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The influence of cold stress and corticosterone pretreatment on norepinephrine and catechol-O-methyltransferase activity in hypothalamus of rat*

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In this study we explored the influence of exposure to cold stress (4 °C) on the norepinephrine (NE) content and the activity of catechol-O-methyltransferase (COMT) in the rat hypothalmus, and the effect of pretreatment with corticosterone (CS, 5 mg/kg, i. p.) on the changes in the NE content and COMT activity induced by stress. A marked decrease of NE content in the hypothalamus was observed during the first 15min of exposure to 4 °C, with a subsequent recovery. The decrease in NE content by stress was blocked by the pretreatment with CS. COMT activity at 15 min of cold exposure decreased by about 15% as compared with the normal level and it remained at the decreased level during the ensuing period up to 90min. CS pretreatment did not affect the decrease in COMT activity induced by cold stress. The decrease of NE content in the hypothalamus by stress may by caused by the increase of turnover rate and its recovery may by due to the elevation of synthesis through the high level of plasma CS in consequence of the increased release. COMT activity is not directly related to NE decrease induced by stress and the plasma CS concentration.

INTRODUCTION

It is generally recognized that the secretion of corticotropin (ACTH) and corticosteroids increases in animals placed in stressful condition, and there is a regulatory mechanism of corticotropin releasing hormone (CRH) and ACTH secretion in the hypothalamo-pituitary-adrenal axis via feedback of plasma corticosteroids concentration. Reacently, brain monoamines, especially norepinephrine (NE) in the hypothalamus, are thought to have a relation in this feedback system. Various forms of stress, such as exposure to cold, formalin injection, forced immobilization or foot electric shock, have been reported to produce a decrease in NE content[1-4] and an increase in metabolic turnover or NE[5-9] in the hypothalamus. The elevation of tyrosine hydroxylase activity has been observed in rats after exposure to cold, forced immobilization or formalin injection[4, 8, 10]. In addition, little or no change in momoamine oxidase activity is reported after the swimming stress[11]. Howevere, reports on changes is changes is catechol-O-methyltransferase (COMT) activity under stress are as yet few.

* 一部は第54回日本薬理学会総会（1981年3月）で発表

The present study was conducted to examine the NE content and COMT activity in the hypothalamus of rats exposed to cold stress (4 °C) and the influence of corticosterone (CS) on the changes of NE and COMT induced by stress.

MATERIALS AND METHODS

Male Sprague-Dawley rats were caged individually in an air-conditioned room (temp. 23±1 °C, relative humidity 55±10%) on the lighting schedule of 6 : 00 ~ 18 : 00 (o' clock) and freely given rat chow (CE-2, Nippon Clea) and water. They were handled enough for more than 2 weeks and used at 9~10 weeks of age for the experiments.

Three groups of rats were prepared. Rats in the first group were exposed to 4 °C for 0, 5, 10, 15, 30, 60 and 90 min. Rats in the second group were pretreated with CS (5 mg/kg, in physiological saline containing 2% ethanol, i. p.) 20 min before

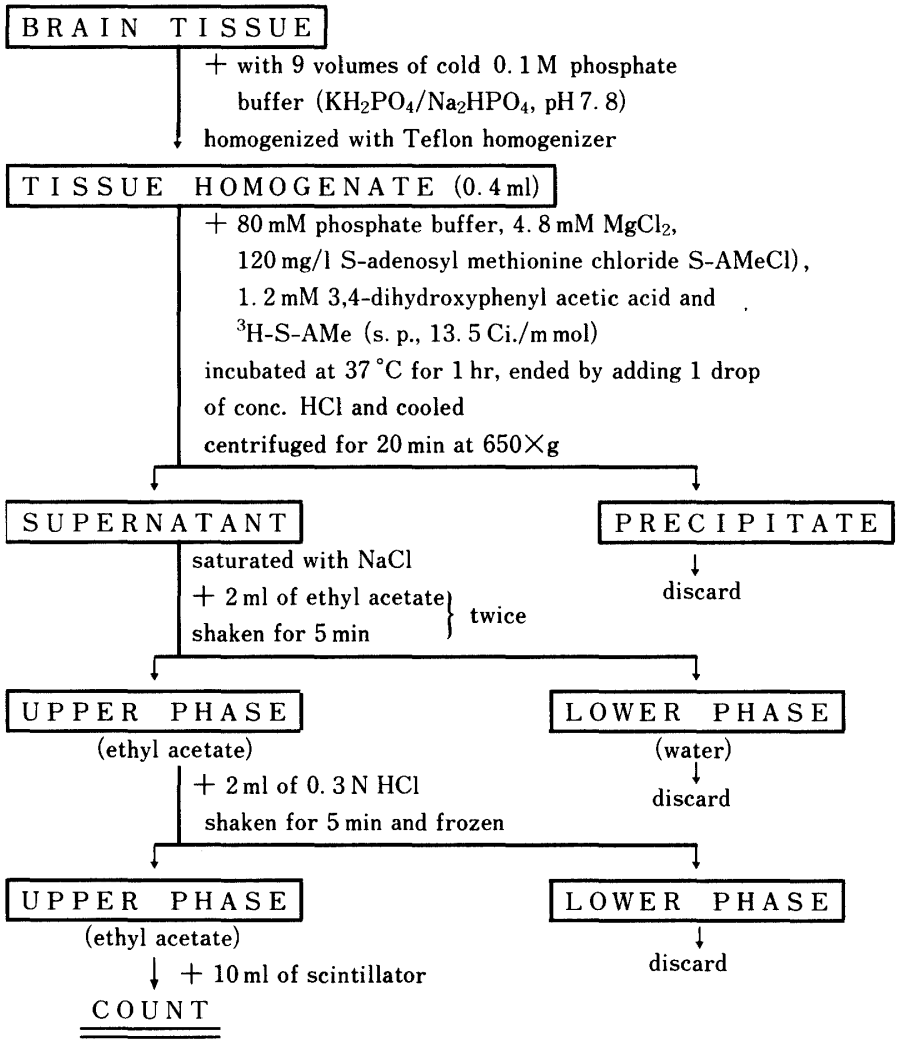

Fig 1 Scheme of the procedure for the determination of COMT activity in the rat brain.

cold exposure. In the third group, rats were administrated with CS without stress.

Rats were decapitated at the same time (10 : 00～11 : 00) in order to avoid variability due to circadian fluctuations, and the hypothalamus was immediately dessected out on the ice-cold glass plate. The tissue was homogenized in 0.4 N $HClO_4$ for the assay of NE, and 0.1 M phosphate buffer (KH_2PO_4/Na_2HPO_4, pH 7.8) for the assay of COMT activity using Teflon homogenizer.

In order to determine NE, the homogenate was centrifuged at 10,000×g for 20 min at 0 °C. The pH of the supernatant was adjusted to 8.25～8.30 with 1 M Tris solution and passed through the glass column packed with 150mg of activated alumina (Merk). NE adsorbed to the alumina was eluted with 2.5 ml of 0.2 N acetic acid at the rate of 0.5 ml/min. The eluate was dried up under the vaccum and dissolved in 0.2ml of 0.004 N Na_2HPO_4.

NE was determined by using fluorodetector connected with high performance liquid chromatography and reaction system (trihydoxyindole method) contrived in our laboratory.[12)]

COMT activity was measured by radiometry based on the procedure described by Broch et al.[13)] (Fig. 1).

NE content was expressed as μg per g wet tissue. COMT activity was expressed as μ moles of the product (^{3}H-methylhydoxyphenyl acetic acid) per g wet tissue per hour.

The statistical differences were defined by the Student "t" test.

RESULTS

1) NE content

The NE content in the hypothalamus in rats exposed to cold stress fell rapidly and reached the lowest level at 15 min of exposure. Thereafter, it tended to return gradually to normal (0 time) level by 90 min. Significant decrease was observed during 5 to 60 min of exposure ($p < 0.05$ or 0.01). The hypothalamic NE content was not affected by administration of CS (5 mg/kg, i. p.). Pretreatment with CS prevented the decrease of NE content by stress and significant difference in NE content was observed at 5, 15, 30 and 60 min of exposure when compared to the group exposed to 4°C without CS pretreatment (Fig 2).

2) COMT activity

The hypothalamic COMT activity in rats placed under a cold stressful condition, declined progressively with time and reached the minimum level (85% of the initial, normal level) at 15min of stress, and it remained at this lowered level during 90min of stress. Although significant decrease in COMT activity was observed at 5 and 10min after CS administration ($p < 0.05$), the same degree of the decrease was observed after vechicle injection (data are not shown).

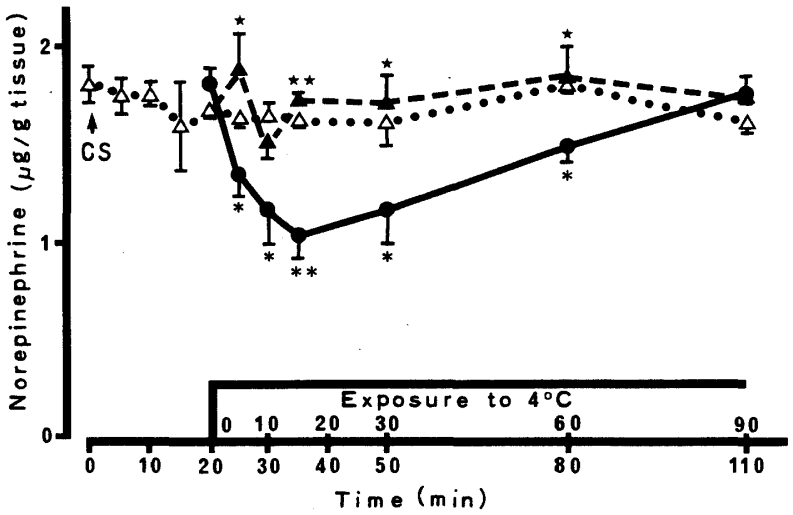

Fig. 2 Changes of norepinephrine (NE) content (μg/g tissue) at various time buring cold stress (4 °C) and effect of corticosterone (CS) on the decreased NE content by stress in the hypothalamus of rats.
Each point and bar represent the mean±SE from 5~6 rats.
*, ** : significant differences from normal (0 time) level ($p < 0.05$, $p < 0.01$), ★, ★★ : significant differences from the group given only cold sress ($p < 0.05$, $p < 0.01$).
●—● : the group of rats exposed to 4°C without CS pretreatment, ▲···▲ : the group of rats pretreated with CS (5 mg/kg, i. p.) 20 min before cold stress, △---△ : the group of rats administrated with CS without stress.

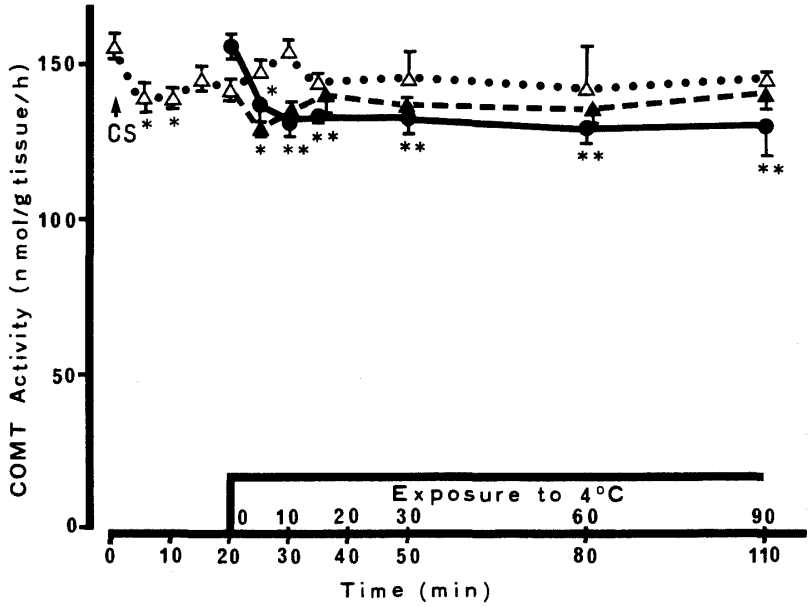

Fig. 3 Catechol-O-methyltransferase (COMT) activity (n mol/h/g tissue) at various time during cold stress (4 °C) and effect of corticosterone (CS) on the decreased COMT activity by stress in the hypothalamus of rats.
Each point and bar represent the mean±SE from 5~6 rats. *, ** : significant differences from normal (0 time) level ($p < 0.05$, $p < 0.01$).
●—● : the group of rats exposed to 4 °C without CS pretreatment, ▲···▲ : the group of rats pretreated with CS (5mg/kg, i. p.) 20min before cold stress, △---△ : the group of rats administrsted with CS without stress.

Thus, the decrease of COMT activity after CS treatment alone seems to be due to injection procedure. The COMT activity in rats exposed to cold stress after pretreatment with CS decreased further at 5 and 10 min of stress, and maintained low level for 30 and 60 min of stress (Fig. 3).

DISCUSSION

In the present study, we observed that the hypothalamic NE content was decreased during the exposure to 4°C and this decrease was blocked by CS (5 mg/kg, i.p.) given 20 min prior to exposure to cold. On the other hand, CS alone decreased COMT activity in the same tissue and did not attenuate the decrease in COMT activity due to cold stress. The NE content during stress showed a tendency to recover after 60 min, whereas the COMT activity remained lowered. From these results and the data in the literatures, we consider that the decrease in hypothalamic NE content by cold stress during the first 5～15 min may be due to the increased secretion from tissue and its recovery in the hypothalamus of rat exposed to cold for more than 60 min may be due to the elevation of NE synthesis through the adaptation to stressful stimuli by increasing release of corticosteroids. Since it is reported that the final metabolite of NE, 3-methoxy-4-hydroxyphenylethylenglycol sulfate, was increased by stress of forced running[3], the decrease of COMT activity found in the present study might be explained as the result of that the final metabolite increased by stress lays traps for the COMT activity participating in the initial stage of NE metabolism. An alternative explanation is that the decrease of COMT activity might by due to secondary affect to the decreased NE content through the homeostatic recovery mechanism.

Ito et al.[14] reported that the secretion of not only ACTH but other hormones such as TSH and prolactin, was increased in the animals placed under stressful conditions. Previous to this phenomenon, it is considered that the secretion of CRH, thyrotropin releasing hormone and prolactin releasing hormone from the hypothalamus is induced. Although data are not shown, we observed that a tendency to increase in protein content in the hypothalamus with time of exposure to cold stress. It suggests a possidility that biochemical changes occurring at the very early stage of stress may stimulate the synthesis of a particular RNA and the subsequent protein synthesis.

It seems to conclude that the decrease in NE content in the hypothalamus of rat by cold stress was blocked by pretreatment with CS, while the hypothalamic COMT activity appears to be not directly related to NE decrease induced by stress and the plasma CS concentration.

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