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BRIEF COMMUNICATION

Blocking Effects of Ethanol on Stress-Induced Activation of Rat Mesoprefrontal Dopamine Neurons

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MATSUGUCHI, N., Y. IDA, I. SHIRAO AND S. TSUJIMARU. *Blocking effects of ethanol on stress-induced activation of rat mesoprefrontal dopamine neurons.* PHARMACOL BIOCHEM BEHAV 48(1) 297–299, 1994.—We investigated the effects of ethanol on stress-induced activation of the brain dopamine (DA) systems in rats. Ethanol (0.5 and 1.0 g/kg) was injected IP 25 min before sacrifice (5 min before 20-min immobilization stress). Ethanol treatment by itself did not affect the levels of either DA or its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), in the mesoprefrontal cortex, cingulate cortex, olfactory tubercle, or caudate putamen. Immobilization stress for 20 min caused increases in DOPAC levels in the prefrontal cortex (160% of control) and cingulate cortex (135% of control), but not in the olfactory tubercle or caudate putamen. The stress had no effects on DA levels in any of the four brain regions studied. Pretreatment with ethanol blocked, in a dose-dependent manner, the stress-induced increases in DOPAC levels in the mesoprefrontal cortex. The present data suggest that ethanol exhibits a blocking effect on stress-induced activation of the mesoprefrontal DA neurons. This blocking effect may be related to the anxiolytic action of ethanol.

Ethanol Mesoprefrontal dopamine neurons DOPAC Immobilization stress

PSYCHOPHYSIOLOGICAL stress models such as 20-min footshock stress (1–3,8,9,12) and short duration immobilization stress (10), in addition to a psychological stress model, conditioned fear (1,4,7), cause selective activation of the mesoprefrontal dopamine (DA) neurons. Anxiolytic benzodiazepines (BZDs) such as diazepam antagonize stress-induced selective activation of the mesoprefrontal DA neurons (2,7,8,9) which results from interaction with BZD receptors (7). Moreover, anxiogenic β-carboline FG 7142, a BZD receptor inverse agonist, selectively activates the mesoprefrontal DA neurons in a manner similar to that produced by stress (6,11). These previous studies raise the hypothesis that the mesoprefrontal dopamine (DA) system may play an important role in the control of negative emotional states (anxiety and/or fear) by interacting with the BZD/GABA receptor-chloride channel complex.

The pharmacological effects of ethanol, e.g., anxiolytic, muscle relaxant, and sedative/hypnotic properties, are thought to be mediated through the BZD/GABA receptor-chloride channel complex (5,12). It has been previously reported that ethanol prevents footshock stress-induced increases in DA metabolism in rat prefrontal cortex by interacting with the BZD/GABA receptor-chloride channel complex (3). In the present study, we used another kind of stress, immobilization, and investigated the effects of ethanol on stress-induced changes in DA metabolic activities in several brain regions by measuring levels of DA and its major metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC).

METHOD

Animals

Male Wistar rats, weighing 190–210 g, were used. Animals were housed in a temperature-controlled room (24 ± 1°C)

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with a 12-h light : dark cycle, and were allowed free access to food and water.

Drugs

Ethanol, at doses of 0.5 g/kg (5% w/v) or 1.0 g/kg (10% w/v), dissolved in physiological saline, was used.

Stress Procedure

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

Experimental Procedure

Forty-eight rats were randomly assigned to one of six groups (n = 8 for each group). Rats in the three nonstressed groups were injected IP with ethanol in saline at either 0.5 g/kg or 1.0 g/kg, or physiological saline vehicle 25 min before sacrifice. The animals in the remaining three stressed groups received identical injections 5 min prior to the 20-min immobilization stress.

Tissue Preparation and Biochemical Assay

Immediately following each experimental procedure, rats were sacrificed by decapitation, and brains were rapidly removed. Four brain regions were dissected on ice for neurochemical analysis: the prefrontal cortex, cingulate cortex, olfactory tubercle, and caudate putamen (1). Dissected brain tissues were stored at −80°C until assay. Within 1 week after the experiment, DA and DOPAC levels in the four brain regions were determined by high performance liquid chromatography with electrochemical detection.

Statistical Analysis

Statistical analysis of the data from each brain region was made by a stress (stressed and nonstressed) × drugs (ethanol 0, 0.5, 1.0 g/kg) factorial analysis of variance (ANOVA) and post hoc Newman-Keuls test for multiple comparisons.

RESULTS

Figure 1 shows the mean (±SEM) of DOPAC levels in four brain regions examined in the present study. ANOVA revealed a significant stress effect on DOPAC levels in the prefrontal cortex, $F(1, 42) = 69.4$, $p < 0.01$, and the cingulate cortex, $F(1, 42) = 43.2$, $p < 0.01$, but not in the olfactory tubercle, $F(1, 42) = 1.3$, and caudate putamen, $F(1, 42) = 1.4$. ANOVA revealed no significant stress effects on DA levels in the prefrontal cortex, $F(1, 42) = 2.7$, or the cingulate cortex, $F(1, 42) = 1.1$. Ethanol treatment showed significant effect on DOPAC levels in the prefrontal cortex, $F(1, 42) = 5.8$, $p < 0.05$.

As shown in Figure 1, ethanol treatment had no effects on DA metabolism in any of four brain regions studied in nonstressed rats. Immobilization stress for 20 min elicited significant increases in DOPAC levels in the prefrontal cortex (160% of control) and cingulate cortex (135% of control), but caused no changes in those levels in the olfactory tubercle and caudate putamen. The stress had no effect on DA levels in the four brain regions examined (data not shown). Pretreatment with ethanol blocked, in a dose-dependent manner, stress-induced increases in DOPAC level only in the prefrontal cortex, significant effects obtained at a dose of 1.0 g/kg.

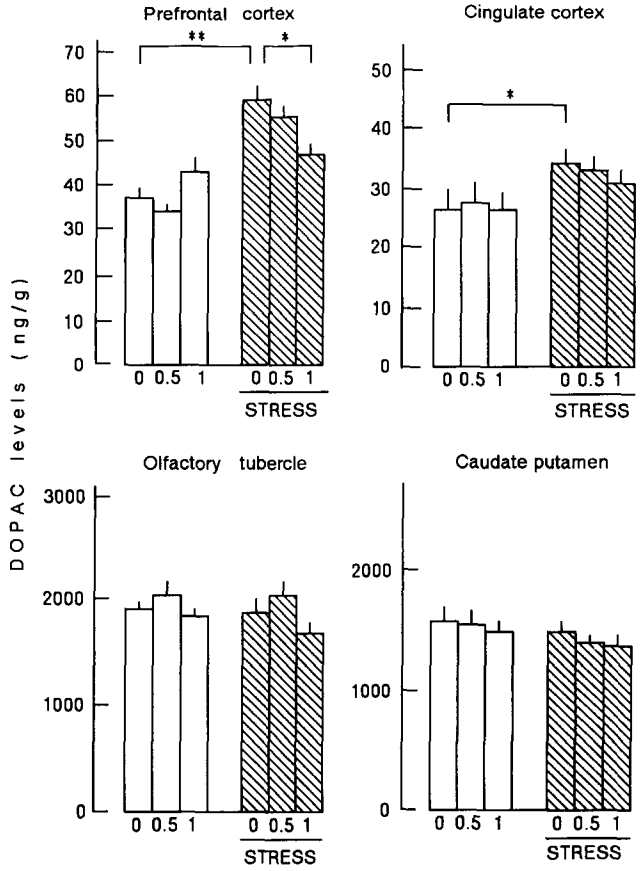

FIG. 1. Effect of ethanol on DOPAC levels (ng/g) in four brain regions of stressed and nonstressed rats. Ethanol at either 0.5 g/kg or 1.0 g/kg in saline, or saline alone were administered IP 25 min before sacrifice (5 min before 20-min immobilization stress). Each value indicates the mean ± SEM of eight rats. The horizontal bar indicates the statistical significance between two groups compared: $*p < 0.05$; $**p < 0.01$.

DISCUSSION

In the present study, immobilization stress for 20 min caused increases in DA metabolism in the prefrontal cortex and cingulate cortex, but not in the olfactory tubercle or caudate putamen, and the degree of increase in the prefrontal cortex was greater than that in the cingulate cortex. These data are in virtual agreement with previous findings that various stressful stimuli, such as 20-min footshock stress (1,2,8,9,12), short-term immobilization stress (10), and conditioned fear (1,4,7), elicit selective activation of the mesoprefrontal DA neurons, although these are accompanied by a lesser degree of activation of DA neurons in other regions, e.g., the cingulate cortex (8), olfactory tubercle (1), and nucleus accumbence (2,12). The present study revealed that ethanol at physiological doses exhibits an attenuating effect on the stress-induced increases in DA metabolism in the prefrontal cortex. Under stress, ethanol seems to elicit a similar pharmacological action on the mesoprefrontal DA neurons to those of anxiolytic BZDs, as reported in previous studies (2,7-9). Ethanol is known to have anxiolytic properties, and its pharmacological actions are induced by influencing the chloride channel modulation at the BZD/GABA receptor-chloride channel complex

(5,13). It has been previously reported that oral treatment with ethanol at 1.2 g/kg prevents increases in DA metabolism induced by 20-min footshock stress in the rat prefrontal cortex; its preventative effects are antagonized by Ro 15-4513, a partial inverse agonist of BZD receptors (3). Taken together, with this previous data, the present data suggest that ethanol could reduce stress-induced activation of the mesoprefrontal DA neurons by interacting with BZD/GABA receptor-chloride channel complex, this effect being likely related to the anxiolytic properties of ethanol.

In conclusion, the present findings suggest that ethanol could block stress-induced activation of the mesoprefrontal DA neurons; this action may be related to the anxiolytic action of ethanol, and support the hypothesis that the mesoprefrontal DA neurons play an important role in the control of negative emotional states (anxiety and/or fear).

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