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Regional Characteristics of Stress-Induced Increases in Brain Noradrenaline Release in Rats

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TANAKA, M., Y. KOHNO, R. NAKAGAWA, Y. IDA, S. TAKEDA, N. NAGASAKI AND Y. NODA. *Regional characteristics of stress-induced increases in brain noradrenaline release in rats.* PHARMACOL. BIOCHEM BEHAV 19(3) 543–547, 1983.—Male Wistar rats were exposed to immobilization stress for various periods (1 to 5 hr) with or without an IP injection of probenecid at 400 mg/kg. The regional characteristics of stress-induced increases in noradrenaline (NA) release in the rat brain related to the time-course of stress were demonstrated by measuring levels of the major metabolite of NA, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4). Increases in MHPG-SO_4 levels occurred mainly within the first hr of stress in the hypothalamus, amygdala and thalamus, while the peak elevations of the metabolite levels were delayed in the hippocampus, cerebral cortex, pons+medulla oblongata and basal ganglia. According to the accumulation of MHPG-SO_4 during each 1-hr period of stress, regional characteristics of NA release were classified into the following four types based upon regions where the most marked increase in MHPG-SO_4 levels occurs mainly: (1) within the first hr of stress (the hypothalamus, amygdala and thalamus), (2) during the first and second hr (the hippocampus and cerebral cortex), (3) during the third hr (the basal ganglia) and (4) to the same extent from the first to the fourth hr of stress (the pons+medulla oblongata). These results suggest that noradrenergic neurons in different brain regions respond differentially to stress and reflect their own characteristic patterns depending upon nature and time-course of the stressor.

Immobilization-stress Noradrenaline release MHPG-SO_4 Rat brain Regional characteristics Probenecid

IT IS well known that various stressful stimuli activate the noradrenergic system in many brain regions in the rat [11]. By measuring levels of noradrenaline (NA) and its major metabolite in the rat brain [10], 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in various brain regions, we have demonstrated that an increase in NA turnover is produced in extended brain regions by a variety of stressors [4, 9, 13, 14, 16]. In general, there appear to be regional characteristics of increases in NA turnover induced by stress in extended brain regions. Psychological stress increases NA turnover preferentially in the hypothalamus and amygdala [4], and a potent blocker of opioids, naloxone, enhances increases in NA turnover induced by immobilization-stress selectively in the hypothalamus, amygdala and thalamus [14]. From these results, we have suggested that increases in NA turnover in these specific areas are involved in the "emotional" responses of rats exposed to these stresses [12, 14, 15]. Moreover, we have suggested that there exist regional characteristics of stress-induced increases in NA turnover related to the time-course of stress when rats are continuously exposed to the same stressor. NA turnover in the hypothalamus, amygdala and thalamus increases in the early phase of stress. In contrast, such a response occurs later in the pons plus medulla oblongata (pons+med.obl.) [13].

In the previous study [13], however, a comparison of NA turnover in brain regions was made only between early and late phases of immobilization-stress. The present study was undertaken to confirm the previous findings and to demonstrate a more detailed picture of regional brain characteristics of increases in NA turnover during 5-hr periods of immobilization-stress. We measured the accumulation of MHPG-SO_4 in 7 brain regions during each 1-hr of stress in rats treated with probenecid, which blocks active transport of MHPG-SO_4 from the brain [8].

METHOD

Male Wistar rats, weighing 180–220 g, receiving a standard diet with water freely available, were housed 4 to each cage (265×425×150 mm standard plastic cage containing wood shavings) in a 12 hr light/dark cycled room (light on at 0700 and off at 1900 hr) at constant temperature (24±1°C) and humidity (50±10%).

Immobilization-stress was produced by enclosing the rats

in a flexible wire mesh (3×3 mm) as described previously [13,14].

Probenecid (a gift from Nippon Merck Banyu, K.K.) was dissolved in 0.1 N NaOH and the pH was adjusted to 8.0, and the drug at 400 mg/kg was injected intraperitoneally.

By balancing the body weight, the rats were divided into eleven groups of 8 rats ranging from A to K as shown in Fig. 1: untreated controls (A); an IP injection of probenecid alone (B); exposure to stress for various durations ranging from 1 to 4 hr (D, F, H and J); stress-exposure for various durations (0 to 4 hr) followed by probenecid injection and a subsequent 1-hr period of immobilization-stress (C, E, G, I and K). Immediately after these treatments, the rats were sacrificed by decapitation and the brains were removed. The brains were dissected into seven discrete regions according to the method of Gispen *et al.* [3] and frozen on solid CO_2. The brain regions dissected were: the hypothalamus, amygdala, thalamus, hippocampus, cerebral cortex, pons+med.obl. and basal ganglia which included the caudate nucleus, putamen and globus pallidus. Blood from the cervical wound was collected into heparinized tubes. The brain tissues and the separated plasma were stored at −45°C until assayed. MHPG-SO_4 levels in the brain regions were assayed by our fluorometric method [5] and plasma corticosterone levels were fluorometrically determined by the method of van der Vies [18].

Time-course changes (1 to 4 hr) in MHPG-SO_4 levels in the various brain regions were compared using changes in the metabolite levels of the rats in groups A, D, F, H and J. For a statistical analysis, the two-tailed Student's *t*-test was employed. The values of MHPG-SO_4 accumulated during 0–1 hr, 1–2 hr, 2–3 hr, 3–4 hr and 4–5 hr of immobilization-stress were obtained by subtractions of the values of rats in groups A, D, F, H and J from those in groups C, E, G, I and K, respectively (See the legend for Fig. 3).

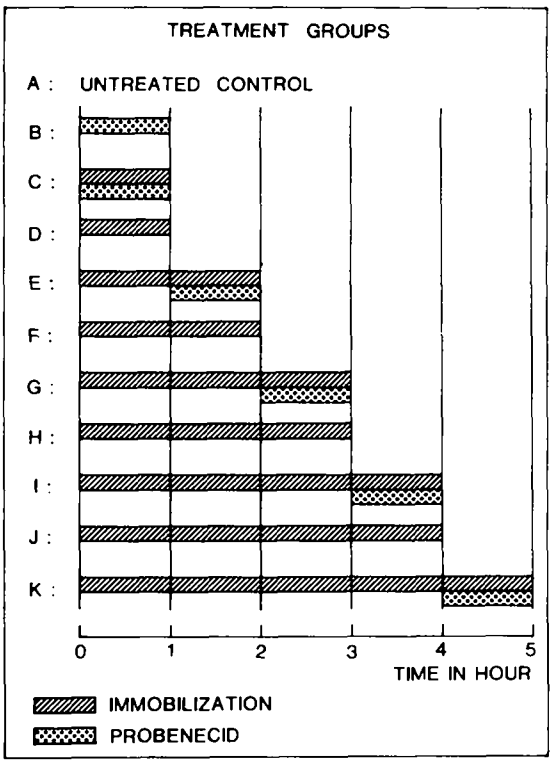

FIG. 1. Treatment groups. Treatment groups consist of eleven groups ranging from A to K. The rats in A group are untreated controls and those in D, F, H, and J groups were only immobilized for 1, 2, 3 and 4 hr, respectively. Probenecid at 400 mg/kg was injected to the animals in B and C groups 1 hr before sacrifice but the rats in C groups were followed by exposure to 1-hr immobilization-stress immediately after the drug injection. The animals in E, G, I and K groups were immobilized for 1, 2, 3 and 4 hr, respectively, and injected with probenecid and continued to be further exposed to the same stress for 1 hr.

RESULTS

MHPG-SO_4 levels were significantly increased in all brain regions examined after exposure to immobilization-stress as compared to untreated controls (Fig. 2). However, the increase in the metabolite levels related to the time-course of stress was different among the brain regions examined. In the hypothalamus, amygdala and thalamus, MHPG-SO_4 levels were markedly elevated within 1 hr of stress, but thereafter, no further increase in the metabolite level was observed, despite continuous exposure to immobilization-stress for a further 3 hr period. In contrast to these three regions, MHPG-SO_4 levels in the hippocampus, cerebral cortex and pons+med.obl. were significantly increased within 1 hr of stress and then progressively continued to increase up to 3 hr of stress. The basal ganglia failed to show significant increase in MHPG-SO_4 levels within 1 hr of stress, however, the metabolite levels were significantly elevated at 2 hr of stress and remained at the same levels up to 4 hr.

The hypothalamus, amygdala and thalamus exhibited very similar time-course changes in accumulation of MHPG-SO_4 (Fig. 3). In these regions, the most marked accumulation of MHPG-SO_4 was produced during the first 1-hr of stress, and the accumulation was markedly higher than that observed during later periods of stress. After the first hr of stress, the accumulation of MHPG-SO_4 was decreased. In the hippocampus, the accumulation was increased from the first 1-hr period to the second 1-hr period of stress and peaked at the second 1-hr period and then gradually decreased to the fifth hr. Similar change was observed in the cerebral cortex; the accumulation of MHPG-SO_4 was increased within the first 1-hr period, remained at the same levels during the second 1-hr period and gradually decreased to the fifth 1-hr period of stress. The pons+med.obl. showed a different pattern of change from the five regions discussed above. In this region, the accumulation peaked during the second 1-hr period of stress, but thereafter remained at the same level even during the fourth 1-hr period and decreased during the fifth hr of stress. In the basal ganglia, the accumulation of MHPG-SO_4 gradually increased, reached a peak during the third 1-hr period, and then gradually decreased to the fifth hr of stress.

Plasma corticosterone levels were significantly elevated within the first 1-hr (31.5±0.76 μg/dl; mean±S.E.M. of 8 rats) as compared to those in untreated controls (10.1±2.92 μg/dl), and remained at slightly decreased levels at 2 hr (25.8±1.76 μg/dl), 3 hr (30.6±1.69 μg/dl) and 4 hr (25.8±1.62 μg/dl). Probenecid injection alone elevated plasma corticosterone levels (29.9±1.81 μg/dl), however, no further increase in the levels was obtained when the drug was injected into the immobilized animals.

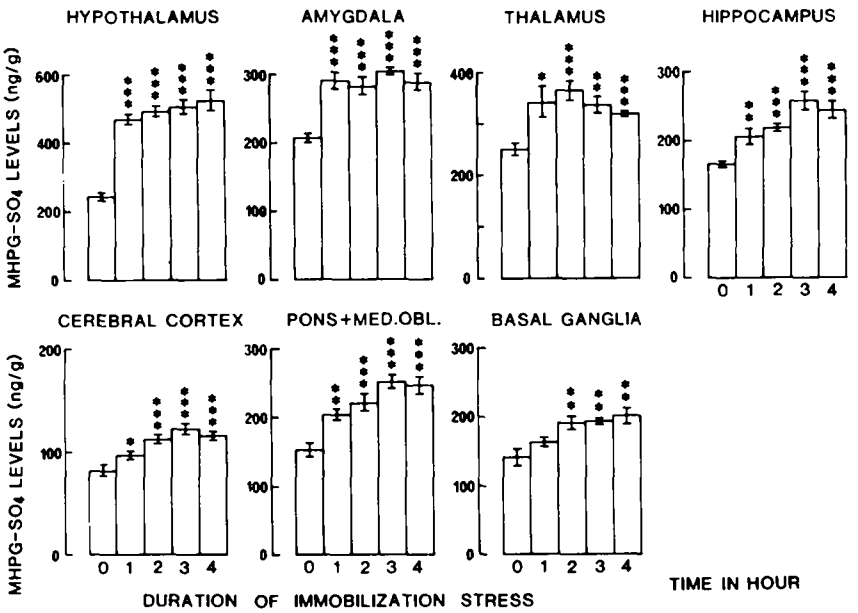

FIG. 2. Effects of immobilization-stress for various durations ranging from 1 hr to 4 hr on 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) levels (ng/g) in the rat brain regions. The value indicates the mean±S.E.M. of 7–8 rats. Statistically significant as compared to those in untreated controls (0 hr): $*p<0.05$, $**<0.01$, $***p<0.001$.

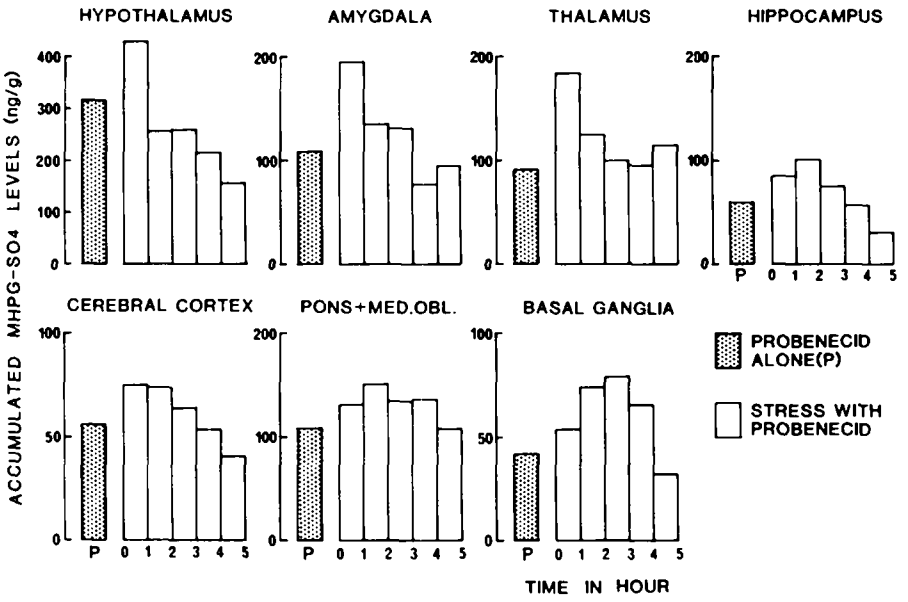

FIG. 3. The accumulated MHPG-SO_4 (ng/g) in the brain regions during each 1-hr period of immobilization-stress ranging from the first hr (0–1 hr) to the fifth hr (4–5 hr). The stippled columns indicate the accumulated MHPG-SO_4 by probenecid alone (1 hr after injection) and the open columns those during each 1-hr period of immobilization-stress. When the mean value of MHPG-SO_4 levels in A–K groups was A–K, respectively, the accumulated value was calculated according to the following formula: ΔMHPG-SO_4(probenecid)=B–A; ΔMHPG-SO_4 (0–1 hr)=C–A; ΔMHPG-SO_4 (1–2 hr)=E–D; ΔMHPG-SO_4 (2–3 hr=G–F; ΔMHPG-SO_4 (3–4 hr)=I–H; ΔMHPG-SO_4 (4–5 hr)= K–J.

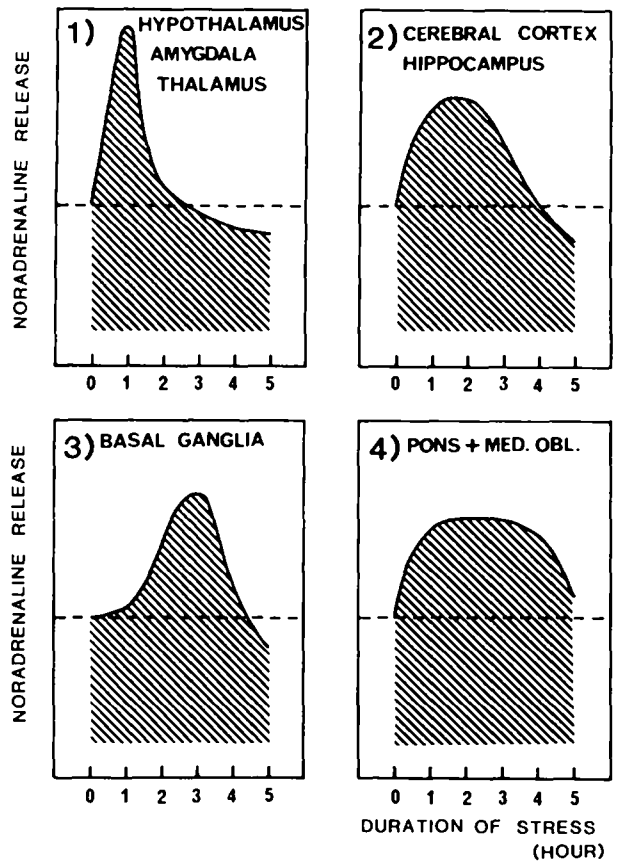

FIG. 4. A schematic presentation of regional characteristics of noradrenaline (NA) release during 5 hr of immobilization-stress. The ordinate indicates NA release and the abscissa the time-course of 5 hr of stress. The horizontal, broken line shows the basal level of NA release, i.e., the natural release of NA at the non-stressed state. The characteristics are divided into the following four types; NA release is enhanced markedly within the first hr of stress (1), during the first and second hr (2), during the third hr (3), and the NA release is continuously enhanced from the first to the fourth hr of stress (4).

DISCUSSION

The present study was consistent with our previous findings [13,14], and showed that immobilization-stress caused increases in MHPG-SO_4 levels in all brain regions examined. As shown in the previous reports [13,14] and a review [11], this result indicates that NA release is enhanced by immobilization-stress in extended brain regions of rats.

However, the present study revealed that the changes in MHPG-SO_4 levels related to the time-course of immobilization-stress were different among the brain regions examined. In the hypothalamus, amygdala and thalamus, the most marked increases in MHPG-SO_4 levels occurred during the first hr but thereafter no definite increases in the metabolite levels were produced even though the stressor was continuously applied (Fig. 2). In contrast, increases in MHPG-SO_4 levels were delayed in four other brain regions.

If, however, immobilization-stress affects the elimination process of MHPG-SO_4 from the brain, these time-related regional neurochemical changes might be unreliable. Probenecid was employed in order to exclude this possibility and to draw a more detailed profile of regional characteristics of NA release during stress. The probenecid study not only supports the view mentioned above but also permits to classify the regional brain characteristics into the following four types: (1) regions where the most marked increase in MHPG-SO_4 levels occurs within the first hr of stress but thereafter either no increase or only slight increase in the metabolite levels is produced (the hypothalamus, amygdala and thalamus), (2) regions where the most marked increase occurs during the first and second hr of stress (the hippocampus and cerebral cortex), (3) regions where the highest increase occurs during the second or third hr of stress (the basal ganglia), and (4) regions where the increase in MHPG-SO_4 levels observed during each 1-hr period is almost the same from the first to the fourth hr of stress (pons+med.obl.).

There might, however, be some problems concerned with the use of probenecid. It has been reported that probenecid at 400 mg/kg dose not completely block the active transport of MHPG-SO_4 [8] and it is unclear whether the drug is uniformly distributed into all brain regions or whether the drug acts equally in all brain regions. This is unlikely to be a serious problem, however, since probenecid is considered to act in the various brain regions in a non-specific manner and our previous study [6] indicates that regionally different NA turnover evaluated by the probenecid method is in good agreement with that estimated by other methods where α-methyl-p-tyrosine or the compounds labelled with radioisotopes are utilized.

It has also been reported that probenecid inhibits the activity of tyrosine hydroxylase, a synthesizing enzyme of catecholamines, in the mouse brain [2] and that a higher dose of probenecid injection by itself is stressful to rats [8] as indicated in the fact that plasma corticosterone levels were elevated by probenecid alone in the present study. The involvement of compounds accumulated non-specifically by the drug in the brain and peripheral tissues might not be excluded as influencing the results. Although these problems should be taken into consideration in the probenecid study, these are unlikely to be serious, since probenecid-injection was made to the rats in each group under the same conditions, and a comparison related to the time-course of stress was made within the same brain regions.

The possibility is also unlikely that the regional characteristics of stress-induced increase in NA release might be merely a reflection of regional differences in NA turnover or in densities of NA varicosities, since both the hypothalamus and amygdala exhibit the same characteristic in spite of the fact that the former has a much higher NA turnover rate than does the latter [1], and that the thalamus and pons+med.obl. show different characteristics in spite of having virtually the same NA concentrations [6]. Thus, the regional characteristics of NA release during stress, observed in this study, are considered to be mainly related to the stress process. Based upon these results, we propose a schema for regional characteristics of NA release in the brain of rats exposed to 5-hr of immobilization-stress as shown in Fig. 4. Early, medium, late and "steady" responding brain regions, based upon the present data, are shown.

We have already suggested that the increased NA release by stress in the hypothalamus, amygdala and thalamus might be involved in the distress-evoked hyperemotional response in rats [15] or in the emergent response including changes in autonomic and endocrine systems [13]. The delayed enhancement of NA release in the cerebral cortex and hip-

pocampus might be involved in coping against aversive stimuli and in memory processes [7], respectively. The continuous enhancement of NA release in the pons+med.obl. might be related to the histological characteristic of this region; that is, this region includes the locus coeruleus which is the cell body site of noradrenergic bundles innervating the various brain regions [17].

In conclusion, the present study demonstrated regional brain characteristics of NA release related to the time-course of immobilization-stress. Little is known, however, about the functional significance of these changes and this is the focus of further investigations.

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