Effect of Monoamine Oxidase A Knockout on Resistance to Long-Term Exposure to Ethanol

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 694-696, June, 2002
Original article submitted March 29, 2002

It was shown that transgenic Tg8 mice with monoamine oxidase A (MAO A) gene knockout demonstrate higher resistance to acute ethanol exposure compared to wild type C3H mice. This difference was observed at the early age (28-30 days). Long-term ethanol treatment changed the resistance to hypnotic, but not hypothermic action of this agent. Seven-day exposure increased the resistance to ethanol-induced narcotic sleep in Tg8 and C3H mice. After 30-day ethanol treatment the duration of narcotic sleep sharply decreased in C3H mice and increased in Tg8 mice, which attested to their decreased tolerance to ethanol.

Key Words: *monoamine oxidase A gene knockout; ethanol tolerance*

It was demonstrated that the central serotoninergic system is involved in the mechanisms of alcohol dependence and tolerance [4]. Moreover, the important role of genotype in the sensitivity to ethanol was established [3]. However, peculiarities of the metabolism and reception in the serotoninergic system determining predisposition and tolerance to alcohol were not studied. Animal models with altered serotoninergic system are useful in this respect.

Monoamine oxidase A (MAO A) knockout Tg8 mice [2] were obtained in 1995 in Curie Institute (France). These mice have no MAO A, the enzyme involved in serotonin and catecholamine metabolism. It should be noted that norepinephrine and dopamine catabolism is catalyzed by MAO A, catechol-O-methyltransferase, and MAO B, while serotonin is primarily catabolized via oxidative deamination catalyzed by MAO A. Tg8 mice are characterized by increased brain content of MAO A substrates, serotonin, norepinephrine, and to a lesser extent, dopamine, and decreased level of their metabolites [2,6].

We previously showed that Tg8 mice demonstrated increased resistance to ethanol [1,7]. However, their sensitivity to long-term ethanol exposure and their sensitivity to ethanol in the postnatal period were not studied. It is well known that long-term ethanol intake changes organism's resistance to this agent. Ethanol resistance also varies in the postnatal ontogeny [9] due to maturation of various neurotransmitter systems [10]. There are data on considerable changes in dopamine and serotonin content and their metabolism in Tg8 mice during ontogeny [2]. The aim of the present study was examination of the effect of MAO A gene knockout on ethanol resistance during its application for different time periods, as well as age-dependent changes in ethanol resistance.

MATERIALS AND METHODS

Experiments were carried out on male Tg8 mice with MAO A gene knockout and parental C3H/He (C3H) mice. All animals were kept under standard vivarium conditions at the Institute of Cytology and Genetics at natural light-dark regimen with free access to water and food. The experiments were carried out in September with 2 age groups: 28-30 days (11-13 g) and 2.5-3.0 months (20-25 g).

For evaluation of the tolerance to narcotic effects of ethanol the mice were intraperitoneally injected with 20% ethanol (5 g/kg). Sleep duration was determined by the recovery of turning over reflex. Rectal temperature

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0007-4888/02/1336-0603$27.00

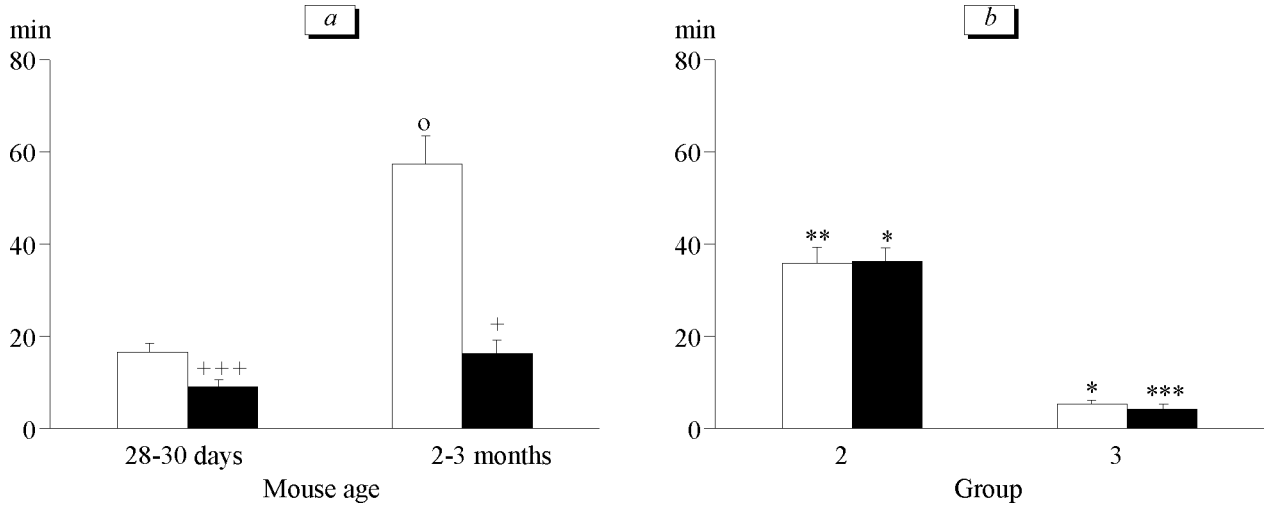

Fig. 1. Sleep duration in intact (*a*) and alcohol-treated (*b*) C3H (open bars) and Tg8 (dark bars) mice. Here and in Fig. 2: °p<0.001 compared to 28-30-day-old mice; *p<0.001, **p<0.01, ***p<0.05 compared to intact mice; ^{+}p<0.001, ^{++}p<0.01, ^{+++}p<0.05 compared to C3H mice.

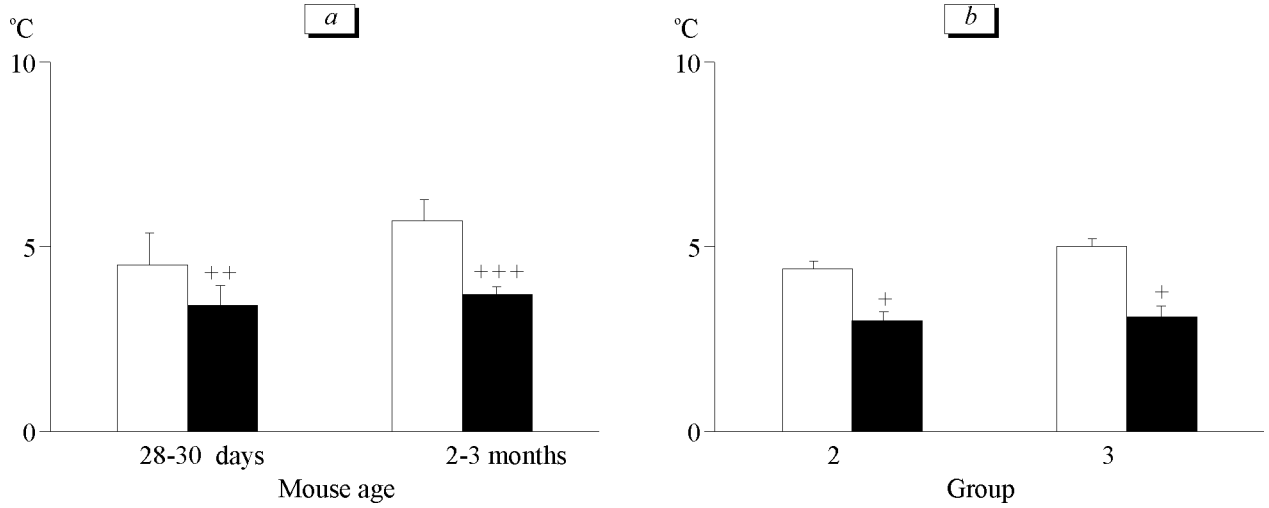

Fig. 2. Maximum decrease in rectal temperature in intact (*a*) and alcohol-treated (*b*) C3H (open bars) and Tg8 (dark bars) mice.

was measured using an electronic thermometer with 20-min intervals for 1 h after alcohol administration.

In group 1 mice, the initial ethanol sensitivity was studied. Group 2 mice had free access to 10% alcohol for 1 month, while group 3 animals received intraperitoneal injections of 20% ethanol for 7 days. Sensitivity to ethanol was assessed by ethanol-induced hypothermia and sleep duration 1 month after exposure to 10% ethanol (group 2) and on day 8 after daily injections (group 3).

Statistical processing of the results was performed by ANOVA test using Origin 5.0 and Statistica 5.5 software.

RESULTS

Both 30-day exposure to ethanol and daily injections for 1 week changed ethanol sensitivity in both mouse strains. It was found that 7- and 30-day ethanol treatment had no effect on ethanol-induced hypothermia in both mouse strain, but changed the duration of narcotic sleep. A pronounced effect of MAO A gene knockout was demonstrated (Fig. 1, 2). Seven-day ethanol treatment 6- and 4-fold decreased the duration of narcotic sleep in C3H (p<0.001, $F_{1.18}$=62.09) and Tg8 (p<0.05, $F_{1.18}$=5.55) mice, respectively, compared to the initial values. Thirty-day treatment significantly decreased sleep duration in C3H mice (p<0.001, $F_{1.18}$=10.68) and increased it in Tg8 mice (p<0.001, $F_{1.18}$=20.87). Increased ethanol resistance in C3H mice can be explained by altered content of biogenic amines in the brain. It was demonstrated that ethanol exposure decreased the content of norepinephrine and dopamine in some brain regions [8]; neurotoxins damaging the catecholamine system decelerate, while serotoninergic neurotoxin 5,7-hydroxytryptamine accelerate the de-

velopment of tolerance to hypnotic effect of alcohol [5]. Different effects of long-term alcohol treatment on ethanol resistance in Tg8 and C3H mice confirm the important role of catecholamines and serotonin in the mechanisms of alcohol tolerance. Decreased resistance to the hypnotic effect of ethanol after long-term treatment in Tg8 mice (in contrast to enhanced resistance in C3H mice) can be explained by impaired metabolism of biogenic amines.

Tg8 mice demonstrated attenuated reaction to ethanol as early as on days 28-30 of life. Ethanol resistance decreased during ontogeny in control mice, which agrees with published data [9,10], whereas in Tg8 mice it did not change.

Thus, MAO A gene knockout mice demonstrate not only increased resistance to acute ethanol exposure, but also altered age dynamics of alcohol tolerance. These data indicate that the development of ethanol resistance is mediated by the system of biogenic amines of the brain.

The study was supported by the Russian Foundation for Basic Research (grant No. 02-04-50018).

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