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EFFECT OF ETHANOL ON BRAIN LEVELS OF DOPAMINE AND ITS METABOLITES IN RATS DIFFERING IN SENSITIVITY TO STRESS

R. Yu. Yukhananov, T. M.-A. Tennila, N. I. Miroshnichenko, V. S. Kudrin, K. S. Raevskii, M. M. Airaksinen, and A. I. Maiskii

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There is experimental evidence that addiction to ethanol and to other narcotics is realized through participation of the brain dopamine system. For instance, dopamine antagonists block voluntary ethanol consumption and destruction of dopamine neurons in the midbrain is accompanied by reduction of voluntary ethanol consumption [9, 11]. Ethanol administration is accompanied by activation of dopamine neurons, especially in those brain regions where their mesolimbic and mesocortical projections terminate [9, 10].

Studies of the effect of ethanol on dopamine metabolism have been undertaken many times but without taking account of differences in the organization of projections of dopaminergic neurons in the CNS or of initial differences between animals.

The aim of this investigation was to study the effect of ethanol on dopamine metabolism in rats differing in their sensitivity to stress. It was shown previously that this difference correlates with the level of ethanol consumption [4]. The animals were subjected to forced swimming and their sensitivity to stress was estimated as the duration of immobilization. Levels of dopamine and its metabolites were determined in the medial prefrontal cortex, nucleus accumbens, and striatum, so that dopamine metabolism could be characterized in mesocortical mesolimbic, and mesostriatal conducting systems [8].

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TABLE 1. Effect of Ethanol (2 g/kg) on Concentration of Dopamine and Its Metabolites in Brain of Rats Differing in Sensitivity to Stress ($M \pm m$)

Preparation	Medial frontal cortex		Nucleus accumbens		Striatum	
	HA	LA	HA	LA	HA	LA
Dopamine						
control	0,28±0,06 (4)	0,33±0,05 (4)	5,52±0,74 (5)	4,92±0,33 (5)	4,77±0,37 (6)	4,15±0,36 (6)
ethanol	0,27±0,05 (6)	0,25±0,02* (6)	4,46±0,4 (7)	5,64±0,33*** (9)	4,82±0,46 (7)	5,42±0,37* (8)
DOPAA						
control	0,12±0,06 (3)	0,23±0,08 (3)	0,84±0,10 (5)	0,99±0,05 (5)	0,53±0,05 (6)	0,47±0,06 (6)
ethanol	0,07±0,02 (5)	0,13±0,03 (5)	0,90±0,12 (7)	0,99±0,06 (9)	0,53±0,05 (6)	0,56±0,04 (9)
HVA:						
control			0,52±0,06 (5)	0,48±0,11 (4)	0,41±0,06 (6)	0,41±0,05 (6)
ethanol			0,46±0,10 (4)	0,58±0,09 (7)	0,45±0,03 (7)	0,44±0,03 (9)

Legend. Values given in ng/mg tissue; $*p < 0.05$, $**p < 0.01$ compared with control, $***p < 0.05$ compared with HA animals. Control animals received water 8 ml/kg perorally. Number of animals given in parentheses.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 220-240 g. The rats were put in a circular plastic basin 50 cm high and 32 cm in diameter, filled to a height of 25 cm with water at a temperature of 20°C. The rats remained in the basin for 600 sec, after which they were transferred to a heated cage for 30 min and then returned to the animal house. The duration of immobilization, i.e., the duration of passive swimming by the rat in the vertical position with its head just projecting above the water surface, was recorded for each rat. Two groups of animals were chosen for the experiments, depending on their duration of immobilization under forced swimming conditions [4]: 1) immobilization for less than 120 sec, and 2) for more than 300 sec. The selected animals were used for later experiments after 10 days. Ethanol was given to the rats perorally in the form of a 25% solution, whereas the control animals received water. The animals were decapitated 1 h after receiving ethanol or water, the brain was quickly cooled on ice, and the medial prefrontal cortex, nucleus accumbens, and striatum were isolated. The medial frontal cortex was isolated as in [12] but the transverse section 2.2 μ thick was taken between 12,500 and 10,300 μ according to the atlas [13]. To isolate the nucleus accumbens a section 2.3 μ thick was taken between 10,300 and 7900 μ, part of striatum was separated along its visible boundaries, and the remainder of the striatum was taken 7900 and 6600 μ. The isolated brain structures were quickly frozen, weighed, and kept in liquid nitrogen. To determine dopamine and its metabolites the brain structures were homogenized in a homogenizer with Teflon pestle revolving at a speed of 3000 rpm. The isolation medium consisted of 0.1 N $HClO_4$, and dioxybenzylamide (100 ng/ml) was added as the internal standard. The samples were centrifuged at 10,000g for 10 min. The supernatant was filtered through millipore filters and applied, in a volume of 20 μl, to a column measuring 4.6 × 250 mm, connected to an LC-4B double amperometric detector and TL-5 cell. A voltage of 850 mV was applied to the working electrode. The stationary phase was RP-18 octadecylsilane and the moving phase was 0.02 M citrate buffer with the addition of 0.3 mM of the organic modifier acetonitrile, pH 3.6 [6]. A "Shimadzu" R1A integrator (Japan) was used to calculate the concentrations of the substances in the sample.

EXPERIMENTAL RESULTS

Two groups of animals were isolated from the population of noninbred rats: with low activity (LA), mean duration of immobilization under forced swimming conditions 90.7 ± 6.3 sec, and with high activity (HA), with duration of immobilization of 340.6 ± 8.1 sec. Determination of the concentration of dopamine and its metabolites revealed no difference between the LA and HA animals in the cortex, striatum, and nucleus accumbens (Table 1). Only a tendency was observed for the concentration of dihydroxyphenylacetic acid (DOPAA) to rise in the medial frontal cortex in the LA animals. The LA and HA animals differed in a number of aspects of their behavior [1, 4, 5], in their sensitivity to psychotropic drugs [3], and in their level of ethanol consumption under conditions of free choice between 15% ethanol solution and water. In the early stages of contact with ethanol, among the LA animals there was a higher percentage of drinkers and a higher average level of ethanol consumption [4,

5]. In a single dose of 2 g/kg ethanol lowered the dopamine level in the medial frontal cortex by 30% and raised it in the striatum of the LA rats; the concentrations of neither DOPAA nor homovanillic acid (HVA) in these brain regions showed any change. Moreover, after ethanol administration the dopamine concentration in the nucleus accumbens was significantly higher in the LA than in the HA animals. In this connection it has to be pointed out that there is solid evidence that the nucleus accumbens is involved in the regulation of voluntary ethanol consumption and in the mechanism of the facilitatory effect of ethanol on the self-stimulation response [9, 10]. It can be tentatively suggested that the differences between the LA and HA animals with respect to ethanol consumption are due to differences in the sensitivity of the mesolimnic structures of the brain to ethanol. The mechanism of action of ethanol on the brain dopamine level can be realized through blocking of recurrent influences and a consequent increase in synthesis of the neurotransmitter, for no change in dopamine metabolism was found in our experiments, as reflected in brain levels of DOPAA and HVA.

Differences found in the effects of ethanol on the dopamine level in the cortex and striatum agree to some extent with data [12] showing a fall of the dopoamine level in the medial frontal cortex after administration of ethanol. The fact is that mesostriatal and mesocortical dopaminergic endings differ in several features, for autoreceptors, in particular, are probably absent in the cortex [7], a fact which may go some way toward explaining the opposite effects of ethanol in the frontal cortex and striatum. Whatever the case the effects of ethanol which we found were manifested only in the LA animals, suggesting that the dopamine system is involved in the action of ethanol on this group of rats. Unlike Fadda's group [11, 12], who used selectively bred rats with a high level of ethanol consumption in their work, we found no effect of ethanol on dopamine metabolism. The disagreement between the results can perhaps be attributed to the heterogeneity of the mechanisms determining the craving for alcohol [2]. In the animals which we studied, craving for ethanol was strong when they were put in individual cages. Such a situation can be regarded as a stress factor. During adaptation the level of voluntary consumption falls. Quite probably in this case ethanol has a stress-protective action, whereas in inbred animals alcohol consumption induces euphoria. It can thus be tentatively suggested that the changes we found in the dopamine concentration largely reflect the action of the dopaminergic systems of the brain in the stress-protective action of ethanol.

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