COMPARISON OF BRAIN BIOGENIC AMINE LEVEL AND BLOOD ETHANOL KINETICS IN C57Bl/6 AND CBA MICE

N. A. Khodorova, N. V. Vlasova, and A. I. Maiskii

UDC 616.83-008.441.13-055.5/.7-092.9-092:616.831.41.-008.94:577.175.82

KEY WORDS: ethanol; pharmacokinetics; blood; serotonin; dopamine; noradrenalin; brain; CBA and C57Bl/6 mice.

Experiments on noninbred male albino rats have shown that animals predisposed to the development of experimental alcoholism are characterized by a high serotonin level in the hypothalamus and brain stem and also by a high rate of ethanol elimination from the blood. Meanwhile animals not predisposed to develop alcoholism have a low serotonin level in their hypothalamus and brain stem and a low rate of ethanol elimination from the blood [1-5]. It was therefore interesting to study whether predisposition to the development of alcoholism is a genetically determined trait.

To study this problem the pharmacokinetics of ethanol removal from the blood and the concentrations of biogenic amines in parts of the brain were determined in C57Bl/6 and CBA inbred mice, characterized by predisposition and absence of predisposition to the development of experimental alcoholism respectively [6, 10, 11].

EXPERIMENTAL METHOD

Experiments were carried out on male inbred C57Bl/6 and CBA mice weighing 20-25 g. The concentrations of serotonin, noradrenalin, and dopamine were determined in parts of the brain: cerebral cortex, hypothalamus, thalamus, and brain stem – by the method in [8, 9], using a spectrofluorometer from Opton (West Germany). The results were subjected to statistical analysis by Student's test [7]. Ethanol kinetics were studied 5, 15, 30, and 90 min after intraperitoneal injection of a test dose (0.1 g/kg) of 25% ethanol solution. Blood (50 μl) was taken from the caudal vein. The ethanol concentration was measured by gas–liquid vapor-phase analysis with a flame-ionization detector, as described previously. The pharmacokinetic parameters were calculated by computer, using a single-component model, analyzing the process of ethanol absorption.

EXPERIMENTAL RESULTS

The results indicate considerable differences in the distribution of biogenic amines among brain structures of intact mice of the standard inbred lines C57Bl/6 and CBA (Table 1). In C57Bl/6 mice the serotonin level in the hypothalamus was 38.7% higher and in the brain stem 22.4% higher than in CBA mice, but in the thalamus it was 21% lower and in the cerebral cortex 12.5% lower; the noradrenalin level was 36.9% higher in the hypothalamus, 36.6% higher in the thalamus, and 20.4% higher in the brain stem than in CBA mice. No significant differences were found between the dopamine concentrations in the brain structures tested in the two lines of mice.

Comparison of the ethanol kinetics in the blood of the two inbred lines of mice revealed differences between them in the rate of absorption and excretion of alcohol (Table 2, Fig. 1). In C57Bl/6 mice the maximal concentration (C_{max}) was reached by the 15th minute (10.3 μmole/ml), whereas in CBA mice it was reached by the 30th minute (10.4 μmole/ml) of the experiment. These data show differences in absorption of ethanol between the two lines, confirmed by values of the absorption constant (K_a): in CBA mice it was twice as high as in C57BL/6 mice ($P < 0.01$). The inbred lines also differed significantly in their constant of alcohol elimination (K_e), which was twice as high in C57Bl/6 mice, and was combined in these animals with high ethanol clearance. This is confirmed by values obtained for the half-elimination period ($T_{1/2}$), which was only half as long for

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. 98, No. 9, pp. 284-285, September, 1984. Original article submitted November 17, 1983.

0007-4888/84/9809-1183$08.50

TABLE 1. Concentrations of Biogenic Amines in Parts of Brain (in μg/g tissue) of C57Bl/6 and CBA Mice

Part of brain	C57Bl/6 mice			CBA mice		
	serotonin	noradrenalin	dopamine	serotonin	noradrenalin	dopamine
Hypothalamus	1,54±0,04	0,89±0,02	1,08±0,03	1,02±0,04*	0,65±0,04*	1,02±0,03
Thalamus	0,65±0,02	0,56±0,01	0,96±0,02	0,72±0,009*	0,41±0,01*	0,96±0,009
Brain stem	0,60±0,009	0,63±0,003	0,79±0,007	0,44±0,008*	0,53±0,009*	0,80±0,01
Cerebral cortex	0,28±0,01	0,27±0,01	0,96±0,02	0,24±0,009*	0,31±0,02	0,97±0,02

Legend. *P < 0.001; here and in Table 2 significance of differences calculated between values for C57Bl/6 and CBA mice.

TABLE 2. Pharmacokinetic Parameters in Blood of Standard Inbred C57Bl/6 and CBA Mice

Genetic line of mice	(K_e)	(K_a)	C_{max}, μmoles/ml	$T_{1/2}$, h^{-1}
C57Bl/6	0,4±0,04***	4,8±0,1**	8,4±0,6	1,7±0,1*
CBA	0,19±0,01	2,2±1,6	10,4±0,7	3,5±1,4

Legend. *P < 0.06, **P < 0.01, ***P < 0.001.

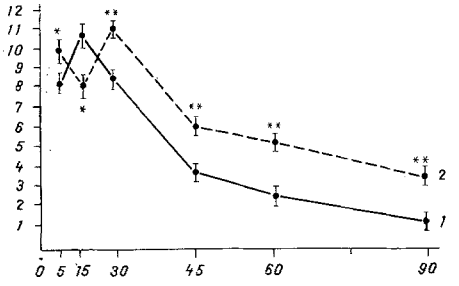

Fig. 1. Blood ethanol concentration as a function of time for standard inbred lines of mice C57Bl/6 (1) and CBA (2). Abscissa, time (in min); ordinate, ethanol concentration (in μmoles/ml). *P < 0.05, **P < 0.01.

C57Bl/6 mice (1.7 h^{-1}) as in CBA mice (3.5 h^{-1}). The data on the pharmacokinetic parameters indicate that C57Bl/6 mice have a higher rate of ethanol elimination from the blood than CBA mice.

The results confirm the principle discovered previously with rats: Animals with a high serotonin level in the hypothalamus and brain stem and with a high rate of elimination of ethanol from the blood are predisposed to the development of experimental alcoholism [1, 4, 5]. This rule, observed in inbred C57Bl/6 mice, is genetically determined [6, 10, 11].

At the same time, it will be noted that animals with a low serotonin level in the hypothalamus and brain stem, and with a reduced rate of ethanol elimination from the blood, as is observed in CBA mice and as was found previously in rats, have no predisposition to the development of experimental alcoholism [5].

LITERATURE CITED

1. S. A. Borisenko, N. S. Tolmacheva, and N. A. Khodorova, in: Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), p. 80.
2. Yu. V. Burov, Vest. Akad. Med. Nauk SSSR, No. 5, 72 (1982).
3. Yu. V. Burov, T. I. Absava, A. B. Kampov-Polevoi, et al., Farmakol. Toksikol., No. 1, 50 (1981).

4. N. V. Vlasova, in: The Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), p. 119.
5. V. N. Zhukov, N. A. Khodorova, and Yu. V. Burov, Byull. Éksp. Biol. Med., No. 7, 35 (1982).
6. A. I. Maiskii and S. V. Shoshina, in: Pharmacology of Experimental Alcoholism [in Russian], Moscow (1982), p. 28.
7. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
8. G. B. Ansell and M. F. Beeson, Anal. Biochem., 23, 196 (1968).
9. R. H. Cox and J. H. Perhach, J. Neurochem., 215, 1770 (1973).
10. A. K. S. Ho and C. S. Tsai, Pharmacol. Biochem. Behav., 3, 1073 (1975).
11. T. Koivula, M. Koivusalo, and K. O. Lindros, Biochem. Pharmacol., 24, 1807 (1975).

HIGH SENSITIVITY OF MITOCHONRDIA IN THE RAT SMALL INTESTINAL MUCOSA TO ADRENALIN

A. M. Babskii and I. V. Shostakovskaya

UDC 612.355.014.21.014.46:615.357.452:577.175.522

KEY WORDS: mitochondria; small intestinal mucosa; oxidative phosphorylation; calcium capacity; adrenalin.

Most research into the effect of adrenalin on mitochondrial metabolism has been undertaken on mitochondria of the liver, which constitute a convenient and stable preparation [3, 5, 8, 15]. Functional characteristics of mitochondria of other, more reactive tissues and the effect of regulating factors on them have received much less study. One of the most reactive tissues is the small intestinal mucosa (SIM), which is sensitive to changes in the sympathicoadrenal system [6]. Probably on account of its increased reactivity, it is difficult to isolate mitochondria from this tissue [1].

In the investigation described below this difficulty was overcome by using new techniques to isolate mitochondria and to work with them. In this way the action of adrenalin on mitochondria of SIM and also on more native liver mitochondria could be studied.

EXPERIMENTAL METHOD

During work with the animals and isolation of mitochondria from the liver and SIM, a combination of conditions was adopted whereby organelles with properties closest to native could be obtained [1, 9]. Experiments were carried out on male Wistar rats weighing 200-220 g. The final dilution of the suspension during keeping was 70-80 mg protein/ml for liver mitochondria and 30-40 mg protein/ml for SIM mitochondria.

The effect of adrenalin on energy metabolism was assessed by pH-metric recording of oxidative phosphorylation [4] and of Ca^{++} uptake in mitochondria [13]. The incubation medium for the liver mitochondria (26°C) consisted of 4-5 mg mitochondrial protein in 1 ml, 150 mM sucrose, 50 mM KCl, 1 mM KH_2PO_4, and 3 mM Tris-HCl, pH 7.4; for SIM mitochondria (28°C) it consisted of 2-2.5 mg mitochondrial protein in 1 ml, 250 mM sucrose, 40 mM KCl, 3 mM KH_2PO_4, and 5 mM Tris-HCl, pH 7.4. Succinate (6 mM) was used as oxidation substrate. The pH of the incubation medium was recorded with an LPU-01 pH-meter and EZ-2 automatic writer. The action of adrenalin on the rate of phosphorylation of added ADP [14], the rate of uptake of Ca^{++}, and the calcium capacity (CC) of the mitochondria was studied [13]. Protein was determined by Lowry's method. Adrenalin was injected intraperitoneally 15 and 30 min and 3 h before sacrifice of the animals, in a dose of 5 μg or 25 μg per 100 g body weight. The β-adrenoblocker inderal, in a dose of 1 mg/100 g body weight, was injected intraperitoneally 30 min before injection of the hormone. Control animals received an injection of an equal volume of 0.9% NaCl solution at the corresponding times.

The results were subjected to statistical analysis by the comparison of pairs method [2].

Laboratory of Functional Biophysics of Mitochondria, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino. Department of Human and Animal Physiology, Ivan Franko L'vov University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 9, pp. 286-288, September, 1984. Original article submitted November 25, 1983.

0007-4888/84/9809-1185$08.50