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Effects of *in vitro* ethanol on the brain cation pump in alcoholics and controls

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Abstract—*In vivo* ethanol exposure reduces *in vitro* Na^+,K^+-adenosine triphosphatase (Na^+,K^+-ATPase) sensitivity to ethanol in some animal models, but very little is known about the effects of ethanol on human brain Na^+,K^+-ATPase. Cerebral cortex homogenates from 13 male alcoholic and 9 control subjects were assayed for K^+-*p*-nitrophenylphosphatase (K^+-*p*NPPase, a measure of Na^+,K^+-ATPase) and Mg^{2+}-*p*NPPase activities at 37° for 20 min in 75 mM imidazole-HCl (pH 7.4), 5 mM *p*-nitrophenylphosphate, 5 mM $MgCl_2$, and 20 mM KCl, with or without 1 mM ouabain. Native K^+-*p*NPPase activities were similar in control and alcoholic brains (61.5 ± 3.5 vs 55.3 ± 3.1 nmol/mg/min). *In vitro* exposure to a near lethal ethanol level (0.5%, or 110 mM) was without effect, whereas 5% ethanol inhibited K^+-*p*NPPase activity by about 28% ($P < 0.001$) in both groups. Both 0.5 and 5% ethanol *in vitro* significantly stimulated Mg^{2+}-*p*NPPase activity (1–2% and 19–20%, respectively). By comparison, mouse brain K^+-*p*NPPase was inhibited significantly by *in vitro* ethanol, and Mg^{2+}-*p*NPPase activity was unaffected. Ethanol levels attainable in humans may not be sufficient to alter significantly brain Na^+,K^+-ATPase activity.

Key words: alcohol; brain; cation pump; Na^+,K^+-adenosine triphosphatase; K^+-activated *p*-nitrophenylphosphatase; tolerance

Na^+,K^+-ATPase* (EC 3.6.1.3) is the cation pump enzyme responsible for maintenance of ion gradients crucial to nerve conduction, co-transport, and cellular isotonicity [1]. In contrast to the numerous studies in nonhuman tissue, there is only one published study to our knowledge on the effects of ethanol on human brain Na^+,K^+-ATPase activity [2]. That study, which was performed approximately 20 years ago, reported that brain Na^+,K^+-ATPase activity was inhibited less than expected by *in vitro* ethanol in one 34-year-old subject who "had a history of chronic alcoholism". Many important species differences are known to exist among enzymes, and it is plausible that human and nonhuman Na^+,K^+-ATPases could differ in several crucial respects. Is susceptibility to inhibition of Na^+,K^+-ATPase by *in vitro* ethanol different in human brain? Does excessive and chronic exposure to ethanol *in vivo* affect the inhibition of Na^+,K^+-ATPase by *in vitro* ethanol in human subjects? To address these questions, we investigated the inhibitory effects of *in vitro* ethanol on the activity of human brain Na^+,K^+-ATPase comparing subjects who met accepted diagnostic criteria for alcoholism with subjects who did not.

Materials and Methods

Human brain was obtained from the National Neurological Research Specimen Bank (VAMC Wadsworth, Los Angeles, CA), sponsored by the NINDS and NIMH, NMSS, the HD Foundation and the Veterans Administration. Frontal, temporal, and parietal cortices were obtained from 22 males, ages 15–75, 13 of whom had documented histories of ethanol abuse, and 10 of these had cirrhosis at autopsy. One "drink" was assumed to contain 0.6 ounces of absolute ethanol, and beer, wine, and distilled spirits were assumed to contain 5, 12, and 40% absolute ethanol, respectively [3]. Autolysis time ranged from 8.5 to 29 hr. Controls died of trauma, cardiac arrest, pneumonia, and cancer. Ten percent (w/v) cerebral cortex homogenates were prepared in 0.32 M sucrose and stored at −70° prior to use. Homogenates from adult mouse brain (CXJ8, Balb/C, SWXJ, and SJL strains) were prepared similarly after decapitation. Protein was measured by the method of Lowry *et al.* [4]. K^+-*p*NPPase activity was assayed [5] after incubation at 37° for 20 min in medium containing 8 μg homogenate protein in (final concentrations) 75 mM imidazole-HCl (pH 7.4), 5 mM ditris *p*-nitrophenylphosphate, 5 mM $MgCl_2$, 20 mM KCl, with or without 1 mM ouabain in a final volume of 80 μL. Reactions were terminated by the addition of 200 μL of 0.117 M NaOH. Reaction product (*p*-nitrophenol) was detected at 410 nm. K^+-*p*NPPase activity was derived from the difference between the values obtained in the presence and absence of ouabain. Mg^{2+}-*p*NPPase activity was defined as the difference between the values obtained in the presence of ouabain and the no-enzyme blank. Data were analyzed by two-tailed *t*-tests, ANOVA, and simple linear regression. Differences were considered significant at the 0.05 level. Data are expressed as means ± SEM.

Results

Native K^+-*p*NPPase (55.3 ± 3.1 and 61.5 ± 3.5 nmol/mg/min) and Mg^{2+}-*p*NPPase activities (46.0 ± 2.0 and 44.7 ± 2.2 nmol/mg/min) were similar in alcoholic and control human brain homogenates, respectively, as were K^+-*p*NPPase/Mg^{2+}-*p*NPPase ratios (1.24 ± 0.10 and 1.39 ± 0.08).

In vitro exposure to 5% (1.1 M) ethanol produced significant ($P < 0.001$) inhibition of human brain K^+-*p*NPPase by 27.5 ± 1.8 and 28.2 ± 1.4%, and stimulation ($P < 0.01$) of Mg^{2+}-*p*NPPase activity by 19 ± 1.4 and 21 ± 1.7%, respectively, for alcoholic and control brain (Table 1). *In vitro* exposure to 0.5% (110 mM) ethanol produced insignificant ($P > 0.05$) inhibition of K^+-*p*NPPase by 0.8 ± 1.4 and 1.4 ± 0.9%, and stimulation ($P < 0.05$) of Mg^{2+}-*p*NPPase by 1 ± 0.6 and 2 ± 0.7%, respectively, for alcoholic and control brain. Human brain K^+-*p*NPPase activity did not correlate with age, with daily or lifetime ethanol intake, time elapsed from the last drink until death, or autolysis time (data not shown).

Mouse brain homogenate K^+-*p*NPPase (50.2 ± 0.43 nmol/mg/min) was more sensitive to *in vitro* ethanol than human K^+-*p*NPPase (Table 1). *In vitro*

* Abbreviations: Na^+,K^+-ATPase, Na^+,K^+-adenosine triphosphatase; K^+-*p*NPPase, K^+-*p*-nitrophenylphosphatase; and Mg^{2+}-*p*NPPase, Mg^{2+}-activated *p*-nitrophenylphosphatase.

Table 1. *In vitro* ethanol exposure and brain *p*NPPase activity

Preparation	% of Native K^+-*p*NPPase		% of Native Mg^{2+}-*p*NPPase	
	0.5% Ethanol	5% Ethanol	0.5% Ethanol	5% Ethanol
Alcoholic human brain homogenate (N = 13)	99.2 ± 1.4	72.5 ± 1.8*	101 ± 0.6†	119 ± 1.4‡
Control human brain homogenate (N = 9)	98.6 ± 0.9	71.8 ± 1.4*	102 ± 0.7†	121 ± 1.7‡
Mouse brain homogenate (N = 4)	95.6 ± 0.8‡	57.0 ± 1.0*	100 ± 1.6	103 ± 3.3

Values are expressed as means ± SEM. Native K^+-*p*NPPase activities were 55.3 ± 3.1, 61.5 ± 3.5, and 50.2 ± 0.43 nmol/mg/min for the human alcoholic, human control, and mouse groups, respectively. Native Mg^{2+}-*p*NPPase activities were 46.0 ± 2.0, 44.7 ± 2.2, and 47.2 ± 1.51 nmol/mg/min, respectively.

* $P < 0.001$ compared with the no-ethanol control.

† $P < 0.05$ compared with the no-ethanol control.

‡ $P < 0.01$ compared with the no-ethanol control.

exposure to 5 and 0.5% ethanol inhibited K^+-*p*NPPase by 43 ± 1.0% ($P < 0.001$) and 4.4 ± 0.8% ($P < 0.01$), respectively, *In vitro* exposure to either 5 or 0.5% ethanol had no effect on Mg^{2+}-*p*NPPase (47.2 ± 1.51 nmol/mg/min) from mouse brain homogenate.

Discussion

Human brain K^+-*p*NPPase activity is remarkably resistant to *in vitro* inhibition by ethanol levels achievable in humans. Mouse brain homogenate K^+-*p*NPPase activity is more sensitive to *in vitro* ethanol exposure than is human brain, although the inhibition is not very great. Other studies have shown minimal inhibition [6–8], no effect [9–11], or even slight stimulation [12] of mammalian brain Na^+,K^+-ATPase at comparable ethanol concentrations *in vitro*.

In vivo ethanol exposure has little effect on native human brain cation pump activities. Available comparable studies are scant. We previously reported that brain K^+-*p*NPPase activity of alcoholic subjects was 9.1% lower than that of controls [13]. In the present study, the values are 10.1% lower, but in neither study was the difference statistically significant. Greater numbers would be needed to clarify this.

Patient histories of ethanol consumption are difficult to quantify, and absence of such history is not proof of abstinence. Most alcoholic subjects had cirrhosis confirmed at autopsy, but their exposure histories varied greatly. Therefore, duration and dose-related effects of ethanol might confound these results when these brains are analyzed as a single group. However, when native activity was plotted versus cumulative or daily ethanol consumption, or time elapsed from the last drink until death, or time of autolysis, no significant correlations were found.

Animal studies report increased native Na^+,K^+-ATPase activity following long-term ethanol exposure [10, 14–18], no change [12, 19–21], or changes attributable to ethanol withdrawal [22].

In this study, cation pump activities of the brains of alcoholic patients were slightly less inhibited by ethanol, as in the case mentioned by Sun and Samorajski [2]. However, in all cases inhibition was minimal and not statistically significant. Unlike Sun and Samorajski [2], we did not find greater sensitivity of Na^+,K^+-ATPase activity to ethanol with increasing age. They used synaptosomal fractions and we used whole brain homogenates. There are pros and cons about the use of homogenates. More purified preparations leave out interfering substances in other fractions. Some of these substances may be relevant, but some could be artifacts of autolysis. Also, although our findings do not support the importance of inhibition of the cation pump in central nervous system deficits associated with ethanol, the high K^+ and low Na^+ conditions used in our assay system are known to minimize inhibition by ethanol [23]. Other studies are needed to address these issues.

Animal studies of the effect of long-term ethanol exposure on the sensitivity of brain Na^+,K^+-ATPase activity to *in vitro* ethanol show no effect [20] or increased [24] or decreased [15, 17, 19] sensitivity.

In summary, this study provides some unique and original information. The human brain cation pump enzyme is resistant to inhibition by physiologically obtainable levels of ethanol, both *in vivo* and *in vitro*. This study also typified the difficulties inherent in human studies: uncertainty about lifelong ethanol consumption, and variations in age, other illnesses, mode of death (controls are usually found among trauma victims), autolysis time, and genotype. While animal studies can control for these variables, interspecies differences make extrapolation to the human situation problematic.

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REFERENCES

1. Sweadner KJ and Goldin SM, Active transport of sodium and potassium ions. *N Engl J Med* **302**: 777–783, 1980.
2. Sun AY and Samorajski T, The effects of age and alcohol on ($Na^+ + K^+$)-ATPase activity of whole homogenate and synaptosomes prepared from mouse and human brain. *J Neurochem* **24**: 161–164, 1975.
3. Cox TC, Jacobs MR, LeBlanc AE and Marshman JA, *Drugs and Drug Abuse*. Addiction Research Foundation, Toronto, Canada, 1987.
4. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
5. Dahl JL and Hokin LE, The sodium-potassium adenosinetriphosphatase. *Annu Rev Biochem* **43**: 327–356, 1974.
6. Israel Y, Kalant H and Laufer I, Effects of ethanol on

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Na, K, Mg-stimulated microsomal ATPase activity. *Biochem Pharmacol* **14**: 1803–1814, 1965.
7. Rangaraj N and Kalant H, Interaction of ethanol and catecholamines on rat brain ($Na^+ + K^+$)-ATPase. *Can J Physiol Pharmacol* **57**: 1098–1106, 1979.
8. Sun AY and Samorajski T, Effects of ethanol on the activity of adenosine triphosphatase and acetylcholinesterase in synaptosomes isolated from guinea-pig brain. *J Neurochem* **17**: 1365–1372, 1970.
9. Rangaraj N and Kalant H, α Adrenoreceptor mediated alteration of ethanol effects on ($Na^+ + K^+$)-ATPase of rat neuronal membranes. *Can J Physiol Pharmacol* **58**: 1342–1346, 1980.
10. Beaugé F, Stibler H and Kalant H, Brain synaptosomal (Na^+ and K^+)-ATPase activity as an index of tolerance to ethanol. *Pharmacol Biochem Behav* **18**: s519–s524, 1983.
11. Nhamburo PT, Salafsky BP, Hoffman PL and Tabakoff B, Effects of short-chain alcohols and norepinephrine on brain (Na^+, K^+)ATPase activity. *Biochem Pharmacol* **35**: 1987–1992, 1986.
12. Aloia RC, Paxton J, Daviau JS, van Gelb O, Mlekusch W, Truppe W, Meyer JA and Brauer FS, Effect of chronic alcohol consumption on rat brain microsome lipid composition, membrane fluidity and Na^+–K^+-ATPase activity. *Life Sci* **36**: 1003–1017, 1985.
13. Bertoni JM and Sprenkle PM, Low level lead inhibits the human brain cation pump. *Life Sci* **48**: 2149–2156, 1991.
14. Guerri C and Grisolia S, Chronic ethanol treatment affects synaptosomal membrane-bound enzymes. *Pharmacol Biochem Behav* **18 (Suppl 1)**: 45–50, 1983.
15. Rangaraj N, Beaugé F and Kalant H, Temporal correlation of changes in rat brain sialic acid and in inhibition of Na^+–K^+ ATPase with ethanol tolerance. *Can J Physiol Pharmacol* **62**: 899–904, 1984.
16. Rangaraj NI and Kalant H, Effect of ethanol tolerance on norepinephrine–ethanol inhibition of (Na^+–K^+)-adenosine triphosphatase in various regions of rat brain. *J Pharmacol Exp Ther* **231**: 416–421, 1984.
17. Swann AC, Brain Na^+, K^+-ATPase: Alteration of ligand affinities and conformation by chronic ethanol and noradrenergic stimulation *in vivo*. *J Neurochem* **47**: 707–714, 1986.
18. Swann AC, Chronic ethanol and (Na^+–K^+)-adenosine triphosphatase: Apparent adaptation in cation binding and enzyme conformation. *J Pharmacol Exp Ther* **232**: 475–479, 1985.
19. Akera T, Rech RH, Marquis WJ, Tobin T and Brody TM, Lack of relationship between brain ($Na^+ + K^+$)-activated adenosine triphosphatase and the development of tolerance to ethanol in rats. *J Pharmacol Exp Ther* **185**: 594–601, 1973.
20. Westcott JY and Weiner H, Effect of ethanol on synaptosomal ($Na^+ + K^+$)-ATPase in control and ethanol-dependent rats. *Arch Biochem Biophys* **223**: 51–57, 1983.
21. Israel MA and Kuriyama K, Effect of *in vivo* ethanol administration on adenosinetriphosphatase activity of subcellular fractions of mouse brain and liver. *Life Sci* **10 (Part II)**: 591–599, 1971.
22. Rangaraj N and Kalant H, Effects of ethanol withdrawal, stress and amphetamine on rat brain ($Na^+ + K^+$)-ATPase. *Biochem Pharmacol* **27**: 1139–1144, 1978.
23. Swann AC, Ethanol and (Na^+, K^+)-ATPase: Alteration of Na^+–K^+ selectivity. *Alcohol Clin Exp Res* **14**: 922–927, 1990.
24. Beaugé F, Fleuret C, Barin F and Nordmann R, Brain membrane disordering after acute *in vivo* administration of ethanol, isopropanol or *t*-butanol in rats. *Biochem Pharmacol* **33**: 3591–3595, 1984.