Elevated Basal Activity of Tryptophan Oxygenase in Alcohol-Preferring C57Bl Mice

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Tryptophan oxygenase activity in alcohol-preferring C57Bl mice and control CBA and DBA/2 mice was studied under nonstressful conditions and after glucocorticoid-induced stress. Elevated basal tryptophan oxygenase activity in C57Bl mice is probably responsible for reduced brain content of tryptophan and serotonin associated with alcohol preference.

Key Words: *tryptophan oxygenase; C57Bl mice*

It has been noted that genetically determined differences in brain serotonin content are associated with predisposition to various behavioral abnormalities such as alcoholism, drug abuse, aggressive behavior, Tourette syndrome, and others [2]. Brain serotonin is synthesized from tryptophan, and oscillations in the blood level of tryptophan are also associated with many above-listed diseases [3]. Blood content of tryptophan is largely determined by liver-specific enzyme tryptophan oxygenase (TDO_2) catalyzing catabolic degradation of 90% dietary tryptophan. Even minor oscillations in TDO_2 activity exert a considerable effect on the rate of serotonin synthesis and, hence, its brain content.

C57Bl mice represent an animal model of predisposition to alcoholism [6]. This strain is characterized by elevated TDO_2 activity [4] and reduced level of brain serotonin [1,8]. It is suggested that the increased TDO_2 activity in these mice is related to chronic elevation of glucocorticoids under conditions of group housing, which results in permanent glucocorticoid induction of TDO_2 gene [1]. At the same time, it cannot be excluded that the decreased level of blood tryptophan and brain serotonin in C57Bl mice originates from constitutive overexpression of TDO_2 gene.

The aim of this study was to determine TDO_2 activity in the liver of alcohol-preferring C57Bl mice and control CBA and DBA/2 mice under nonstressful conditions [1].

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MATERIALS AND METHODS

Experiments were carried out on 12 adult C57Bl, CBA, and DBA/2 mice (20-25 g) fed a standard laboratory diet. Five days before decapitation the animals were housed individually to exclude stress induced by group housing. Some animals received intraperitoneal dexamethasone (3 μg) 1 h before decapitation to induce glucocorticoid stress. After decapitation, blood was collected and stabilized with 50 μl 0.5 M sodium citrate, the liver was perfused with 1.15% KCl. Liver samples (250-280 mg) were homogenized in a glass homogenizer with 10 volumes of cold 0.05 M phosphate buffer (pH=7.0). The homogenates were centrifuged at 2000*g* and 0°C for 20 min and the supernatant was used for enzyme assay.

TDO_2 activity was determined as described earlier [7] with some modifications. The supernatant (1 ml) and reaction mixture (0.8 ml) containing 2.25 mg/ml L-tryptophan and 0.375 mg/ml horse methemoglobin in 0.05 M phosphate buffer (pH=7.0) were intensively shaken for 5 min for oxygenation and incubated for 45 min at 37°C. The reaction was stopped with 0.5 ml 12.5% trichloroacetic acid, the mixture was centrifuged, pH was adjusted to 7.0 with 1 M NaOH, and the concentration of kynurenine was determined spectrophotometrically at 365 nm. From these data TDO_2 activity was calculated and expressed in nmol/mg protein/h.

Tyrosine aminotransferase (TAT) activity was measured as previously described [4]. A reaction mixture (1.6 ml) containing 7 mM L-tyrosine, 12 mM α-keto-

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TABLE 1. TDO_2 and TAT Activities and Blood Content of Glucocorticoids in Different Mouse Strains ($M \pm m$)

Index	C57Bl	CBA	DBA/2
Control level of corticosterone, μg %	0.93±0.22	0.90±0.28	1.03±0.29
TDO_2 activity, nmol/mg protein/h			
control	21.9±2.3	13.6±1.2**	15.5±0.6*
glucocorticoid induction	62.5±5.3	56.2±3.1	44.3±4.5
TAT activity, mmol/g protein/h			
control	4.50±0.37	4.60±0.21	5.00±0.38
glucocorticoid induction	11.70±0.79	16.90±0.90	14.30±0.51

Note. *$p<0.01$, **$p<0.05$ in comparison with C57Bl.

glutarate, and 60 μM pyridoxal 5'-phosphate in 0.125 M phosphate buffer (pH=7.6) and 200 μl homogenate were incubated for 15-min, the reaction was stopped with 140 μl 10 M KOH and after 30 min the mixture was centrifuged at 2000*g* for 20 min. Optical density of the supernatant was measured at 331 nm, TAT activity was calculated and expressed in mmol/g protein/h.

The blood content of corticosterone was determined by competitive protein binding [9]. Both standard and test samples were deproteinized at 70°C for 10 min to remove endogenous transcortin. [1,2,6,7-^{3}H]-Corticosterone (Amersham) and steroid-free rat plasma as a source of transcortin were added to the samples. Unbound hormone was removed by centrifugation dextran-coated charcoal. The supernatant was transferred to scintillation vials with dioxan scintillator and radioactivity was measuring. Hormone content was calculated from the calibration curve.

The data were processed statistically using Student's *t* test.

RESULTS

Unlike CBA and DBA/2, C57Bl mice are characterized by predisposition to alcoholism, which manifests itself in intense ethanol consumption under conditions of free choice between 8% ethanol and water and in a 4-5-fold increase in ethanol intake after its intravenous injection [6]. This strain is also characterized by low levels of brain serotonin and blood tryptophan and high TDO_2 activity, which is considered to be the main factor determining C57Bl mice predisposition to alcoholism [1]. It has been previously suggested that the observed characteristics result from high sensitivity to stress and permanent glucocorticoid induction of TDO_2 [1]. Therefore, we studied TDO_2 activity under conditions excluding stress and measured blood content of glucocorticoid hormones. CBA and DBA/2 mice without genetic predisposition to alcoholism served as the control.

Under nonstressful conditions we found no significant differences in blood corticosterone between animals of different strains (Table 1). However, TDO_2 activity in the liver of C57BL mice was significantly higher than in CBA and DBA/2 mice. Under these conditions the three mouse strains showed no significant difference in TAT activity, which is also a liver-specific enzyme regulated by glucocorticoids [9].

Thus, the increased activity of TDO_2 and related decrease in brain serotonin are constitutive characteristics of C57Bl mice rather than the result of stress. Since in all mouse strains TDO_2 has similar amino acid sequence (AMLB; ID MM24493; AC U224493), the observed difference could be most reasonably explained by increased expression of TDO_2 gene, probably due to peculiar organization of TDO_2 regulatory sites in C57Bl mice.

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