Relationship between Alcohol Motivation and Ethanol Sensitivity and Blood and Brain Catecholamine Content in the Progeny of Rats Preferring and Nonpreferring Alcohol

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A system of neurochemical signs indicating predisposition to high ethanol consumption is formed in the third generation descendants of ethanol-preferring male rats. Risk-group males are detected (with 95% probability) by the content of dopamine and norepinephrine and their ratio in the brain. Alcohol motivation depends on blood epinephrine content reflecting individual sensitivity to stress.

Key Words: *ethanol; predisposition; motivation; catecholamines; stress*

Genetically determined predisposition to ethanol consumption plays an important role in the development of alcoholism [2], however, biological mechanisms of inheriting predisposition to ethanol consumption in humans with high genetic risk are not quite clear. Therefore, study of the mechanisms of realization of hereditary predisposition to ethanol consumption in the progeny of high-risk animals remains an important task.

Catecholamine- and, primarily, dopaminergic mechanisms of neurotransmission are directly involved in the mechanisms of hereditary ethanol craving and formation of mental and physical alcohol dependence [9,10]. An important component of the effect of ethanol is stimulation of the release and degradation of catecholamine in brain compartments associated with the limbic system and regulating the emotional status. The severity of disorders associated with these processes and formation of compensatory mechanisms maintaining functional activity of the brain are closely related to catecholamine metabolism [1]. The intensity of catecholamine metabolism can be indirectly evaluated by brain content of dopamine (DA), norepinephrine (NE), and NE/DA ratio [6].

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We studied signs of inheriting predisposition to development of pathological alcohol craving in male rats.

MATERIALS AND METHODS

Experiments were carried out on 2 groups of randombred male rats from intact females mated with males preferring (group 1, n=45) and nonpreferring ethanol (group 2, n=28) in 2 previous generations.

Individual ethanol consumption was evaluated at the age of 2.5-3 months. The animals were placed for 10 days under conditions of free choice between 10% ethanol and water (2-bottle test). Daily ethanol consumption for each animal was expressed as the percentage of fluid drunk over 24 h. This parameter served as the measure of alcohol motivation. Each group of males was ranked by individual levels of alcohol motivation and then divided into subgroups with low, medium, and high level of motivation (up to 33%, 33-66%, and more than 66% ethanol in the total daily volume of fluid, respectively).

One month after the 2-bottle test, individual sensitivity to the hypnotic effect of ethanol was tested by a single intraperitoneal injection of ethanol in a dose of 2 g/kg in 20 males of group 1 and 12 males of group 2. The hypnotic effect was evaluated by the

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duration (minutes) of lateral posture; the absence of lateral posture indicated resistance to the hypnotic effect of ethanol. Other males were injected intraperitoneally with normal saline in the same volume. The rats were decapitated 45 min after injection of ethanol or normal saline, blood concentrations of DA, NE, and epinephrine, and hypothalamic concentrations of DA and NE were measured by high-performance liquid chromatography and spectrofluorometry [5,12].

The compared parameters were expressed in percent of their means in group 2 males. The data were processed by Student's *t* and Fisher's tests, correlation coefficients were calculated, variation series were equalized by the weighed moving average [8], and the type of relationship between the level of alcohol motivation and brain and blood catecholamine contents was determined using multiple correlation-regression analysis [7].

RESULTS

Groups 1 and 2 rats differed by the sensitivity to the hypnotic effect of ethanol. The dependencies of the duration of ethanol-induced sleep on the level of alcohol motivation were opposite in groups 1 and 2.

In males with low motivation (13.7±3.7) the duration of ethanol-induced sleep was 3.3±2.1 min in group 1 and 21.0±4.6 min in group 2 (t=3.50; p<0.01). In animals with medium (47.9±4.1%) and high (84.0±5.3%) level of alcohol motivation the duration of sleep was 13.7± 4.8 and 2.4±1.6 min, respectively. In group 2 males with medium and high level of alcohol motivation the duration of sleep was 6.0±3.7 and 12.0±7.2 min, respectively.

This relationship between the hypnotic effect of ethanol and the level of alcohol motivation in group 1 males is represented by a curve reflecting positive correlation at low motivation level (r=0.65; p<0.05) and negative correlation at high level of motivation (r=-0.79; p<0.01). An opposite trend was observed in group 2 males, but we failed to confirm it by correlation analysis.

Rats of both groups differed significantly by the content of DA in the hypothalamus (355±29 and 657±35 ng/g, respectively (t=6.64; p<0.001), while the con-

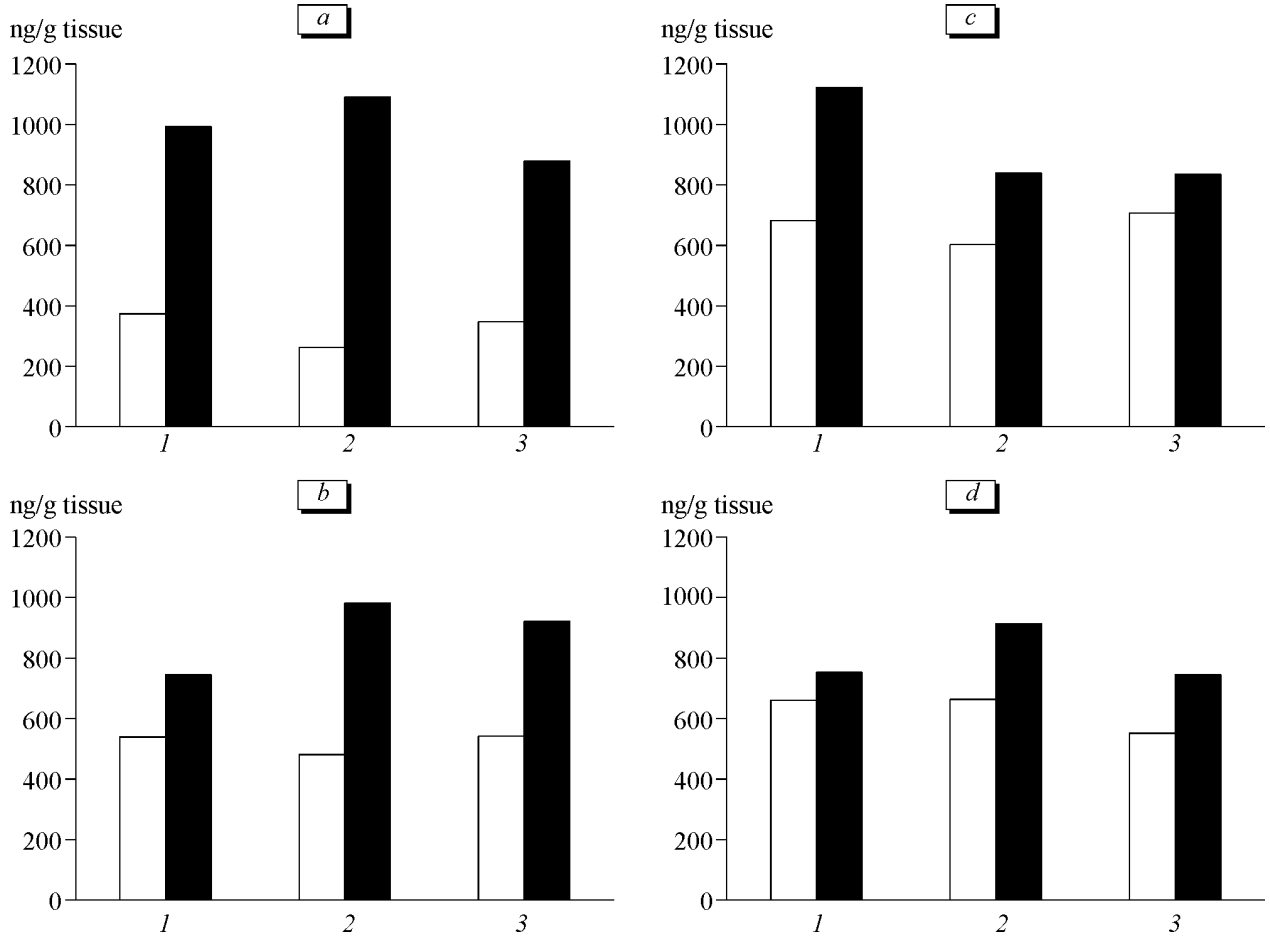

Fig. 1. Content of dopamine (light bars) and norepinephrine (dark bars) in rats with low (*1*), mean (*2*), and high (*3*) levels of alcohol motivation. *a*) males preferring ethanol (group 1); *b*) males nonpreferring ethanol (group 2); *c*, *d*) values in groups 1 and 2, respectively, after a single dose of ethanol (2 g/kg).

tent of NE was almost the same (1019±64 and 986±68 ng/g). An acute hypnotic dose of ethanol increased the content of DA, but not NE in group 1 males (Fig. 1, *a*). On the contrary, in group 2 males acute injection of ethanol did not increase DA content (Fig. 1, *b*).

Since DA content in the hypothalamus of group 1 males is low in comparison with group 2 animals, the mean NE/DA ratio is 2-fold higher in group 1 than in group 2 (3.02±0.25 and 1.51±0.06, respectively, t=5.87, p<0.001), though the contents of NE are high in both groups.

The NE/DA ratio in group 1 males varied depending on the level of alcohol motivation. Increasing motivation from low to medium was associated with an increase of NE/DA ratio from 2.69±0.20 to 4.14±0.13 (t=6.08; p<0.001), but this parameter decreased to 2.55±0.54 with further increase of motivation (t=2.86; p<0.05). For group 1 males the shape of the curve reflecting the relationship between NE/DA ratio and level of alcohol motivation coincided with the curve reflecting the relationship between the level of alcohol motivation and sensitivity to the hypnotic effect of ethanol (Fig. 2, *a*).

In group 2 the relationship between NE/DA ratio and the level of alcohol motivation was linear and did not coincide with the curve reflecting changes in the duration of ethanol-induced sleep (Fig. 2, *b*). The NE/DA ratio in subgroups with low, medium, and high motivation decreased from 1.65±0.13 to 1.52±0.06 and 1.35±0.08. Individual NE/DA values varied in group 2 within ±2.41 standard deviation from the mean, which with 95% certainty distinguishes them from similar parameters in group 1 males.

After ranging individual NE/DA values in groups 1 and 2 only 1 of 22 males was transferred into group 2 instead of group 1, which means that the error did not exceed 5%. Hence, males with hereditary predisposition to ethanol were detected by this parameter.

The relationship between the relative blood content of DA, NE, and epinephrine and the level of alcohol motivation was detected in group 1 males at high (86.6±5.2%) and low (2.9±0.8%) values of motivation. The following multiple regression equations were deduced for this purpose:

$$M=28.71+0.405DA-0.018NE+0.012E$$

for ethanol preferring animals and

$$M=1.49+0.015DA-0.0006NE+0.0004E$$

for animals nonpreferring ethanol, where M is the level of alcohol motivation and E is blood epinephrine content.

A lower percentage of DA and higher content of epinephrine were detected in the blood of ethanol-preferring males of group 1 (Fig. 3). This can serve as a sign of high intensity of metabolic transformations of catecholamines under the effect of ethanol in rats drinking much in comparison with little drinking animals.

Hence, a system of signs of predisposition to develop pathological craving for alcohol, related to the level of alcohol motivation, develops in the 3rd generation males with changed sensitivity to the hypnotic effect of ethanol; this refers these males to the risk group.

It is known that "male" ethanol abuse is rather often inherited from fathers by sons, but not by daughters [3]. Evaluation of the risk for the sons should be based on the data on the relatives in at least two

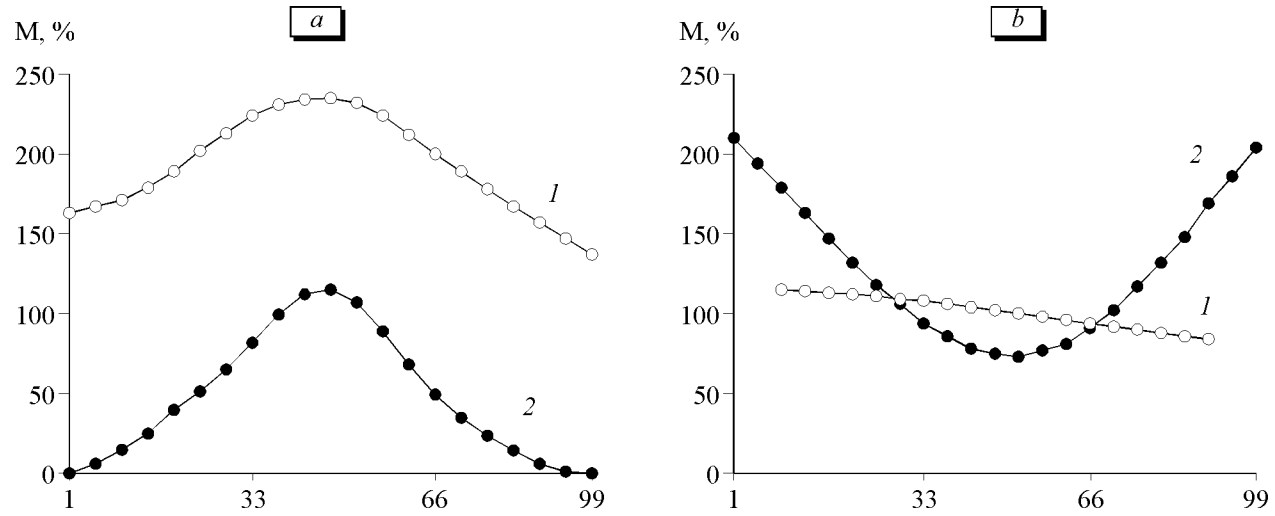

Fig. 2. Relationship between alcohol motivation and NE/DA ratio and rat sensitivity to hypnotic effect of ethanol. Abscissas: individual NE/DA ratios (*1*) and duration of ethanol-induced sleep (*2*) in males of group 1 (*a*) and 2 (*b*) expressed in percent of their means in group 2; ordinates: level of alcohol motivation (M).

previous generations [13]. This prompted us to choose the third generation descending from male albino rats preferring ethanol under conditions of free choice in two previous generations as experimental model of hereditary transfer of pathological craving for ethanol to males.

According to the laws of inheritance, signs of predisposition to ethanol craving are inherited in the third generation only by some individuals. This prompted us to divide the 3rd generation males into 3 subgroups distinctly differing by the quantity of consumed ethanol and correlate alcohol motivation to the parameters characterizing activity of the catecholaminergic mechanisms in the brain.

Identification of signs of predisposition to ethanol craving as a risk factor of alcohol dependence in animal experiments is rather difficult. Behavioral reactions of heterogeneous Wistar rats, including ethanol searching behavior as a sign of predisposition to develop pathological craving for ethanol, were studied as a high-risk marker [11]. This approach provided data on reactivity of various biochemical systems, including catecholaminergic systems of the brain, to acute ethanol loading.

Our results indicate that the system of signs of predisposition to ethanol craving, coupled with the level of alcohol motivation, starts to form as early as in the 3rd generation, at the very beginning of purposeful selection of males preferring ethanol: rat tolerance to the hypnotic effect of ethanol, decreased DA level in the brain, selective reactivity of DA-ergic system to acute ethanol intake, and the NE/DA ratio indirectly reflecting the intensity of catecholamine metabolism in the brain. NE/DA ratio between 2.55± 0.54 and 4.14±0.13 indicates individuals with hereditary predisposition to ethanol craving among the 3rd generation males.

One of the factors promoting the formation of stable ethanol craving is high sensitivity to stress; adaptation to stress directly correlates with catecholamine content [4]. In our experiments high level of epinephrine in the blood of rats drinking much ethanol in the group of animals genetically predisposed to ethanol consumption indicates their emotional tension and high sensitivity to stress.

Some individuals descending from males nonpreferring ethanol in the two previous generations also developed ethanol craving. However these animals did not exhibit the system of signs of predisposition to pathological craving related to the level of alcohol motivation.

These findings suggest that the NE/DA ratio in the brain can be regarded as a potential marker of hereditary predisposition to ethanol consumption when forming risk groups.

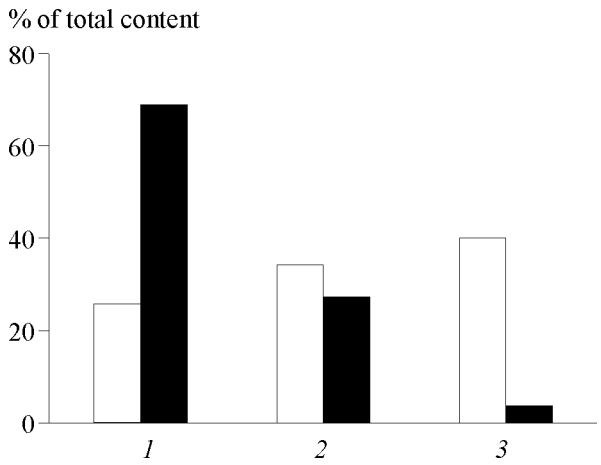

Fig. 3. Relationship between the level of ethanol consumption and blood content of DA (*a*), NE (*2*), and norepinephrine (*3*) in rats preferring (light bars) and nonpreferring ethanol (dark bars).

The realization of hereditary predisposition to ethanol consumption in the risk group individuals depends on environmental factors, specifically on stress sensitivity, which can be evaluated by high epinephrine level in the blood.

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