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Acetate metabolism in brain mechanisms of adaptation to ethanol

Yuri Kiselevski[1], Nicholas Oganesian[1], Sergey Zimatkin[1], Andrzej Szutowicz[2], Stefan Angielski[2], Pavel Niezabitowski[3], Wojciech Uracz[3], Ryszard Gryglewski[3]

[1] Department of Clinical Biochemistry, Grodno State Medical University, Grodno, Belarus
[2] Department of Clinical Biochemistry, Gdansk Medical University, Gdansk, Poland
[3] Department of Pharmacology, College of Medicine, Jagiellonian University, Cracow, Poland

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Summary

Background: The aim of this study was to estimate the role of acetate-induced metabolic changes in brain mechanisms of resistance to the narcotic effect of ethanol.

Material/Methods: Wistar rats were treated daily with ethanol (3.5 g/kg i.p. for 7 days). During alcohol treatment, the duration of ethanol-induced sleep was decreased. Levels of acetate, acetyl-CoA, adenosine, and the pool of AMP+ADP, and the activity of acetyl-CoA synthetase and 5'-nucleotidase in the brain were measured. Synaptosomal adenosine and acetylcholine release were measured in the presence of acetate, adenosine and 2-chloroadenosine.

Results: The concentration of acetate was higher in all investigated brain regions of ethanol rats in comparison to controls. The activity of acetyl-CoA synthetase and 5'-nucleotidase, as well as the levels of adenosine and the pool of AMP+ADP were raised in cerebral cortex of ethanol rats. The synaptosomal level of adenosine was higher in control rats. Acetate causet a 3-fold increase of extrasynaptosomal adenosine level. Synaptosomes from ethanol rats showed higher rates of Ca-dependent releases of acetylcholine. 2-chloradenosine resulted in inhibition of synaptic acetylcholine release only in control rats.

Conclusions: Ethanol causes an increase of acetate in brain and adenosine level in cerebral cortex. Acetate causes an increase of the extrasynaptic adenosine level. Prolonged ethanol treatment results in an increase of synaptic Ca-dependent acetylcholine release. Seven-day treatment with ethanol eliminates the inhibitory effect of 2-chloroadenosine – A1 adenosine receptor agonist on synaptic Ca-dependent acetylcholine release.

key words: **resistance to ethanol • acetate metabolism • adenosine • acetylcholine release**

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Author's address: Dr. Yuri Kiselevski, PhD, MD, Gorki Str, 80, Grodno Medical University, Grodno, 230015, Belarus, email: kiselevyu@yahoo.com

BACKGROUND

Several studies have suggested that acetate may affect the CNS during ethanol consumption via an adenosine-mediated mechanism [1,2]. This has been proven by the correlation between ethanol-induced changes in the characteristics of adenosine receptors, adenosine uptake and release in the brain and certain physiological effects of ethanol and acetate [3].

Acetate is a short-chain fatty acid formed in the liver and released into the general circulation following ethanol injection. The plasma acetate level increases after ethanol injection in humans and animals [3]. Acetate readily crosses the blood-brain barrier and is actively metabolized in the brain [4]. The main entry point for acetate into metabolism in vertebrates is its conversion to acetyl-CoA by acetyl-CoA synthetase (EC 6.2.1.1.) This reaction utilizes adenosine triphosphate (ATP) and yields adenosine 3.5-monophosphate (AMP) [5]. The acetyl-CoA which is formed from acetate can be used for energy generation via TCA; on the other hand, acetyl-CoA is utilized in the brain's cholinergic neurons for acetylcholine (ACh) synthesis. It has been shown that extracellular acetate is accumulated by cholinergic nerve terminals for ACh formation and release [6]. ACh release in the cortex is associated with both behavioral activation and cortical activation, as determined by electroencephalography (EEG) [7]. The rate of efflux of ACh in the cortex is greatest during periods of desynchronized, high-frequency EEG activity, such as that observed during waking and paradoxical sleep, and the lowest during slow-wave sleep. [8]. Factors that modulate the activity of cholinergic neurons could, therefore, alter cortical Ach release and have a major effect on the cortical arousal and behavioral state. Purine nucleoside adenosine is thought to be a modulator of neuronal activity, including cholinergic neurotransmission [9]. In addition, pharmacological experiments suggest that adenosine is a sleep factor, since systemic or intracerebroventricular injections promote sleep and decrease wakefulness [10]. Several studies have shown that some of the central depressant effect of ethanol can be inhibited by adenosine receptor blockers [1]. This confirms the role of adenosine in the central depressant actions of ethanol. Considering the fact that ethanol consumption induces an increase in the adenosine level, it is possible to suppose that the hypnotic effect of ethanol is mediated by adenosine.

The aim of this study was to estimate the role of brain acetate-induced metabolic changes in mechanisms of resistance to the narcotic effect of ethanol.
In order to achieve this aim, we attempted to estimate:
1) the effect of chronic ethanol injection on brain acetate and adenosine levels;
2) the effect of acetate on synaptic adenosine production and release;
3) the effect of adenosine and its receptor agonist on synaptic acetylcholine release in cerebral cortex of alcohol-treated rats.

MATERIAL AND METHODS

Male Wistar rats weighing 100–120 g were used in all studies. The animals were fed standard chow and housed in group cages in an air-conditioned room with a cycle of 12 h light/12 h dark. The rats were divided into 3 groups according to the duration of ethanol-induced sleep time (after an acute test dose of ethanol: 25% solution, 3.5 g/kg i.p.):
- long-sleeping (LS – 178.5±36.6 min);
- medium-sleeping (MS – 113.8±9.3 min);
- short sleeping (SS – 69.2±11 min).

In further experiments we used only MS rats, which made up 70% of the general population. After 14 days of recovery, the MS animals were treated with a daily i. p. injection of 25% ethanol solution, 3.5 g/kg of body weight. The duration of ethanol-induced sleep time was decreased from 108.8±14.3 min. to 13±9 min. by the seventh day of alcohol treatment. The control group received i. p. injections of 0.9% NaCl solution. The rats were sacrificed by decapitation 24 h after the seventh injection of ethanol (for ethanol animals) or NaCl solution (for the control group). Their brains were rapidly removed at the 0–4°C. The brain regions (cerebral cortex, hypothalamus, striatum, medulla oblongata) were dissected, and remained frozen in liquid nitrogen for determination of acetyl-CoA synthetase and 5'nucleotidase activities, as well as the concentration of acetate, acetyl-CoA and adenosine.

The determination of acetyl-CoA synthetase activities was performed in homogenates using the citrate synthetase method [11]. The general activity of 5'nucleotidase was determined in homogenates of brain regions by UV methods with adenosine deaminase and glutamate dehydrogenase [12].

The level of acetyl-CoA was measured by the enzymatic method [13]. The concentration of acetate was determined by gas chromatography (HP 6890) [14]. The concentration of adenosine and the ADP+AMP pools were determined by HPLC (Beckman Gold System) [15].

For metabolic study, nerve terminals were isolated from pooled homogenates of cerebral cortex from 5 animals immediately after dissection. The synaptosomal fraction from cerebral cortex was isolated by differential centrifugation and flotation in Ficoll gradient [16]. The synaptosomes were suspended in 320 mM buffered sucrose. They were incubated for 30 min. at 37°C with shaking at 100 cycles min^{-1}, 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. Quantal ACh release was stimulated by addition of 1.0 mM $CaCl_2$. Adenosine and 2-chloroadenosine were added to the incubation medium (final concentration 10 μM) to study the effects of adenosine on ACh release. Incubation was stopped by centrifugation at 15000 × g for 3 min. in Eppendorf tubes. The adenosine

concentration in the supernatant and pellet was measured using luciferin-luciferase luminometry [17]. The determination of acetylcholine was performed in supernatant by luminometric detection (LKB Pharmacia) [18].

The concentration of protein was measured according to the Bradford method [19].

Data are presented as mean ± SD. The Mann-Whitney test was used to compare the alcohol-treated group with the controls for metabolic experiments, and the Student test was used for homogenate experiments. The level of significance was set at 0.05.

RESULTS

Table 1 shows that 7-day alcohol treatment resulted in increased acetate concentration in all researched brain regions (cerebral cortex, hypothalamus, striatum and medulla oblongata). The rate of rise of the acetate level was higher in cerebral cortex than in other researched brain regions: about 80% for cerebral cortex, and about 50% for hypothalamus, striatum and medulla oblongata. Simultaneously, there were no significant changes in the brain level of acetyl-CoA in rats treated with alcohol as compared to control animals (Table 1). The activities of acetyl-CoA synthetase and 5'nucleotidase, as well as the levels of adenosine and the pool of AMP+ADP were raised in alcohol-treated rats only in cerebral cortex (Table 1), and showed no significant difference in hypothalamus, striatum and medulla oblongata.

The synaptosomal level of adenosine was higher in control rats than in animals treated with alcohol (Figure 1). In the presence of acetate in the incubation medium there was a 3-fold increase in the release fo synaptosomal adenosine (Figure 2).

7-day alcohol administration caused no significant changes in resting (Ca^{2+}-nondependent) ACh release in synaptosomes (Figure 3). By contrast, in the presence of Ca^{2+} the synaptosomes from ethanol-treated rats showed higher rates of Ca^{2+}-dependent release of ACh (Figure 3). 10 μM of adenosine had no influence on Ca^{2+} stimulated ACh release from the neural endings of either control or experimental rats. However, 10 μM of 2-cloradenosine resulted in inhibition of synaptic ACh release only in control rats, but had no influence on this parameter in animals treated with alcohol (Figure 3).

DISCUSSION

Since acetate is an endogenous metabolite that is considered non-toxic, little attention has been given to the possibility that it may contribute to the intoxicating effects of ethanol. Although earlier studies have suggested that plasma acetate level increased 6 times 30 min. after a single intravenous dose of 0.5 g/kg ethanol in rats [20], there are no data concerning the influence ethanol consumption on the level of acetate in brain [21]. The present data have shown that chronic alcohol treatment resulted in an increased acetate level in brain. As acetate readily crosses the blood-brain barrier, the most probable reason for the rise of brain acetate concentration in alcohol-treated rats is its delivery with blood flow from peripheral tissues, mainly from the liver, which is the main ethanol-metabolizing organ.

The utilization of acetate by tissues [22] and acetate uptake by neurons [6] is dependent on the activities of acetyl-CoA synthetase. It is known that this enzyme is substrate-inducible, but as the acetate level increases both in blood and in brain after ethanol injections, the activation of acetyl-CoA synthetase would be expected. We have shown that 7-day alcohol treatment results in the activa-

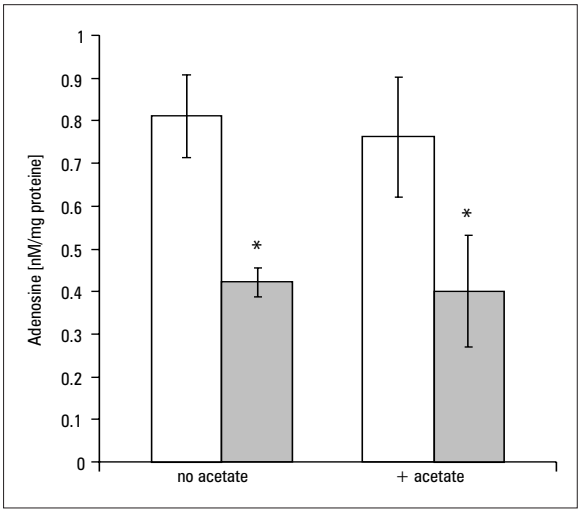

Figure 1. Effects of 2.5 mM acetate on adenosine concentration in cerebral cortex synaptosomes of control (open column) and alcohol (solid column) rat. Results are means ± SD from 5 experiments done in duplicate. * Significantly different from respective no-acetate conditions, $p<0.05$.

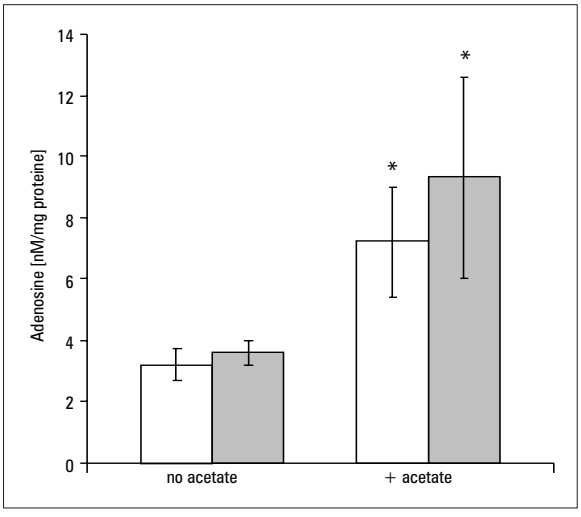

Figure 2. Effects of 2.5 mM acetate on extrasynaptical adenosine concentration in control (open column) and alcohol (solid column) rat. Results are means ± SD from 5 experiments done in duplicate. * Significantly different from respective no-acetate conditions, $p<0.05$.

Table 1. Effect of 7-day ethanol treatment on the concentration of acetate, acethyl-CoA, adenosine, pools of ADP+AMP, activities of acetyl-CoA synthetase and 5'nucleotidase in four brain regions (mean±SD of 6–10 rats).

Parameter *(Units)*	Groups	Brain regions			
		Cerebral cortex	Hypoth	Striatum	Medulla oblongata
Acetate	Control	0.31±0.1	0.37±0.07	0.32±0.06	0.32±0.07
(mM/g of tissue)	Alcohol	0.62±0.14*	0.56±0.1*	0.48±0.06*	0.51±0.1*
Acetyl-CoA	Control	82.2±19.1	60.2±7.5	69.3±15.8	91.8±13.6
(nM/g of tissue)	Alcohol	88.3±8.8	62.5±11.2	75.3±37.4	97.3±16.4
Acetyl CoA synthetase	Control	2.56±0.27	2.45±0.27	2.8±0.2	2.97±0.17
(nM/mg protein min^{-})	Alcohol	3.48±0.3*	2.96±0.54	3.15±0.3	3.3±0.15
5'nucleotidase	Control	15.8±4.0	16.3±2.6	13.6±1.2	21±6.4
(nM/mg protein min^{-})	Alcohol	33.3±10.0*	15.4±3.9	16±6.2	15±3.8
AMP+ADP	Control	2.097±0.5	2.7±0.3	2.6±0.35	1.9±0.19
(µM/g of tissue)	Alcohol	2.89±0.2*	2.9±1.1	2.5±0.5	1.7±0.5
Adenosine	Control	0.31±0.03	0.29±0.15	0.42±0.04	0.23±0.03
(µM/g of tissue)	Alcohol	0.41±0.05*	0.26±0.09	0.44±0.021	0.23±0.06

* ignificantly different from respective controls, $p<0.05$;

Hypoth – Hypothalamus

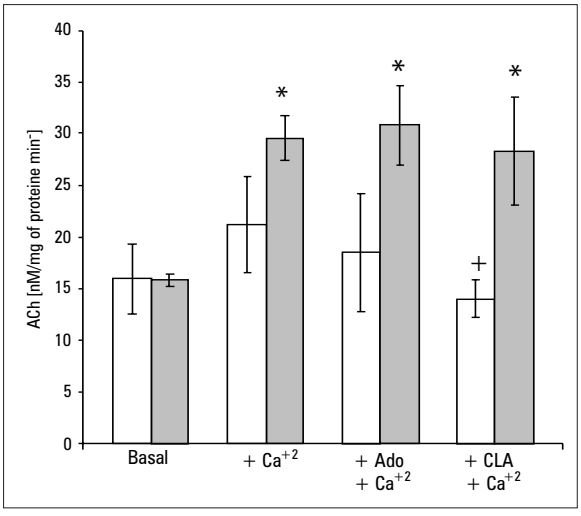

Figure 3. Effects of 10 µM adenosine (ado) and 10 µM 2-cloroadenosine (CLA) on Ca^{+2} stimulated acetylcholine (ACh) release in brain synaptosomes from control (open column) and alcohol (solid column) rat. Results are means ± SD from 5 experiments done in duplicate. * Significantly different from respective controls, $p<0.05$; $^{+}$ Significantly different from respective no 2-chloradenosine conditions, $p<0.05$.

tion of acetyl-CoA synthetase in cerebral cortex. Early studies demonstrated that the activities of acetyl-CoA synthetase were also increased in rat liver and heart after chronic ethanol treatment [23]. In one study it was shown that alcohol and acetate produce an increase of the acetyl-CoA level in cultured hepatocytes [5]. On the other hand, there was no such effect of ethanol on brain acetyl-CoA level in the present study, which is consistent with other reports [24]. One possible explanation for this fact is that the stability of the acetyl-CoA level is critically important for the majority of metabolic processes, and significant oscillations of this parameter *in vivo* are inadmissible.

There is much experimental evidence that ethanol and/or acetate cause an increase of the adenosine level, both in the peripheral and central nervous systems [1]. This is made possible by means of transformation of acetate into acetyl-CoA. The AMP formed from this transformation is subsequently converted to adenosine by 5'nucleotidase. We have found that chronic alcohol treatment results in increasing adenosine concentration in cerebral cortex only. This is in agreement with the rising activities of acetyl-CoA synthetase and 5'nucleotidase also in the same brain region during treatment with alcohol. Simultaneously, several studies have demonstrated that ethanol can increase the extracellular adenosine level by inhibiting its transport [25]. Decreasing adenosine concentration in the cortical nerve endings of alcohol-treated rats is probably the result of impairment of the neuronal mechanism of adenosine uptake by ethanol.

Fredholm et al. have shown that 20 mM ethanol, as well as 5 mM acetate, did cause enhancement of the efflux of adenosine from brain slice [26]. We found a similar effect in cortical synapses. 2.5 mM acetate caused a 3-fold increase of extrasynaptical adenosine concentration. These data justify the supposition that acetate, like ethanol, can block adenosine transport into neuronal cells. Together, these results suggest that ethanol and acetate produce an increased extracellular adenosine level by increasing its production and/or inhibiting adenosine uptake. A prolonged increase of adenosine level in brain during chronic alcohol consumption can lead to decreased sensitivity of the adenosine receptor. Dar et al. have suggested that the adenosine A1 receptor is desensitized by chronic ethanol treatment [3]. Adenosine modulates an 'inhibitory tone' in the CNS and depresses synaptic transmission of various neuromediators, including acetylcholine, by means of activation of the presynaptic A1 receptor [27]. The acceleration of Ca-dependent acetylcholine release from the nerve ending of rats treated with ethanol, according to our findings, is probably caused by elimination of adenosine's 'inhibitory tone' due to desensitization of the presynaptic adenosine A1 receptor. The results of our experiment suggest that the inhibitory effect of selective A1 receptor agonist – 2

chloroadenosine on Ca-stimulated synaptic acetylcholine release is lost during 7-day ethanol injection. It is known that adenosine is a somnogenic factor [28]. According to different experiments, adenosine promotes sleep, at least partly, by activating A1 receptors located on neurons in the basal forebrain and the presynaptic membrane of the nerve endings of these neurons in the cerebral cortex [29]. Accordingly, a decreased duration of ethanol-induced sleep for 7 day treatment with alcohol may result from A1 adenosine receptor desensitization, which is caused by acetate-induced prolonged high extracellular adenosine level.

CONCLUSIONS

1. Ethanol causes an increase of acetate in brain and adenosine level in cerebral cortex.
2. Acetate causes an increase of extrasynaptic adenosine level.
3. Prolonged ethanol treatment results in increased release of synaptic Ca-dependent acetylcholine.
4. Seven-day ethanol treatment eliminates inhibition of the 2-chloroadenosine – A1 adenosine receptor agonist on synaptic Ca-dependent acetylcholine release.

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