Pharmacology Biochemistry & Behavior, Vol. 14, pp. 729–732, 1981. Printed in the U.S.A.

Regional Responses of Rat Brain Noradrenergic Neurones to Acute Intense Stress

RYOICHI NAKAGAWA,[1] MASATOSHI TANAKA, YASUKO KOHNO, YASUTAKA NODA* AND NOBUYUKI NAGASAKI

*Department of Pharmacology and *Institute of Animal Experiment, Kurume University School of Medicine, Kurume 830, Japan*

Received 14 October 1980

NAKAGAWA, R., M. TANAKA, Y. KOHNO, Y. NODA AND N. NAGASAKI. *Regional responses of rat brain noradrenergic neurones to acute intense stress.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 729–732, 1981.—Contents of noradrenaline (NA) and its principal metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), in six brain regions of the rat were monitored simultaneously during 180 min of acute intense stress, i.e., electric tail shock under immobilization. In the hypothalamus and amygdala, NA contents decreased rapidly, and subsequently remained at the decreased levels while MHPG-SO_4 contents increased progressively. The hippocampus and cerebral cortex showed more delayed changes in NA and MHPG-SO_4 contents than the above regions. In the pons+med. obl., no decreases of NA contents were observed at any time, but MHPG-SO_4 contents increased significantly. Neither NA nor MHPG-SO_4 content changed significantly in the basal ganglia except for a transient increase of NA. These results suggest that, during acute intense stress, each brain region responds differently and the NA content is maintained at a decreased level despite continuously enhanced release of the amine.

Noradrenaline 3-Methoxy-4-hydroxyphenylethyleneglycol sulfate Acute intense stress
Regional difference Maintenance of NA content

ACUTE stress has been shown to induce excitation of brain noradrenergic neurone and enhance release of noradrenaline (NA) from the nerve ending. Increased levels of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4), a principal metabolite of released NA [19,25], were observed after cold exposure [16], electric foot shock [12,22], and hind-limb ischemia [24]. We reported that immobilization combined with electric foot shock for 180 min resulted in a decrease of NA content associated with a marked increase of MHPG-SO_4 level in the rat whole brain, but immobilization or foot shock alone did not affect NA content despite a significant increase of MHPG-SO_4 level [17]. This finding suggests that a depletion of NA results from strongly enhanced release of NA after the combined stress. In addition, the stress produced remarkable lesions in gastric mucosae associated with a reduced NA content in the stomach [17]. These marked changes in central and peripheral organs indicate that the combined stress used in the previous study is extremely intense. There have been few studies, however, on simultaneous changes in NA and MHPG-SO_4 contents in brain regions under the intense stress situation. The present study was undertaken to clarify the regional characteristics in the response of brain noradrenergic neurone to electric tail shock under immobilization considered to be the same intense stress.

METHOD

Animals

Forty-eight male Wistar rats weighing 200–250 g were housed 4 per plastic cage (210×320×135 mm) at near constant temperature (24 ± 1°C). The rats were provided with food and water ad lib and kept under a 12 hr light/dark cycle.

Stress Procedure

As shown in Fig. 1, each rat was immobilized with a wire net of 3 mm mesh and received an electric shock through an apparatus attached to a tail, based on the method of Weiss [31]. The shock was delivered using a constant voltage stimulator in square-wave pulses (5 Hz) for 5 sec every 30 sec at 15 V intensity (AC, about 2–3 mA). The stress was applied to eight animals for 15, 30, 60, 120 and 180 min, respectively. Immediately after the stress, rats were decapitated, but eight rats in the control group were sacrificed just before the stress.

Assay of NA and MHPG-SO_4 Contents in Brain Regions

The brain was removed immediately after the decapitation, then divided on the ice plate by the method of Gispen *et al.* [7] into six regions: hypothalamus, amygdala, hippocam-

[1]To whom reprint requests should be addressed.

Copyright © 1981 ANKHO International Inc.—0091-3057/81/050729-04$00.90/0

pus, cerebral cortex, pons+med. obl., and basal ganglia. These samples were kept in −40°C until assayed. Contents of NA and MHPG-SO_4 were determined simultaneously by a sensitive, fluorometrical method developed by Kohno *et al.* [11].

Statistical Evaluation

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

RESULTS

Figure 2 represents changes in contents of NA and MHPG-SO_4 in six brain regions under the acute intense stress situation. In the hypothalamus, rapid and remarkable changes in both NA and MHPG-SO_4 contents were observed. As early as 15 min, the NA content decreased markedly by 37.6% and the MHPG-SO_4 level increased significantly by 27.1%. NA concentration continued to decrease until 60 min (47.7%); thereafter it remained at this level until 120 min. By contrast, MHPG-SO_4 levels increased progressively from 60 min (101.9%) to 180 min (162.9%). During this period, the MHPG-SO_4 level increased on the course of the stress without further decrease of the NA content. Similarly, the amygdala demonstrated the same rapid decrease of NA content by 17.8% at 15 min, and MHPG-SO_4 level showed a modest increase after 30 min and reached a statistical significance at 60 min. NA concentration remained at a depressed level, 29.4 to 33.3% for the duration, while the MHPG-SO_4 level increased progressively from 30 min (12.4%) to 180 min (47.7%). In the cerebral cortex, NA content decreased by 22.7% and MHPG-SO_4 level increased by 15.5% at 60 min. Here, too, NA concentration remained at a depressed level while MHPG-SO_4 showed a progressive increase. The hippocampus, however, showed delayed changes in both NA and MHPG-SO_4 contents when compared with the above regions; at 120 min, NA content had decreased by 27.9% and MHPG-SO_4 showed an increase of 16.8%. In the pons+med. obl., no significant decreases of NA contents were observed at any time although MHPG-SO_4 levels increased significantly from 60 min (34.0%) to 180 min (70.6%). Neither NA nor MHPG-SO_4 content changed significantly in the basal ganglia except for a significant and transient increase of NA content by 36.7% at 15 min.

DISCUSSION

A number of studies have investigated the relationship between the types of stress situations and their influence on the brain noradrenergic neurone. Generally, NA contents decrease in most brain regions with acute stress, but the degree of decrease is quite variable and appears to depend on the nature of the stress. Maynert and Levi [15] reported decreases of NA content in the whole brain correlated with duration and frequency of electric foot shock to rats for 60 min, and they suggested that NA depletion in the brain follows according to the intensity of stress. Our previous study [17] suggests that the combined stress, which is considered extremely intense, severely depressed NA content by a strongly enhanced release of the amine. During intense stress, the NA depletion in the brain has been shown to reflect NA release which exceeds amine synthesis [2, 20, 21].

The present study subjected to the same intense combined stress, i.e., electric tail shock under immobilization, demonstrated a distinctive response of noradrenergic

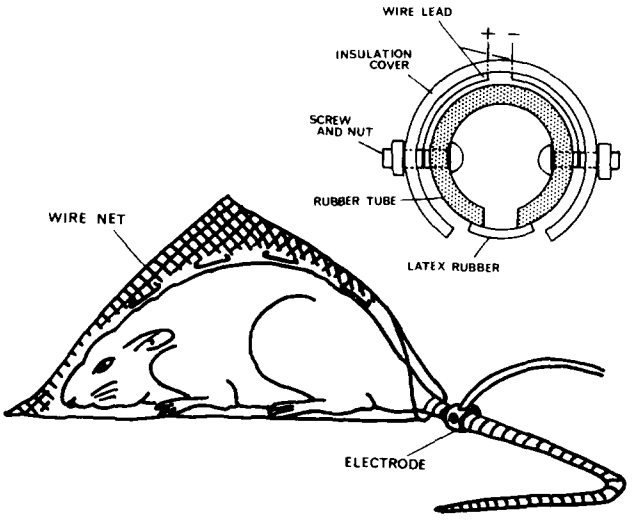

FIG. 1. An immobilized rat with an electrode. Each rat was immobilized with a wire net of 3 mm mesh and received electric shock through an electrode attached to a tail. The right upper diagram represents a tail electrode at frontal view.

neurone in each brain region. The hypothalamus showed rapid and remarkable changes in both NA and MHPG-SO_4 contents. This finding is consistent with previous observations that NA contents decreased more markedly in the hypothalamus [30] or in the hypothalamic nuclei [13,18] after short-term stresses, and that increases of MHPG-SO_4 levels were more remarkable in this region than the other regions after foot shock [22] or hind-limb ischemia [24]. The characteristic response of noradrenergic neurone to the stress in this region is interesting with respect to the evidence that the hypothalamus has a primary role on emotions, pituitary-adrenocortical axis, and the autonomic nervous system. The occurrence of the same rapid change in NA content in the amygdala suggests that a close relationship exists between this region and the hypothalamus, a functional relationship which reflects the response to stressful conditions, as shown in previous neuroendocrinological studies [1,10]. In the hippocampus, changes in both NA and MHPG-SO_4 contents were delayed when compared with the above regions. The reason for the delayed response in this region is not known, but may be due to the involvement of the hippocampus in functions other than emergency reaction to stress, such as the continuation of normal social behavior or the regulation of balance between physiological response systems, as suggested by Isaacson [8] and Ely *et al.* [6]. In the pons+med. obl., no decreases of NA contents were observed at any time despite significant increases of MHPG-SO_4 levels indicating enhanced release of NA. It may be that NA concentration is adequately maintained by an increased synthesis of the amine. Evidence for this comes from studies on tyrosine hydroxylase, the rate-limiting enzyme of NA synthesis, which demonstrate higher activity of this enzyme in the brainstem under stressful [33] or non-stressful condition [4]. In the basal ganglia, no increases of MHPG-SO_4 levels occurred at any time. This finding suggests that the stress did not induce enhancement of NA release in this region. The cause of an early increase of NA content is not known, but the accumulation of NA associated with a transient decrease of MHPG-SO_4 level has been reported under immobilization stress [26].

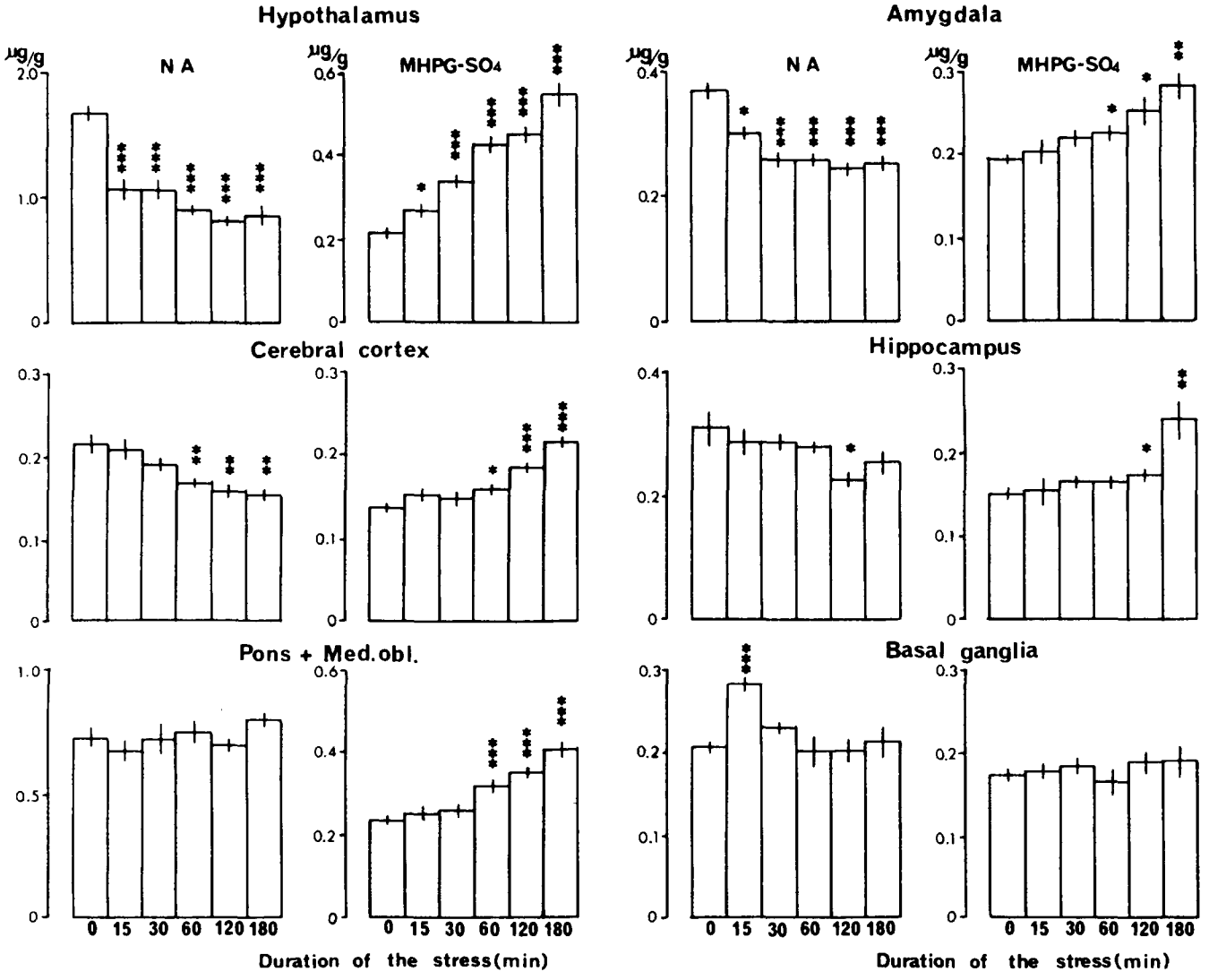

FIG. 2. Changes in contents of NA and MHPG-SO_4 in various brain regions of rat during 180 min of electric tail shock under immobilization; each bar represents a mean ± SEM of eight rats. The asterisk above the bar indicates the statistical significance as compared with control rat (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

In the present study, it is notable that NA contents decreased and remained at the lower levels despite progressive increases of MHPG-SO_4 contents in the hypothalamus, amygdala, and cerebral cortex. Suppressed elimination of MHPG-SO_4 by the stress appears to be excluded. The previous study employed probenecid, an inhibitor of active transport of the metabolite, has shown that the accumulated levels of MHPG-SO_4 in rat brain regions without probenecid were well related to those with probenecid under immobilization [26]. Therefore, in these regions, the continuously enhanced release of NA is considered to be elicited by the intense stress, however, NA contents were maintained after the reductions to some extents. Previous studies also reported similar changes in NA contents associated with continuously enhanced turnover of NA during foot shock [27] and foot shock under immobilization [2]. We propose the existence of a supplementary regulation system to protect NA store from exhaustive depletion in the brain resulting from continuously enhanced release of NA by the stress. A mechanism for this system has not been precisely understood. However, the system may have a relationship to an enzyme which synthesizes NA, for example, tyrosine hydroxylase or dopamine-β-hydroxylase. It is well documented that chronically-repeated stress enhances the activity of tyrosine hydroxylase in the brain [14, 18, 23, 29, 32, 33]. It is of interest, however, that other studies [3,9] have reported an accelerated tyrosine hydroxylation in the brain after acute stresses. Davis [5] suggested the possibility that an allosteric change in brain tyrosine hydroxylase may increase the affinity for oxygen, allowing greater catecholamine synthesis. Thierry *et al.* [28] showed that newly synthesized NA in the functional pool of the brainstem is increased and selectively utilized during acute foot shock stress. It is considered that this new, available NA may contribute to the maintenance of NA concentration in the brain under acute stress situation.

REFERENCES

1. Allen, J. P. and C. F. Allen. Role of the amygdaloid complexes in the stress-induced release of ACTH in the rat. *Neuroendocrinology* **15:** 220–230, 1974.
2. Bliss, E. L., J. Ailion and J. Zwanziger. Metabolism of norepinephrine, and serotonin and dopamine in the rat brain with stress. *J. Pharmac. exp. Ther.* **164:** 122–134, 1968.
3. Brown, R. M., S. R. Snider and A. Carlsson. Changes in biogenic amine synthesis and turnover induced by hypoxia and/or foot shock stress. II. The central nervous sytem. *J. Neural Transm.* **35:** 293–305, 1974.
4. Bucopoulos, N. and R. K. Bhatnagar. Correlation between tyrosine hydroxylase activity and catecholamine concentration or turnover in brain regions. *J. Neurochem.* **29:** 639–643, 1977.
5. Davis, J. N. Brain tyrosine hydroxylation: Alteration of oxygen affinity in vivo by immobilization or electroshock in the rat. *J. Neurochem.* **27:** 211–215, 1976.
6. Ely, D. L., E. G. Greene and J. P. Henry. Effects of hippocampal lesion on cardiovascular, adrenocortical and behavioral response pattern in mice. *Physiol. Behav.* **18:** 1075–1083, 1977.
7. Gispen, W. H., P. Schotman and E. R. de Kloet. Brain RNA and hypophysectomy: A topographical study. *Neuroendocrinology* **9:** 285–296, 1972.
8. Isaacson, R. L. *Inhibition and Learning*. New York: Academic Press, 1972, p. 497.
9. Kenessey, A. and Z. Huszti. *Catecholamines and Stress*. Oxford: Pergamon Press, 1976, p. 331.
10. Knigge, K. M. Adrenocortical response to stress in rats with lesions in hippocampus and amygdala. *Proc. Soc. exp. Biol. Med.* **108:** 18–21, 1961.
11. Kohno, Y., K. Matsuo, M. Tanaka and N. Nagasaki. Simultaneous determination of noradrenaline and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in discrete brain regions of the rat. *Analyt. Biochem.* **97:** 352–358, 1979.
12. Korf, J., G. K. Aghajanian and R. H. Roth. Increased turnover of norepinephrine in the rat cerebral cortex during stress: Role of locus coeruleus. *Neuroendocrinology* **12:** 933–938, 1973.
13. Kvetnánský, R., M. Palkovits, A. Mitro, T. Torda and L. Mikalaj. Catecholamines in individual hypothalamic nuclei of acutely, and repeatedly stressed rats. *Neuroendocrinology* **23:** 257–267, 1977.
14. Lamprecht, F., B. Eichelman, N. B. Thoa, R. B. Williams and I. J. Kopin. Rat fighting behavior: Serum dopamine-β-hydroxylase and hypothalamic tyrosine hydroxylase. *Science* **177:** 1214–1215, 1972.
15. Maynert, E. W. and R. Levi. Stress-induced release of brain norepinephrine and its inhibition by drugs. *J. Pharmac. exp. Ther.* **143:** 90–95, 1964.
16. Meek, J. L. and N. H. Neff. The rate of formation of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate in brain as an estimation of the rate of formation of norepinephrine. *J. Pharmac. exp. Ther.* **184:** 570–575, 1973.
17. Nakagawa, R., M. Tanaka and N. Nagasaki. Remarkable responses of brain monoamine neurones, changes in peripheral noradrenaline contents and the gastric mucosae under intense stress situation. *Kurume med. J.* **24:** 117–125, 1978.
18. Palkovits, M., R. M. Kobayashi, J. S. Kizer, D. M. Jacobowitz and I. J. Kopin. Effect of stress on catecholamines and tyrosine hydroxylase activity in individual hypothalamic nuclei. *Neuroendocrinology* **18:** 144–153, 1975.
19. Schanberg, S. M., G. Breece, J. J. Schildkraut, E. K. Gordon and I. J. Kopin. 3-Methoxy-4-hydroxyphenylglycol sulfate in brain and cerebrospinal fluid. *Biochem. Pharmac.* **17:** 2006–2008, 1968.
20. Stolk, J. M., R. L. Conner, S. Levine and J. D. Brachas. Brain norepinephrine metabolism and shock-induced fighting behavior in rats: Differential effect on shock and fighting on the neurochemical response to a common foot shock stimulus. *J. Pharmac. exp. Ther.* **190:** 193–209, 1974.
21. Stone, E. A. Accumulation and metabolism of norepinephrine in rat hypothalamus after exhaustive stress. *J. Neurochem.* **21:** 589–601, 1973.
22. Stone, E. A. Effect of stress on sulfated glycol metabolites of brain norepinephrine. *Life Sci.* **16:** 1725–1730, 1975.
23. Stone, E. A., L. S. Freedman and L. E. Morgano. Brain and adrenal tyrosine hydroxylase activity after chronic foot shock stress. *Pharmac. Biochem. Behav.* **9:** 551–553, 1978.
24. Stoner, H. B. and A. Hunt. *Catecholamines and Stress*. Oxford: Pergamon Press, 1976, p. 113.
25. Sugden, R. F. and D. Eccleston. Glycol sulfate ester formation from [^{14}C] noradrenaline in brain and the influence of a COMT inhibitor. *J. Neurochem.* **18:** 2461–2468, 1971.
26. Tanaka, M., Y. Kohno, R. Nakagawa, S. Takeda and N. Nagasaki. *Abstr. 12th CINP Congress*. Oxford: Pergamon Press, 1980, p. 339.
27. Taylor, K. M. and R. Laverty. The metabolism of tritiated DA in regions of the rat brain in vivo. *J. Neurochem.* **16:** 1367–1376, 1969.
28. Thierry, A. M., G. Blanc and J. Glowinski. Effect of stress on the disposition of catecholamine localized in various interneuronal storage forms in the brainstem of the rat. *J. Neurochem.* **18:** 449–461, 1971.
29. Thonen, H. Induction of tyrosine hydroxylase in peripheral and central adrenergic neurones by cold exposure of rats. *Nature* **228:** 861–862, 1970.
30. Vellucci, S. V. The effect of ether stress and betamethasone treatment on the concentration of norepinephrine and dopamine in various regions of the rat brain. *Br. J. Pharmac.* **60:** 601–605, 1977.
31. Weiss, J. M. A tail electrode for unrestrained rats. *J. exp. Analysis Behav.* **10:** 85–86, 1967.
32. Weiss, J. M., I. Howard, L. A. Pohorecky, J. Brick and N. E. Miller. Effect of chronic exposure of stressors on avoidance-escape behavior and on brain norepinephrine. *Psychosom. Med.* **37:** 522–534, 1975.
33. Zigmond, R. E., F. Schon and L. L. Iversen. Increased tyrosine hydroxylase activity in the locus coeruleus of rat brain stem after reserpine treatment and cold stress. *Brain Res.* **70:** 547–552, 1974.