arise in the myocardium as a result of acute (myocardial infarction) or chronic (ischemic heart disease) course of the disease.

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EFFECT OF NALOXONE ON ETHANOL-INDUCED MEMBRANE-BOUND ENKEPHALIN CONVERTASE ACTIVATION IN THE RAT MESENCEPHALON AND HYPOTHALAMUS

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The brain enkephalin system may be involved in the pathogenesis of alcoholism through its role both in the regulation of pathological addiction to ethanol and also, evidently, in the formation of tolerance to it [6, 9]. Recent investigations have revealed several general principles which suggest that the formation of these symptom-complexes is associated with lowering of the sensitivity of the system for enkephalin formation and release to the stimulating action of ethanol [1, 5, 10]. However, the fine mechanisms of the action of ethanol on enkephalin metabolism and, consequently, on the processes lying at the basis of adaptation of enkephalin neurotransmission to ethanol, require a closer study. One interesting object from this point of view is enkephalin convertase (carboxypeptidase E, H; E.C. 3.4.17.10). This carboxypeptidase B-like peptide hydrolyse catalyzes the final stage of processing of enkephalins, removing the hexapeptide precursor arginine or lysine from the C-end of the molecule, and activated by cobalt ions [7, 8, 12]. It was shown previously that as a result of chronic alochol intake by rats the activity of this enzyme in the brain changes significantly, and that the most marked changes are observed in the mesencephalon and hypothalamus [2]. The absence of any such effect in vitro [2] suggests that changes in enkephalin convertase activity in alcoholic intoxication may be quite complex in nature and may be connected with adaptation of the enkephalin system to long term exposure to ethanol.

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TABLE 1. Effect of Chronic Administration of Ethanol and Naloxone on Enkephalin Convertase Activity in Mesencephalon (including hypothalamus) in Rats (M ± m)

Experimental conditions	Enkephalin convertase activity, pmoles/min/mg protein	
	soluble	membrane bound
Water (i/g) – physiological saline (i/p)	929±96(5)	441±20 (6)
Water (i/g) – naloxone (i/p)	918±35 (6)	463±16 (6)
Ethanol (i/g) – physiological saline (i/p)	954±43 (5)	571±20* (5)
Ethanol (i/g) – naloxone (i/p)	917±35 (5)	447±23** (5)

Legend. Number of animals in each group shown between parentheses: 1) Significance of differences from "water – physiological saline" group ($p < 0.01$), **) Significance of differences from "ethanol – physiological saline" group ($p < 0.01$).

The aim of this investigation was to study the effect of naloxone, an opioid receptor antagonist, on activity of membrane-bound and soluble forms of enkephalin convertase in the mesencephalon (including the hypothalamus) of rats with chronic exposure to alcohol and control rats.

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-220 g were used. The rats were divided into four groups. Animals of group 1 were given a 20% solution of ethanol in a daily dose of 9-15 g/kg, according to the schedule described previously [14], by means of a gastric tube three times a day (at 8 a.m. and 2 and 8 p.m.) for four days. Every day 30 min before the first and last doses of ethanol, these animals received an intraperitoneal injection of naloxone (2 mg/kg) in the form of a solution in 0.9% NaCl. Instead of naloxone, rats of group 2 were given an intraperitoneal injection of physiological saline. Instead of the ethanol solution, rats of groups 3 and 4 received the equivalent volume of distilled water. The animals of groups 3 were given naloxone intraperitoneally, whereas those of group 4 were given physiological saline, as indicated above. After 16-20 h, at the end of the course of ethanol administration, the rats were decapitated and the mesencephalon (including the hypothalamus) quickly removed. The brain regions thus obtained were frozen and kept until use at −20°C for not more than one month. Soluble and membrane fractions were obtained as follows. Brain samples were homogenized in 100 volumes of 10 mM Tris-HCl buffer, containing 1 mM EDTA (pH 7.4), and incubated at 37°C for 4 h. This procedure activates enkephalin convertase and depresses cobalt-nonstimulated carboxypeptidase B-like activity [3]. The homogenate thus treated was centrifuged at 30,000*g* (30 min, 4°C). The residue and supernatant were diluted with 10 mM Na-acetate buffer with 1 mM EDTA (pH 6.0) to a protein concentration of 0.18-0.21 mg/ml, and used as preparations of soluble and membrane-bound forms of enkephalin convertase [2]. Enkephalin convertase activity was determined from the rate of formation of Dns-Phe-Leu from a Dns-Phe-Leu-Arg substrate [8], as described previously [2], and expressed in pmoles product formed during 1 min, calculated per milligram protein. The Dns-Phe-Leu and Dns-Phe-Leu-Arg were synthesized and generously provided by Candidate of Chemical Sciences V. N. Kalikhevich (St. Petersburg State University). The protein concentration in the samples was determined by Lowry's method. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Table 1 gives the results of determination of enkephalin convertase activity in the mesencephalon (including the hypothalamus) of rats after administration of ethanol and naloxone. Administration of alcohol for 4 days to the animals clearly led to increased activity of the membrane-bound form of enkephalin convertase in this part of the brain but had no effect on the activity of the soluble form of the enzyme. Meanwhile, after combined administration of ethanol and naloxone, activity of the enzyme corresponded to the control level. Injection of naloxone alone, into rats, incidentally, did not change activity of either the soluble or the membrane-bound form of the enzyme.

The results of the present study are in good agreement with those obtained previously on activation of the membrane-bound form of enkephalin convertase in the mesencephalon and hypothalamus after alcoholic intoxication for 6 weeks [10], and they thus demonstrate that an enzyme-activating effect can be obtained by the use of a shortened program of alcoholization. Incidentally the degree of activation of the enzyme (in the combined region of mesencephalon + hypothalamus) observed in the present investigation (29%), is comparable with that described previously for the hypothalamus (29%) but considerably lower than that in the mesencephalon (63%) [2]. It can be tentatively suggested that this difference may be due to differences in the duration of chronic alcoholic poisoning and, consequently, it may be connected with adaptation of the brain enkephalin system to the action of ethanol.

Pharmacologic activity of the opioid receptor antagonist naloxone suggests two possible points of view regarding the mechanism of prevention of the activating action of ethanol on activity of the membrane-bound form of enkephalin convertase by this compound. First, the effect of naloxone may be caused by opioid receptor blockade per se, i.e., it may be due to the consequences of hypostimulation of receptors. Second, the protective action of naloxone may be caused by prevention of hyperstimulation of opioid receptors. The absence of changes in enkephalin convertase activity in response to administration of naloxone alone indicates that prevention of the activating action of alcohol on the enzyme by it may be realized in accordance with a second mechanism. In this basis it can be concluded that activation of membrane-bound enkephalin convertase by ethanol takes place on account of hyperstimulation of opioid receptors by opiate-active compounds. This conclusion is in good agreement with data on the activating action of ethanol on the formation of enkephalins and on the rate of their supply to enkephalin receptors [1, 5, 6]. An increase in activity of the membrane-bound form of enkephalin convertase as a result of long-term administration of ethanol can therefore be regarded as a compensatory reaction of the enkephalin forming system to an increase in the steady-state enkephalin concentration close to enkephalin receptors. Undoubtedly under these circumstances the possibility cannot be ruled out that agonists of nonpeptide nature, such as certain tetrahydroisoquinolines [4, 13] and, possibly, endogenous morphine [11, 15], may make a definite contribution to the process of opioid receptor stimulation. Whatever the case, however, our results are evidence of the existence, at least in chronic alcohol intoxication, of a pathway of regulation of enkephalin convertase activity, mediated by opioid receptors. In this case it is still a puzzle whether this regulation takes place within the boundaries of the enkephalin synapse alone, or whether it involves a more complex mechanism with the participation of extra neurons.

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