacrifice. All rats were sacrificed at

[1]Supported by N.S.E.R.C. A8072 and by the Matsumae International Foundation grants to the first author.

TABLE 1

Na LEVELS (ng/g WET WEIGHT) IN EIGHT BRAIN REGIONS OF WIRE MESH-RESTRAINED, SUPINE-RESTRAINED AND NON-RESTRAINED CONTROL RATS

Region	Wire Mesh Restraint	Supine Restraint	Non-Restrained Control
Hypothalamus	1541.5 (178.4)*	1724.8 (84.2)	1784.1 (65.4)
Amygdala	414.3 (9.3)*	404.8 (31.2)†	470.2 (22.3)
Hippocampus	139.4 (7.8)	117.8 (6.7)*	153.3 (5.0)
Thalamus	310.6 (11.6)	324.9 (19.6)	323.7 (18.6)
Midbrain	446.3 (17.2)	464.3 (17.7)	458.1 (18.6)
Pons + Medulla Oblongata	591.1 (17.2)	564.9 (22.3)	548.4 (19.4)
Cerebral Cortex	184.4 (7.8)	181.8 (6.5)	163.1 (6.7)
Basal Ganglia	201.7 (18.3)	227.8 (6.2)	178.7 (13.3)

Each value is expressed as the mean ± S.E. for 10 rats.
*Significantly different from control; $p<0.05$, Tukey test following ANOVA.
†Significantly different from control; $p<0.01$, Tukey test following ANOVA.

TABLE 2

MHPG-SO_4 LEVELS (ng/g WET WEIGHT) IN EIGHT BRAIN REGIONS OF WIRE MESH-RESTRAINED, SUPINE-RESTRAINED AND NON-RESTRAINED CONTROL RATS

Region	Wire Mesh Restraint	Supine Restraint	Non-Restraint Control
Hypothalamus	405.2 (53.1)†	317.3 (8.04)*	279.7 (25.2)
Amygdala	220.5 (33.0)†	227.7 (42.8)†	67.5 (20.2)
Hippocampus	193.1 (33.6)†	94.1 (13.5)*	68.1 (9.3)
Thalamus	517.5 (70.2)	794.1 (188.4)*	581.6 (29.9)
Midbrain	154.6 (12.3)*	161.3 (6.4)*	117.9 (10.7)
Pons + Medulla Oblongata	146.4 (13.1)	164.4 (9.1)*	126.3 (15.7)
Cerebral Cortex	78.1 (4.7)	94.3 (2.0)*	68.4 (2.9)
Basal Ganglia	418.5 (79.8)†	522.3 (66.9)†	250.2 (50.7)

Each value is expressed as the mean ± S.E. for 10 rats.
*Significantly different from control; $p<0.05$, Tukey test following ANOVA.
†Significantly different from control; $p<0.01$, Tukey test following ANOVA.

the same time of day (1000 to 1200 hr). Following the 2 hr restraint period, all rats were decapitated and the brains rapidly removed. The stomachs were also removed and inspected for the number of ulcers, lesions, or erosions. Eight brain regions (hypothalamus, amygdala, hippocampus, thalamus, midbrain, pons plus medulla oblongata, cerebral cortex and basal ganglia) were dissected over frozen CO_2 by the method of Gispen *et al.* [3]. Blood from the cervical wound was collected into heparinized tubes and centrifuged. Separated plasma and brain tissues were stored at −45°C until assayed (within ten days). Contents of NA and MHPG-SO_4 in the brain regions were analyzed fluorometrically by the method of Kohno *et al.* [10]. Plasma corticosterone levels were measured fluorometrically by a slight modification of the method of van der Vies [16].

RESULTS

Tables 1 and 2 illustrate levels of NA and MHPG-SO_4, respectively, in the wire mesh-restrained, supine-restrained, and non-restrained animals. Wire mesh-restrained animals showed significant NA depletion in the hypothalamus and amygdala (86.4% and 88.1% of control values, respectively). Supine-restrained rats showed significant NA depletion in the amygdala and hippocampus (86.1% and 76.8% of control values, respectively). With regard to MHPG-SO_4 levels, wire mesh-restrained rats showed significant elevations in the hypothalamus and hippocampus (145% and 284% of control values, respectively), while supine-restrained rats showed significant MHPG-SO_4 elevations in the amygdala, basal ganglia and thalamus (337%, 209%, and 137% of control val-

TABLE 3

SUMMARY OF STOMACH CONDITIONS AND PLASMA CORTICOSTERONE LEVELS

Treatment	No. of Rats	No. of Rats With Ulcers	Mean (±S.E.) Ulcers per Rat	Mean (±S.E.) Plasma Corticosterone (μg/dl)
Supine Restraint	10	7*	1.0 (0.31)	64.8 (2.54)†
Wire Mesh Restraint	10	3	0.3 (0.16)	55.6 (2.00)†
Non-Restrained Control	10	0	0.0 (0.00)	27.6 (7.46)

*$p<0.05$ (χ^2 test).
†$p<0.01$ Different from control (Tukey test following ANOVA).

ues, respectively). As shown in Table 3, both forms of restraint produced gastric ulcers. However, supine restraint produced a greater incidence and frequency of ulcers, $\chi^2(2)=7.47$, $p<0.01$. Plasma corticosterone was markedly and significantly elevated in both groups of restrained rats, relative to controls, $F(2,27)=19.22$, $p<0.001$.

DISCUSSION

Stress-related enhancement of brain NA turnover (decreased NA level but increased MHPG-SO_4 level) [10] has been reported elsewhere. However, these studies examined primarily electric footshock stress [14] or the chronic procedure of activity-stress [15]. It is interesting that a short-term, intense stressor such as restraint is capable of producing marked and differential regional brain noradrenaline changes. Most clearly influenced by both forms of restraint in the present study were the hypothalamus, amygdala, hippocampus and basal ganglia. In comparison, the more severe and chronic procedure of activity-stress exerted the greatest effects on noradrenaline turnover in the cerebral cortex, midbrain, thalamus, hippocampus and pons plus medulla oblongata [15]. In addition, "psychological" stress, in the form of exposing rats to the sight, sound, and odor of other rats being shocked, also produced most marked NA changes in the amygdala and hypothalamus [9]. It may be that the acute-chronic distinction often applied to various physical and psychological stressors is better reflected by effects on regional central neurochemical changes than by peripheral disease effects. Chronic stress procedures (activity-stress), appear to involve a greater number of brain regions and produce more marked NA turnover (accelerated NA release and consequently lower NA levels and higher MHPG-SO_4 levels) than do acute stress treatments (restraint). In both of these procedures, however, massive gastric glandular ulcers are seen. Perhaps (1) the number of brain regions involved and (2) the intensity of involvement of these regions, as reflected by NA turnover, are sensitive indices of the intensity of a particular stressor and may determine the behavioral and pathophysiological responses of an organism to that stressor. We are currently examining the influence of central NA agonists and antagonists on restraint-stress-induced regional brain NA turnover and its relation to peripherally manifested "stress-related" gastric disease.

ACKNOWLEDGEMENTS

We thank Miss S. Takeda and Mrs. M. Kato for expert technical assistance and Nippon Roche K. K. for a generous gift of MHPG-SO_4.

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