Effect of Ethanol on 5-Hydroxytryptamine Turnover in Rat Brain[1] (34723)

GERTRUDE M. TYCE, EUNICE V. FLOCK, WILLIAM F. TAYLOR, AND CHARLES A. OWEN, JR.

Mayo Clinic and Mayo Foundation: Section of Biochemistry and of Medical Statistics, Rochester, Minnesota 55901

The narcotic action and the toxicity of ethanol are markedly potentiated by modest doses of 5-hydroxytryptamine (5-HT) (1), and it has been suggested that some of the toxic effects of ethanol may be due to its influence on 5-HT metabolism. In peripheral tissues, ethanol induces a shift in the metabolism of 5-HT so that 5-hydroxytryptophol (5-HTOH) is found instead of 5-hydroxyindoleacetic acid (5-HIAA) (2–4). However, in the brains of ethanol-intoxicated rats, no obvious alterations were found in the metabolism of 5-HT-^{14}C injected into the caudate nucleus (5). Also, the metabolism of 5-HT by brain slices was essentially unaffected by the presence of ethanol in the incubate (6). It is possible, however, that the toxicity of ethanol might be mediated by a change in the turnover of 5-HT in brain, and the present investigation was designed to study this.

Methods. Sprague-Dawley rats maintained on a Rockland rat diet and weighing 250 to 340 g were used. Under ether anesthesia, polyethylene tubing was inserted into the tail vein so that injections could be made, and in some of the rats the femoral artery was cannulated so that blood samples could be collected. The rats rapidly recovered from this brief anesthesia, but 4 to 5 hr were allowed to elapse before injections were made. Ethanol (3.3 g/kg) was injected intraperitoneally, as a 25% solution in saline, to fed rats. At 15 and 75 min after injection the rats were killed, an aliquot of blood was removed by cardiac puncture, and the brains were removed and frozen in powdered Dry Ice. Control rats (not given an injection) were killed and the brains were removed in a similar manner.

Turnover of 5-HT was measured by the method of Tozer and co-workers (7). The rates of increase in concentration of 5-HT and of decrease in concentration of 5-HIAA were measured after the administration of a monoamine oxidase inhibitor (*N*-benzyl-*N*-methyl-2-propynylamine; pargyline), 75 mg/kg, intravenously as a 2.5% solution in isotonic saline during 2 min to control rats and to ethanol-treated rats at 15 min after alcohol administration. Immediately before pargyline injection or at intervals afterward, the rats were anesthetized with pentobarbital (Nembutal), aliquots of blood were removed by cardiac puncture, and the brains were removed and frozen in powdered Dry Ice.

Concentrations of 5-HT and 5-HIAA in brain were measured fluorometrically after extraction by the procedure of Quay (8). Ethanol concentrations were measured in perchloric acid extracts of whole blood and in Somogyi filtrates of brain by the alcohol dehydrogenase method (9).

Results. Effect of ethanol on concentration of 5-hydroxyindoles in brain. There was no apparent effect of ethanol on the concentration in brain of either 5-HT or 5-HIAA. In the brains of 10 normal rats the mean concentration of 5-HT was 0.62 μg/g (SD, 0.06). At 10 and 70 min after the injection of ethanol the concentration of 5-HT was not different from these normals (0.63 μg/g; SD, 0.05; $n = 8$; and 0.61 μg/g; SD, 0.03; $n = 6$, respectively). The mean concentration of 5-HIAA in the brains of 10 control rats was 0.49 μg/g (SD, 0.03) and was not significantly changed at 10 and 70 min after the injec-

[1] This investigation was supported in part by Research Grant NB-4004 from the National Institutes of Health, Public Health Service.

tion of ethanol (0.52 μg/g; SD, 0.05; $n = 8$; and 0.51 μg/g; SD, 0.02; $n = 6$, respectively).

Toxicity of ethanol and pargyline. No deaths occurred among 41 rats given pargyline and no ethanol. However, 10 of 41 rats died when given the same dose of pargyline 15 min after ethanol; 9 of these rats died within 6 min after the injection of pargyline and the other died 45 min after. Fifteen rats were treated with ethanol alone and one of these rats died 60 min after the injection.

Concentrations of ethanol in blood and brain. During the first 100 min after the injection of ethanol, the ethanol concentration varied from 2.90 to 4.78 mg/ml in the blood of eight rats and from 2.42 to 3.88 mg/g in their brains. There was no significant difference between the concentrations of ethanol in blood or in brains of four of these rats which had received both ethanol and pargyline and four which had received ethanol alone (Table I). There was significant variation among the rats and a significant decrease of ethanol concentration in blood with time. (This was based on a standard analysis of variance method. p values for variation among rats and for time, <0.005).

Changes in 5-HIAA in brain. With passage of time after the administration of pargyline, the concentrations of 5-HIAA appeared to decrease exponentially in normal and in ethanol-intoxicated rats (Fig. 1).

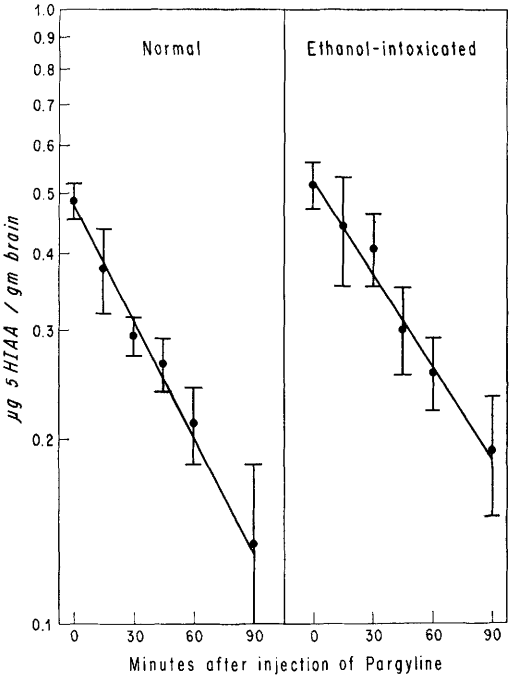

FIG. 1. Concentration of 5-HIAA in brains of normal and ethanol-intoxicated rats at different times after intravenous injection of pargyline (75 mg/kg), shown as means ± SD of 6 to 10 determinations. Least squares regression equations are: for normal rats, $\log_{10}Y = -0.371X - 0.321$; for ethanol-intoxicated rats, $\log_{10}Y = -0.301X - 0.277$.

Lines of best fit were drawn by the method of least squares using $\log_{10}$ of 5-HIAA versus time; the fractional rate of decrease in 5-HIAA concentration (k) is $\log_e 10$ (= 2.3)

TABLE I. Mean Ethanol Concentrations in Blood and Brain of Rats After Injection of Ethanol With and Without Pargyline.[a]

Tissue	Time after ethanol (min)	Ethanol concentration (mg/ml or mg/g)[b]			
		With pargyline		Without pargyline	
		Mean	SD	Mean	SD
Blood	25	3.62	0.25	3.86	0.65
	40	3.32	0.24	3.73	0.54
	55	3.28	0.40	3.51	0.51
	70	3.18	0.31	3.49	0.64
	100	2.90	0.40	3.29	0.77
Brain	100	2.76	0.26	2.99	0.69

[a] Ethanol (3.3 g/kg) given intraperitoneally; pargyline (75 mg/kg) given intravenously 15 min after administration of ethanol.

[b] Four determinations for each mean.

TABLE II. Turnover Rates of 5-HT in Brain in Normal and Ethanol-Intoxicated Rats Measured from Decrease in 5-HIAA Concentration After Monoamine Oxidase Inhibition by Pargyline.

Condition of rats	5-HIAA concentration in brain before pargyline			Rate constant of 5-HIAA decrease after pargyline		Turnover rate of 5-HT (μg/g/hr)[b]
	Mean	SD	n[a]	k (hr^{-1})	n	
Normal	0.49	0.03	10	0.85	47	0.38
Ethanol-intoxicated	0.52	0.05	8	0.69[c]	36	0.33

[a] n is number of animals in each group.

[b] Product of concentration and rate constant of decrease of 5-HIAA, corrected for difference in molecular weights of 5-HT (176) and 5-HIAA (191) [5-HIAA concentration × k × (176/191)].

[c] For difference from normal, $p < 0.05$.

times the slope of this line. There was a small but significant difference ($p < 0.05$) between the fractional rate of decrease of 5-HIAA concentration in the ethanol-intoxicated rats (0.69) and that in the normal rats (0.85) calculated by the method outlined by Steel and Torrie (10) (Table II). The turnover rate of 5-HT in the brain was 0.38 μg/g/hr in normal rats and 0.33 μg/g/hr in the ethanol-intoxicated rats.

Accumulation of 5-HT after pargyline administration. Figure 2 shows how 5-HT concentration in brain changed with time among normal and ethanol-intoxicated rats after the administration of pargyline. The trend, while upward in both cases, appeared to have some curvature in the normals. The trend for intoxicated rats was irregular but no curvature was apparent.

When a straight line and a parabola were fitted to the normal data by the method of least squares, a parabola fitted significantly better ($p < 0.005$), so it would appear justified to state that the increase among normals was steeper during the first 30 min (0.54 μg/g/hr) than during the next 60 min (0.22 μg/g/hr). The overall rate of increase of 5-HT during the entire 90 min among the intoxicated rats (0.27 μg/g/hr) was less than that in the normals (0.33 μg/g/hr), but not significantly so. However, the rate of increase of 5-HT during the entire 90 min in the ethanol-intoxicated rats was significantly less ($p < 0.01$) than the more rapid rate of increase of 5-HT during the first 30 min after pargyline in the normals [$p < 0.001$ by analysis of variance according to Kendall (11)].

Comment. The similarity in concentration of 5-HT in the brains of ethanol-intoxicated and normal rats confirms the observations by Pscheidt and associates (12) and Duritz and Truitt (13). However, Gursey and Olson (14) reported that administration of ethanol

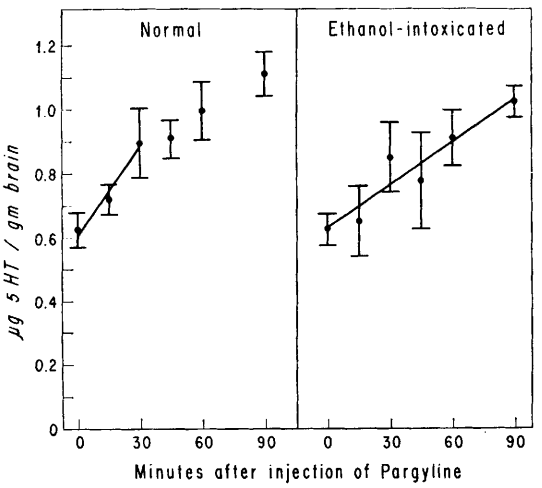

FIG. 2. Concentration of 5-HT in brains of normal and ethanol-intoxicated rats at different times after intravenous injection of parglyline (75 mg/kg), shown as means ± SD of 6 to 10 determinations. Least squares regression equations, calculated for 0 to 30 min after injection of pargyline in normal rats and 0 to 90 min after injection of pargyline in ethanol-intoxicated rats, are: for normal rats, $Y = 0.544X + 0.613$; for ethanol-intoxicated rats, $Y = 0.265X + 0.634$.

to rabbits, at a dose of 2 g/kg, decreased the concentration of 5-HT in the brain stem to 40% of control levels in 30 min, and Bonnycastle and co-workers (15) reported that 5-HT was increased by 56% in rat brain 70 min after the intraperitoneal injection of ethanol at 4.5 g/kg. The lack of change in the concentration of 5-HIAA in brain in ethanol-intoxicated rats suggested that ethanol does not induce a shift in the metabolism, in the brain, of endogenous 5-HT toward 5-HTOH. It has previously been shown that the metabolism of exogenous 5-HT-^{14}C is the same in ethanol-intoxicated rats as in normal rats (5).

When determined by the rate of 5-HIAA decrease in brain after the administration of pargyline, the turnover of 5-HT in brains of control rats (0.38 μg/g/hr) was similar to that reported by Tozer and associates (7) (0.41 μg/g/hr). Alcohol intoxication resulted in a modest decrease in the relative rate of decrease of 5-HIAA concentration in brain after administration of pargyline. When the turnover of 5-HT in brains of control rats was determined by the rate of increase in 5-HT concentration after the administration of pargyline, it was more rapid in the first 30 min (0.54 μg/g/hr) than was reported by Tozer and co-workers (7) (0.44 μg/g/hr); after 30 min the concentration of 5-HT in brain increased more slowly (0.22 μg/g/hr). Differences in concentrations of 5-HT and in the increases in 5-HT concentration in brain after pargyline administration have been reported in different strains of Sprague-Dawley rats (16). In the ethanol-intoxicated rats, the amounts of 5-HT were significantly lower and the 5-HT concentration in brain increased more slowly after pargyline administration. Further experiments would be required to determine whether the inhibitory effect of ethanol on the synthesis of 5-HT was on the decarboxylation of 5-hydroxytryptophan or on the hydroxylation of tryptophan, which is the rate-limiting step in the synthesis of 5-HT.

It is not known to what extent the decreased turnover of 5-HT may contribute to the narcotic action of ethanol. Diaz and associates (17) have previously shown that another central nervous system depressant, ether, had an opposite effect on 5-HT turnover, increasing the turnover rate of 5-HT in brain by 50%; however, the turnover rate of 5-HT in brain was unchanged by cyclopropane or halothane anesthesia.

The monoamine oxidase inhibitor administered after ethanol resulted in the death of one fourth of the animals treated. Severe hypertensive episodes and a number of deaths have been reported after the ingestion of alcohol or of certain foods (notably cheeses) by patients receiving monoamine oxidase inhibitors (18). The toxicity of the alcoholic beverages has been attributed to the pressor effects of tyramine which is present in amounts up to 25 μg/ml in some beers and wines (19) and which normally would be rapidly degraded by monoamine oxidase. In the present experiments, ethanol *per se* was found to be much more toxic when administered with a monoamine oxidase inhibitor than when administered alone, but this toxicity did not result from an inhibition of the metabolism of ethanol by pargyline. It seems unlikely that it would result from the rather modest effects that ethanol exerted on the turnover of 5-HT. 5-HT condenses readily with aldehydes to form β-carbolines *in vitro*. Further experiments are required to determine whether such reactions can occur *in vivo* and whether the carbolines formed are toxic. 5-Methoxytryptamine has been shown to condense with acetaldehyde *in vivo* after the administration of monoamine oxidase inhibitor to form 1-methyl-6-methoxy-1,2,3,4-tetrahydro-2-carboline (20). Several other β-carboline bases (harmine, harmaline, 6-methoxytetrahydroharman) have been shown to elicit hallucinations in man in doses greater than 4 mg/kg (21).

Summary. In rats which had received an intoxicating dose of ethanol, the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the brain were not different from concentrations in brains of normal rats. The turnover of cerebral 5-HT, determined from rates of increase in concentration of 5-HT or of decrease in concentration of 5-HIAA after the administration of a monoamine oxidase inhib-

itor, was modestly decreased during ethanol intoxication.

1. Rosenfeld, G., Quart. J. Stud. Alc. **21**, 584 (1960).

2. Davis, V. E., Brown, H., Huff, J. A., and Cashaw, J. L., J. Lab. Clin. Med. **69**, 132 (1967).

3. Feldstein, A., Hoagland, H., Freeman, H., and Williamson, O., Life Sci. **6**, 53 (1967).

4. Tyce, G. M., Flock, E. V., and Owen, C. A., Jr., (Abstr.) Fed. Proc., Fed. Amer. Soc. Exp. Biol. **27**, 400 (1968).

5. Tyce, G. M., Flock, E. V., and Owen, C. A., Jr., Mayo Clin. Proc. **43**, 668 (1968).

6. Eccleston, D., Reading, W. H., and Ritchie, I. M., J. Neurochem. **16**, 274 (1969).

7. Tozer, T. N., Neff, N. H., and Brodie, B. B., J. Pharmacol. Exp. Ther. **153**, 177 (1966).

8. Quay, W. B., Anal. Biochem. **5**, 51 (1963).

9. Bonnichsen, R., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.; transl. by D. H. Williamson), p. 285. Academic Press, New York (1963).

10. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics: With Special Reference to the Biological Sciences." McGraw-Hill, New York (1960).

11. Kendall, M. G., "The Advanced Theory of Statistics," 1st ed., Vol. 2, p. 222. Griffin, London (1946).

12. Pscheidt, G. R., Issekutz, B., Jr., and Himwich, H. E., Quart. J. Stud. Alc. **22**, 550 (1961).

13. Duritz, G., and Truitt, E. B., Jr., Biochem. Pharmacol. **15**, 711 (1966).

14. Gursey, D., and Olson, R. E., Proc. Soc. Exp. Biol. Med. **104**, 280 (1960).

15. Bonnycastle, D. D., Bonnycastle, M. F., and Anderson, E. G., J. Pharmacol. Exp. Ther. **135**, 17 (1962).

16. Miller, F. P., Cox, R. H., Jr., and Maickel, R. P., Science **162**, 463 (1968).

17. Diaz, P. M., Ngai, S. H., and Costa, E., Anesthesiology **29**, 959 (1968).

18. Davies, E. B., Lancet **2**, 691 (1963).

19. Horwitz, D., Lovenberg, W., Engelman, K., and Sjoerdsma, A., J. Amer. Med. Ass. **188**, 1108 (1964).

20. McIsaac, W. M., Biochim. Biophys. Acta **52**, 607 (1961).

21. Naranjo, C., *in* "Ethnopharmacologic Search for Psychoactive Drugs: Proceedings of a Symposium Held in San Francisco, California, January 28-30, 1967" (D. H. Efrom, ed.), Public Health Service Publication No. 1645, pp. 385–391. U.S. Department of Health, Education, and Welfare, Public Health Service, Washington, D.C. (1967).

Received Jan. 14, 1970. P.S.E.B.M., 1970, Vol. 134.