INTERACTION OF ETHANOL WITH OPIATE RECEPTORS

R. Yu. Yukhananov, A. I. Maiskii, and Yu. V. Burov

UDC 612.822.014.467:547.943/.014.46:615.917:547.262

KEY WORDS: ethanol; opiate receptors; alcoholization.

The many effects of ethanol in the body include its action on the endogenous opioid system. According to one view, interaction of ethanol with opiate receptors is indirect in character and is maintained by the formation of cyclic derivatives of biogenic amines, with a molecular structure similar to that of endogenous and exogenous ligands of opiate receptors, *in vivo* in the course of chronic alcoholization [3]. Meanwhile the effect of the ethanol molecule itself on functional properties of opiate receptors has not been adequately studied.

In the investigation described below the action of ethanol on membrane-bound opiate receptors was studied.

EXPERIMENTAL METHOD

Male albino rats weighing 180-200 g, receiving a standard dry pellet diet, were used. The animals' predisposition to ethanol consumption was demonstrated on the basis of the duration of ethanol anesthesia [1]. Chronic alcoholization was carried out by allowing free choice between 15% ethanol solution and water [2]. To isolate biological material containing opiate receptors the rats were decapitated, brain tissue was removed, homogenized quickly in Tris-buffer (pH 7.4), and centrifuged for 20 min at 50,000g [7]. The residue was resuspended in Tris-buffer and incubated for 60 min at 25°C. The homogenate was then recen-

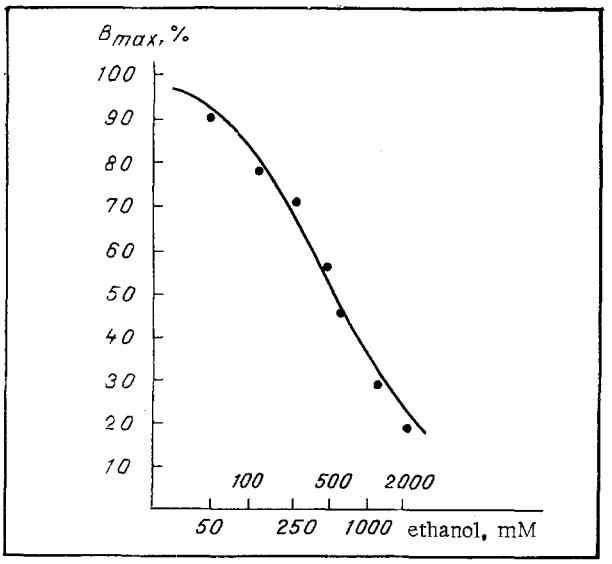

Fig. 1. Inhibition of naloxone binding with opiate receptors of rat brain at 37°C by ethanol. Abscissa, ethanol concentration (in mM); ordinate, percentage inhibition of naloxone binding (naloxone binding in the absence of ethanol taken as 100%).

Department of Neuropharmacology, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 588-591, November, 1985. Original article submitted January 18, 1985.

0007-4888/85/0011-1555$09.50

TABLE 1. Effect of Ethanol on Stereospecific Binding of Naloxone in Whole Rat Brain Homogenate *in Vitro*

Experimental conditions	Number of experiment	ID_{50} for ethanol, mM
Short-sleeping rats	4	671±81
Long-sleeping rats	4	402±58*
Alcoholization for 3.5 months		
experiment	3	433±63
control	3	352±58
Alcoholization for 10 months		
experiment	4	421±42
control	4	494±50

Legend. Rats of the same age as the experimental animals, but with no contact with ethanol, used as the control. Naloxone concentration 0.8-1 nM. *P < 0.05 compared with short-sleeping animals.

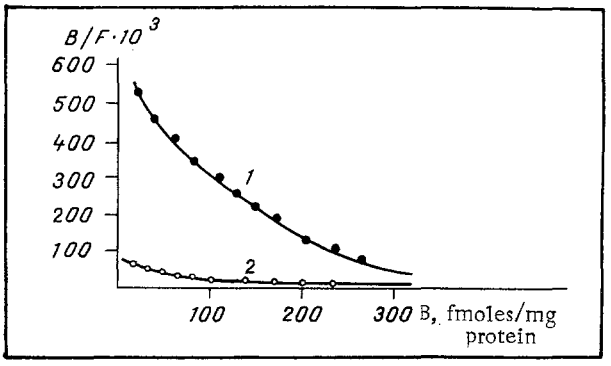

Fig. 2. Scatchard plot of naloxone binding with rat brain opiate receptors at 37°C. Abscissa, specific binding of ^{3}H-naloxone with opiate receptors (B) in fmoles/mg protein; ordinate, ratio of quantity of naloxone specifically bound with opiate receptors (B) to naloxone concentration in incubation medium (F). 1) In absence of ethanol in incubation medium, 2) in presence of 0.5 M ethanol.

trifuged for 20 min at 50,000g and the residue was resuspended in Tris-buffer and kept at −20°C until required for use. Free and bound ligand (N-allyl-2,3-^{3}H-naloxone, from Amersham Corporation, England) were separated by means of GF/B filters. Inhibitor analysis was carried out by log-logit transformation. Scatchard plot analysis was carried out by method in [6]. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Ethanol at 37°C was shown to produce dose-dependent inhibition of binding of ^{3}H-naloxone with opiate receptors (Fig. 1). ID_{50} (the ethanol concentration reducing naloxone binding by 50%) under these conditions was 462 mM. A considerable reduction of stereospecific binding was recorded when the ethanol concentration in the incubation medium was as low as 50 mM. When this experiment was repeated with the temperature of the incubation medium at 0°C, it was found that ethanol within the concentration range from 50 to 1000 mM did not affect binding of ^{3}H-naloxone with opiate receptors. Temperature-dependent inhibition of ligand–receptor binding suggests that ethanol does not compete for the stereospecific binding site of ^{3}H-naloxone. The explanation of the inhibitory properties of ethanol on the opiate system must be sought, in our view, in the ability of the alcohol molecule to modify the structure of the lipids of biological membranes. In the presence of ethanol, mobility of the lipid bilayer and fluidization of the membranes have been shown to be enhanced [9]. We know that the lipid matrix of biological membranes can exist in two interconvertible states: solid-crystalline and liquid-crystalline; this phase transition, moreover, takes place within the temperature range from 17 to 20°C. It can be tentatively suggested that at 0°C,

when the membrane lipids are in a solid crystalline state, ethanol does not penetrate inside the membrane and does not exhibit its action on the receptor. To study the problem of whether chemical modification of biological membranes takes place under the influence of ethanol, the reversibility of the effects of alcohol was tested. For this purpose, after preincubation of synaptic membranes at 37°C the incubation mixture was cooled to 0°C. Under these conditions ethanol, even in a concentration of 1 M, did not affect ^{3}H-naloxone binding. Consequently, the action of ethanol is not due to the formation of stable chemical bonds with protein and lipid components of the membranes.

Subsequent Scatchard plot analysis showed that ethanol, in a concentration close to ID_{50} (0.5 M) inhibits binding of ^{3}H-naloxone with high-affinity opiate receptors virtually completely. It must be pointed out that although the number of high-affinity binding sites was sharply reduced, their affinity for the ligand remained unchanged (Fig. 2). The total number of binding sites in the presence and absence of ethanol did not differ statistically significantly, and was 453 and 549 fmoles/mg protein respectively. It can be postulated that ethanol promotes interconversion of high- and low-affinity binding sites for ^{3}H-naloxone, while leaving the total number of binding sites unchanged. The possibility of such interconversion between different types of receptors was demonstrated previously during a change in ionic composition of the incubation medium [4]. It can thus be concluded from the results that the ethanol molecule has a differential effect on opiate receptors with different affinity for naloxone. The greatest changes were recorded during interaction between ethanol and high-affinity opiate receptors, which can be classed in the μ-type.

During development of dependence on ethanol the lipid composition of the biological membranes changes: The latter plays an important role in the mechanisms of development of tolerance and of disturbances of normal functioning of neuroregulatory systems [9]. There is experimental proof that the development of tolerance is causally connected with an increase in the content of saturated fatty acids in the composition of the membrane lipids [10]. Our results confirm these views indirectly. The study of the action of ethanol on ^{3}H-naloxone binding with opiate receptors in their predisposition to the development of experimental alcoholism, showed than ethanol inhibits to a greater degree the binding of ^{3}H-naloxone with membranes isolated from the brain of animals predisposed to ethanol consumption (Table 1). On the basis of previous data, according to which rats predisposed to the development of experimental alcoholism are less sensitive to the action of alcohol on body temperature [5] and to its analgesic effect [8] than animals not so predisposed, it can be postulated that differences between these groups of animals are determined by differences in the structure or lipid composition of their synaptic membranes. The different effects of ethanol on ^{3}H-naloxone binding in a system *in vitro* can be similarly explained.

Analysis of the inhibitory action of ethanol on ^{3}H-naloxone binding in animals at different stages of experimental alcoholism [2] revealed no differences between the control (with no contact with ethanol) and experimental animals after 3.5 and 10 months of voluntary alcoholization (Table 1). These data are evidence that the formation of physical dependence, which takes place in rats after consumption of ethanol solution under free choice conditions for 10 months, is unconnected with any change in the sensitivity of opiate receptors to ethyl alcohol. Meanwhile differences in such sensitivity of these receptors found in predisposed rats or, conversely, in rats not predisposed to the development of experimental alcoholism, justifies the conclusion that a receptor component is involved in the biochemical mechanisms of the primary intensity of alcohol motivation. The absence of any differences in sensitivity of opiate receptors at stages of established mental and physical dependence on ethanol during the modeling of experimental chronic alcoholism does not, at the same time, rule out the participation of the opioid system in the formation of the disease. This is shown, in particular, by our data on changes in the concentrations of endogenous opioid neuropeptides in different parts of the brain in rats at different stages of experimental alcoholism [2].

LITERATURE CITED

1. Yu. V. Burov, T. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol Toksikol., No. 1, 50 (1981).
2. Yu. V. Burov, R. Yu. Yukhananov, and A. I. Maiskii, Byull. Éksp. Biol. Med., No. 7, 48 (1983).
3. K. Blum, M. G. Hamilton, and J. E. Wallse, in: Alcohol and Opiates, K. Blum (ed.), New York (1977), pp. 203-231.

4. W. Bowen, S. Gentleman, M. Herkenham, et al., Proc. Natl. Acad. Sci. USA, 78, 4818 (1981).
5. J. Brick and G. P. Horowitz, Pharmacol. Biochem. Behav., 16, 473 (1982).
6. H. A. Feldman, Anal. Biochem., 48, 317 (1972).
7. M. V. Fischel and R. Medzihradsky, Molec. Pharmacol., 20, 269 (1981).
8. H. J. Fridman, M. B. Bass, and D. Lester, Pharmacol. Biochem. Behav., 13, 773 (1981).
9. D. B. Goldstein and J. H. Chin, Fed. Proc., 40, 2073 (1981).
10. J. M. Littleton, Adv. Biol. Psychiat., 3, 75 (1979).

PHARMACOKINETICS OF SERUM PHOSPHOCREATINE IN MAN, DOG, AND RABBIT

N. I. Afonskaya, I. I. Shepeleva,
E. P. Anyukhovskii, M. B. Samarenko,
L. A. Makhotina, and V. A. Saks

UDC 615.224:546.18/.033.018.5+
616.154.94:615.224:546.18

KEY WORDS: phosphocreatine; pharmacokinetics; man; experimental animals; intravenous infusion.

Investigation of the metabolism of the ischemic heart and of ultrastructural injuries to its cells at the stage of transition of these injuries into irreversible have clearly demonstrated that the time of this transition coincides with a reduction in the concentration of high-energy phosphates below a critical level [3, 5, 6]. Phosphocreatine, which participates in the mechanism supplying energy for contraction as an intracellular carrier of energy, also has been shown to have a significant protective action on the ischemic myocardium [4, 7]. Before analogous investigations of the protective action of phosphocreatine can be conducted *in vivo*, however, further information is required on the pharmacokinetics of phosphocreatine in the blood, so that the possible effective doses of this substance can be accurately determined.

In the present investigation the pharmacokinetics of phosphocreatine in human, canine, and rabbit blood plasma was studied.

EXPERIMENTAL METHOD

Experiments were carried out on healthy rabbits and dogs anesthetized with pentobarbital (25 mg/kg body weight, intravenously). Phosphocreatine (disodium salt) in physiological saline was injected intravenously in the form of a bolus or by infusion. At the times indicated in Figs. 1 and 2, 6 ml of blood was taken from the carotid artery and kept on ice until the end of the experiment, when plasma was obtained from it by standard methods. To determine the concentrations of creatine and phosphocreatine in the plasma, it was extracted in 6% $HClO_4$ (1 ml plasma and 0.5 ml of acid) and in 35% methanol. The plasma was then centrifuged for 10 min at 8000 rpm in a U2-21 centrifuge (Beckman, USA), with UA-20 rotor. The supernatant was poured off and neutralized with 5% K_2CO_3 (to pH 7.0) and recentrifuged under the same conditions. The supernatant was again poured off and concentrations of creatine and phosphocreatine in it were determined colorimetrically [2]. Phosphocreatine was converted into free creatine by hydrolysis in 0.1 M HCl for 10 min, after which total creatine was determined colorimetrically. Free creatine was determined in parallel tests without hydrolysis, and the phosphocreatine concentration was calculated as the difference between total and free creatine.

The pharmacokinetics of phosphocreatine in man was determined by taking 6-ml blood samples from the subclavian vein by means of a catheter at times after a single intravenous injection of Neoton (Schiapparelli Farmaceutici, Turin, Italy) or after infusion of this substance into the cubital vein.

All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 591-593, November, 1985. Original article submitted August 9, 1984.

 0007-4888/85/0011-1558$09.50