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Alterations of cerebral protein kinase activity following ethanol administration

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Although it has been shown that ethanol induces various biochemical changes in the central nervous system (CNS) [1, 2], exact biochemical mechanisms underlying known effects of this agent on the CNS remain to be elucidated. In previous reports [3–5], we have shown that continuous oral administration of ethanol induces an increase of the content of adenosine cyclic 3′,5′-monophosphate (cyclic AMP) as well as the formation of cyclic AMP via the activation of adenylate cyclase (E.C. 4.6.1.1) in the mouse cerebral cortex. In addition it was also found that stimulatory effects of added biogenic amines on the formation of cyclic AMP in cerebral cortical slices are reduced significantly in these ethanol treated animals [3]. On the other hand, it seems well established that cyclic AMP-dependent protein kinase (E.C. 2.7.1.37), which is specifically stimulated by low concentrations of cyclic AMP, may have an important role in the regulation of brain functions, possibly by phosphorylating of neuronal membranes [6]. These facts suggest that the increased cerebral metabolism of cyclic AMP found in animals treated continuously with ethanol may be coupled with the increase of cyclic AMP-dependent protein kinase activity.

In this paper we describe effects of acute and continuous oral administrations of ethanol on cyclic AMP-dependent protein kinase activity in the mouse cerebral cortex.

Swiss albino male mice weighing 25–35 g were used in all experiments. For examining the effect of acute administration of ethanol (4 g/kg of body wt), 20% (w/v) solution of ethanol in physiological saline was administered intraperitoneally (i.p.) and animals were sacrificed 60 min after the injection. Control animals received an equivalent amount of physiological saline and were handled exactly the same as ethanol-treated groups. Continuously ethanol-treated mice received orally a liquid diet [3–5] containing 6% ethanol (daily ethanol dose: 18–25 g/kg), while control animals were fed with a liquid diet containing sucrose isocaloric to ethanol. Subcellular fractions from the mouse cerebral cortex were prepared according to the procedure of Gray and Whittaker [7].

Protein kinase activity was measured according to the radiometric method of Miyamoto *et al.* [8]. The incubation medium contained 100 mM sodium acetate buffer (pH 6.0), 1 mM magnesium acetate, 10 mM sodium fluoride, 2 mM theophylline, 0.3 mM EGTA, 0.5 mg histone (Sigma Type II-A), 5 μM [γ-^{32}P]-ATP (S.A. 0.4 Ci/m-mole) and enzyme preparation (15–30 μg of particulate proteins). Reaction was carried out in the presence or absence of 10^{-6} M cyclic AMP at 30° for 60 sec. The reaction was terminated by the addition of 2 ml of the solution containing 5% (w/v) trichloroacetic acid, 0.25% phosphotungstic acid and 0.06 N sulfuric acid. After adding 0.1 ml of 0.68% (w/v) bovine serum albumin to promote the precipitation, the tubes were centrifuged. After two washings of the pellet, by dissolving in 0.1 ml of 1 N NaOH and reprecipitating by the addition of 2 ml of the solution used for the termination of the reaction, the precipitate was finally dissolved in 0.1 ml of 1 N NaOH. Each sample was placed in 15 ml of scintillator (consisting of 500 ml of toluene, 500 ml of ethyleneglycol monoethyl ether, 4 g of 2,5-diphenyloxazol and 0.2 g of 1-4-bis-2 (5-phenyloxazolyl) benzene), and the radioactivity was measured using a Packard Tri-Carb liquid scintillation spectrometer, model 3390. The activity of cyclic AMP-dependent protein kinase was expressed as pmoles of ^{32}P incorporated into histone (calculated by the difference between the rate of phosphorylation in the presence and absence of cyclic AMP)/mg protein/min. Protein content was determined by the method of Lowry *et al.* [9].

Table 1 shows protein kinase activity in crude mitochondrial (P_2) and microsomal (P_3) fractions of the cerebral cortex measured at 60 min after the administration of a single dose of ethanol (4 g/kg, i.p.) or saline. Both basal and cyclic AMP-dependent activities of the enzyme in these fractions were not significantly altered by the treatment with ethanol. Similarly, *in vitro* addition of ethanol to the assay medium up to 2.5%, which is approximately 7–8 times higher than the blood levels of ethanol found in acutely treated animals, failed to modify the enzyme activity, although 5 and 10% ethanol showed a significant inhibition of cyclic AMP-dependent protein kinase activity.

In contrast with the data obtained in acute experiments, continuous oral administrations of ethanol for 2 weeks showed a tendency to increase the basal and cyclic AMP-dependent activities of protein kinase in both P_2 and P_3

Table 1. Effect of acute administration of ethanol (4 g/kg, i.p.) on protein kinase activity in subcellular fractions of mouse cerebral cortex

	Protein kinase activity* (pmoles/mg protein/min ± S.E.M.)			
	Control		Ethanol-treated	
Fraction	Basal†	cAMP-dependent‡	Basal†	cAMP-dependent‡
P_2	1300 ± 438	627 ± 300	1196 ± 267	640 ± 343
P_3	1626 ± 473	464 ± 97	2272 ± 345	983 ± 301

* Each value represents mean ± S.E.M. obtained from 4–5 separate experiments.

† Expressed as pmoles of ^{32}P incorporated into histone/mg protein/min in the absence of added cyclic AMP.

‡ Calculated by the difference of pmoles of ^{32}P incorporated into histone in the presence and absence of added cyclic AMP.

Table 2. Effect of continuous oral administration of ethanol (2 weeks) on protein kinase activity in subcellular fractions of mouse cerebral cortex

Fraction	Protein kinase activity* (pmoles/mg protein/min ± S.E.M.) Control Basal†	Control cAMP-dependent‡	Ethanol treated Basal†	Ethanol treated cAMP-dependent‡
P_2	2173 ± 311	447 ± 126	3015 ± 557	751 ± 185
P_3	3146 ± 405	771 ± 370	3739 ± 680	1608 ± 559

* Each value represents mean ± S.E.M. obtained from 4–6 separate experiments. For † and ‡, see the footnote of Table 1.

fractions, but these changes were not statistically significant (Table 2). Slight increase of the basal activity found in ethanol-treated groups may be explicable by the fact that cyclic AMP content as well as the activity of adenylate cyclase in the brain is increased in these animals [4]. By subfractionating the P_2 fraction we have found, however, a significant increase of the synaptosomal activity of cyclic AMP-dependent protein kinase in continuously ethanol treated animals (Fig. 1). This increase found in synaptosomes was returned to the normal range of the enzyme activity at 7 days after the removal of ethanol from the diet.

Concerning the role of cyclic AMP-dependent protein kinase in nervous systems, Greengard *et al.* [10] proposed such a hypothesis that the stimulated protein kinase may cause a change in the ionic permeability of neuronal membranes by phosphorylating of specific membraneous proteins. If this is the case, the observed increase in synaptosomal protein kinase activity following a continuous administration of ethanol may cause alterations in the functions of synapses by changing the state of phosphorylation and ionic permeability of synaptic membranes. Present results suggest such a change may involve in the formation and/or maintenance of ethanol dependence. Studies for clarifying biochemical mechanisms underlying the activation of adenylate cyclase [4, 5] and protein kinase activities following continuous administrations of ethanol are under way in our laboratory.

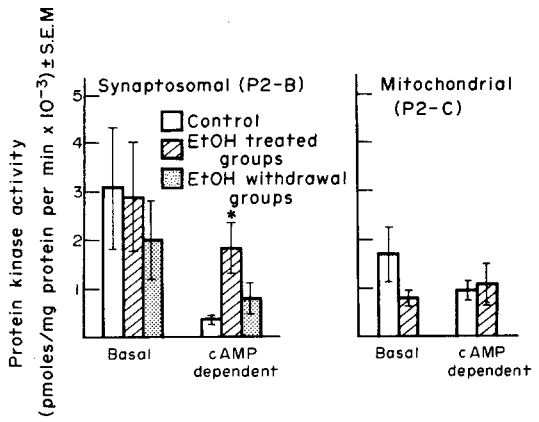

Fig. 1. Effects of continuous oral administration and withdrawal of ethanol on protein kinase activity in submitochondrial fractions from mouse cerebral cortex. Ethanol (18–25 g/kg/day) was administered by a liquid diet method (see text for details) for 2 weeks. The activity in ethanol withdrawal groups was measured 7 days after the termination of ethanol administration. Control groups received a liquid diet containing sucrose isocaloric to ethanol for 2 weeks. Each value represents mean ± S.E.M. obtained from 4–6 separate experiments. * Differs from the control, $P < 0.02$.

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REFERENCES

1. H. Wallgren, in *Handbook of Neurochemistry* (Ed. A. Lajtha) Vol. 6, p. 509. Plenum Press, New York (1971).
2. H. Kalant, *Fedn Proc.* **34,** 1930 (1975).
3. M. A. Israel, H. Kimura and K. Kuriyama, *Experientia* **28,** 1322 (1972).
4. K. Kuriyama and M. A. Israel, *Biochem. Pharmac.* **22,** 2919 (1973).
5. K. Kuriyama and M. A. Israel, in *Hormones and Brain Function* (Ed. K. Lissak) p. 293. Plenum Press, New York (1973).
6. T. A. Langan, in *Advances in Cyclic Nucleotide Research* (Eds. P. Greengard and G. A. Robison) Vol. 3, p. 99. Raven Press, New York (1973).
7. E. G. Gray and V. P. Whittaker, *J. Anat.* **96,** 79 (1962).
8. E. Miyamoto, J. F. Kuo and P. Greengard, *J. biol. Chem.* **244,** 6395 (1969).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
10. P. Greengard, D. A. McAfee and J. W. Kebabian, in *Advances Cyclic Nucleotide Research* (Eds. P. Greengard and G. A. Robison) Vol. 1, p. 337. Raven Press, New York (1972).