Pergamon

Pharmacology Biochemistry and Behavior, Vol. 52, No. 4, pp. 845–848, 1995

0091-3057/95 $9.50 + .00

0091-3057(95)00186-7

BRIEF COMMUNICATION

Cognitive Function in Rats With Alcohol Ingestion

H. SASAKI,*[1] Y. MATSUZAKI,* T. NAKAGAWA,* H. ARAI,* M. YAMAMA,* K. SEKIZAWA,* Y. IKARASHI† AND Y. MARUYAMA†

*Department of Geriatric Medicine, Tohoku University, School of Medicine, Sendai 980 Japan
†Department of Neuropsychopharmacology (Tsumura), Gunma University School of Medicine, Maebashi, Gunma, 371 Japan

Received 12 September 1994

SASAKI, H., Y. MATSUZAKI, T. NAKAGAWA, H. ARAI, M. YAMAMA, K. SEKIZAWA, Y. IKARASHI AND Y. MARUYAMA. *Cognitive function in rats with alcohol ingestion.* PHARMACOL BIOCHEM BEHAV 52(4) 845–848, 1995. – The effect of alcohol ingestion on learning disturbances was tested in rats. Rats were fed either an alcohol solution or a nonalcohol solution. The concentration of acetylcholine in the whole brain was significantly lower in rats fed with alcohol than rats fed without alcohol. Passive avoidance learning shows a lower tendency in rats with alcohol compared to rats without alcohol, but the alcohol and control groups did not differ in passive avoidance learning. We suggest that alcohol may disturb acetylcholine metabolism in the brain.

Alcohol Acetylcholine Cognitive function Passive avoidance learning Rats

A GREAT deal of research has been conducted concerning cognitive impairment and the consumption of alcohol, and it is now well established that excessive drinking and cognitive impairment are related. Chronic alcohol abuse, even in younger individuals, may lead to mild and nonprogressive intellectual impairment, and about 10% of alcoholics show obvious signs of dementia (8). Computer tomography scans show that about 50% of the remainder have structural brain damage, although routine psychological tests detect no abnormality (18). Freund (6) and Walker et al. (21) detected behavioral defects in mice, demonstrated by maze learning and avoidance, when on a normal diet after having previously been fed a nutritionally controlled diet together with ethanol. Morphological abnormalities were also detected. Golgi impregnation studies showed a loss of dendritic spines on hippocampal pyramidal and dentate gyrus granule cells in mice (17) and reduced numbers of these neurons in rats (20). Observations on cortical damage related to chronic alcoholism in man are difficult to interpret. Couville (4) considered that alcoholism is a frequent cause of diffuse cerebral atrophy, whereas many other investigations have not confirmed this view.

Fully satisfactory evidence for such alcoholic intellectual deterioration is so far lacking. It has been demonstrated that there is a specific deficiency in acetylcholine (ACh), choline (Ch) acetyltransferase, and acetylcholinesterase (AChE) in autopsy material from patients with Alzheimer's disease (5). The severity of dementia is correlated with the neuropathologic indicator of cholinergic losses (15). We speculated that if chronic alcohol abuse brought cognitive dysfunction, ACh in the brain tissue might be reduced. We adopted animal models fed with alcohol and with normal diet and tested whether ACh deprivation is one of the reasons for cognitive impairment.

[1] To whom requests for reprints should be addressed.

METHOD

Animals

Studies were carried out using 4-week-old male Wistar rats in cages under 12 L : 12 D conditions. The rats were divided into two groups. The first group (control, $n = 20$) was given free access to water, as well as a commercial diet (CE-2, Nihon CLEA, Tokyo, Japan). The second group (alcohol, $n = 33$) was also given free access to water and the diet, except that they were also given free access to a 15% aqueous ethanol solution from 0800 to 1800 h in place of the water. Each rat in the alcohol group drank an average 8 ml of the 15% alcohol solution and average of 7 ml of water every day. When the rats became 24 weeks old (after 20 weeks on the diet with or without alcohol), they were trained and tested using the passive avoidance test for cognitive responses. All experiments were performed between 1600 and 2000 h.

Spontaneous Movement in Rats

The spontaneous movements of the rats in the two groups were measured for 1 h using an Animex counter (Animex III, Shimazu Co.), 1 week after the 15% alcohol solution was taken away from the second group. Two groups of five rats were selected. All of these rats were different from those used for passive avoidance.

Passive Avoidance Learning in Rats

Passive avoidance learning was carried out, according to the step-through procedure, in the two groups of rats drinking either water ($n = 10$) or alcohol ($n = 18$) 1 week after the alcohol solution was taken away from the alcohol group (11). The apparatus consisted of two compartments, one illuminated [300 × 250 mm; light (60 W), with a height of 200 mm to the top of the chamber], the other dark (200 × 150 mm, with a height of 200 mm to the top of the chamber). The compartments were separated by a guillotine door (70 × 100 mm). A rat was placed into the illuminated, safe compartment and could enter the dark compartment through the door and stand on a grid floor. Once all four paws were on the grid, a foot shock with a constant current (0.3 mA) and constant voltage (50 V, 50 Hz) was delivered to the floor grid for 3 s. The rat could escape the shock only by stepping back into the illuminated, safe side. Although the rats quickly escaped the shock administered, we could not measure shock duration. Passive avoidance learning was repeated on the second, third, and fourth days in the same way as in the first trial, and the response latency in entering the dark compartment was measured. Results on latency time of step-through were recorded for each experiment. The maximal latency time of those rats that did not move into the dark compartment during the observation period was set at 300 s.

Determination of Brain ACh and Choline (Ch)

Brain ACh and Ch were measured in the two groups of rats drinking either water ($n = 5$) or alcohol ($n = 10$), which were used in the passive avoidance learning and spontaneous movement tests. One week after the last learning trial, rats that were kept free from alcohol were killed by microwave irradiation (microwave device NJE 2603 10kW, New Japan Radio, Tokyo, Japan) at 9.0 kW for 0.75–1.15 s, which raised the brain temperature to 95.0 ± 1.7°C, resulting in the complete cessation of enzyme activity as an acetylcholine esterase and prohibited degradations of ACh and Ch (9,12). The brain was removed from the skull and was homogenized with a mixture of 1 ml 0.05 M perchloric acid ($HClO_4$) and 10 nmol/10 μl EHC using an ultrasonic cell disrupter (model US-300T, Nissei, Tokyo, Japan). The homogenate was centrifuged at 10,000 × g at 4°C for 15 min. The supernatant was filtered through a 0.45-μm millipore filter and then 5 μm supernatant was injected into a liquid chromatography with an electrochemical detection (LCEC) system to measure ACh and Ch (10,16). Tissue pellets obtained by centrifugation for the determination of protein were stored at −85°C until analysis. For assaying the protein concentration, a solution of 1 N NaOH was added to the pellets, for the preparation of a final sample (10 ml), and homogenized. The homogenate obtained from brain tissues was diluted with 1 N NaOH at rates of 5, 3, 1, 2, 3, 2, and 10-fold, respectively. Using a Bio-Rad protein assay kit (Bio-Rad Labs, Richmond, CA), 0.1 ml of each of the above diluted homogenates was used for assaying the protein concentration based upon the method of Bradford (1). Bovine serum albumin was used as the standard. The LCEC system consisted of an LClOOP pump (Yokogawa Co., Ltd., Tokyo, Japan), an LClOOS injector with 20 μl sample loop (Yokogawa), an LC-4A amperometric detector with platinum electrodes [Bioanalytical System (BAS), West Lafayette, IN], and an LClOOW/F-PC work station (Yokogawa) for LC data processing. The analytical column was the BAS Acetylcholine Separation Column. A glassy carbon column was used as the precolumn, and an immobilized column containing AChE and Ch oxidase was used as the postcolumn. Analytical column temperature was set at 35°C (with a BAS Temperature Controller LC22A). The mobile phase was 0.05 M phosphate buffer, pH 8.4, containing 1 nM $EDTD_2Na$ and 0.4 mM sodium 1-octanesulfonate (SOS). The flow rate was set at 0.8 ml/min. The electrode potential was set at +0.5 V against an Ag/AgCl reference electrode for the detection of hydrogen peroxide. The principle of the technique is based upon the separation of ACh and Ch in the separation column, followed by an enzymatic conversion through reaction with AChE and Ch oxidase to hydrogen peroxide, which is detectable electrochemically by a platinum electrode.

Determination of Blood Ethanol Concentration

Blood ethanol concentrations were measured in two groups of rats drinking either water ($n = 5$) or alcohol ($n = 10$), which were different from those used for spontaneous movement or passive avoidance learning. When the rats became 24 weeks old (after 20 weeks on the diet with or without alcohol), 2 ml of blood was sampled by cardiac puncture. Blood samples were taken at 1800 h during free access to alcohol in the second group (alcohol, $n = 5$), and during free access to water in the first group (water, $n = 5$). Blood samples were also taken 1 week after the second group ($n = 5$) ceased to drink alcohol. Blood ethanol concentration was measured using a radiative energy attenuation technique (3).

Reagents

ACh iodide and Ch iodide were purchased from the Sigma Chemical Co. (St. Louis, MO). Ethylhomocholine(EHC) iodide as an internal standard (IS), was synthesized from dimethyl-3-amino-1-propanol (Sigma) and iodoethane (Sigma) in the Department of Neuropsychopharmacology (Tsumura), Gunma University, School of Medicine. Other reagents for extraction and chromatography were of the highest available purity and purchased from commercial sources.

Statistics

Data are reported as means ± SD. Statistical analysis was performed by ANOVA and Duncan's multiple range test for passive avoidance learning, and the Student's *t*-test was adopted for comparisons of spontaneous movements and ACh and Ch contents in the brain. Significance was accepted at $p < 0.05$.

RESULTS

Spontaneous movements of rats in the control group ($n = 5$) and alcohol group ($n = 5$) were 523192 (mean ± SD) and 586234, respectively. They were not significantly different.

On the first day, there was no significant difference in latency times between the two groups. The latency of rat entrance into the dark compartment increased on subsequent test days for the two groups ($p < 0.01$) (Fig. 1). From the second to the fourth day, the latency times in control rats ($n = 10$) showed a tendency to be higher than those of the alcohol group ($n = 18$), but they were not significantly different. Brain weights of the two groups of rats are listed in Table 1. Brain weights were not different between the two groups. Concentrations of Ch in rat brain tissues were not significantly different between the two groups. Concentrations of ACh in rat brain tissues of the control group ($n = 5$) was significantly higher than that of alcohol group ($n = 10$) $p < 0.05$).

Blood alcohol concentrations were 13 ± 4 (mean ± SD) mg/dl during free access to alcohol in the second group ($n = 5$) and less than 6 mg/dl in the first group ($n = 5$) and 1 week after cessation of alcohol intake in the second group ($n = 5$).

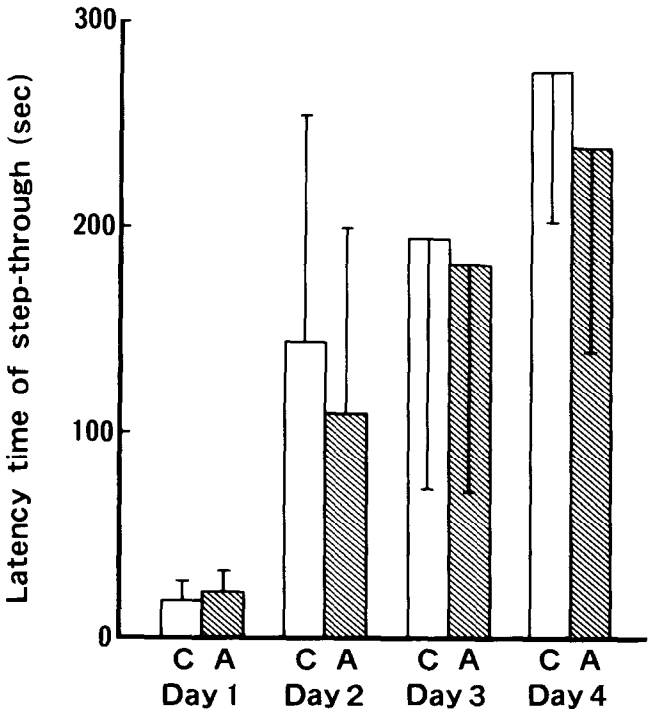

FIG. 1. Passive avoidance learning was performed on rats fed with alcohol ($n = 18$) (A) and without alcohol ($n = 10$) (C). The latency times of step-through during passive avoidance learning from the first to the fourth days in 24-h intervals are compared between the two groups. They were not significantly different in the 4 days. Statistical analysis was performed by ANOVA and Duncan's multiple range test. *F*-Value of ANOVA was 20.6 ($p < 0.001$).

TABLE 1

CONCENTRATIONS OF CHOLINE (Ch) AND ACETYLCHOLINE (Ach) IN RAT WHOLE BRAIN

	Control ($n = 5$)	Alcohol Addicted ($n = 10$)
Body weight (g)	260 ± 61	26.59 ± 59
Brain weight (g)	1.58 ± 0.07	1.62 ± 0.06
Protein (mg/g tissue weight)	102.5 ± 2.4	103.5 ± 4.5
Ch (pmol/mg protein)	179.8 ± 11.3	156.8 ± 35.0
Ach (pmol/mg protein)	338.1 ± 17.2	297.9 ± 30.6*

Each value is expressed as mean ± SD, *$p < 0.05$.

DISCUSSION

We found that chronic alcohol intake reduces brain ACh in rats. Cognitive function shows a tendency to be lower in the rats fed with alcohol than in those without alcohol, but the alcohol and control groups did not differ in passive avoidance learning. The impairment of cognitive function in rats fed with alcohol has been reported and the degree of impairment increases with a duration of alcohol consumption from 3–5 months and is essentially irreversible (7). Brain ACh concentrations measured by bioassay were reportedly lowered by 38% in rats drinking 15% aqueous ethanol solution for 22 weeks (13). In similar experiments, coenzyme A, cholinesterase, and choline transacetylase were also lowered (19). However, measurements of ACh in the brain tissue were not suitable and not reliable in previous reports (13,19). This is the first report of a reduction in ACh in the rat's brain when the animal is fed alcohol.

We do not know the reasons for no significant difference in cognitive functions between the two groups with alcohol and without alcohol. The behavioral disturbances induced by chronic ethanol treatment are persistent and unrelated to the transient residual effects of acute ethanol withdrawal. Deficiencies have been documented 2–18 weeks after ethanol removal in mice exposed to alcohol for 5 months (21). The disturbance of brain function associated with tolerance and physical dependence, expressed as the ethanol withdrawal syndrome, is thought to last for a few days, while the brain damage associated with chronic ethanol exposure may be permanent (21). We studied shuttle-box avoidance learning 1 week after the cessation of alcohol. Therefore, ethonal withdrawal syndrome may not seriously influence the present results. Furthermore, the lapse of 1 week from the behavioral experiments may not influence on the brain ACh. Probably the doses of alcohol in rats in the present study may be insufficient to influence passive avoidance learning but sufficient enough to reduce brain Ach. Shuttle-box avoidance learning in mice is not affected by 5 weeks of ethanol treatment, but 3, 5, or 7 months of ethanol treatment produces progressive avoidance learning (7). Rats in the present study were fed alcohol for 5 months, but drinking alcohol was limited to daytime hours. Therefore, alcohol intoxication would not be enough to reduce passive avoidance learning. In the present experiment, ethanol administration results in an average daily intake of approximately one-third of that in the previous experiments (21). A concentration of alcohol of 100 mg/dl or over suggest intoxicating doses in acute stage in human subjects (14). Con-

centration of alcohol in the present experiment seems to be lower than that which induces acute stage intoxication in human subjects. Further, alcohol intoxication may reduce both brain ACh and passive avoidance learning. However, the present passive avoidance procedure is characterized as a broad test for learning, memory, performance-enhancing, cognition-activating, or psychostimulant activity (2). We could not deny these factors in the present step-through procedure.

Alcohol has been reported to be toxic to the nervous system. Chronic excessive ingestion of ethanol is directly associated with serious neurological and mental disorders (e.g., brain damage, memory loss, sleep disturbances, and psychoses). In addition, nutritional and vitamin deficiencies, incident to poor food intake or faulty gastrointestinal function seem to cause many neuropsychiatric syndromes that are common in alcoholics, such as Wernick's encephalopathy, Korsakoff's psychosis, polyneuritis, and nicotinic encephalopathy (14). The rats with alcohol were given free access to food and ingested an average of 12 ± 2 g (mean ± SD) of food every day, which was comparable to the rats without alcohol. Therefore, nutritional and vitamin deficiencies would not seem to be the reasons for the present results.

In summary, the present results suggest that chronic alcohol ingestion reduces brain ACh by direct intoxications to the brain and not by nutritional and vitamin deficiencies. Disturbances in cognitive functions might be influenced by additional alcohol consumption.

ACKNOWLEDGEMENTS

We thank Mr. G. Crittenden for the English correction and Ms. J. Takamatsu for typing the manuscript. This work was supported by a SRF grant for Biomedical Research.

REFERENCES

1. Bradford, M. M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. Anal. Biochem. 72:248-254; 1976.
2. Butler, D. E.; Poschel, B. P. H.; Marriotl, J. G. Cognition-activating properties of 3-(aryloxy) pyridines. J. Med. Chem. 24: 346-350; 1981.
3. Carey, P. L.; Whitter, P. D.; Johnson, C. A. Abbott radiative energy attenuation method for quantifying ethanol evaluated and compared with gas-liquid chromatography and the Du Pont. Clin. Chem. 30:1867-1870; 1984.
4. Courville, C. B. The effect of alcohol on the nervous system in man. Los Angeles: San Lucas Press; 1955.
5. Davis, P.; Maloney, A. J. F. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet 2:1403; 1976.
6. Freund, G. Chronic central nervous system toxicity of alcohol. Annu. Rev. Pharmacol. 13:217-227; 1973.
7. Freund, G.; Walker, D. W. Impairment of avoidance learning by prolonged ethanol consumption in mice. J. Pharmacol. Exp. Ther. 17: 284-292; 1971.
8. Horvath, T. B. Clinical spectrum and epidemiological features of alcohol dementia, In: Rankin, J. G., ed. Alcohol, drugs and brain damage. Toronto: Addiction Research Foundation of Ontario; 1975:1-16.
9. Ikarashi, Y.; Maruyama, Y.; Stavinoha, B. Study of the use of the microwave magnetic field for the rapid inactivation of brain enzyme. Jpn. J. Pharmacol. 35:371-387; 1984.
10. Ikarashi, Y.; Sasahara, T.; Maruyama, Y. Determination of choline and acetylcholine levels in rat brain regions by liquid chromatography with electrochemical detection. J. Chromatogr. 322: 191-199; 1985.
11. Jarvik, M. E.; Kopp, A. An improved one-trial passive avoidance learning situation. Psychol. Rep. 21:221-224; 1967.
12. Maruyama, Y.; Ikarashi, Y.; Blank, C. L. Evaluation of various microwave instruments for rapid inactivation of brain enzymes. Biogen. Amines 4:55-71; 1987.
13. Moss, J. N.; Smyth, R.-D.; Beck, H.; Martin, G. J. Ethanol impact on brain acetylcholine and its modification by cysteine. Arch. Int. Pharmacodyn. 168:235-238; 1967.
14. Murdoch Ritchie, J. The aliphatic alcohols. In: Gilman, A. G.; Goodman, L. S.; Gilman, A., eds. The pharmacological basis of therapeutics, 6th ed. New York: Macmillan Publishing Co.; 1980: 376-390.
15. Perry, E. K.; Tomlinson, B. E.; Blessed, G.; Bergmann, K.; Gibson, P. H.; Perry, R. H. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br. Med. J. 2:1457-1459; 1978.
16. Potter, P. E.; Meck, J. L.; Neff, N. H. Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem. 41:188-194; 1983.
17. Riley, J. N.; Walkerr D. W. Morphological alterations in hippocampus after longterm alcohol consumption in mice. Science 201: 646-648; 1978.
18. Ron, M. A.; Acker, W.; Shaw, G. K.; Lishman, W. A. Computerised tomography of the brain in chronic alcoholism. Brain 105: 497-514; 1982.
19. Smyth, R. D.; Beck, H. The Effect of time and concentration of ethanol administration on brain acetylcholine metabolism. Arch. Int. Pharmacodyn. 182:295-299; 1969.
20. Walker, D. W.; Barnes, D. E.; Zornetzer, S. F.; Hunter B. E.; Kubanis, P. Neuronal loss in hippocampus produced by prolonged ethanol consumption in rats. Science 209:711-713; 1980.
21. Walker, D. W.; Hunter, B.; Abraham, W. Neuroanatomical and functional deficits subsequent to chronic ethanol administration in animals. Alcoholism 5:267-282; 1982.