Pharmacology Biochemistry & Behavior, Vol. 30, pp. 769–773. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Effect of Acute Ethanol Administration on Noradrenaline Metabolism in Brain Regions of Stressed and Nonstressed Rats

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Received 26 November 1987

SHIRAO, I., A. TSUDA, Y. IDA, S. TSUJIMARU, H. SATOH, M. OGUCHI, M. TANAKA AND K. INANAGA. *Effect of acute ethanol administration on noradrenaline metabolism in brain regions of stressed and nonstressed rats.* PHARMACOL BIOCHEM BEHAV 30(3) 769–773, 1988.—The effects of ethanol on noradrenaline (NA) metabolism of brain regions in stressed and nonstressed rats were investigated. Male Wistar rats were injected IP with either saline, or ethanol at 0.5 g/kg or 2 g/kg, 5 min before exposure to 1-hr immobilization stress. Levels of NA and its major metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) in various brain regions and plasma corticosterone levels were fluorometrically determined. Immobilization stress caused significant increases in MHPG-SO_4 levels in all brain regions examined, i.e., the hypothalamus, amygdala, hippocampus, cerebral cortex and locus coeruleus (LC) region. In nonstressed rats, ethanol significantly increased MHPG-SO_4 levels in the hypothalamus, hippocampus and cerebral cortex, but not in the amygdala or in the LC region. In stressed rats, ethanol attenuated stress-induced increases in MHPG-SO_4 levels preferentially in the amygdala and LC region, but not in the remaining three regions. Although ethanol per se dose-dependently elevated plasma corticosterone levels in nonstressed rats, ethanol at 2 g/kg attenuated the stress-induced elevation of corticosterone. These results suggest that the attenuating effect of ethanol on stress-induced increases in NA turnover in the amygdala and LC region might be related to the stress-relieving properties of this drug.

Ethanol Stress Brain noradrenaline metabolism Amygdala MHPG-SO_4 Rats

IT has been well established that immobilization stress increases noradrenaline (NA) release in extended brain regions and elevates plasma corticosterone levels in rats [19–21]. These increases in NA release caused by stress are attenuated by morphine in the hypothalamus, thalamus, amygdala, hippocampus and midbrain [22], and by diazepam, a benzodiazepine anxiolytic, in the hypothalamus, amygdala, hippocampus, locus coeruleus (LC) region and cerebral cortex [8]. From these results, we have suggested that stress-induced increases in NA release in the hypothalamus and amygdala are closely related to the provocation of fear and/or anxiety, and that their attenuation by diazepam or morphine results in the alleviation of these emotions [6, 20, 22, 23].

Together with the fact that ethanol has anxiety-reducing properties [1,2], the possibility rises that ethanol could attenuate stress-induced increases in NA release in some brain regions. This hypothesis is partly supported by the reports that ethanol not only prevents decreases in NA contents caused by stress in the hypothalamus, thalamus-midbrain and hippocampus [4,12], but also attenuates stress-induced elevations of plasma corticosterone levels in the rat [14].

However, there are few reports wherein the effects of ethanol on stress-induced increases in NA release were studied by measuring the metabolite levels of NA in the rat brain. Furthermore, in these reports, the number of brain regions was limited and the amygdala, considered to be one of the most important regions for regulating emotion, was not studied. Accordingly, the present study investigated the effects of ethanol on NA metabolism in extended brain regions including the amygdala, in both stressed and nonstressed rats by simultaneously measuring levels of NA and its major metabolite in the rat brain, 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4).

METHOD

Subjects

Forty-eight male Wistar rats, weighing 180–200 g, were used as subjects. Rats received standard lab food and water

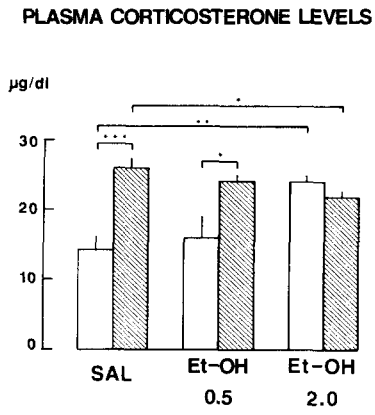

FIG. 1. Effects of ethanol on plasma corticosterone levels (μg/dl) in nonstressed and stressed rats. Each value indicates the mean± S.E.M. of 8 rats. Open bars, nonstressed rats; slashed bars, stressed rats. Abbreviations: SAL, saline; Et-OH 0.5, ethanol 0.5 g/kg; Et-OH 2.0, ethanol 2.0 g/kg. The horizontal bar indicates the statistical significance between the two groups compared. Levels of significance are: $*p<0.05$, $**p<0.01$, $***p<0.001$.

freely available and were housed 4 to a cage (265×425×150 mm standard plastic cage containing wood shavings) at constant room temperature (25±1°C) and humidity (50±10%) under a 12 hour (0700 to 0900) light-dark cycle.

Procedure

Stress procedure. Immobilization stress was accomplished by enclosing rats in flexible wire mesh (3×3 mm) initially formed into a cone and then bent to conform to the size of the individual rats.

Drug. Ethanol at doses of 0.5 g/kg (5% w/v) or 2.0 g/kg (20% w/v) dissolved in physiological saline was used.

Experimental procedure. By balancing body weights, animals were allocated to six groups of eight rats each. For the first three nonstressed groups, either ethanol at 0.5 g/kg, 2 g/kg, or saline was injected intraperitoneally (IP) 65 min before sacrifice. The animals in the remaining three stressed groups received identical injections 5 min prior to the 1-hr immobilization stress period. All experimental procedures were carried out between 1000 and 1400, since we found no diurnal variations of either NA or MHPG-SO_4 contents during this period [11].

Tissue Preparation and Biochemical Determination

Immediately after each treatment, rats were sacrificed by decapitation. The brain was rapidly removed and dissected into discrete brain regions according to the method of Gipsen *et al.* [6] and frozen on solid CO_2. The regions dissected were: the hypothalamus, amygdala, hippocampus and cerebral cortex. The LC region was also dissected out by the method of Reis and Ross [17]. Blood was collected from cervical vessels into heparinized tubes. Separated plasma and brain tissues were stored at −45°C until assayed. Plasma corticosterone levels were determined fluorometrically by the modified method of van der Vies [24]. NA and MHPG-SO_4 levels in the brain regions were determined simultaneously by our fluorometric method [10].

Statistical Analysis

Factorial ANOVA followed by post hoc Tukey's tests, where significant main effects were detected with significance considered at the 0.05 level, were used for statistical analyis.

RESULTS

As shown in Fig. 1, ethanol significantly elevated plasma corticosterone levels in the nonstressed group as compared with saline group. With the exception of the group pretreated with ethanol at 2.0 g/kg, immobilization stress caused significant elevations of plasma corticosterone levels as compared with the respective nonstressed controls. In the stressed rats, ethanol at 2 g/kg significantly reduced elevations of plasma corticosterone levels as compared with the stressed-saline rats. In all regions examined, immobilization stress caused significant elevations of MHPG-SO_4 levels as compared with the respective nonstressed saline control groups (Fig. 2). In the nonstressed rats, ethanol significantly and dose-dependently elevated MHPG-SO_4 levels in the hypothalamus, hippocampus and cerebral cortex, but the drug did not affect stress-induced increases in MHPG-SO_4 levels in these regions. In contrast, in the amygdala and LC region, ethanol per se did not affect the MHPG-SO_4 levels in the nonstressed rats, but ethanol at a dose of 0.5 g/kg and 2 g/kg reduced stress-induced increases in MHPG-SO_4 levels in the amygdala and LC region, respectively.

In the hypothalamus, immobilization stress caused a significant reduction of NA levels in the stressed-saline group, as compared with the respective nonstressed-saline group. However, ethanol did not affect NA levels in the stressed-saline group. For the remaining regions, no significant changes in NA levels were observed in terms of effects of ethanol and stress.

DISCUSSION

Immobilization stress for 1 hr significantly elevated plasma corticosterone levels, and ethanol at a dose of 2.0 g/kg significantly attenuated these elevations. This finding is consistent with previous reports [9,14], suggesting that ethanol has a stress-relieving effect.

Immobilization stress also caused significant increases in MHPG-SO_4 levels in all five brain regions examined. NA levels, however, tended to be reduced by stress in most brain regions, although statistically significant differences were obtained only in the hypothalamus. These results, indicating that immobilization stress increases NA release in several brain regions, are consistent with our previous reports [8, 19, 22]. Although ethanol by itself increased NA release in these regions, ethanol had no effects on stress-induced increases in NA release in the hypothalamus, hippocampus and cerebral cortex. In contrast, in the amygdala and LC region, ethanol attenuated the stress-induced increases in NA release despite the fact that the drug did not affect NA release in the nonstressed condition. These findings clearly reveal that the effects of ethanol are differentially produced according to both the animal situation, i.e., presence or absence of stress, and the particular brain regions examined.

It has been reported that ethanol reverses the stress-induced reduction of NA content in the hypothalamus, thalamus-midbrain, hippocampus and striatum [4,12]. In the present study, such severe reductions of NA content were not observed. This discrepancy probably results from differ-

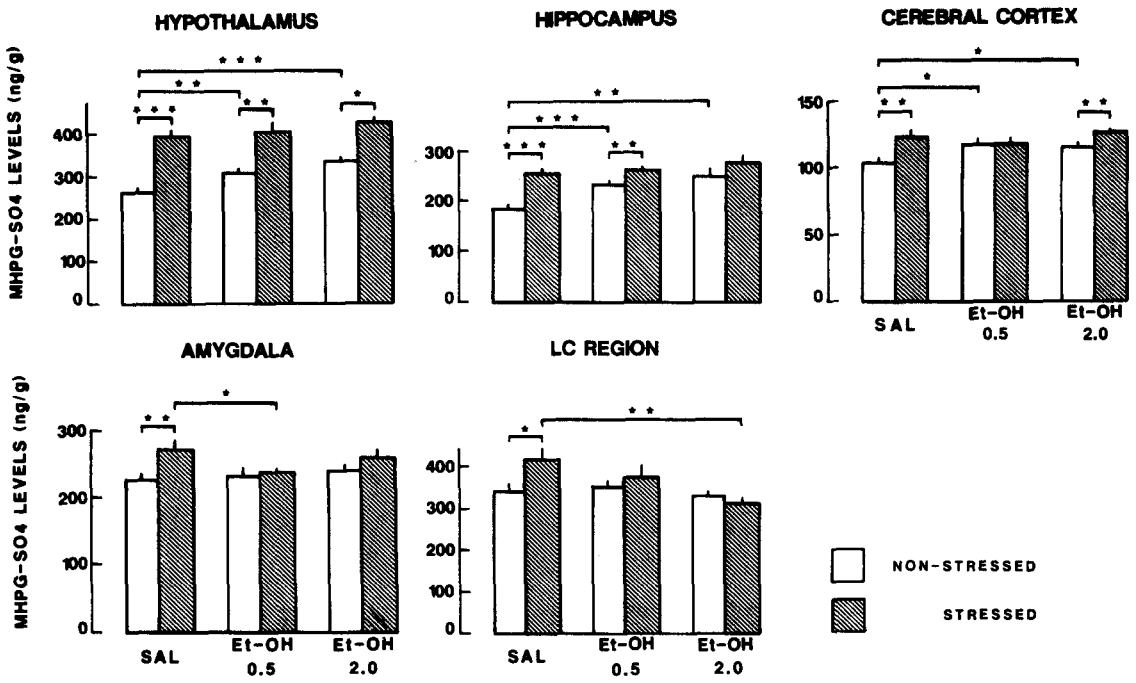

FIG. 2. Effects of ethanol on 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) levels (ng/g) in brain regions in nonstressed and stressed rats. Each value indicates the mean±S.E.M. of 8 rats. Abbreviations: SAL, saline; Et-OH 0.5, ethanol 0.5 g/kg; Et-OH 2.0, ethanol 2.0 g/kg. The horizontal bar indicates the statistical significance between the two groups compared. Levels of statistical significance are: $*p<0.05$, $**p<0.01$, $***p<0.001$.

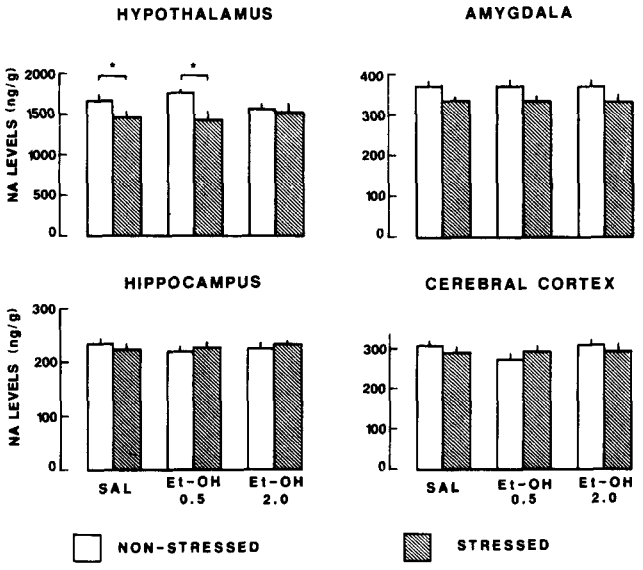

FIG. 3. Effects of ethanol on noradrenaline (NA) levels (ng/g) in brain regions in nonstressed and stressed rats. Each value indicates the mean±S.E.M. of 8 rats. Abbreviations: SAL, saline; Et-OH 0.5, ethanol 0.5 g/kg; Et-OH 2.0, ethanol 2.0 g/kg. The horizontal bar indicates the statistical significance between the two groups compared. A level of statistical significance is: $*p<0.05$.

ences in the stress situation employed. The De Turck *et al.* [4] and Kuriyama *et al.* [12] stress situations were produced by taping the paws of the animal and immobilization in water, respectively. These stressors are likely more severe than that employed in our work [7]. The present finding, that the attenuating effect of ethanol on increases in NA release was observed only in the amygdala and LC regions, suggests that these particular brain regions are more critically involved in mediating the attenuating effect of ethanol on stress responses.

Morphine, an opiate which acts to relieve distress-evoked hyperemotionality, attenuates immobilization stress-induced increases in NA release not only in the amygdala but also in the hypothalamus, thalamus, hippocampus and midbrain [22]. Diazepam, a typical anxiolytic agent, also attenuates such increases of NA release not only in the amygdala but also in the hypothalamus, hippocampus, cerebral cortex and LC region [8]. Taken together, these results clearly demonstrate that morphine, diazepam and ethanol attenuate immobilization stress-induced increases in NA turnover through a common mechanism, likely via the amygdala.

Microinjection of morphine into the amygdala abolishes emotional responsiveness [18] and reduces anxiety as measured by the social interaction test in rats [5]. Electrical stimulation of the LC, which increases noradrenergic activity in many brain regions including the amygdala which is innervated by the LC [3], produces behavioral effects of anxiety [16]. These effects are attenuated by both morphine and diazepam [8,22]. The increases in anxiety which are associated with an increase in LC activity are attenuated by ethanol which depresses the activity of these neurons [13,15]. Together with these findings and with the present results, it is suggested that increases and decreases in the activity of noradrenergic neurons in the amygdala are closely related to the production and attenuation of emotionality, respectively.

In conclusion, this study indicates that the effect of ethanol on brain NA release is different depending upon whether or not the animals are under a stressful situation and that ethanol attenuates stress-induced increases in NA release specifically in the amygdala and LC regions, which might be related to the stress-reducing effects of ethanol.

ACKNOWLEDGEMENTS

We are grateful to Ms. S. Takeda for her technical assistance and to Dr. G. B. Glavin for his kind proofreading and also to Nippon Roche K.K. for a generous supply of MHPG-SO_4.

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