Development of Alcohol Motivation in Rats of a Heterogenous Population

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The indices of ethanol or water preference under conditions of free choice and the duration of ethanol-induced sleep were measured in rats from a heterogenous population before and after compulsory alcoholization. The development of alcohol motivation was shown to be accompanied by the impairment of orosensory recognition of the strength of tested alcohol solutions (5, 10, and 15%) and the appearance of significant positive correlations between the index of preference and the dose of alcohol consumed. The sensitivity to hypnotic effects of ethanol was found to be unstable.

Key Words: *preference, motivation, tolerance, ethanol*

One of the typical features of animals disposed to spontaneous ethanol consumption is weakness of their adaptive behavior, which manifests itself in a low competitiveness in the struggle for biologically significant goals [1]. Ethanol normalizes their adaptive behavior, and thereby these animals develop a pathological inclination to alcohol faster than those which initially rejected ethanol and consumed water. However, our study of the water/alcohol preference in rats belonging to a heterogenous population, which is close to the natural one [3], made it possible to doubt such a simple explanation of biological adaptation to alcohol.

Previously, we have found that rats showing initial preference for 5% ethanol are characterized by a higher utilization of aldehydes in the liver largely attributable to increased activity of aldehyde dehydrogenase with a high K_m. The data on an accelerated utilization of dicarbonic intermediates and a low level of endogenous ethanol allowed us to formulate the concept of "dicarbonic starvation" as a preference-inducing factor [5]. Short alcoholization eliminates the difference between water consumers (WCs) and ethanol consumers (ECs), producing a sort of metabolic correction of this "starvation" by alcohol [6].

We have suggested that these metabolic characteristics constitute the basis for subsequent development of alcohol motivation in ECs. However, in the course of six months' compulsory alcoholization by 15% and 20% ethanol the coefficients of preference in WCs and ECs became equal, and the strategy of biochemical adaptation underwent important qualitative changes. Thus, ECs intensify integrated activity of enzymes involved in aldehyde metabolism in the hepatocyte compartments, while WCs strengthen the interaction between aldehyde dehydrogenases of different cerebral structures.

The revealed peculiarities of metabolic adaptation to alcohol are attributed only to the extreme groups of animals (in terms of the sign of preference) which comprise only 5-10% of the total heterogenous population. These two strategies of adaptive changes reflect only evolutional diversity, while about 80% of the population constitute the rats with an intermediate index of preference (initially, alcohol does not mean anything significant for them). They will apparently adapt themselves to ethanol by a common, conservative route. Therefore, the present study was aimed at investigating the dynamics of the preference and tolerance indices within the whole

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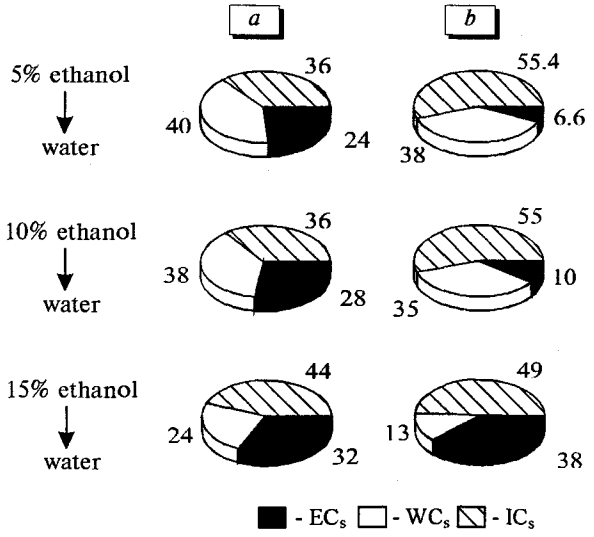


Fig. 1. Distribution of ethanol or water preferring rats (ECs and WCs, respectively) within a heterogenous population under conditions of free choice between ethanol solutions and water (in percent of general sample). ICs=the group of rats showing no preference. Here and in Fig. 2: (*a*) test 1; (*b*) test 2 in a month after alcohol intoxication (15% ethanol solution as a single source of drinking for one month).

population, including both extreme and intermediate groups. Correlation analysis [4] was applied as an additional tool to investigate the relations between the indices.

MATERIALS AND METHODS

Experiments were carried out on 60 outbred male albino rats (purchased from Rappolovo breeding center) with an initial body weight 80 to 120 g. They received a standard laboratory diet. Water or ethanol preference was tested in individual feed-providing cages under conditions of free choice between water and three ethanol solutions (5, 10 and 15%) with a week interval between tests. Each test lasted for 48 h. The coefficient of preference was calculated from the following formula:

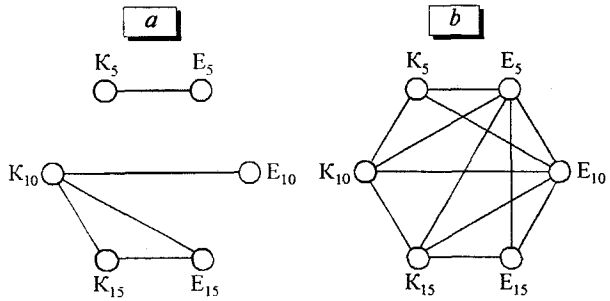


Fig. 2. Correlation ($p<0.01$) between ethanol preference and consumption during the tests. K_5, K_{10}, K_{15}, — the index preference of 5, 10 and 15% ethanol solutions, respectively; E_5, E_{10} and E_{15} — the level of consumption (g/kg body weight per day) of the same solutions.

$$K = \frac{V(\text{ethanol solution}),\ \text{ml}}{V(\text{water}),\ \text{ml} + V(\text{ethanol solution}),\ \text{ml}}.$$

The animals with $K \geq 0.7$ were considered to be ECs, while those with $K \leq 0.2$ were classified as WCs. According to the three ethanol concentrations, the rats were divided into 6 groups: WC_5, WC_{10} and WC_{15} or EC_5, EC_{10} and EC_{15} The rats with the coefficient values ranging from 0.2 to 0.7 were grouped into intermediate consumers (IC). Alcohol consumption during the test was calculated as the amount of absolute alcohol, g/kg body weight, per day.

The level of alcohol tolerance was measured a week after the first test.

Ethanol in a dose of 3.5 g/kg was administered intraperitoneally as a 20% solution (w/v), and the following indices were measured: (1) latency, sec (the time period from the injection to lying down); (2) turnover reaction, sec (the time period from the injection to a loss of spatial orientation; (3) the time of ethanol-induced sleep, min. The animals were classified as short or long sleepers, if the period of sleep was less than 10 min or more than 66 min, respectively.

After these tests, the rats were placed into common cages (10 rats/cage) and kept for a month on a standard laboratory diet with a 15% ethanol solution to drink. The mean ethanol consumption per day was assessed in the middle of this period. Ethanol was replaced by water for the following three weeks which were a "withdrawal period". Repeated testing of preference and tolerance was performed according to the initial protocol.

RESULTS

Distribution of the preference characteristics revealed by the free choice test is presented in Table 1. The data expressed as a percentage of the total number of rats are illustrated by Fig. 1, *a*.

As every rat was labeled, we could follow the changes in preference during the experiment and determine the stability (St) of phenotypic characteristics using the following formula:

$$St = \frac{\text{the number of rats with the same preference}}{\text{the number of rats with inverted preference}}.$$

Primary analysis of the preference characteristics in the heterogenous population showed a comparatively low level of ethanol preference stability when tested with increasing alcohol concentrations: $St_{WC}=31.25$; $St_{EC}=0.66$; $St_{IC}=1.7$.

Repeated tests performed after a month of compulsory 15% ethanol drinking and three weeks of the

TABLE 1. Distribution of the Preference Indices at the First and Second Testing

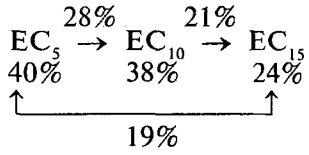
Rats	Test 1 (n=60)			Test 2 (n=53)		
	ethanol concentration, %					
	5	10	15	5	10	15
WCs	4	6	23	13	15	17
ECs	23	21	8	21	20	13
ICs	33	33	29	19	18	23

withdrawal period revealed the indications of alcohol motivation development (Fig. 1, *b*).

The stability of ethanol preference increased twofold as revealed by separate tests with increasing alcohol concentrations: St_{WC}=0.86; St_{EC}=1.4; St_{IC}=0.85.

This process was accompanied by impaired recognition of the solutions' strength, i.e., by a decrease in ethanol sensitivity.

The heterogenous population of rats exhibited more pronounced manifestations of aversive reactions to ethanol as compared to the first test. In the tests with 5% and 10% ethanol solutions the number of WCs increased nearly fourfold (from 6.6 to 24%) and threefold (from 10 to 28%), respectively. However, the test with the 15% solution showed a relatively stable ethanol preference with only a minor (from 38 to 32%) decrease. More than 20 % of the rats developed persistent alcohol motivation and preferred any alcohol solution to water.



By the end of the experiment the number of animals with K=0.5, i.e., indifferent to water or alcohol, increased twofold.

It is necessary to note that the IC rats (according to test 1) represented the principal subject of adaptive modulation, developing either addiction or aversion to alcohol. Both ECs and WCs, as revealed in test 2, included each about 18% of the former ICs. Therefore, in further experiments designed to study the adaptive responses to alcohol (development of alcohol motivation or aversion), it is advisable to use rats with an intermediate initial preference index.

Thus, alcoholization with the following withdrawal modified the preference pattern splitting and stabilizing the phenotypic characteristics of alcohol preference or aversion, and forming the group of rats with persistent alcohol motivation.

Correlation analysis (p<0.01) of the relationship between the preference indices and the dose of ethanol consumed during the first test revealed five positive correlations (Fig. 2). At the first exposure ethanol seemed to be perceived only as a new stimulus, different for different ethanol concentrations (5, 10, and 15%). The second test demonstrated a sharp increase in positive correlations regardless of ethanol concentration (Fig. 2). During the 2.5 months, which elapsed between tests 1 and 2, the body weight in the WC and EC rats increased by nearly the same value (58 and 54 g, respectively). It can be suggested, therefore, that alcohol motivation is favorable for the manifestations of specific pharmacological properties of the consumed alcohol. On this condition ethanol loses the quality of a new situational stimulus, and multiple contacts with it

TABLE 2. The Indices of Tolerance to Alcohol in Rats before and after Ethanol Injection (3.5 g/kg, $M\pm m$)

Index		Groups					
		WCs_5	ECs_5	WCs_{10}	ECs_{10}	WCs_{15}	ECs_{15}
Body weight increase, g	before	157.1±3.9	156.6±9.3	157.0±9.0	149.4±8.8	162.9±7.2	155.0±19.0
	after	217.1±12.9	207.5±11.9	221.0±15.7	206.6±11.3	222.8±12.1	207.5±24.2
Latency, sec	before	180.0±48.9	128.6±35.7	180.0±50.2	142.5±33.9	140.0±45.8	100.0±52.9
	after	240.0±53.9	240.0±37.6	240.0±37.9	195.0±43.5	260.0±42.4	270.0±17.3
Turnover reaction, sec	before	104.3±28.9	115.7±23.4	126.0±39.2	135.5±12.9	71.7±27.5	131.7±32.2
	after	145.7±22.9	115.6±6.2	75.0±17.1	145.6±12.5	98.3±23.5	126.3±8.0
Sleep duration, min	before	15.4±8.3	41.3±12.3	24.0±10.3	38.5±10.9	11.7±6.4	43.3±21.7
	after	56.6±25.6	42.6±17.8	65.2±35.9	66.1±25.4	61.7±23.4	67.7±31.5

trigger the adaptive mechanisms of metabolic and neuronal tolerance. This suggestion is supported by our data on the impaired orosensory recognition of the ethanol solution strength in tests with increasing concentrations combined with an increased rate of aversive responses within the population.

The first testing of hypnotic effects of alcohol showed the following results: 34% of animals did not fall asleep or slept less than 10 min; 32% of rats slept more than 60 min, and the time of sleep of the remaining 34% varied within these limits (intermediate sleepers). The testing repeated in 2.5 months revealed 27% of short sleepers and 29% of long sleepers. It should be noted that the group of short sleepers included $^1/_3$ of rats initially classified as intermediate sleepers. The final group of long sleepers included 43% of the former long sleepers; 37% of the former short sleepers and 20% of the former intermediate sleepers. The same results have been reported for mice [2]. Thus, due to high variability of the duration of ethanol-induced sleep the primary testing gives no way of determining the risk groups or prognostic features for the development of ethanol tolerance or sensitivity as a result of multiple contacts with alcohol.

The groups of ECs and WCs, formed on the basis of the initial preference or aversion to ethanol, included animals with various durations of ethanol-induced sleep; hence, they did not differ by the mean indices of tolerance (Table 2). From phenotypic analysis of preference and tolerance within the given population makes it can be concluded that they independently contribute to the development of alcohol motivation following compulsory alcohol intoxication.

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