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Time-Related Differences in Noradrenaline Turnover in Rat Brain Regions by Stress

MASATOSHI TANAKA, YASUKO KOHNO, RYOICHI NAKAGAWA, YOSHISHIGE IDA, SHIGEKO TAKEDA AND NOBUYUKI NAGASAKI

Department of Pharmacology, Kurume University, School of Medicine, Kurume 830, Japan

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TANAKA, M., Y. KOHNO, R. NAKAGAWA, Y. IDA, S. TAKEDA AND N. NAGASAKI. *Time-related differences in noradrenaline turnover in rat brain regions by stress.* PHARMAC. BIOCHEM. BEHAV. 16(2) 315–319, 1982.—Male Wistar rats were stressed by immobilization from 15 to 180 min and the effect on noradrenaline (NA) and 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) contents in eight discrete brain regions were determined. NA levels significantly decreased and MHPG-SO_4 levels increased in the hypothalamus, amygdala, thalamus, hippocampus, pons+med.obl. and cerebral cortex. By contrast, the basal ganglia exhibited increases in NA levels and transient decreases in MHPG-SO_4 levels. The midbrain failed to show significant alterations. The most rapid and marked increase in MHPG-SO_4 level was found in the hypothalamus. When rats were exposed to stress after treatment with probenecid 400 mg/kg, the hypothalamus and amygdala showed greater accumulations of MHPG-SO_4 in the early phase of stress, while the pons+med.obl. and basal ganglia in the later phase. The other regions showed virtually the same accumulations. These results suggest that NA release is enhanced by immobilization in the six regions mentioned above and that response of NA neurons occurs rapidly in the hypothalamus and amygdala but is delayed in other regions.

Noradrenaline 3-Methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) Immobilization stress
Rat brain Regional difference Time-related difference Plasma corticosterone

VARIOUS forms of stressful stimuli have been shown to affect biogenic amines in the brain, although the literature contains some conflicting views. In general, a variety of acute stresses have been found to result in decreased noradrenaline (NA) concentrations and increased NA turnover as reviewed by Stone [18]. However, most studies of stress effects on brain catecholamine dynamics employed techniques that determine the altered catecholamine levels induced by stress alone and/or by stress modified by certain chemical substances such as synthesis inhibitors. Few studies have been made on changes in metabolite levels of NA caused by acute stress in an extended number of brain regions.

NA in the rat brain is predominantly metabolized to form the sulfate conjugates of 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG-SO_4) and 3,4-dihydroxyphenylethyleneglycol (DHPG-SO_4) [15, 16, 19]. The production of MHPG-SO_4 appears to be directly related to the functional activity of central noradrenergic neurons [17]. Recently, by measuring both NA and MHPG-SO_4 levels in 15 brain areas, we have reported that regional levels of MHPG-SO_4 in the brain are also indicative of NA turnover [9].

The purpose of the present study is to clarify the regional characteristics of brain NA metabolism in the rat after various periods of immobilization stress by measuring simultaneously NA and MHPG-SO_4 contents using the sensitive fluorometric assay method developed by us [8].

METHOD

Male Wistar rats weighing 180–220 g were used in all experiments. The animals were housed 4 to a cage (265×425×150 mm standard plastic cage containing wood shavings) in a 12-hour (7:00 a.m. to 7:00 p.m.) light/dark cycled room at constant temperature (24±1°C) and humidity (50±10%). Food and water were provided ad lib. Immobilization stress was employed by enclosing animals in a flexible wire mesh initially formed into a cone and then bent to conform to the size of the individual animals.

Two separate studies were performed. In the first study, rats were stressed by immobilization for periods of 0 (control), 15, 30, 60, 120 or 180 min. In the second study, which was undertaken to confirm the regional differences in time-related MHPG-SO_4 formations during the same immobilization stress as employed in the first study, probenecid (400 mg/kg, IP) was injected to prevent active transport of MHPG-SO_4 from brain tissue [11]. The drug-induced accumulations of MHPG-SO_4 were then compared for two different phases of stress: 45 min initial stress, designated in this paper as "early" and 45 min continued stress after prior 90 min stress, designated as "late." Rats were allocated to one of five groups: untreated control; probenecid injection 45 min before sacrifice; probenecid injection with subsequent 45 min immobilization; 90 min immobilization; and 90 min immobilization followed by probenecid injection with subsequent 45 min immobilization.

Immediately after treatment, the rat was sacrificed by decapitation, and the brain was rapidly dissected into discrete brain regions according to the method of Gispen *et al.* [4] and frozen on solid CO_2. Brain regions dissected were: the pons and medulla oblongata (pons+med.obl.), hypothal-

amus, thalamus, midbrain (not included in the second study), hippocampus, amygdala, cerebral cortex, and basal ganglia which included caudate nucleus, putamen and globus pallidus.

For the first study, blood was collected from the cervical vessels into heparinized tubes. Separated plasma and brain tissues were stored at −45°C until assayed. Plasma corticosterone levels were determined by the modified method of van der Vies [21]. NA and MHPG-SO_4 levels were determined simultaneously according to our fluorometric method [8]. In the second study, only MHPG-SO_4 levels were measured.

All experiments were performed between 10:00 a.m. and 2:00 p.m., considering the diurnal variations in plasma corticosterone and brain NA contents. For a statistical analysis, Student's *t*-test (two-tailed) was employed.

RESULTS

The corticosterone levels in plasma were significantly elevated within 15 min of immobilization as indicated in Fig. 1. Subsequently, corticosterone levels continued to increase, reached a peak level at 60 min and although decreased slightly by 180 min, remained significantly elevated over those observed in control animals.

Immobilization stress produced significant decrease in NA contents in six of the eight discrete brain areas examined (Fig. 2). In the hypothalamus, the NA content already decreased significantly within 15 min of stress and appeared to decrease again after 30 min ($p<0.10$). Between 60–180 min of stress, almost the same degree of significant reduction in NA

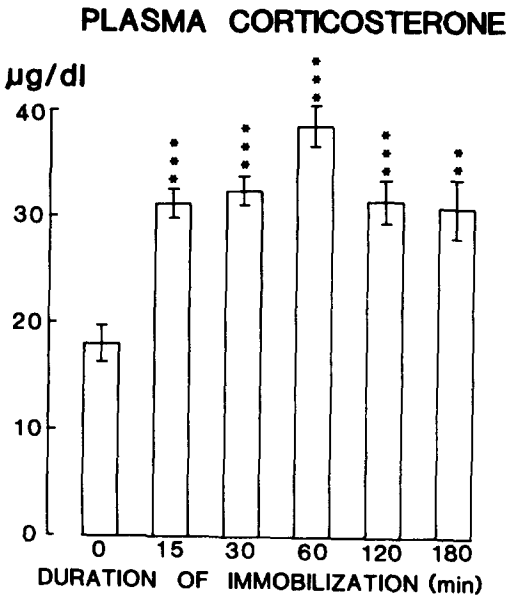

FIG. 1. Changes in the plasma corticosterone level in the rat exposed to immobilization stress for various periods. Each value indicates the mean ± SEM of 8 rats. Significantly different from controls (0 min); $^{**}p<0.01$, $^{***}p<0.001$.

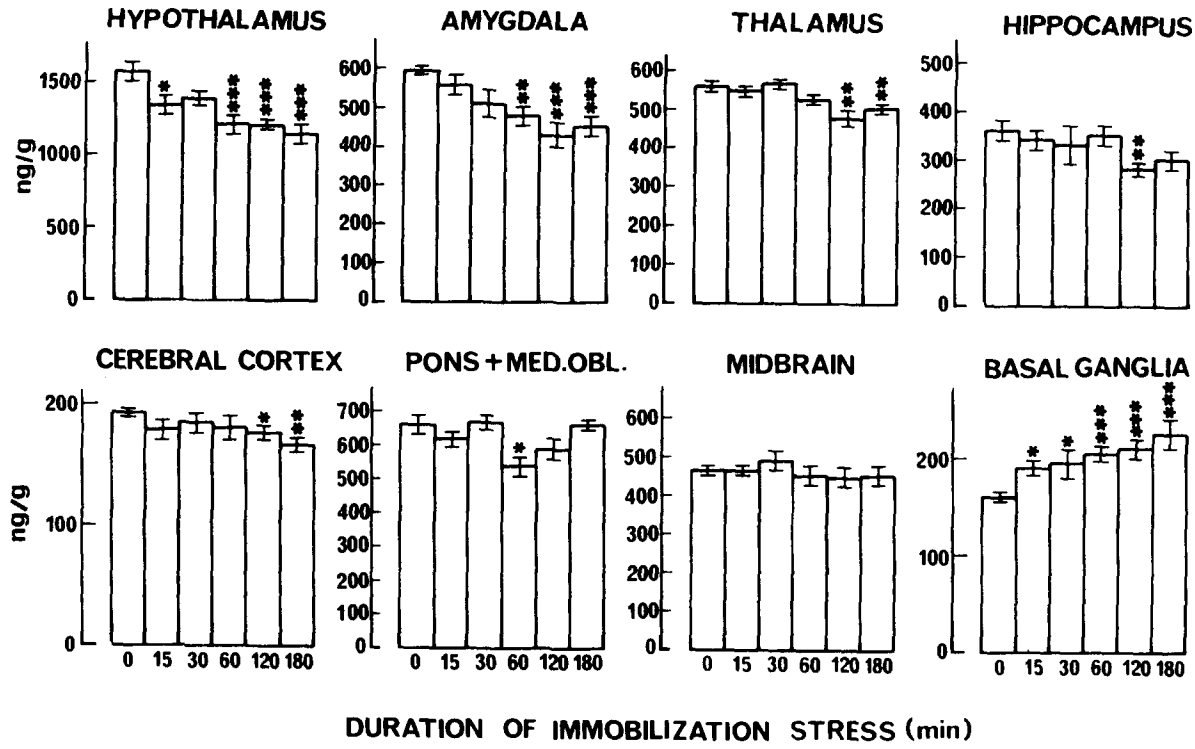

FIG. 2. Changes in the NA levels (ng/g) in the eight discrete brain regions in the rat exposed to immobilization stress for various periods. Each value indicates the mean ± SEM of 8 rats. Significantly different from controls (0 min); $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

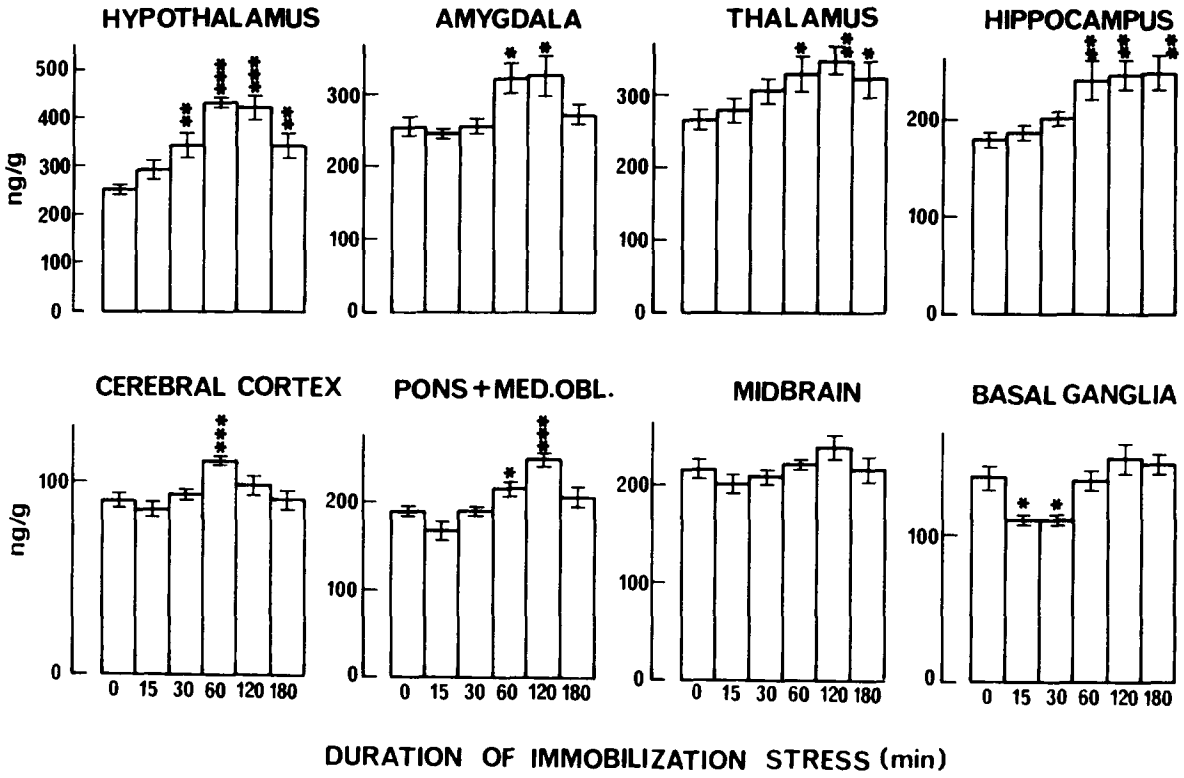

FIG. 3. Changes in the MHPG-SO_4 levels (ng/g) in the eight discrete brain regions in the rat exposed to immobilization stress for various periods. Each value indicates the mean ± SEM of 7–8 rats. Significantly different from controls (0 min); $*p<0.05$, $**p<0.01$, $***p<0.001$.

contents was observed (23–27% of the control value). In the amygdala, NA levels showed a tendency to decrease within 30 min ($p<0.10$) and showed a statistically significant decrease between 60–180 min to almost the same extent as that observed in the hypothalamus. The thalamus and cerebral cortex exhibited significant decreases in NA contents at 120 and 180 min. Similar changes were found in the hippocampus, with a significant decrease at 120 min and a tendency to decrease at 180 min ($p<0.10$). The NA levels in the pons+med.obl. decreased significantly at 60 min, and the midbrain failed to show any significant alterations in NA contents. By contrast, in the basal ganglia NA contents were significantly elevated within 15 min of immobilization and continued to increase thereafter.

As shown in Fig. 3, the most rapid and marked change in MHPG-SO_4 levels was observed in the hypothalamus. The metabolite level appeared to increase at 15 min ($p<0.10$), then rose significantly between 30–180 min, with the peak elevation at 60 min (73% of controls). MHPG-SO_4 levels in the amygdala were elevated significantly at 60 and 120 min (27% and 28%, respectively). The thalamus and hippocampus showed significant elevations in MHPG-SO_4 contents at 60 min (25%, 36%), 120 min (31%, 38%) and 180 min (22%, 41%), respectively. The MHPG-SO_4 level in the cerebral cortex was elevated significantly at 60 min (22%) but at no other time. In the pons+med.obl., significant elevation of MHPG-SO_4 contents occurred at 60 and 120 min (14% and 31%, respectively), with the peak at 120 min. No significant alterations in MHPG-SO_4 levels were observed in the midbrain. By contrast, the basal ganglia showed a significant decrease in MHPG-SO_4 levels at 15 and 30 min (20%) as compared with controls, and although metabolite levels tended to increase at 120 min, no significant changes were observed either at 120 or 180 min.

The results of the second study are indicated in Table 1. In all the brain regions examined, statistically significant increases in MHPG-SO_4 contents occurred 45 min after probenecid injection. MHPG-SO_4 contents accumulated by probenecid treatment alone (Table 1, B-A) were the highest in the hypothalamus and relatively lower in the hippocampus, cerebral cortex and basal ganglia. Accumulated levels of MHPG-SO_4 in rats injected with probenecid and subsequently stressed for 45 min (early phase, Table 1, C-A) were compared to those in rats immobilized for 90 min, then injected with probenecid and subsequently stressed for 45 min more (late phase, Table 1, E-D). The brain areas where accumulations of MHPG-SO_4 were greater during the early phase were the hypothalamus and amygdala. The pons+med.obl. and basal ganglia showed opposite results, with accumulations of MHPG-SO_4 greater during the late phase. Virtually the same degree of accumulation of MHPG-SO_4 for both stress periods was observed in the hippocampus, cerebral cortex and thalamus.

DISCUSSION

The present study revealed that immobilization stress produced reductions of NA contents in the hypothalamus,

TABLE 1

ACCUMULATED VALUES OF MHPG-SO_4 CONTENTS (ng/g) IN BRAIN REGIONS OF THE RAT INDUCED BY PROBENECID WITH OR WITHOUT IMMOBILIZATION STRESS

Treatment	Control	Probenecid 45 min	Probenecid + Stress 45 min	Stress 90 min	Stress 90 min + Probenecid + Stress 45 min	ΔMHPG-SO_4 B-A[a]	ΔMHPG-SO_4 C-A[a]	ΔMHPG-SO_4 E-D[a]
Hypothalamus	236 ± 15	462 ± 52[b]‡	587 ± 22[c]*	458 ± 19[b]‡	655 ± 42[d]‡	226	351	197
Amygdala	143 ± 6	215 ± 11[b]‡	248 ± 9[c]*	197 ± 8[b]‡	254 ± 13[d]†	72	105	57
Pons+med.obl.	159 ± 10	229 ± 11[b]‡	253 ± 9	212 ± 13[b]†	329 ± 20[d]‡	70	94	117
Hippocampus	108 ± 6	141 ± 9[b]‡	178 ± 7[c]†	134 ± 9[b]*	207 ± 12[d]‡	33	70	73
Thalamus	292 ± 13	446 ± 51[b]†	454 ± 24	387 ± 17[b]‡	548 ± 33[d]†	154	162	161
Cerebral cortex	128 ± 6	175 ± 7[b]‡	185 ± 5	150 ± 8[b]*	220 ± 12[d]‡	47	57	70
Basal ganglia	134 ± 13	179 ± 17[b]*	183 ± 17	162 ± 14	254 ± 15[d]‡	45	49	92

A: control, B: probenecid 45 min, C: probenecid + stress 45 min, D: stress 90 min, E: stress 90 min + probenecid + stress 45 min.

Each value represents the mean ± S.E.M. of 7–8 rats. Ninety min of stress (D group) caused significant elevations of MHPG-SO_4 levels in all regions except the basal ganglia, as comapred with controls (A group).

[a]The values obtained by subtracting the levels of the latter from the former indicate accumulations of MHPG-SO_4 by probenecid alone (B-A), by initial 45 min immobilization with probenecid (C-A, "early phase" of stress) and by later 45 min immobilization with probenecid after prior immobilization for 90 min (E-D, "late phase" of stress).

[b]Significantly different from controls (A). [c]Significantly different from group B. [d]Significantly different from group D.

Significance levels are; *$p<0.05$, †$p<0.01$, ‡$p<0.001$.

amygdala, thalamus, pons+med.obl., hippocampus and cerebral cortex. These results are consistent with the findings of several authors who have reported decreases in regional NA contents in the rat brain [2, 7, 12, 14] and in some brain nuclei [10,13] after a variety of stressful stimuli.

Furthermore, levels of MHPG-SO_4, the major metabolite of NA in rat brain [15,16] which has been considered to reflect NA released in the brain by neuronal activity [19] were elevated significantly in those brain areas where NA contents were significantly reduced after immobilization stress. These results are partly consistent with previous observations of levels in the hypothalamus and brain stem using ^{3}H-NA or $Na_2{}^{35}SO_4$ [17], of endogenous MHPG-SO_4 levels in the medulla-pons elevated by swimming stress [1], and of elevated levels in many brain regions induced by intense stress as reported by us [12].

The findings that immobilization stress caused not only a decrease in NA levels but also an increase in MHPG-SO_4 levels in six out of eight regions studied strongly suggest that utilization of NA in these areas is enhanced by stress. Increased release of NA exceeding synthesis seems to result in reduction of NA contents [2,12].

However, in these brain areas there were time-related differences in the responsiveness of NA neurons to stress. The most rapid and marked response of NA neurons was found in the hypothalamus, as shown by the fact that NA levels decreased and MHPG-SO_4 increased within 30 min of immobilization and that the peaked values of NA reduction and of MHPG-SO_4 elevation were the highest among the regions examined. In contrast to changes within the first 60 min, no further changes occurred either in NA or in MHPG-SO_4 levels at 120 and 180 min. This indicates that NA release in the hypothalamus occurs mainly within the first 60 min of immobilization. This is supported by the finding in the second study that probenecid-induced accumulation of MHPG-SO_4 during the "early" 45 min stress was almost twice that of the "late" 45 min stress following previous 90 min stress.

Similar time-related changes in NA neurons seen in the hypothalamus were found in the amygdala, i.e., NA contents appeared to decrease within 30 min, and probenecid-induced accumulation of MHPG-SO_4 during the early phase of stress was almost twice that of the late phase.

When compared with the hypothalamus and amygdala, the response of NA neurons in the other four regions was delayed. In the hippocampus, NA levels declined at 120 min, and MHPG-SO_4 levels still remained elevated at 180 min. The probenecid study showed almost the same accumulation of the metabolite in both early and late phases of stress in this region. Similar responses of noradrenergic neurons were found in the thalamus. Similarly, the NA reduction in the cerebral cortex was delayed; however, a significant elevation of MHPG-SO_4 level was seen only at 120 min. The pons+med.obl. also exhibited slightly delayed enhancement of NA release as indicated by the finding that MHPG-SO_4 accumulation induced by probenecid was greater during the late phase of stress than during the early phase.

These results indicate that in these brain areas there exist time-related differences in enhancement of NA turnover induced by stress. These regional differences are unlikely to be due to regionally different densities of noradrenergic nerve terminals, since the amygdala and hippocampus contain virtually the same contents of NA and MHPG-SO_4 and are innervated by dorsal noradrenergic bundles from the locus coeruleus [20], although the present results suggest that noradrenergic terminals in the former respond to stress more rapidly than do those in the latter. The regionally different enhancement of NA turnover, which are related to time-course of stress, might be involved in the regionally different function mediated by noradrenergic system in these areas during the course of immobilization stress.

In contrast to these six regions, MHPG-SO_4 levels in the

basal ganglia decreased transiently, while NA levels increased. Changes in MHPG-SO_4 levels were observed only at 15 and 30 min of immobilization, furthermore, probenecid-induced accumulation of MHPG-SO_4 during the late phase of immobilization was twice that of the early phase, which was virtually the same as that accumulated by probenecid alone. These findings indicate that release of NA in the basal ganglia is reduced in the early phase of immobilization. The significance of transient inhibition of NA release in this region remains uncertain; however, it is noteworthy that immobilization caused reduction in NA release in the basal ganglia.

The changes in both plasma corticosterone and hypothalamic MHPG-SO_4 levels exhibited a striking resemblance in their time course. An inhibitory role of the central noradrenergic system on secretion of CRF or ACTH [3,6] and an increase in NA turnover in the hypothalamus induced by ACTH [5] have been described. If these reports are taken into consideration, the increased NA release in the hypothalamus in the present study might be involved in the regulation mechanism of ACTH or CRF secretion.

The present study has clarified that NA release is enhanced by acute immobilization-stress in the hypothalamus, amygdala, cerebral cortex, pons+med.obl., thalamus and hippocampus, but is decreased transiently in the basal ganglia during the early phase of stress, and that there exist some regional differences in time-related responses of noradrenergic neurons to the continuous stress, i.e., NA neurons in the hypothalamus and amygdala respond relatively rapidly, while response in the other regions is delayed slightly. This was confirmed by the second probenecid-study. These regional differences might be related to serial, physiological, regionally different functions involved in stress-induced emergency responses such as emotion, arousal level, and endocrine and autonomic nervous system responses.

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