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Acetate-Dependent Mechanisms of Inborn Tolerance to Ethanol

Sergey M. Zimatkin[1,*], Nikolay A. Oganesian[1], Yury V. Kiselevski[1] and Richard A. Deitrich[2]

[1]Grodno State Medical University, 80 Gorkogo Street, Grodno 230015, Belarus and [2]Department of Pharmacology, University of Colorado, Denver, CO, USA

*Corresponding author: Tel.: +375-152-742492; Fax: +375-152-335341; E-mail: zimatkin@grsmu.by

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Abstract — **Aims:** To clarify the role of acetate in neurochemical mechanisms of the initial (inborn) tolerance to ethanol. **Methods:** Rats with low and high inborn tolerance to hypnotic effect of ethanol were used. In the brain region homogenates (frontal and parietal cortex, hypothalamus, striatum, medulla oblongata) and brain cortex synaptosomes, the levels of acetate, acetyl-CoA, acetylcholine (AcH), the activity of pyruvate dehydrogenase (PDG) and acetyl-CoA synthetase were examined. **Results:** It has been found that brain cortex of rats with high tolerance to hypnotic effect of ethanol have higher level of acetate and activity of acetyl-CoA synthetase, but lower level of acetyl-CoA and activity of PDG. In brain cortex synaptosomes of tolerant rats, the pyruvate oxidation rate as well as the content of acetyl-CoA and AcH synthesis were lower when compared with intolerant animals. The addition of acetate into the medium significantly increased the AcH synthesis in synaptosomes of tolerant, but not of intolerant animals. Calcium ions stimulated the AcH release from synaptosomes twice as high in tolerant as in intolerant animals. Acetate eliminated the stimulating effect of calcium ions upon the release of AcH in synaptosomes of intolerant rats, but not in tolerant animals. As a result, the quantum release of AcH from synaptosomes in the presence of acetate was 6.5 times higher in tolerant when compared with intolerant rats. **Conclusion:** The brain cortex of rats with high inborn tolerance to hypnotic effect of ethanol can better utilize acetate for the acetyl-CoA and AcH synthesis, as well as being resistant to inhibitory effect of acetate to calcium-stimulated release of AcH. It indicates the metabolic and cholinergic mechanisms of the initial tolerance to ethanol.

INTRODUCTION

Animals and human have a large variability in their tolerance to ethyl alcohol. This depends mainly on the differential individual sensitivity of their CNS to ethanol (Draski and Deitrich, 1996). One of the most commonly used indicators of the behavioral response to high ethanol doses in animals is the sedative or hypnotic effect of ethanol. The hypnotic effect often referred to as 'sleep' is measured by the duration of loss of the righting reflex after a test dose of ethanol (Deitrich, 1993).

The mechanisms of inborn tolerance to hypnotic effect of alcohol are not completely understood. Ethanol potentiates the gamma-aminobutyric acid (GABA) agonist-dependent increase in Cl^- influx in cortical and cerebellar membranes only in animals with low tolerance to hypnotic effect of ethanol (high alcohol sensitive (HAS) rats and LS mice) when compared with low sensitive lines (low alcohol sensitive (LAS) rats and SS mice) (Allan *et al.*, 1991). It supports the putative role of the sensitivity of the GABA-operated Cl^- channels in the hypnotic effect of ethanol. It was found by electrophysiology that Purkinje neurons of cerebellum of HAS rats and LS mice are more sensitive to depressant effect of ethanol, when compared with LAS rats and SS mice (Palmer *et al.*, 1987). The mouse and rat lines have no differences in the peripheral ethanol metabolism or the whole brain aldehyde dehydrogenase (ALDH) activity. However, Purkinje neurons of cerebellum of LAS rats and SS mice have higher ALDH activity, when compared with HAS rats and LS mice, thus implicating acetaldehyde or acetate in the hypnotic effects of ethanol (Zimatkin and Deitrich, 1995). Significant differences in ALDH activity were found earlier in specific brain structures of AT and ANT rats genetically differing in sensitivity to alcohol-induced motor incoordination (Zimatkin and Lindros, 1989). In the previous investigations, we found also the lower ethanol oxidation rate and accumulation of ethanol-derived acetaldehyde in brain structures of animals with higher inborn tolerance to hypnotic effect of ethanol (Zimatkin *et al.*, 2001a, b).

The second metabolite of ethanol (acetate) can also mediate some behavioral effects of alcohol (Brundege and Dunwiddie, 1995; Kiselevski *et al.*, 2003), especially its rate-suppressing effects, such as sedation, ataxia or motor slowing (Arizzi *et al.*, 2003; Correa *et al.*, 2003). Acetate easily penetrates through the blood–brain barrier from the periphery and is metabolized in brain (Subramanian and Mead, 1971). It has been shown that rat brain sections can utilize the radioactively labeled acetate for acetylcholine (AcH) synthesis (Dolezal and Tucek, 1981). But acetate, normally, is a minor precursor of acetyl residue for AcH biosynthesis in brain slices and synaptosomes when compared with glucose and acetylcarnitine (Dolezal and Tucek, 1981; Lefresne *et al.*, 1977).

The brain acetate can be derived from the peripheral metabolism of ethanol or produced directly in the brain during oxidation of ethanol (acetaldehyde) *in situ* (Zimatkin *et al.*, 2006). In addition, there are several sources of endogenous acetate in brain tissues. It can get into the brain from the periphery as it is produced by the anaerobic bacteria in the gut as well as in the course of oxidation of endogenous acetaldehyde in the body (Ostrovsky, 1986). The main sources of the local acetate in the brain are: oxidation of endogenous acetaldehyde by ALDH, hydrolysis of AcH by cholinesterase and acetyl-CoA by acetyl-CoA hydrolase (Quraishi and Cook, 1972).

Acetyl-CoA is the integral substrate for the basic and energy metabolism as well as a donor of acetyl groups for AcH synthesis in a brain (Tucek, 1983). Activity of pyruvate dehydrogenase (PDG), rate of pyruvate utilization and synthesis of acetyl-CoA as well as transport of acetyl-CoA into cytosol serve as the limiting steps in formation of AcH (Szutowicz and Bielarczyk, 1991). Oxidative decarboxylation

of pyruvate in PDG reaction is known to be the main source of acetyl-CoA in brain tissue. The additional, minor source of acetyl-CoA in a brain is its formation from acetate in acetyl-CoA synthetase reaction. Acetate-derived acetyl-CoA is the main point of entrance of two-carbonic residue of ethanol into a basic metabolism. Acetate serves as a linkage between the ethanol metabolism and cholinergic neurotransmitter system (Carroll, 1997), which is one of the main component of tolerance to alcohol (Nevo and Hamon, 1995).

As it is known, glucose is the main substrate for energy metabolism in the brain. Acute ethanol and acetate administration in low doses decrease the glucose uptake and metabolism in rat brain, especially in brain cortex (Pawlosky *et al.*, 2010). Acute ethanol administration even in low doses decreases glucose utilization in the human brain. The absence of cognitive performance in those experiments is explained by the possible shift in the substrate for energy metabolism from glucose to acetate (Volkow *et al.*, 2006).

Adenosine is one of the best known modulators of cholinergic activity (Dunwiddie and Masino, 2001). The stimulation of A_1 receptors inhibits, whereas stimulating of A_2 receptors activates cholinergic neurotransmission (Dunwiddie and Fredholm, 1997). The effect of adenosine as an inhibiting neuromodulator is implemented through the A_1-receptors which are localized on the presynaptic membrane. These receptors are known to be excessively present on axonal projections of cholinergic neurons in rat brain cortex (Olah and Stiles, 1995).

It has been demonstrated that acetate increase the level of adenosine in blood plasma and other tissues of the body, including brain (Puig and Fox, 1984). We have found that acetate significantly (2-fold) increases the release of adenosine from rat brain cortex synaptosomes (Kiselevski *et al.*, 2003). One of the reasons for that is the increase of adenosine production in the course of acetate activation in acetyl-CoA synthetase reaction (Yamamoto *et al.*, 2005). The inhibitory effect of adenosine upon the cholinergic neurotransmission has been well documented and is known to be a physiologic one (Bouron, 2001; Materi *et al.*, 2000).

The sensitivity of mice with genetically high tolerance to hypnotic effect of ethanol (SS) to agonists of A_1 receptors is much lower when compared with LS mice (Proctor *et al.*, 1985; Smolen and Smolen, 1991). It can be explained by the lower density of A_1 receptors in the brain of SS mice (Fredholm *et al.*, 1985).

We have suggested that acetate participates in the central mechanisms of inborn tolerance to hypnotic effect of alcohol through the specificities of the initial and ethanol-induced acetate-related basic and AcH metabolism and cholinergic activity in the brain cortex.

MATERIALS AND METHODS

Animals, chemicals and experimental design

Male Wistar rats were obtained from the breeding colony of the Grodno State Medical University. A total of 90 Wistar rats (140–180 g) were tested for the differences in sensitivity to hypnotic effect of ethanol. Twenty percents of ethanol was administered in the dose of 3.5 g/kg, intraperitoneally (i.p.), and the duration of alcohol-induced sleep was measured by the interval from the loss of righting response to its recovery. The animals were divided into Wistar short-sleeping (wSS, duration of sleep was 55 ± 11 min) and Wistar long-sleeping (wLS, duration of sleep was 220 ± 8 min) groups. Two weeks after the testing, seven rats from each group received intraperitoneal injection of 20% ethanol solution (3.5 g/kg) 1 h before the decapitation; another seven animals from each group received an injection of 0.85% solution of NaCl (served as control). One hour after the injections, rats were decapitated, their brains were quickly excised and brain regions (frontal and parietal cortex, hypothalamus, striatum, medulla oblongata) separated, frozen and stored in liquid nitrogen before the examination. All experimental procedures complied with European Community Council Directive (86/609/EEC) for care and use of laboratory animals. Protocols were reviewed and approved by the Ethical Committee of the Grodno State Medical University.

The brain samples from 10 rats of the genetically selected lines with different inborn sensitivity to the hypnotic effect of ethanol (LAS and HAS) were obtained from the University of Colorado Alcohol Research Center, frozen in liquid nitrogen and shipped to Grodno (Medical University) in dry ice for the examination.

All chemicals were obtained from Sigma-Aldrich.

Biochemical assay

The concentration of acetate in homogenates of brain regions was determined by gas chromatography (HP 6890) (Giles *et al.*, 1986); the level of acetyl-CoA was measured spectrophotometrically (Szutowicz and Bielarczyk, 1987) as well as activity of PDG and acetyl-CoA synthetase (Szutowicz *et al.*, 1981).

Synaptosomes were isolated from the homogenates of brain cortex of another six wSS and six wLS rats immediately after dissection. The synaptosomal fraction from cerebral cortex was isolated by differential centrifugation and flotation in Ficoll and Saccharose gradient (Szutowicz *et al.*, 1977). The synaptosomes were suspended in 320 mM buffered sucrose. Synaptosomal suspension, containing 2.5–3 mg of protein, incubated for 30 min at 37°C was shaken at 100 cycles/min in depolarizing medium containing a final volume of 2.0 ml: 20 mM Na-HEPES, 1.5 mM Na phosphate (final pH 7.4), 90 mM NaCl, 30 mM KCl, 2.5 mM Na-pyruvate (or 2.5 mM K-acetate), 2.5 mM Na-*L*-malate, 0.01 mM choline chloride, 0.01 mM eserine sulfate, 320 mM sucrose and 2.5–3.0 mg of synaptosomal protein. The basal release (proportional to the synthesis) of AcH was measured in the presence of pyruvate and malate, the stimulated release of Ach in the presence of 1.0 mM $CaCl_2$. Quantal AcH release was calculated as stimulated minus basal release. Enzymatic technique was applied to examine the level of pyruvate and acetyl-CoA in deproteinized samples (Szutowicz and Bielarczyk, 1987). The AcH was determined by luminometry (LKB Pharmacia) (Ricny *et al.*, 1986).

Statistics

The data are presented as mean ± SD. One-way analysis of variance (for comparison of groups) and Pearson correlation were used; *post hoc* comparisons were performed using Scheffe test. The differences were considered significant at

Table 1. Concentration of acetate in the brain regions of rats with different inborn tolerance to hypnotic effect of ethanol before and 1 h after the acute alcohol administration

Groups	Acetate (mmol/g of tissue)				
	Frontal cortex	Parietal cortex	Hypothalamus	Striatum	Medulla oblongata
wLS	0.40 ± 0.07	0.42 ± 0.02	0.24 ± 0.05	0.48 ± 0.07	0.39 ± 0.08
wSS	0.62 ± 0.08*	0.45 ± 0.08	0.24 ± 0.03	0.52 ± 0.06	0.36 ± 0.05
wLS + Eth	0.72 ± 0.07†††	0.81 ± 0.11†††	0.47 ± 0.15†	0.85 ± 0.21†††	0.83 ± 0.25††
wSS + Eth	1.0 ± 0.15$^{\dagger\dagger\dagger x}$	0.92 ± 0.09†††	0.48 ± 0.08††	0.88 ± 0.07††	0.86 ± 0.19††

Data are mean ± standard deviation; $n = 7$. wLS, long sleeping rats; wSS, short sleeping rats; +Eth, 60 min after intraperitoneal administration of 3.5 g/kg of ethanol.
*$P < 0.05$ when compared with wLS rats.
$^{\dagger}P < 0.05$.
$^{\dagger\dagger}P < 0.001$.
$^{\dagger\dagger\dagger}P < 0.01$.when compared with the corresponding group without ethanol.
$^{x}P < 0.05$ when compared with wLS +ethanol rats (Scheffe test).

Table 2. Concentration of acetyl-CoA in the brain regions of rats with different inborn tolerance to hypnotic effect of ethanol

Groups	Acetyl-CoA (nmol/g of tissue)				
	Frontal cortex	Parietal cortex	Hypothalamus	Striatum	Medulla oblongata
wLS	98.8 ± 19.0	98.4 ± 17.2	69.8 ± 5.6	83.1 ± 19.1	75.1 ± 14.3
wSS	75.9 ± 9.0*	80.3 ± 9.3	76.6 ± 6.9	79.0 ± 8.3	76.2 ± 12.1
wLS+Eth	84.1 ± 15.8	98.8 ± 15.5	63.7 ± 14.2	73.3 ± 10.9	111.1 ± 16.6†††
wSS+Eth	101.3 ± 8.3	116.2 ± 9.7	73.6 ± 20.2	72.9 ± 19.6	96.9 ± 7.2

Data are mean ± standard deviation; $n = 7$. wLS, long sleeping rats; wSS, short sleeping rats; +Eth, 60 min after intraperitoneal administration of 3.5 g/kg of ethanol.
*$P < 0.05$ when compared with wLS rats.
$^{\dagger\dagger\dagger}P < 0.001$ when compared with the corresponding group without ethanol (Scheffe test).

$P < 0.05$. Computer program STATISTICA 6.0 was used in this study.

RESULTS

A heterogeneous distribution of endogenous acetate in the brain regions of wLS ($F = 10.5$; $P < 0.001$) and wSS ($F = 30.6$; $P < 0.001$) rats was found. Hypothalamus has the lowest acetate level in both groups of animals. wSS rats have higher basal level of acetate in frontal brain cortex when compared with wLS rats ($F = 21.8$; $P < 0.001$; Table 1). Acute administration of ethanol resulted in practically doubling of acetate levels in all brain regions studied in both groups of animals ($F = 15.3$–93.4; $P < 0.01$), but the acetate level in frontal cortex of wSS rats still be a higher when compared with wLS animals ($F = 30.8$; $P < 0.001$; Table 1).

The basal concentration of acetyl-CoA (the integral substrate for basic metabolism and the main donor of acetyl groups for AcH synthesis) was specifically lower in frontal cortex of wSS rats when compared with wLS animals ($F = 7.1$; $P < 0.05$; Table 2). Acute ethanol administration increased the concentration of acetyl-CoA in brain frontal and parietal cortex of wSS ($F = 25.5$; $P < 0.001$ and $F = 48.9$; $P < 0.001$, respectively), but not in wLS rats. As a result, it eliminates the differences between groups (Table 2). On the contrary, ethanol increased the acetyl-CoA level in medulla oblongata of wLS ($F = 20.9$; $P < 0.001$; Table 2).

The activity of PDG, in the brain cortex of wSS rats was lower when compared with wLS rats ($F = 18.05$; $P < 0.001$; Table 3). On the contrary, the activity of acetyl-CoA synthetase (the alternative source of acetyl-CoA) in brain cortex of wSS and LAS rats was higher when compared with wLS and HAS animals, respectively ($F = 10.1$; $P < 0.01$ and $F = 11.3$; $P < 0.01$, respectively) (Table 3).

Table 3. Activity of PDG and acetyl-CoA in brain cortex of rats with different inborn tolerance to hypnotic effect of ethanol

Groups	N	Enzyme activities (nmol/min/mg of protein)	
		PDG	Acetyl-CoA synthetase
wLS	14	26.3 ± 1.4	3.3 ± 0.2
wSS		18.8 ± 0.8*	4.2 ± 0.2*
HAS	10	28.5 ± 2.6	2.8 ± 0.3
LAS		20.7 ± 3.5	5.1 ± 0.6[a]

Data are mean ± standard deviation.
*$P < 0.05$ when compared with wSS rats.
[a]$P < 0.01$ when compared with HAS rats (Scheffe test).

Similar to the homogenates, in brain cortex synaptosomes of wSS rats the pyruvate oxidative rate was lower when compared with wLS animals (10.8 ± 2.7–16.6 ± 7.03; $F = 4.5$; $P < 0.05$; according to Scheffe test $P < 0.001$) and the level of acetyl-CoA in wSS was lower when compared with wLS animals (23.3 ± 3.7–34.4 ± 5.3; $F = 23.5$; $P < 0.001$; according to Scheffe test $P < 0.001$).

The basal (total) release (proportional to the synthesis) of AcH from the brain cortex synaptosomes in wSS rats was 2.5 times as lower as in wLS rats ($F = 32.5$; $P < 0.001$; Table 4). Calcium ions stimulate the AcH release from synaptosomes of wLS (60%); $F = 14.1$; $P < 0.01$) and wSS

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The basal (total) release (proportional to the synthesis) of AcH from the brain cortex synaptosomes in wSS rats was 2.5 times as lower as in wLS rats ($F = 32.5$; $P < 0.001$; Table 4). Calcium ions stimulate the AcH release from synaptosomes of wLS (60%); $F = 14.1$; $P < 0.01$) and wSS

Table 4. The release of AcH from brain cortex synaptosomes in rats with different inborn tolerance to hypnotic effect of ethanol

Condition of incubation	Groups	
	wLS	wSS
	Basal (total) AcH release (pmol/min/mg protein)	
2.5 mmol of pyruvate + 2.5 mmol of malate	15.8 ± 1.62	6.2 ± 0.43**
2.5 mmol of pyruvate + 2.5 mmol of malate + 1 mmol Ca^{+2}	25.3 ± 1.9††	13.6 ± 2.4**†
2.5 mM of pyruvate + 2.5 mM of malate + 2.5 mM of acetate	13.4 ± 2.1	10.1 ± 2.3#
2.5 mM of pyruvate + 2.5 mM of malate + 2.5 mM of acetate + 1 mM Ca^{+2}	15.03 ± 1.1	20.95 ± 2.1*#
	Quantal AcH release (pmol/min/mg protein)	
2.5 mmol of pyruvare + 2.5 mmol of malate	9.5 ± 1.07	7.4 ± 2.3
2.5 mM of pyruvare + 2.5 mM of malate + 2.5 mM of acetate	1.63 ± 1.3#	10.8 ± 0.47**

Data are mean ± standard deviation; $n = 6$.
*$P < 0.05$.
**$P < 0.01$, when compared with wLS rats.
†$P < 0.05$.
††$P < 0.01$ when compared with animals of the corresponding group without addition of calcium ions to the medium.
#$P < 0.05$ when compared with animals of the corresponding group without addition of acetate to the medium (Scheffe test).

rats (119%) $F = 14.1$ and 8.8, respectively; $P < 0.01$). Consequently, the quantum release of AcH (Ca-stimulated minus basal levels) from brain cortex synaptosomes in wSS and wLS rats became quite similar (Table 4).

The addition of acetate into the incubation medium increased the synthesis of AcH by synaptosomes of wSS ($F = 5.7$; $P < 0.05$), but did not change it in wLS rats. As a result, it eliminated the differences in basal AcH release (synthesis) in brain cortex synaptosomes of wSS and wLS rats (Table 4). In the presence of acetate, calcium ions still significantly stimulated the synthesis of AcH in wSS ($F = 12.0$; $P < 0.05$), but not in wLS rats. As a result, the stimulated synthesis of AcH in wSS became even higher than in wLS rats (Table 4). Consequently, the quantal release of AcH from brain cortex synaptosomes in wSS rats slightly increased, but significantly decreased in wLS animals ($F = 10.5$; $P < 0.05$). As a result, in the presence of acetate a quantum release of AcH from synaptosomes in wSS rats become 6.5 times as high as in wLS animals ($F = 26.4$; $P < 0.01$; Table 4).

DISCUSSION

We have found the heterogeneous distribution of the endogenous (basal) acetate in brain regions. It supports the idea of the local origin (at least partially) of acetate in the brain. The higher basal level of acetate in frontal cortex of wSS rats can be explained by the inborn specificities of acetaldehyde and acetate metabolism, in particular, higher activity of ALDH in the brain cortex structures when compared with wLS (Zimatkin, 2008). The cause of alcohol-induced increase of acetate concentration in the brain can be its transport with the blood from the periphery, mainly from the liver, the major ethanol-oxidative organ, as well as the local oxidation in the brain itself (Zimatkin *et al.*, 2006).

We have found significantly lower level of acetyl-CoA in brain cortex of wSS, when compared with wLS rats. Acute ethanol administration increased the concentration of acetyl-CoA in brain cortex of wSS, but not of wLS rats and eliminated the comparative deficiency of acetyl-oA in brain cortex of wSS rats. It indicates the inborn acetyl-CoA deficiency in the brain cortex of wSS rats and its compensation from ethanol-derived acetate. Acetate-derived acetyl-CoA is the main point of entrance of two-carbonic residue of ethanol into a basic metabolism. Probably wSS rat brains can utilize acetate for acetyl-CoA production better then wLS and it may be one of the reasons of their higher tolerance to hypnotic effect of ethanol.

Oxidation of pyruvate is the main source of acetyl-CoA in brain tissue. It is confirmed by high positive correlation between PDG activity and acetyl-CoA content in rat brain regions in our animals ($r = 0.845$; $P = 0.002$). wSS rats are characterized by the initially low activity of PDG when compared with wLS rats. It can be the reason of the initial comparative deficiency of acetyl-CoA in brain cortex of wSS rats. The similar decreased activity of PDG has been found in the brain cortex of LAS when compared with HAS rats.

On the contrary, both wSS and LAS rats have higher initial activity of acetyl-CoA synthetase in the brain cortex. But that enzyme can be the alternative source of acetyl-CoA. In our experiments with acute alcohol administration, the % of increase of acetyl-CoA level in the brain regions positively correlated with the activity of acetyl-CoA synthetase, which catalyses the acetyl-CoA synthesis directly from acetate. Therefore, the higher initial activity of acetyl-CoA synthetase in wSS rat's brain cortex can be the reason of the higher increase of acetyl-CoA after an acute alcohol administration in these animals. Therefore, the acute alcohol administration eliminates the initial comparative deficiency of acetyl-CoA in brain cortex of wSS rats. It seems to be that the wSS rats are better adapted to utilize acetate instead of pyruvate for acetyl-CoA formation for general and energy metabolism in brain cortex, when compared with wLS rats.

The data obtained in our study demonstrate a reduced capability to utilize pyruvate and much lower level of acetyl-CoA in wSS rat brain cortex synaptosomes. This indicates a less efficient system of synthesis of acetyl-CoA from pyruvate, and, as a result, the lower initial AcH synthesis in wSS rats.

Our data demonstrate that the addition of acetate to the incubation medium of wLS brain cortex synaptosomes inhibits both the basal and calcium-stimulated release of AcH. On the contrary, in wSS rats, acetate significantly activates these processes. This indicates that in wSS rats acetyl-CoA,

formed from acetate, can be a source of acetyl residues for biosynthesis of AcH under the conditions of insufficiency of the major, PDG, pathway.

It is known that acute ethanol administration even in low doses decreases glucose utilization in the brain. The absence of cognitive performance in those experiments is explained by the possible shift in the substrate of energy metabolism from glucose to acetate (Volkow *et al.*, 2006). Our data have confirmed that suggestion and explain possible neurochemical mechanisms of that phenomenon.

The different response of AcH synaptosomal release to calcium in the brain cortex in wSS and wLS rats in the presence of acetate can be mediated by adenosine, which is one of the best known modulators of cholinergic activity (Dunwiddie and Masino, 2001). The stimulation of A_1 receptors inhibits, while stimulating of A_2 receptors activates cholinergic neurotransmission (Dunwiddie and Fredholm, 1997). Effect of adenosine as an inhibiting neuromodulator is implemented through the A_1-receptors that are localized on the presynaptic membrane. These receptors are known to be excessively present on axonal projections of cholinergic neurons in rat brain cortex (Olah and Stiles, 1995).

It has been demonstrated that acetate increases the level of adenosine in blood plasma and in other tissues of the body, including brain (Puig and Fox, 1984). We have found that acetate significantly (2-fold) increases the release of adenosine from rat brain cortex synaptosomes (Kiselevski *et al.*, 2003). One of the reasons for that is the increase of adenosine production in the course of acetate activation in acetyl-CoA synthetase reaction (Yamamoto *et al.*, 2005). Therefore, it may be suggested that the addition of acetate into the incubation medium can increase the level of extrasynaptosomal adenosine, which by means of modulating effect in wLS rats upon A_1 receptors in the synaptosomes, leads to inhibition of calcium-stimulated release of AcH. The inhibitory effect of adenosine upon the cholinergic neurotransmission has been well documented and is known to be a physiologic one (Bouron, 2001; Materi *et al.*, 2000).

The sensitivity of mice with genetically high tolerance to hypnotic effect of ethanol (SS) to agonists of A_1 receptors is much lower when compared with LS mice (Proctor *et al.*, 1985; Smolen and Smolen, 1991). It can be explained by the lower density of A_1 receptors in the brain of SS rats (Fredholm *et al.*, 1985). It also may be the reason of the lower initial inhibitory effect of acetate in wSS rats, but it has not been proved yet.

CONCLUSIONS

The wSS rats with high inborn tolerance to hypnotic effect of ethanol have higher level of acetate and a lower level of acetyl-CoA in brain cortex when compared with wLS rats with lower tolerance to ethanol. Acute ethanol administration increases the acetate concentration in all brain regions in both wSS and wLS rats and eliminates the comparative deficiency of acetyl-CoA in brain cortex of wSS rats.

The activity of PDG in a brain cortex of rats with high inborn tolerance to hypnotic effect of ethanol was lower when compared with rats with low tolerance. There were the opposite differences in activity of acetyl-CoA synthetase.

wSS rat brain cortex synaptosomes have initially lower PDG activity, acetyl-CoA and AcH levels. In addition, synaptosomes of wSS rats more actively use the acetyl group of acetate in synthesis of AcH. As a result, acetate eliminates the deficiency of acetyl-CoA and AcH in wSS rat brain cortex synaptosomes, when compared with wLS rats. It seems that wSS rats are better adapted to utilize acetate instead of pyruvate for basic and energy metabolism and AcH synthesis in brain cortex, when compared with wLS rats. It can be the first, metabolic, mechanisms of inborn tolerance to ethanol.

Synaptosomes of wSS rats are more sensitive to stimulating effect of calcium to AcH release when compared with wLS. Consequently, the quantum release of AcH (Ca-stimulated minus basal release) from brain cortex synaptosomes in wSS and wLS rats is quite similar.

The addition of acetate into the incubation medium modulates a calcium-stimulated synaptosomal release of AcH in wSS and wLS rats in opposite directions. Acetate increases the stimulating effect of calcium to the quantal AcH release in wSS, but not in wLS rats. As a result, in the presence of acetate a quantum release of AcH from synaptosomes in wSS rats became 6.5 times as high as in wLS rats. This can be another, cholinergic, mechanism of the inborn tolerance to ethanol.

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REFERENCES

Allan AM, Mayes GG, Draski LJ. (1991) Gamma-aminobutyric acid activated chloride channels in rats selectively bred for differential acute sensitivity to alcohol. *Alc Clin Exp Res* **15**:212–18.

Arizzi M, Correa M, Betz AJ *et al.* (2003) Behavioral effects of intraventricular injections of low doses of ethanol, acetaldehyde and acetate in rats; studies with low and high rate operant schedules. *Behav Brain Res* **471**:203–10.

Bouron A. (2001) Modulation of spontaneous quantal release of neurotransmitters in the hippocampus. *Prog Neurobiol l* **63**:613–35.

Brundege JM, Dunwiddie TV. (1995) The role of acetate as a potential mediator of the effects of ethanol in the brain. *Neurosci Lett* **186**:214–18.

Carroll P. (1997) Evidence to suggest that extracellular acetate is accumulated by rat hippocampal cholinergic nerve terminals for acetylcholine formation and release. *Brain Res* **753**:47–55.

Correa M, Arizzi MN, Betz A *et al.* (2003) Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res Bull* **62**:197–202.

Deitrich RA. (1993) Selective breeding for initial sensitivity to ethanol. *Behav Genet* **23**:153–62.

Dolezal V, Tucek S. (1981) Utilization of citrate, acetylcarnitin, acetate, pyruvate and glucose for the synthesis of acetylcholine in rat brain slices. J *Neurochem* **36**:1323–30.

Draski LJ, Deitrich RA. (1996) Initial effects of ethanol on the central nervous system. In Deitrich RA, Erwin WG (eds). *Pharmacological Effects of Ethanol on the Nervous System*. Boca Ratton: CRC Press, 227–50.

Dunwiddie TV, Fredholm BB. (1997) Adenosine neuromodulation. In Jacobson KA, Jarvis MF (eds). *Purinergic Approaches in Experimental Therapeutics*. New York: Wiley Liss, 359–82.

Dunwiddie TV, Masino SA. (2001) The role and regulation of adenosine in the central nervous system. *Annu Rev Neurosci* **24**:31–55.

Fredholm BB, Zahniser NR, Weiner GR *et al.* (1985) Behavioral sensitivity to PIA in selectively bred mice is related a number of A1 adenosine receptors but not to cyclic AMP accumulation in brain slices. *Eur J Pharmacol* **111**:133–6.

Giles HG, Meggiorini S, Vidins EI. (1986) Semiautomatic analysis of ethanol and acetate in human plasma by head spice gas chromatography. *Fisiol Pharmacol* **64**:717–9.

Kiselevski Y, Oganesian N, Zimatkin S *et al.* (2003) Brain acetate metabolism in mechanisms of adaptation to ethanol. *Med Sci Monit* **9**:178–82.

Lefresne P, Hamon M, Beaujouan JC *et al.* (1977) Origin of the acetyl moiety of acetylcholine synthesized in rat striatal synaptosomes. *Biochimie* **59**:197–215.

Materi LM, Rasmuson DD, Semba K. (2000) Inhibition of synaptically evoked cortical acetylcholine release by adenosine: an *in vivo* microdialysis study in the rat. *Neuroscience* **97**:219–26.

Nevo L, Hamon M. (1995) Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. *Neurochem Int* **26**:305–36.

Olah ME, Stiles GL. (1995) Adenosine receptor subtypes: characterization and therapeutic regulation. *Annu Rev Pharmacol Toxicol* **35**:581–606.

Ostrovsky YM. (1986) Endogenous ethanol—its metabolic, behavioral and biomedical significance. *Alcohol* **4**:239–47.

Palmer MR, Wang Y, Fossom LH *et al.* (1987) Genetic correlation of ethanol-induced ataxia and cerebellar Purkinje neuron depression among inbred strains and selected lines of rats. *Alcohol Clin Exp Res* **11**:494–501.

Pawlosky RJ, Kashiwaya Y, Srivastava S *et al.* (2010) Alterations in brain glucose utilization accompanying elevations in blood ethanol and acetate concentrations in the rat. *Alcohol Clin Exp Res* **34**:375–81.

Proctor WR, Baker RC, Dunwiddie TV. (1985) Differential CNS sensitivity to PIA and theophylline in long-sleep and short-sleep mice. *Alcohol* **2**:287–91.

Puig JG, Fox IH. (1984) Ethanol-induced activation of adenosine nucleotide turnover. Evidence for a role of acetate. *Clin Invest* **74**:936–41.

Quraishi S, Cook RM. (1972) Utilization of fatty acid in remnants. IV. Relative activities of acetyl CoA synthetase and acetyl CoA hydrolase in mitochondria and intracellular localization of acetyl CoA synthetase. *Agric Food Chem* **20**:91–5.

Ricny J, Coupek J, Tucek S. (1986) Determination of acetylcholine and choline by flow-injection with immobilized enzymes and fluorimetric or luminometric detection. *Analyt Biochem* **176**: 221–7.

Smolen TN, Smolen A. (1991) Purinergic modulation of ethanol-induced sleep time in long-sleep and short-sleep mice. *Alcohol* **8**:123–30.

Subramanian C, Mead GF. (1971) Rapid uptake of 1-14C-acetate by the adult rat brain 15 seconds after carotid injection. *Biochim Biophis Acta* **248**:41–7.

Szutowicz A, Bielarczyk H. (1991) Relationships between pyruvate oxidation, Acetyl-CoA compartmentalization and acetylcholine synthesis in nerve terminals. In Bisswanger H, Ulrich J (eds). *Biochemistry and Physiology of Thiamine Diphosphate Enzymes*. Weinheim: VCH Verlagsgesellshaft.

Szutowicz A, Lysiak W, Angelski S. (1977) The effect of *l-l* hydroxycitrate on piruvate metabolism in rat brain synaptosomes. J *Neurochem* **29**:375–8.

Szutowicz A, Stepien M, Piec G. (1981) Determination of pyruvate dehydrogenase and acetyl-CoA synthetase activities using citrat-synthetase. *Analyt Biochem* **115**:81–7.

Szutowicz A, Bielarczyk H. (1987) Elimination of CoASH interference from acetyl-CoA cycling assay by maleic anhydride. *Anal Biochem* **164**:292–4.

Tucek S. (1983) Acetylcoenzyme-A and the synthesis of acetylylcholine in neurons: review of recent progress. *Gen Physiol Byophys* **2**:313–24.

Volkow ND, Wang GJ, Franceschi D *et al.* (2006) Low doses of alcohol substantially decrease glucose metabolism in the human brain. *Neuroimage* **29**:295–301.

Yamamoto T, Moriwaki Y, Takahashi S. (2005) Effect of ethanol on metabolism of purine bases (hypoxanthine, xanthine, and uric acid). *Clinica Chimica Acta* **356**:35–57.

Zimatkin SM. (2008) *Brain Aldehyde Dehydrogenases and Their Role in Alcoholism Pathogenesis: Research Monograph*. Grodno: GGMU (in Russian), 314.

Zimatkin S, Deitrich R. (1995) Aldehyde dehydrogenase activities in the brains of rats and mice genetically selected for different sensitivity to alcohol. *Alcohol Clin Exp Res* **19**:1300–6.

Zimatkin SM, Lindros KO. (1989) A histochemical study of the distribution of aldehyde dehydrogenase activity in brain structures of rats with genetically different alcohol-related behavior. *Alcohol* **6**:321–5.

Zimatkin SM, Liopo AV, Slychenkov VS *et al.* (2001a) Relationship of brain ethanol metabolism to the hypnotic effect of ethanol. I: Studies in outbred animals. *Alcohol Clin Exp Res* **25**:976–81.

Zimatkin SM, Liopo AV, Satanovskaya VI *et al.* (2001b) Relationship of brain ethanol metabolism to the hypnotic effect of ethanol. II: Studies in selectively bred rats and mice. *Alcohol Clin Exp Res* **25**:982–8.

Zimatkin SM, Pronko SP, Vasiliou V *et al.* (2006) Enzymatic mechanisms of ethanol oxidation in the brain. *Alcohol Clin Exp Res* **30**:1500–5.