The manifestation of cryoresistance by Na^+-reconstituted red cells is thus connected to a greater degree with changes in the inner surface of the plasma membrane.

It can be tentatively suggested that disturbance of the permeability of red cell membranes after rapid freezing and thawing is largely dependent on the initial state of the plasma membrane.

The author is grateful to E. V. Chaplai for expert assistance.

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TYROSINE HYDROXYLASE ACTIVITY IN THE BRAIN OF RATS WITH DIFFERENT LEVELS OF INITIAL ALCOHOL MOTIVATION

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UDC 612.822.1.015.1:577.152.199.1].014.46:547.262

KEY WORDS: alcohol; alcohol motivation; tyrosine hydroxylase; rat hypothalamus.

Investigations have shown that animals of the same population differ in their attitude to alcohol [2, 3]. The activating effect of alcohol on the structure of positive reinforcement at the hypothalamic level has been shown to be related to a high level of alcohol intake in a certain number of animals [1]. In other animals, by contrast, absence of any activating effect of alcohol on the structure of positive reinforcement correlated with a low level of alcohol consumption. The development of a liking for alcohol in rats can thus be taken to depend on its effect on the structure of positive reinforcement.

Considering that the chief neurochemical substrate of the structures of positive reinforcement is the catecholaminergic system of the brain [11-13], in the investigation described below tyrosine hydroxylase (TH) activity in the hypothalamus was studied in rats with different levels of initial alcohol motivation.

EXPERIMENTAL METHOD

To determine the initial level of alcohol motivation in rats without prolonged contact with this substance, a method based on the difference in the rate of alcohol metabolism in rats with high and low initial levels of alcohol motivation was used. Animals with a mean duration of sleep of 80 min (predisposed to taking alcohol) and 180 min (rejecting alcohol) were selected for the experiments after intraperitoneal injection of a 25% solution of ethanol in a dose of 4.5 g/kg body weight.

Noninbred male albino rats weighing 200-250 g were used.

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 0007-4888/81/9105-0608$07.50

TABLE 1. Kinetic Characteristics of Hypothalamic TH of Rats with High and Low Alcohol Motivation (M ± m)

Group of animals	K_m for tyrosine ($DMPH_4$ 0.25 mM), in mM	V_{max}, nanomoles dopa/min/mg protein	K_m for $DMPH_4$ (tyrosine 0.16 mM), in mM	V_{max}, nanomoles dopa/min/mg protein
Rats with low alcohol motivation	0,301 ± 0,030	13,43 ± 2,60	0,460 ± 0,095	16,3 ± 6,1
Rats with high alcohol motivation	0,267 ± 0,013	12,64 ± 3,25	0,335 ± 0,031*	12,2 ± 0,6

*$P < 0.05$ for comparison of two groups of animals.

To study the TH response, the brain tissue to be tested was homogenized in 10 volumes of cold 0.05 M K-phosphate buffer, pH 6.0, with 0.2% Triton X-100. The homogenate was centrifuged for 10 min at 15,000g. The supernatant, with a protein concentration of 4.5-5.0 mg/ml, was used for the determination of TH activity; this was done by measuring the increase in dopa by a colorimetric method [5] after incubation of the samples for 15 min in 0.1 M K-phosphate buffer, pH 6.1, at 30°C. The substrate used was L-tyrosine-HCl (from Sigma, USA), and 6,7-dimethyl-5,6,7,8-tetrahydropterine ($DMPH_4$), from Sigma, USA, was used as the coenzyme for the reaction. The Michaelis constants (K_m) and maximal reaction velocity (V_{max}) were calculated from graphs of reaction velocity as a function of substrate and coenzyme concentrations by the method in [7]. Protein was determined by Lowry's method [8].

EXPERIMENTAL RESULTS

The kinetics of the TH reaction in the hypothalamus of rats with different levels of initial alcohol motivation was studied in each animal separately. As Table 1 shows, hypothalamic TH from rats with a high level of initial alcohol motivation had a lower K_m for the coenzyme of the reaction ($DMPH_4$) than hypothalamic TH from rats rejecting alcohol. This evidently may also explain the increase in hypothalamic TH activity observed in rats with a high initial alcohol motivation. The values of V_{max} and K_m for tyrosine and V_{max} for $DMPH_4$ did not differ statistically significantly in the animals of the two groups. The value of K_m is determined, of course, by the whole range of physicochemical properties of the enzyme. Chemical modification of the enzyme, with a change in its quaternary structure or conformational state, leads to a change in the affinity of the enzyme for various ligands, and this is reflected in the value of K_m.

The difference in the values of K_m for $DMPH_4$ in the animals of the two experimental groups thus indicates an initial difference in the properties of TH in the animals with high and low alcohol motivation. Animals with an initially high level of alcohol motivation were distinguished by high activity of TH, which is an important part of the catecholaminergic system. This explains the mechanism of realization of the activating effect of alcohol on the structure of positive reinforcement, discovered in rats predisposed to alcohol consumption [1], and it is confirmed by data in the literature showing that substances which modify activity of the catecholaminergic system have a similar action on alcohol consumption by animals, on the one hand, and on the self-stimulation reaction on the other hand. For instance, there is evidence [11] that the TH inhibitor α-methyltyrosine inhibits the self-stimulation reaction and reduces alcohol intake; 6-hydroxydopamine, which causes degeneration of catecholaminergic neurons, leads to total suppression of the self-stimulation reaction and to a decrease in the alcohol consumption of animals [6, 9, 10].

Comparison of the results of the present experiments with data in the literature thus leads to the conclusion that a definite level of activity of the catecholaminergic system is necessary for the development of a positive emotional state under the influence of alcohol. Analysis of data in the literature [4] shows that the change in K_m for the pterine coenzyme of TH in physiological experiments is usually observed during a change in activity of the presynaptic receptors which regulate the activity of this enzyme through a feedback mechanism. The results suggest that in animals with different levels of initial alcohol motivation there may be differences in the activity of the catecholaminergic system. In animals with high activity of the catecholaminergic system, alcohol consumption causes activation of structures of positive reinforcement and the development of a positive emotional state, and as a result of this, it causes an urge for further consumption of alcohol. In animals with low activity of the catecholaminergic system this does not happen and they refuse to take alcohol.

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EFFECT OF TAURINE ON PROPERTIES OF GUANYLATE CYCLASE OF THE SARCOPLASMIC RETICULUM OF THE HEART

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UDC 612.172.014.21:576.311.33].014.46:547.436

KEY WORDS: sarcoplasmic reticulum; guanylate cyclase; taurine.

The role of taurine in the regulation of cardiac contractile activity is interesting to the cardiologist. Contractile processes in muscle tissue are known to be regulated by the intracellular distribution of calcium ions. There is evidence to suggest that the role of taurine is to control the movement of Ca^{++} in myocardial cells [3, 6]. Outflow of Ca^{++} and its accumulation in the sarcoplasmic reticulum (SPR) of the heart are processes which determine contraction and relaxation of the myocardium.

It has been shown on skeletal and cardiac muscles that with an increase in the taurine concentration the inflow of Ca^{++} into SPR and its binding are increased [4, 7]. In addition to these observations it has been found that in the presence of taurine activity of transport Ca-ATPase in SPR isolated from the rat and dog heart is unchanged [2, 5]; taurine does not affect ATP-dependent Ca^{++} transport [14].

High activity of guanylate cyclase (GC) in the SPR of the heart has recently been reported [11, 15]. The connection between GC activity and Ca^{++} transport in the microsomes of the heart is not yet clear, but it seems that the cyclic guanosine monophosphate (GMP) formed as a result of the guanylate cyclase reaction must influence movement of Ca^{++} in the SPR of the myocardial cells, if it is recalled that cyclic GMP increases the outflow of Ca^{++} from smooth muscle microsomes [1]. It can be tentatively suggested that the effect of taurine mentioned above on the inflow and binding of Ca^{++} in the SPR is effected through GC.

In the present investigation the effect of taurine was studied on GC activity in SPR isolated from the guinea pig heart.

EXPERIMENTAL METHOD

Fresh hearts, washed with physiological saline to remove blood, were homogenized in a Virtis-45 homogenizer (15 sec at 4500 rpm and 15 sec at 71,000 rpm) in isolation medium containing 10% sucrose, 25 mM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 91, No. 5, pp. 555-557, May, 1981. Original article submitted August 21, 1980.

0007-4888/81/9105-0610$07.50