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Membrane Na^+K^+ATPase Activity: Changes Using an Experimental Model of Alcohol Dependence and Withdrawal[1]

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COWAN, C. M., J. O. CARDEAL AND E. A. CAVALHEIRO. *Membrane Na^+K^+ATPase activity: Changes using an experimental model of alcohol dependence and withdrawal.* PHARMAC. BIOCHEM. BEHAV. 12(3) 333–335, 1980.—An alcohol dependent state was induced in rats via gastric intubation. Alcohol was given three times daily for seven days in increasing doses, the final dose and concentration varying from rat to rat, being adjusted according to weight loss and state of intoxication. After seven days dosing, alcohol was withdrawn and twenty hours later an audiogenic stimulus was given to induce convulsions. Na^+K^+ATPase activity was measured in the hippocampus of rats during alcohol administration and during withdrawal. Animals which received twenty-one doses of alcohol showed a significant increase in Na^+K^+ATPase activity compared with controls. On withdrawal of alcohol, the enzyme activity fell, but remained higher than control values. In the withdrawal groups, membrane Na^+K^+ATPase levels were increased significantly compared to control levels in the order: no convulsion <with convulsion < postconvulsion. It is concluded that Na^+K^+ATPase activity is modified during chronic alcohol administration and during seizures induced after alcohol withdrawal by audiogenic stimulation.

Membrane ATPase Alcohol dependence Alcohol withdrawal Hippocampus Audiogenically induced seizures

ETHANOL and other alcohols have been shown to inhibit Na^+K^+ATPase activity *in vitro* [10,11] while chronic administration has been shown to stimulate the activity of this enzyme [13,22], though the effects vary somewhat depending on the species of animal used [7]. Various experimental methods have been designed to induce a state of physical dependence in animals, using intragastric administration of alcohol via an implanted cannula in dogs [4]; gastric intubation of ethanol in 2–3 daily doses to monkeys [3]; liquid diet with 35% of calories derived from ethanol given to mice [6] and inhalation of ethanol vapour in rats [22]. Using these methods one can study abstinence seizures which may be induced by withdrawal of the dependent drug and provide information regarding the pathophysiological and possible chemical mechanisms of the origin of seizure activity.

Ethanol is a general CNS depressant thought to act at the level of the neuronal membrane. The inhibitory potency of alcohols correlates with their effectiveness as depressants [2,12] and it is possible that the inhibition of membrane Na^+K^+ATPase is a factor in the depression of nerve cell activity by drugs, such as alcohol. We have induced an alcoholic dependent state in rats via gastric intubation and, on withdrawal of alcohol, convulsions have been induced in some of the rats by audiogenic stimulation. The activity of membrane Na^+K^+ATPase has been measured during the chronic administration of alcohol and also during the withdrawal phase.

METHOD

Male Wistar albino rats, weighing between 200 and 300 g and housed in individual cages, were given, by gastric intubation, 3 g/kg of alcohol as a 15% (w/v) solution in water, once every eight hours. Water and food were available ad lib throughout the experiment. Animals were also given a liquid diet three times per day to avoid weight loss. One hour after the administration of each dose, the presence/absence of various behavioural paradigms were tested: gripping reflex, ataxia, righting reflex and coma. If the animal was not sufficiently intoxicated, the concentration of the following dose was increased by 2.5%. The animals were given alcohol for seven days, after which it was withdrawn. Twenty hours after withdrawal of the alcohol the rats were individually stimulated audiogenically to induce convulsions. The following groups of animals were used: (1) Control Group 1—no alcohol or audiogenic stimulation. (2) Control Group 2—no

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TABLE 1

MEMBRANE Na^+K^+ATPASE ACTIVITY OF RAT HIPPOCAMPUS DURING CHRONIC ADMINISTRATION OF ALCOHOL AND DURING WITHDRAWAL

		Withdrawal		
Control	21 doses	No convulsion	Convulsion	Post-convulsion
0.81 ± 0.02 (n=20)	1.18 ± 0.07* (n=10)	0.86 ± 0.02*† (n=5)	0.92 ± 0.03*† (n=5)	1.06 ± 0.06*‡ (n=5)

Data expressed (mean ± SD) in μmoles orthophosphate release/hour/mg protein.
Control values are mean of control 1 and 2 ± SD.
*Significantly different compared to control values for $p<0.01$.
†Significantly different compared to alcoholic animals for $p<0.001$.
‡Significantly different compared to alcoholic animals for $p<0.01$.

alcohol but each animal individually received one min of audiogenic stimulation. Control rats were given an equal volume of equicaloric sucrose solution. (3) Experimental group which received 21 doses of alcohol and were killed one hour after the last dose. (4) Experimental groups during withdrawal (20 hr after the last dose of alcohol): (a) without audiogenic stimulation (not convulsive). (b) killed during the clonic phase of the audiogenically induced convulsion (convulsive). (c) killed 10 min after the end of the convulsion (postconvulsive). The animals were killed by decapitation and the brains removed.

Enzyme Activity

The left and right hippocampi were dissected and weighed and individually homogenized in icecold Tris buffer, pH 7.4 (0.1 ml/mg fresh tissue). Membrane Na^+K^+ATPase activity was determined, based on the method of Marichich and Nasello [19] and Rapport [21]. 0.15 ml samples of the homogenate were incubated in a medium containing 130 mM NaCl, 20 mM KCl, 6 mM $MgCl_2$. $6H_20$, 1 mM EDTA and 3 mM ATP in 40 mM Tris buffer, pH 7.4. Half of the tubes contained, in addition, 1 mM oubain. The final volume of the tubes was 1 ml and the enzyme reaction started by addition of the homogenate. The tubes were incubated at 37°C for 20 min and the reaction stopped by plunging the tubes into ice and adding 0.1 ml of trichloroacetic acid 10%. The orthophosphate concentration in each tube was determined by standard spectrophotometric procedure [6], the tubes being read at 660 nm. All determinations were done in duplicate. Membrane Na^+K^+ATPase activity was calculated by the difference between total ATPase activity and that in the tubes containing oubain. Results were expressed as μmoles orthophosphate released/hour/mg protein, the protein concentration being determined by the modification of the Lowry method by Hartree [9]. Statistical analysis was by Student *t*-test.

RESULTS

The results are shown in Table 1. There is a marked increase in membrane Na^+K^+ATPase activity after chronic administration of alochol. During abstinence enzyme activity is still higher than that of the controls although not as much as during chronic administration. All rats developed a tolerance to ethanol, the final dose needed to maintain an alcoholic state being considerably higher than that at the start of the experiment. The final dose varied considerably from one animal to another and the volume was modified according to weight loss during alcohol dosing (Table 2).

TABLE 2

MEAN BODY WEIGHT ± SD, IN GRAMS, OF CONTROL RATS AND OF RATS SUBMITTED TO CHRONIC ETHANOL ADMINISTRATION

	Initial weight	*Final* weight
Controls (20)	232.0 ± 3.7	234.8 ± 4.9
Alcoholics (25)	242.8 ± 7.8	225.9 ± 6.7

N in parentheses. All control vs alcoholic differences not significant at a 0.05% level in a *t* test.

On withdrawal of alcohol the behaviour of the animals became markedly changed. Twenty hours after withdrawal the animals were hyperexitable, showing tremor, piloerection, bulging eyes and reacted excessively to any noise. On auditory stimulation the animals convulsed. The auditory stimulus was applied for 1 min and the animals reacted rapidly to the stimulus, running to and fro along the cage and jumping and eventually fell in a tonic extension followed by clonus of the head and fore and hind paws. Respiratory rhythm was broken and recovery was not achieved for some minutes. The auditory stimulus alone had no effect on the enzyme activity showing no change between control Groups 1 and 2.

DISCUSSION

The hippocampus has a lower seizure threshold compared to other areas of the brain which has been related to its small extracellular space [8,14]. Na^+K^+ATPase has been shown to increase significantly in the cortex and hippocampus of cats during chronic alcohol administration but not in other brain areas [16]. Our experiments also show an increased Na^+K^+ATPase activity during chronic administration of alcohol in the hippocampus of rats. All rats developed tolerance to the alcohol which resulted in the appearance of withdrawal symptoms after administration was stopped.

Activity of Na^+K^+ATPase is affected in various ways depending on the experimental method. *In vitro* various authors have shown that ethanol has an inhibitory effect on the activity of this enzyme [7, 11, 15], whereas chronic administration, in rats, has been shown to increase Na^+K^+ATPase activity [13, 18, 22].

Rangaraj and Kalant [20] observed no increase in Na^+K^+ATPase activity of whole brain homogenates during chronic alcohol treatment but enzyme activity was increased during withdrawal. The difference observed during the chronic phase could be explained by the fact that small increase in hippocampal enzyme activity may not be detected when whole brain homogenates are used.

The decrease in enzyme activity on withdrawal of alcohol administration was somewhat expected since the observed stimulatory effect of alcohol has been removed. The increase of enzyme activity during the audiogenically induced convulsion was also expected since various papers have been published relating convulsive activity and increased Na^+K^+ATPase activity [1,19]. Due to the controversial findings relating enzyme activity during the postconvulsive period [17,21] it is difficult to conclude anything concerning the postconvulsive increase. However, it has been suggested that the increased enzyme activity would be the result of norepinephrine stimulation during the withdrawal reaction [20] which could not only last longer than but could also be increased by the convulsion. Comparison of our results with those of similar studies of epileptic convulsions should be interpreted with caution because of the differences between the two types of convulsions.

REFERENCES

1. Bignami, A., G. Palladini and G. Venturini. Effect of cardiazol on Na^+K^+ activated adenosine triphosphatase of the rat brain in vivo. *Brain Res.* **1:** 413–414, 1965.
2. Davis, P. W. and T. M. Brody. Inhibition of (Na^+K^+)-activated adenosine triphosphatase activity in rat brain by substituted phenothiazines. *Biochem. Pharmac.* **15:** 703–710, 1966.
3. Ellis, F. W. and J. R. Pick. Experimentally induced ethanol dependence in rhesus monkeys. *J. Pharmac. exp. Ther.* **175:** 88–93, 1970.
4. Essig, C. F. and R. C. Lam. Convulsions and hallucinatory behaviour following alcohol withdrawal in the dog. *Archs Neurol.* **18:** 626–632, 1968.
5. Fiske, C. H. and Y. Subbarow. The colorimetric determination of phosphorus. *J. biol. Chem.* **66:** 375–393, 1925.
6. Freund, G. Alcohol withdrawal syndrome in mice. *Archs Neurol.* **21:** 315–320, 1969.
7. Goldstein, D. B. and Y. Israel. Effects of ethanol on mouse brain (Na^+K)-activated adenosine triphosphatase. *Life Sci.* **2:** 957–963, 1972.
8. Green, J. The hippocampus. *Physiol. Rev.* **44:** 561–580, 1964.
9. Hartree, E. F. Determination of protein: A modification of the Lowry method that gives a linear photometric response. *Analyt. Biochem.* **48:** 422–427, 1972.
10. Israel, Y., H. Kalant and J. Laufer. Effects of ethanol on $Na^+K^+Mg^{++}$ stimulated microsomal ATPase activity. *J. Biochem. Pharmac.* **14:** 1803–1814, 1965.
11. Israel, Y., H. Kalant and A. E. LeBlanc. Effects of lower alcohols on potassium transport and microsomal adenosine activity of rat cerebral cortex. *Biochem. J.* **100:** 27–33, 1966.
12. Israel, Y. and I. Salazar. Inhibition of brain microsomal adenosine triphosphatase by general depressants. *Arch. Biochem. Biophy.* **122:** 310–317, 1967.
13. Israel, Y., H. Kalant, A. E. LeBlanc, J. C. Bernstein and I. Salazar. Changes in cation transport and (Na^+K^+)-activated adenosine triphosphatase produced by chronic administration of ethanol. *J. Pharmac. exp. Ther.* **174:** 330–336, 1970.
14. Izquierdo, I., A. G. Nasello and E. S. Marichich. Effects of potassium on rat hippocampus: the dependence of hippocampal evoked and seizure activity on extracellular potassium levels. *Arch. Int. Pharmacodyn. Ther.* **187:** 318–328, 1972.
15. Kalant, H. and I. Israel. Effects of ethanol on active transport of cations. In: *Biochemical Factors in Alcoholism,* edited by R. P. Maikel. New York: Pergamon Press, 1967, pp. 25–37.
16. Knox, W., R. G. Perrin and A. K. Sen. Effect of chronic administration of ethanol on (Na^+K^+)-ATPase activity in six areas of the cat brain. *J. Neurochem.* **19:** 2881–2884, 1972.
17. Kryzhanovsky, G. N., A. M. Golenda, V. Scevison and R. N. Glebov. The activity of Na^+K^+ATPase and acetylcholinesterase of the membrane structures of rat brain and spinal cord during the convulsive seizure. *Bull. Eksp. Biol. Med.* **82:** 1051–1052, 1976.
18. LeBlanc, A. E., H. Kalant, R. J. Gibbins and N. D. Berman. Acquisition and loss of tolerance to ethanol by the rat. *J. Pharmac. exp. Ther.* **168:** 244–250, 1969.
19. Marichich, E. S. and A. G. Nasello. Epilepsy and adenosine triphosphate (ATP): effect of electrical stimulation and high potassium perfusion on hippocampal ATP content. *Brain Res.* **57:** 409–416, 1972.
20. Rangaraj, N. and H. Kalant. Effects of ethanol withdrawal, stress and amphetamine on rat brain Na^+K^+-ATPase. *Biochem. Pharmac.* **27:** 1139–1144, 1978.
21. Rapport, R. L., B. Harris, P. Friel and A. Ojemann. Human epileptic brain: Na^+K^+ATPase activity and phenytoin concentrations. *Archs Neurol.* **32:** 549–553, 1975.
22. Roach, M. K., M. M. Khan, R. Coffman, W. Pennington and D. L. Davis. Brain Na^+K^+-activated adenosine triphosphatase activity and neurotransmitter uptake in alcohol-dependent rats. *Brain Res.* **63:** 323–329, 1973.