Biochemical Pharmacology, Vol. 22, pp. 2919–2922. Pergamon Press, 1973. Printed in Great Britain.

SHORT COMMUNICATIONS

Effect of ethanol administration on cyclic 3′,5′-adenosine monophosphate metabolism in brain

(*Received* 26 *January* 1973; *accepted* 9 *May* 1973)

CYCLIC 3′,5′-adenosine monophosphate (cyclic AMP) has been established as an intracellular mediator of the action of a number of amines and polypeptide hormones[1–3] and may play an important role in the function of the central nervous system.[4,5] Although it has been shown that ethanol induces alterations in the function of the endocrine system[6,7] and the metabolism of biogenic amines in the brain,[8] little information is available concerning ethanol induced changes in the metabolism of cyclic AMP in the brain. In this report we describe the effects of chronic and acute administration of ethanol on cyclic AMP metabolism in the brain.

Swiss female albino mice weighing 25–30 g were used in all experiments. Mice were fed continuously with a liquid diet to study the effect of chronic ethanol administration. Ethanol treated animals received a diet of 61% commercial Metrecal, 6% (w/v) ethanol, and distilled water as previously described.[7–9] Control groups received exactly the same amount of liquid diet containing sucrose isocaloric to the ethanol intake in the experimental mice. The average daily intake per mouse of liquid diet containing 6% ethanol was 7–9 ml, a daily ethanol dose of 16–21 g/kg body wt in the ethanol treated animals. Animals in both the ethanol treated and control groups were maintained at 30° under exactly the same handling and environmental conditions. Mice were treated with an intraperitoneal injection of ethanol (4 g/kg body wt) or isocaloric sucrose in experiments in which the acute effects of ethanol administration were studied.

Adenylate cyclase activity in homogenates of cerebral cortex, prepared by gentle hand homogenization, was measured by the chromatographic separation of cyclic ^{3}H-AMP formed from ^{3}H-ATP as described by Krishna *et al.*[10] For standard experiments, incubations were carried out for 7 min at 30° and terminated, after the addition of 10 μmoles of carrier cyclic AMP, by immersion in a boiling water bath for 2 min. For the measurement of radioactivity, an aliquot of sample was added to 10 ml of counting fluid containing 20% (v/v) Bio-Solv solubilizer, Formula BBS-3 and counted in a Beckman LS-200 liquid scintillation spectrometer with automatic quenching correction. Cyclic 3′,5′-nucleotide phosphodiesterase (phosphodiesterase) activity in homogenate preparations of cerebral cortex prepared in 0·32 M sucrose was assayed according to the method of Weiss.[11] The inorganic phosphate liberated from cyclic AMP was determined spectrophotometrically by the method of Swanson *et al.*[12] Tissue blanks for each determination were assayed by an identical procedure except that cyclic AMP was omitted from the reaction mixture.

Tissue cyclic AMP content was measured by the radioimmunoassay method of Steiner *et al.*[13] Control and ethanol treated animals were frozen intact in liquid nitrogen and cerebral hemispheres were immediately dissected. While still frozen, each cerebral hemisphere was placed in 0·5 ml of boiling 0·05 M sodium acetate buffer (pH 6·2) and boiled for 10 min, after which it was homogenized. The final volume was adjusted to 1·0 ml by the addition of acetate buffer, and each sample was centrifuged at 1000 *g* for 15 min. Cyclic AMP was determined in 0·1 ml of the supernatant. The ^{125}I-labelled cyclic AMP derivative and anti-cyclic AMP antiserum were purchased from Collaborative Research (Waltham, Massachusetts), and the goat anti-rabbit Ig G from Schwarz Biochemicals (Orangeburg, New York).

Protein content[14] and blood serum level of ethanol[15] were determined spectrophotometrically.

Table 1 shows the effect of acute and chronic ethanol treatments on adenylate cyclase and phosphodiesterase activities in the cerebral cortex. Adenylate cyclase and phosphodiesterase activities, enzymes responsible for the formation and degradation of cyclic AMP respectively, were not affected significantly by acute ethanol administration. It is now well known that the activity of adenylate cyclase is activated by sodium fluoride.[16] No alteration of adenylate cyclase activity by acute ethanol treatment, however, was found in the presence of sodium fluoride (10 mM). By contrast, adenylate cyclase activity in cerebral cortex was activated following the chronic ingestion of ethanol. Adenylate cyclase activity increased by 50, 39 and 53 per cent at 1, 2 and 3 weeks of continuous ethanol ingestion respectively. In the presence of 10 mM sodium fluoride, however, no differences in adenylate cyclase activity were found between control and chronically treated ethanol groups. Adenylate cyclase activity as a function of time and protein concentrations is shown in Fig. 1. Adenylate cyclase activity in the

TABLE 1. EFFECT OF ACUTE AND CHRONIC ETHANOL TREATMENTS ON ADENYLATE CYCLASE AND PHOSPHODIESTERASE ACTIVITIES IN MOUSE CEREBRAL CORTEX

	Time after injection (hr)	Enzyme activity (nmoles/mg protein/min) ± S.D.			
		Adenylate cyclase		Phosphodiesterase	
		Control	EtOH treated	Control	EtOH treated
			Acute*		
	0	0·18 ± 0·01	—	59·7 ± 5·6	
	1·5	0·17 ± 0·02	0·18 ± 0·01	58·6 ± 9·8	58·1 ± 2·6
	3·0	0·17 ± 0·04	0·19 ± 0·02	58·4 ± 5·3	57·7 ± 2·5
	6·0	0·19 ± 0·03	0·18 ± 0·03	58·0 + 2·8	53·7 ± 2·5
+ NaF†	0	0·39 ± 0·08			
	1·5	0·41 ± 0·07	0·39 ± 0·09		
	3·0	0·37 ± 0·06	0·39 ± 0·07		
	6·0	0·38 ± 0·06	0·40 ± 0·06		
			Chronic		
	Treatment (weeks)				
	1	0·18 ± 0·01	0·27 ± 0·04‡	57 ·6 ± 2·9	59·2 ± 4·0
	2	0·18 ± 0·02	0·25 ± 0·02‡	59·7 ± 10·7	56·6 ± 6·5
	3	0·17 ± 0·03	0·26 ± 0·03‡	58·6 ± 8·2	59·2 + 3·7
+ NaF†	1	0·39 ± 0·01	0·41 ± 0·02		
	2	0·39 ± 0·07	0·40 ± 0·06		
	3	0·37 ± 0·07	0·40 ± 0·06		

* Four g/kg of ethanol was injected intraperitoneally. The ethanol levels in blood serum at 1·5, 3·0 and 6·0 hr after the injection were 310, 178 and 23 mg % respectively.

† NaF (10 mM) was added *in vitro* to the reaction mixture for adenylate cyclase assays. The results in this table are the mean ± S.D. obtained from five separate determinations.

‡ $P < 0·01$.

control was decreased after 10 min incubation, but repeated and careful examination failed to reveal such a decline of enzyme activity in chronically treated ethanol groups. Neither acute nor chronic ethanol treatment altered phosphodiesterase activity in cerebral cortex (Table 1).

Figure 2 shows the effect of acute and chronic ethanol treatments on the cyclic AMP level in cerebral hemisphere. Cerebral cyclic AMP content was significantly increased by 64, 49 and 14 per cent following 1, 2 and 3 weeks of ethanol ingestion, while no alteration was found in acutely treated groups.

In vitro addition of ethanol up to 20 mg/ml, a dose approximately 7–8 times higher than the anesthetic dose of ethanol, had no effect on either adenylate cyclase or phosphodiesterase activities.

Steady state cyclic AMP levels increased in brain preparations from mice chronically administered ethanol. This change was accompanied by activation of adenylate cyclase, but not by any detectable alteration of phospodiesterase activity. It is generally considered that phosphodiesterase governs the tissue level of cyclic AMP.[17] However our data suggest that in mouse brain some changes in cyclic AMP levels are most probably related to alterations in adenylate cyclase activity rather than phosphodiesterase activity. If phosphodiesterase is really the rate limiting step for maintaining steady state levels of cyclic AMP in the brain, chronic ethanol treatment may affect selectively a cyclic AMP pool in the brain relatively independent of phosphodiesterase activity. One of the interesting findings in this study is that increases in adenylate cyclase activity following chronic ethanol treatment was not distinguishable after the addition of sodium fluoride to the assay system. The mechanism of activation of adenylate cyclase by sodium fluoride remains unclear. However, it has been suggested that sodium fluoride activates the enzyme following interaction with the catalytic system (possibly located in the plasma membrane) by dissociating an inhibitory complex.[16] Furthermore the sodium fluoride acti-

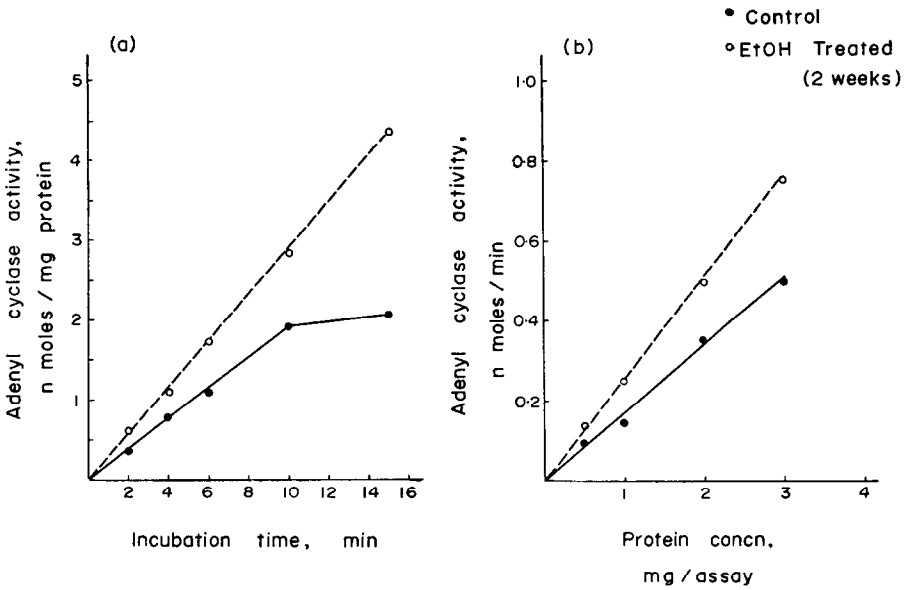

FIG. 1. Effect of chronic ethanol treatment (2 weeks) on adenylate cylase activity in mouse cerebral cortex. For the assay of a series, 1 mg of enzyme protein was used. All values are the averages of three separate experiments.

vated enzyme is more stable at 40° than the native enzyme.[16] The increase in adenylate cyclase activity which was not distinguishable in the presence of sodium fluoride and the finding that brain adenylate cyclase from chronically ethanol treated animals was more stable than the enzyme from control animals suggests that the effect of chronic ethanol treatment on the enzyme may involve a mechanism similar to fluoride activation—possibly an effect on the inhibitor–catalytic component complex. Our previous findings[18] indicated that chronic ethanol treatment also decreased hormonal sensitivity of

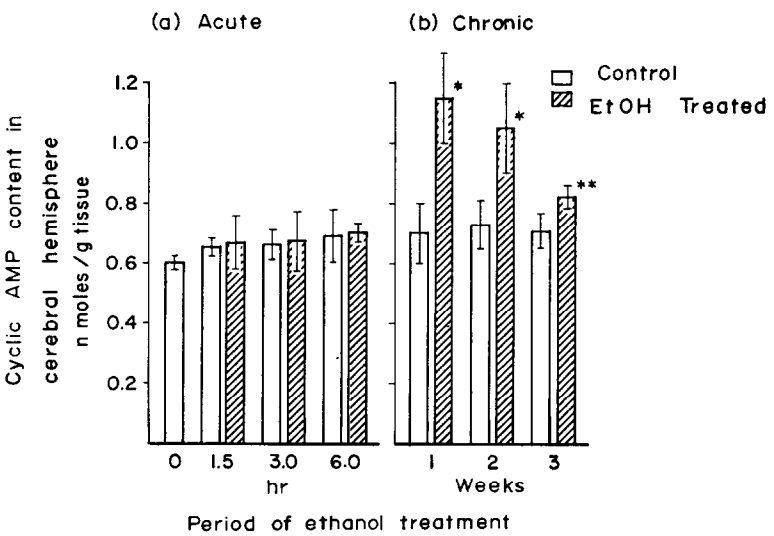

FIG. 2. Effect of acute and chronic ethanol treatments on cyclic AMP content of the cerebral hemisphere. * $P < 0.01$, ** $P < 0.05$. The average ± S.D. obtained from 10 separate determinations is shown.

the cyclic AMP system in slices of cerebral cortex. Considering the model of adenylate cyclase proposed by Robison *et al.*[19] that depicts the enzyme as an integral part of plasma membrane with a receptor–regulatory component exposed on the outer surface and a catalytic component exposed on the inner surface, chronic ethanol treatment may alter both receptor–regulatory and catalytic unit of brain adenylate cyclase.

Ethanol induced changes in adenylate cyclase activity as well as decreased hormonal sensitivity of the cyclic AMP synthesizing system in brain slices[18] are demonstrable only after chronic ethanol treatment suggesting that the changes may not be due to the direct effect of ethanol on the cyclic AMP system but rather may be related to the complex processes of ethanol dependence or habituation. Recent studies in our laboratory[20,21] have shown that chronically administered morphine also induces the activation of brain adenylate cyclase activity and the loss of respondence of cyclic AMP synthesizing system toward various biogenic amines. This fact indicates that the observed alterations in cyclic AMP metabolism in brain are not specific for chronic ethanol treatment. However, both ethanol and morphine are known to be capable of inducing dependence and alterations in the function of endocrine system.[6,22,23] These findings suggest that continuous activation of the hypothalamic–pituitary–adrenal endocrine axis induces an increase in brain cyclic AMP via adenylate cyclase activation and may contribute to the establishment of dependence by these drugs.

Acknowledgement—This work was supported in part by Research grants from the Japanese ministry of education (NO 744032, 1972) and United States Public Health Service (MH 18663 and MH 16477).

Department of Pharmacology, Kyoto Prefectural University of Medicine, Kamikyo-Ku, Kyoto 602, *Japan*
and
Division of Neuropharmacology, Department of Psychiatry, State University of New York, Downstate Medical Center, Brooklyn, New York, U.S.A.

KINYA KURIYAMA
MARK A. ISRAEL*

REFERENCES

1. E. W. SUTHERLAND and T. W. RALL, *Pharmac. rev.* **12,** 265 (1960).
2. R. W. BUTCHER, G. A. ROBISON, J. G. HARDMAN and E. W. SUTHERLAND, *Advan. enz. Reg.* **6,** 357 (1968).
3. B. MCL. BRECKENRIDGE, *Ann. rev. Pharmac.* **10,** 19 (1970).
4. G. R. SIGGINS, R. J. HOFFER and E. E. BLOOM, *Science, N.Y.* **165,** 1018 (1969).
5. D. A. MCAFEE, M. SCHORDERET and P. GREENGARD, *Science, N.Y.* **171,** 1156 (1971).
6. F. W. ELLIS, *J. Pharmac. exp. Ther.* **153,** 121 (1966).
7. K. KURIYAMA, P. Y. SZE and G. E. RAUSCHER, *Life Sci.* **10,** (pt. 2), 181 (1971).
8. K. KURIYAMA, G. E. RAUSCHER and P. Y. SZE, *Brain Res.* **26,** 450 (1971).
9. G. FREUND, *Arch. Neurol.* **21,** 315 (1969).
10. G. KRISHNA, B. WEISS and B. B. BRODIE, *J. Pharmac. exp. Ther.* **163,** 379 (1968).
11. B. WEISS, *J. Neurochem.* **18,** 469 (1971).
12. P. D. SWANSON, H. F. BRADFORD and H. MCILWAIN, *Biochem. J.* **92,** 237 (1964).
13. A. L. STEINER, D. M. KIPNIS, R. UTIGER and C. PARKER, *Proc. natn. Acad. Sci. U.S.A.* **64,** 367 (1969).
14. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
15. R. BONNISCHEN, in *Method of Enzymatic Analysis* (Ed. H. U. BERGMEYER; transl. D. H. WILLIAMSON), p. 285. Academic Press, New York (1963).
16. J. P. PERKINS and M. M. MOORE, *J. biol. Chem.* **246,** 62 (1971).
17. W. Y. CHEUNG, *Advan. Biochem. Pharmac.* **3,** 51 (1970).
18. M. A. ISRAEL, H. KIMURA and K. KURIYAMA, *Experientia* **28,** 1322 (1972).
19. G. A. ROBISON, R. W. BUTCHER and E. W. SUTHERLAND, *Ann. N.Y. Acad. Sci.* **139,** 703 (1967).
20. K. NAITO and K. KURIYAMA, *Jap. J. Pharmac.* **23,** 274 (1973).
21. K. NAITO, K. NAKAGAWA, H. KIMURA and K. KURIYAMA, *Bull. Jap. Neurochem. Soc.* **11,** 123 (1972). (In Japanese).
22. J. W. SLOAN, in *Narcotic Drugs, Biochemical Pharmacology* (Ed. D. H. CLOUET) p. 262. Plenum Press, New York (1971).
23. R. GEORGE, in *Narcotic Drugs, Biochemical Pharmacology* (Ed. D. H. CLOUET) p. 283. Plenum Press, New York (1971).

* Present address: Department of Medicine, Children's Hospital Medical Center, Boston, Massachusetts, 02115, U.S.A.