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The Effects of Ethanol on Cerebral Regional Acetylcholine Concentration and Utilization[1] (40330)

TELFAIR H. PARKER, RODERICK K. ROBERTS, GEORGE I. HENDERSON, ANASTACIO M. HOYUMPA, JR., DENNIS E. SCHMIDT, AND STEVEN SCHENKER

Departments of Medicine and Pharmacology, Vanderbilt University School of Medicine, Veterans Administration Hospital, and Tennessee Neuropsychiatric Institute, Nashville, Tennessee 37203

The precise cerebral mechanism(s) of the acute effects of alcohol (ethanol) on the brain are still uncertain, but an alteration in neurotransmitter balance has been proposed as one possibility (1). Unfortunately, the available data concerning the level and metabolism of most key putative neurotransmitters following the acute and chronic administration of alcohol are conflicting (1). This may be due to species differences, dose of alcohol given, acute or chronic ethanol administration, brain area(s) assayed, and other methodologic difficulties.

The effect of acute and chronic alcohol exposure on cerebral acetylcholine (ACh) is controversial and incompletely defined (1). The aims of the present study were (i) to determine the effects of increasing acute oral doses of alcohol on regional cerebral ACh levels, (ii) to correlate the brain ACh concentrations with blood alcohol levels, (iii) to measure regional cerebral ACh utilization rates in rats at blood ethanol levels seen during modest human inebriation when rat brain ACh levels are essentially unaltered, and (iv) to assess the effects of prolonged oral alcohol consumption on brain ACh levels in rats. The results of these studies are the basis of this report.

Experimental procedures. Nonfasted female Sprague–Dawley rats, weighing 200 to 250 g, and female Swiss albino mice, weighing 20 to 25 g, were used for the acute alcohol and acetaldehyde experiments. Alcohol was diluted with saline to give a 25% solution (v/v) and was given by gavage to rats as a single oral dose of 3 to 7 g/kg body wt. Mice received orally 20% ethanol as single doses of 1.5 or 3 g/kg. Controls for the 3 g/kg alcohol dose received orally an equal volume of saline or isocaloric glucose and were sacrificed at the appropriate time. Since glucose and saline controls in these studies gave the same brain ACh values, in other acute experiments wherein net brain ACh levels were measured (Tables I and II) only saline controls were used. Acetaldehyde was dissolved in saline and was given to rats as 40 mg/kg intravenously 15 min before sacrifice; this dose has previously been reported to lower brain ACh in mice (2).

For the chronic alcohol studies, Sprague–Dawley female rats weighing between 200 and 250 g were paired, one receiving ethanol and the second serving as a pair-fed control. All rats were maintained on a 12-hr light–dark cycle in stainless-steel cages. They received the Lieber–DeCarli liquid diet containing either 6% (v/v) ethanol or isocalorically balanced maltose-dextrins for pair-fed controls as previously described. The rats were sacrificed after 5 weeks on alcohol. The growth curves and blood alcohol levels of these animals have been reported previously (3).

In both acute and chronic studies ACh levels in the various brain regions were determined by pyrolysis–gas chromatography (4) following head-focused microwave sacrifice (5). The landmarks for identifying cortex, corpus striatum, midbrain, and brainstem have also been described by us earlier (6).

In order to estimate relative ACh turnover, the rate of decline of ACh levels following inhibition of ACh synthesis by hemicholinium-3 (HC-3) was determined. This rate of decline of ACh has been shown to be dependent upon neuronal firing rate of cholinergic neurons (7–9) and therefore appears to

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TABLE I. THE EFFECT OF ORAL ACUTE ALCOHOL ADMINISTRATION ON REGIONAL CEREBRAL ACETYLCHOLINE LEVELS IN RATS.[a]

Alcohol dose and time of sacrifice		Brain areas assayed				Blood alcohol level (mg/100 ml, mean ± SE)
		Cortex	Corpus striatum	Midbrain	Brainstem	
		(nmoles/g wet wt, mean ± SE)				
3 g/kg, 45 min	Alcohol (6)	27.4 ± 0.3	81.8 ± 2.0*	43.1 ± 1.4	29.6 ± 1.2	172 ± 14
	Saline control (5)§	26.1 ± 1.3	71.7 ± 4.3	40.2 ± 2.0	28.6 ± 2.1	—
	Glucose control (5)	26.1 ± 0.8	75.3 ± 2.7	41.9 ± 1.0	30.6 ± 0.4	—
3 g/kg, 90 min	Alcohol (5)	27.1 ± 1.0**	80.9 ± 4.1	37.5 ± 1.5	29.1 ± 0.4**	179.2 ± 16.0
	Saline control (5)§	23.7 ± 0.4	72.2 ± 1.9	38.3 ± 1.7	26.4 ± 0.7	—
	Glucose control (5)	24.9 ± 0.8	73.2 ± 4.8	38.0 ± 0.6	26.8 ± 0.8	—
3 g/kg, 150 min	Alcohol (5)	27.3 ± 1.2	79.4 ± 3.8	39.9 ± 0.6	28.2 ± 0.7	136 ± 13
	Saline control (6)§	26.0 ± 0.9	71.2 ± 2.6	39.7 ± 1.1	30.2 ± 1.4	—
	Glucose control (6)	24.6 ± 0.7	75.7 ± 3.2	41.0 ± 1.6	29.9 ± 1.8	—
4 g/kg, 45 min	Alcohol (5)	25.1 ± 1.0	90.7 ± 5.4**	37.3 ± 1.5	29.1 ± 1.9	219 ± 32
	Saline control (3)§§	22.2 ± 0.9	69.0 ± 1.3	35.8 ± 0.5	28.7 ± 0.9	—
5 g/kg, 45 min	Alcohol (4)	24.4 ± 1.2	87.3 ± 6.9**	38.3 ± 1.1	28.7 ± 0.8	278 ± 34
	Saline control (3)§§	22.2 ± 0.9	69.0 ± 1.3	35.8 ± 0.5	28.7 ± 0.9	—
6 g/kg, 45 min	Alcohol (5)	30.5 ± 1.0†	87.1 ± 2.8**	42.3 ± 2.5	31.4 ± 1.2	439 ± 31
	Saline control (3)§§	26.5 ± 1.3	70.1 ± 3.3	39.1 ± 2.3	29.4 ± 0.5	—
7 g/kg, 45 min	Alcohol (5)	33.5 ± 1.7**	101.5 ± 6.0**	43.9 ± 1.4‡	31.2 ± 1.2	463 ± 30
	Saline control (3)§§	26.5 ± 1.3	70.1 ± 3.3	39.1 ± 2.3	29.4 ± 0.5	—

[a] Statistical information:

* $p < 0.05$, one-tailed test.

** $p < 0.05$, two-tailed test.

† $p > 0.05$ vs three saline controls assayed on the same day but <0.05 vs pooled saline controls.

‡ $p < 0.05$, one-tailed test, vs saline group ($n = 3$) assayed on same day and <0.05, two-tailed, vs all 45-min saline midbrain control data.

§ In rats receiving 3 g/kg alcohol, the saline and glucose values for each time interval and each area of brain were comparable ($p > 0.05$) and were pooled ($n = 10$–12) for statistical analysis.

§§ Saline groups for 4 and 5 g/kg alcohol and for 6 and 7 g/kg alcohol groups, respectively, were the same. In all instances where the saline controls for a given result consisted of only three rats assayed on the same day, comparison of the same alcohol data vs *all* pooled appropriate saline data ($n = 17$) confirmed the statistical interpretation derived from the three saline controls alone.

TABLE II. THE EFFECT OF ACUTE ORAL ALCOHOL ADMINISTRATION ON CEREBRAL REGIONAL ACETYLCHOLINE LEVELS IN MICE.

Dose of alcohol	Brain region				Blood alcohol (mg/100 ml)
	Cortex‡	Corpus striatum	Midbrain	Brainstem	
	(nmoles/g wet wt)				
1.5 g/kg (7)†	25.5 ± 1.4	56.9 ± 2.7*	28.5 ± 1.0	28.2 ± 1.2	134 ± 10
3 g/kg (7)†	35.1 ± 1.6*	68.3 ± 4.4*	38.2 ± 3.4*	28.1 ± 3.1	332 ± 17
Saline control (6)†	21.9 ± 0.9	47.4 ± 0.9	23.9 ± 2.2	27.7 ± 3.9	—

† Sacrifice 45 min after alcohol or saline administration.

‡ Mean ± SE.

* $p < 0.05$ vs saline controls.

be a valid index of cholinergic function. Briefly, rats were implanted with intraventricular polyethylene cannula as described by Robison *et al.* (10). Following 3 to 5 days of recovery, 20 μg of HC-3 dissolved in water was administered to rats given ethanol (3 g/kg orally) or to controls which received isocaloric glucose. Previous studies (11) have shown that this dose of HC-3 produces a linear decline in brain ACh in the areas studied over 45 min without mortality. The time of administration of HC-3 was varied relative to the time of alcohol administration so as to allow analysis of brain ACh at 0, 15, 30, and 45 min after HC-3 in both ethanol-treated rats and controls. The blood ethanol during

this period averaged 170 ± 17 mg/100 ml (mean ± SE). Declines in ACh in each brain region were converted into slopes by regression analysis, giving the relative turnover rate. Blood alcohol in rats and mice in the acute studies and in the chronic studies were measured by the alcohol dehydrogenase method (12).

Statistical analysis of brain ACh data in the acute studies was carried out by Student's t test, correlations between alcohol dose and ACh levels over the whole alcohol dose range by regression analysis, and the comparison of ACh slopes by analysis of covariance (13). The data were considered statistically significant with a p value of <0.05.

Results. Table I shows the effects of acute oral alcohol administration in various doses on the ACh concentration of several brain regions in rats. With 3 g of alcohol/kg body wt, blood alcohol levels were achieved at 45, 90, and 150 min, which roughly correspond to the concentration of alcohol considered legally intoxicating in man. With this dose, especially at 90 min when the mean blood alcohol was 179 mg/100 ml, cerebral regional ACh levels tended to be slightly higher than in controls, but this was not uniform in all brain areas and was statistically significant in only a few of them (wherein the control values tended to be lower). With increasing doses of alcohol of 4 to 7 g/kg the blood alcohol level rose progressively as one would expect ($r = 0.945$, $p < 0.001$) and brain ACh also tended to increase gradually (Table I). Again, however, the rise was modest and was not present in all brain areas studied even at very high blood alcohol concentrations. For cortex and corpus striatum the relationship between alcohol dose and increase in ACh was significant ($r = 0.53$, $p < 0.001$ and $r = 0.729$, $p < 0.001$, respectively) while the correlation for midbrain and brainstem was not significant ($r = 0.265$, $p = 0.118$ and $r = 0.260$, $p = 0.142$, respectively). As is shown in Table II, doses of 1.5 and 3 g/kg of alcohol which gave mean blood alcohol levels of 134 and 332 mg/100 ml in mice at 45 min also tended to increase brain ACh levels and this was especially evident with the higher dose. There was no change in brainstem ACh with alcohol administration. By contrast, a single dose of acetaldehyde had no effect on regional brain ACh levels in rats (Table III). In addition, chronic administration of ethanol orally in a liquid diet for 5 weeks did not alter brain ACh concentration in rats (Table IV). This type of alcohol intake has been shown in previous studies to involve an average daily consumption of 3.7 ml of absolute alcohol per rat and gives blood alcohol levels of 70 to 200 mg/100 ml. At the time of brain assay for ACh (about 11:00 AM), with the animals fasted since 8:00 AM, the blood alcohol levels were essentially undetectable.

As a more sensitive index of possible derangement of ACh metabolism, the utilization rate of ACh was studied regionally in the brain at blood alcohol levels which coincide with human legal intoxication (approx 170 mg/100 ml) and which give essentially no evidence of alteration of net brain ACh in

TABLE III. THE EFFECT OF ACETALDEHYDE ON REGIONAL CEREBRAL ACETYLCHOLINE LEVELS IN RATS.

	Cortex*	Corpus striatum	Midbrain	Brainstem
Acetaldehyde† (7)	24.5 ± 0.8	75.7 ± 3.1	39.6 ± 1.9	28.8 ± 1.1
Saline (3)	28.2 ± 0.8	73.4 ± 6.1	37.9 ± 1.8	29.5 ± 0.7

† 40 mg/kg given iv. Rats sacrificed 15 min later. The acetaldehyde-injected rats had brain acetylcholine levels comparable to control values ($p > 0.05$) (see also control values in Table I).

* Mean ± SE, nmoles/g wet wt.

TABLE IV. THE EFFECT OF CHRONIC† ALCOHOL INGESTION ON REGIONAL CEREBRAL ACETYLCHOLINE LEVELS IN RATS.

	Cortex*	Corpus striatum	Midbrain	Brainstem
Alcohol‡ (8)	28.8 ± 2.0	71.3 ± 5.1	37.4 ± 1.2	29.1 ± 1.1
Pair-fed control (8)	28.9 ± 0.8	69.2 ± 3.5	39.4 ± 0.9	29.9 ± 1.7

† Five weeks of oral alcohol intake (see Experimental Procedures).

‡ None of the alcohol values were statistically significantly different from appropriate control data.

* Mean ± SE, nmoles/g wet wt.

rats (3 g/kg of ethanol at 45 min, Table I). As is shown in Table V, the rate of utilization of ACh was significantly decreased with this dose of ethanol in the cortex and midbrain. In the corpus striatum and brainstem the values tended to be lower in the alcohol group but this did not reach statistical significance.

Discussion. The present study clearly shows in both rats and mice that acute oral administration of ethanol increases significantly the concentration of ACh in some areas of the brain (Tables I and II). The changes, however, were only modest and occurred primarily at high blood ethanol concentrations. Our data are in agreement with prior observations carried out with a single large dose of ethanol (6 g/kg, po) (14) but do not confirm the studies of Rawat (2) who found depressed levels of ACh in whole brain of mice given 3 g/kg of ethanol. This discrepancy may perhaps best be explained by the use of brain from mice rapidly frozen in liquid nitrogen in the latter study (2); such a sacrifice procedure is known to result in partial degradation of ACh and indeed the levels of ACh in that study were less than half of those obtained by microwave fixation of brain. In our studies the corpus striatum shows the most consistent ACh increase with ethanol, perhaps because it has the most rapid ACh turnover rate (Table V). Both the cortex and midbrain ACh, however, were also affected by alcohol, especially at higher blood alcohol levels. It appears, therefore, that the ethanol effect on brain ACh is a general one.

The mechanism(s) by which ethanol may increase brain ACh is still uncertain. Rawat reasoned that acetaldehyde generated from ethanol metabolism may combine with sulfhydryl groups of coenzyme A and thus decrease the precursor pool for ACh synthesis (2). In our studies, utilizing the same acetaldehyde protocol, no change in brain ACh was noted (Table III). Thus, while blood and brain acetaldehyde concentrations were not measured and it is possible that higher doses of acetaldehyde or administration of this drug over a prolonged time would exert some effect, our data with the single bolus of acetaldehyde do not support such a hypothesis. Against the acetaldehyde concept (2) are not only the observations that brain ACh increased, and not decreased, with alcohol administration but also the extensive *in vitro* and *in vivo* data with brain exposed to ethanol (15–17). In these studies, wherein no significant acetaldehyde is generated, alcohol inhibited the release of ACh from cerebral cortical slices and the mesencephalic reticular formation. These data clearly indicate that alcohol per se exerts an inhibitory effect on ACh release from brain. Our *in vivo* measurements of ACh utilization (Table V) (to our knowledge not previously carried out) showed a statistically significant decreased ACh turnover after ethanol administration in cortex and midbrain. In the corpus striatum and brainstem there was a tendency to a lower ACh utilization but this did not show statistical significance. This is consistent with the slight net accumulation of ACh in most of these areas with this low dose of alcohol. Conceivably at higher blood and brain alcohol levels a greater effect on ACh utilization would be shown. The changes observed here by us and by others (1) on brain ACh with ethanol are most consistent with the concept of Nikander and Wallgren (18) that alcohol

TABLE V. THE EFFECT OF ACUTE ORAL ALCOHOL ADMINISTRATION ON REGIONAL CEREBRAL ACETYLCHOLINE UTILIZATION.

	Control†	Alcohol†	Decrease in alcohol group (%)	p value
	(nmoles/g brain/min)			
Cortex	0.43 ± 0.03 (33)*	0.30 ± 0.03 (32)	30.2	<0.001
Corpus striatum	1.23 ± 0.08 (36)	1.09 ± 0.09 (38)	10.9	>0.10
Midbrain	0.54 ± 0.05 (35)	0.39 ± 0.03 (37)	28.3	<0.02
Brainstem	0.17 ± 0.05 (28)	0.10 ± 0.04 (31)	39.8	>0.10

† Alcohol given to rats as 3 g/kg orally while controls received isocaloric glucose in an equal volume of saline. All data are given as the mean ± SE turnover rate for the number of animals shown. For technique used to measure turnover rate see Experimental Procedures.

* Overall number of samples assayed over 45 min, with 4 to 6 specimens at each time interval.

inhibits the action potential in brain. This may be mediated by a direct effect of alcohol on ionic conductance in the neuronal membrane (18, 19) and/or may be exerted at the presynaptic level (19). The net effect would be, as reported here, decreased utilization resulting in an accumulation of ACh. A precise quantitative stoichiometry between net ACh levels and its turnover, however, may not occur due to compartmentation of ACh in brain. No significant effects of alcohol on cerebral acetyltransferase or acetylcholinesterase activity have been reported (20). The effect of alcohol on brain ACh is not unique for this sedative and is shared by higher alcohols and barbiturates (19). Our observation (Table IV) that chronic alcohol intake does not alter brain ACh levels when alcohol is not present in blood implies that it is the presence of high concentrations of ethanol and not the duration of its administration which is relevant. An alternate interpretation, for which there are good data (17, 21), and which is not addressed by these studies, is that with chronic alcohol use the brain becomes insensitive or less sensitive to the effects of ethanol on ACh turnover.

The functional significance of ethanol-induced changes in brain ACh is uncertain. Erickson and Burnam (22) have shown that physostigmine shortens ethanol-induced sleep-time in mice and these studies have been confirmed by others (23). However, ethanol-induced EEG synchrony, an index of cerebral depression, could not be correlated with brain ACh changes after ethanol (16) and the use of various drugs which alter cerebral ACh status did not predictably alter behavioral depression (24). Finally, physostigmine appears to be a relatively nonspecific analeptic since it may reverse sedation induced by diazepam (unpublished observations) and other sedatives. Thus, the present study documents an increase of brain ACh and its decreased utilization with high levels of alcohol, but the functional significance of these findings remains to be established.

Summary. This study assessed the effect of alcohol, given as single increasing doses or chronically, on regional cerebral acetylcholine concentration. In the acute studies in both rats and mice, brain acetylcholine rose significantly, but modestly, at higher blood ethanol concentrations. This effect was most consistent in the corpus striatum. At low blood alcohol levels, when brain acetylcholine levels were unaltered, the utilization rate of acetylcholine decreased in all brain areas and this was statistically significant in the cortex and midbrain. By contrast, in rats exposed to chronic oral ethanol intake but studied when blood alcohol was normal, brain acetylcholine was unaltered. These data are most consistent with the concept that alcohol directly depresses neuronal function resulting in decreased release (utilization) of acetylcholine and at high alcohol concentrations induces a modest accumulation of acetylcholine in brain.

1. Noble, E. P., and Tewari, S., *in* "Metabolic Aspects of Alcoholism" (C. S. Lieber, ed.), p. 149. University Park Press, Baltimore (1977).
2. Rawat, A. K., J. Neurochem. **22,** 915 (1974).
3. Henderson, G. I., and Schenker, S., Res. Commun. Chem. Pathol. Pharmacol. **16,** 15 (1977).
4. Schmidt, D. E., Speth, R. C., Welsch, F., and Schmidt, M. J., Brain Res. **38,** 377 (1972).
5. Schmidt, D. E., Neuropharmacology **15,** 77 (1976).
6. Vorhees, C. V., Schmidt, D. E., Barrett, R. J., and Schenker, S., J. Nutr. **107,** 1902 (1977).
7. Rommelspacher, H., Goldberg, A. M., and Kuhar, M. J., Neuropharmacology **13,** 1015 (1974).
8. Rommelspacher, H., and Kuhar, M. J., Brain Res. **81,** 243 (1974).
9. Rommelspacher, H., and Kuhar, M. J., Naunyn-Schmiedeberg's Arch. Pharmacol. **287,** Suppl. 1, R 10 (1975).
10. de Balbian Verster, F., Robison, G. A., Hengeveld, C. A., and Bush, E. S., Life Sci. **10,** 1395 (1971).
11. Schmidt, D. E., and Buxbaum, D. M., Brain Res. in press.
12. Stiles, D. J., Batsakis, B., Kremers, B., and Briere, R. O., Amer. J. Clin. Pathol. **46,** 608 (1966).
13. Jerome, C. R., *in* "Statistical Inference," p. 313. Edwards Bros., Ann Arbor, Mich. (1966).
14. Hunt, W. A., and Dalton, T. K., Brain Res. **109,** 628 (1976).
15. Kalant, H., and Grose, W., J. Pharmacol. Exp. Ther. **158,** 386 (1967).
16. Erickson, C. K., and Graham, D. T., J. Pharmacol. Exp. Ther. **185,** 583 (1973).
17. Clark, J. W., Kalant, H., and Carmichael, F. J., Canad. J. Physiol. Pharmacol. **55,** 758 (1977).
18. Nikander, P., and Wallgren, H., Acta Physiol. Scand. **80,** 27A (1970).
19. Israel, Y., Carmichael, F. J., and Macdonald, J. A., *in* "Advances in Experimental Medicine and Biology" (M. Gross, ed.), Vol. 59, p. 55. Plenum Press, New York (1975).

20. Kalant, H., Israel, Y., and Mahon, M. A., Canad. J. Physiol. Pharmacol. **45,** 172 (1967).
21. Kalant, H., and Grose, W., J. Pharmacol. Exp. Ther. **158,** 386 (1968).
22. Erickson, C. K., and Burnam, W. L., Agents and Actions **2,** 8 (1971).
23. Berman, M. L., and Harbison, R. D., J. Pharmacol. **18,** 184 (1976).
24. Graham, D. T., and Erickson, C. K., Psychopharmacologia **34,** 173 (1974).

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