Alcohol Drinking: Abnormal Intake Caused by Tetrahydropapaveroline in Brain

Abstract. *Tetrahydropapaveroline (THP), a dopamine-dopaldehyde condensation product, was delivered directly into the cerebral ventricle of rats automatically every 15 minutes for 12 days. The animals were given access to both water and ethyl alcohol, the latter being presented in 12 concentrations from 3 to 30 percent. Within 3 to 6 days of the start of the infusion of THP, the rats, which normally rejected alcohol, drank alcohol solutions in increasingly excessive amounts; this was accompanied by symptoms that were similar to those of withdrawal and intoxication. These results provide evidence that an abnormal metabolite in the brain may produce the addictive state caused by alcoholic beverages.*

Condensation products of biogenic amine metabolism, formed in the presence of ethyl alcohol, have been implicated in the addictive liability of alcoholic beverages. One of the tetrahydroisoquinolines, tetrahydropapaveroline (THP), is a biosynthetic precursor of the opiate alkaloid morphine (*1, 2*). The production of tetrahydroisoquinolines derived from catecholamines may be induced by acetaldehyde, the first metabolite of ethanol (*2, 3*). In addition, these substances are formed both in vitro and in vivo in the brain as well as in peripheral tissue (*4*).

We now present evidence that if THP is present in the brain of a rat for a protracted period of time, the animal will drink excessive amounts of alcohol in a free-choice situation where water is also available. In fact, in rats of a strain which shows aversion to alcohol even in low concentrations (*5*), alcohol is consumed volitionally to the point of ataxia, intoxication-like behavior, and withdrawal-like symptoms.

Initially, we determined individual preferences for alcohol in rats of the Sprague-Dawley strain by the two-choice, three-bottle technique (*6*): one calibrated drinking tube contains water; another contains a solution of alcohol that is increased stepwise on 12 successive days from a concentration of 3 to 30 percent; and the third bottle is empty. The animals are given free access to the two fluids, as well as to powdered Wayne Lab Blox, and measures of food and fluid intake are recorded at the same time every day.

To mimic the state of metabolic disturbance within the brain occasioned by the chronic drinking of alcohol (*7*), we infused amine metabolites repeatedly into the cerebral ventricle of each rat. After the initial alcohol preference test, we anesthetized each of 20 rats to implant a 20-gauge steel guide tube stereotaxically so that the tip rested just above the lateral cerebral ventricle (*8*). Two days later, a 27-gauge injector needle mounted within a teflon swivel assembly (*9*) was lowered into the guide and fixed so that the needle penetrated the ventricular lumen. The external end of the needle was connected by way of fine polyethylene tubing to a multiple infusion pump in such a way that the rat had complete freedom of movement in its open-topped, high-sided cage (*10*). Then, every 15 minutes for 12 days, a solution of THP (Hoffmann–LaRoche) in 1 μl of cerebrospinal fluid (CSF) was injected automatically over a 13-second interval; this volume is easily accommodated in the ventricular lumen and no pathological signs were evident (*11*).

Two dose rates of THP hydrobromide were used, 0.02 μg/μl or 2.0 μg/μl (*12*), both doses being prepared as the free base in an osmotically balanced artificial CSF (*13*) with 0.1 mg/ml of ascorbic acid added. The solutions were delivered into the ventricles of two groups of rats. In a third group of rats, the CSF was infused alone. A fourth group consisted of an anatomical control in which THP infusions failed to enter the rats' cerebral ventricles (*14*). After all the rats had been infused for 48 hours (192 injections) without access to alcohol, the second 12-day alcohol preference test was begun. Infusions continued throughout the sequence. Blood alcohol concentrations were determined enzymatically (*15*) in selected THP-infused animals during their peak alcohol drinking periods as well as at 8:00 a.m. and 8:00 p.m. on the final days of the preference sequence. Evidence of withdrawal symptoms was recorded according to the criteria of Majchrowicz and others (*16*) which include audiogenic seizures induced by the jingling of keys or an alarm bell in an enclosed chamber.

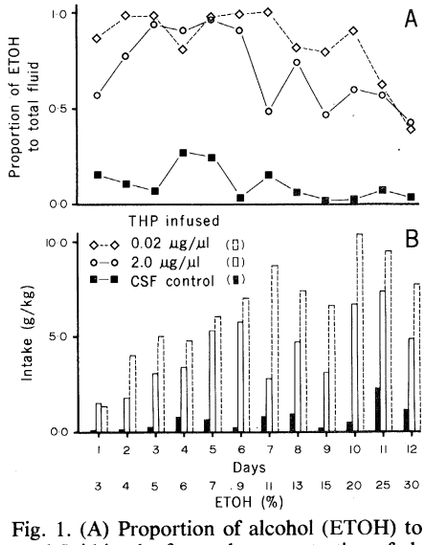

Fig. 1. (A) Proportion of alcohol (ETOH) to total fluid intake for each concentration of alcohol offered (see abscissa) on each day of the 12-day test sequence. Rats were infused every 15 minutes during these 12 days with THP at a rate of 0.02 μg/μl (N = 4), or 2.0 μg/μl (N = 5), or with CSF (N = 4). (B) Corresponding mean amount of alcohol (expressed as grams per kilogram of body weight) consumed by these groups on each day of the alcohol test sequence.

As shown by the proportional measure depicted in Fig. 1A, when 2.0 μg of THP were infused into the ventricle, the rats selected, on most days, half or more of their fluid intake as alcohol, up to the 30 percent concentration. Those rats given 0.02 μg of THP drank alcohol almost exclusively up to the 25 percent concentration on day 11 of the perfusion sequence. The proportion of alcohol preferred over water (Fig. 1A) in both groups given THP was significantly greater than that of the controls ($P < .01$, Newman-Keuls test). Most unusual was the progressive quantities of alcohol consumed. Figure 1B shows that as the concentration of the fluid was elevated on each day, the amount (in grams per kilogram of body weight) of alcohol consumed by the THP-infused rats increased over days in spite of alcohol's progressively aversive taste (*17*). Again, the rats receiving THP on the 0.02 μg/μl schedule drank greater volumes of alcohol ($P < .01$, Newman-Keuls test) than those given the 2.0 μg/μl dose rate of THP (Fig. 1B), perhaps because of an unknown inhibitory or counteractive effect of the higher dose rate.

Table 1 shows that as the choice of alcohol concentrations entered the 11 to 30 percent range, the action of THP became even more pronounced than with the lower concentrations. Further, the mean preference thresholds (*18*) of alcohol versus water for the two ventricularly infused THP groups (Table 1) are to our knowledge the highest ever reported (*17*) at 24.6 percent (2.0 μg/μl dose rate) and 27 percent (0.02 μg/μl dose rate). These remarkable preferences for alcohol persist beyond the time that infusions are stopped and are even manifest against saccharin solutions (*19*). Two other dose rates, 1.0 μg/μl (N = 3) and 0.2 μg/μl (N = 3), of THP were infused intraventricularly according to the same regimen; the mean proportions of alcohol to

water were 0.72 and 0.53 during the preference sequence, whereas the absolute intakes of alcohol were 6.8 and 3.7 g/kg per day, respectively. These values were likewise significantly above those of the controls ($P < .01$).

During the second half of the 12-day preference sequence, several THP-infused rats drank 13 to 16 g/kg on given days in the 11 to 30 percent range of alcohol solutions. Marked ataxia and other intoxication-like symptoms were clearly visible. Also, the blood alcohol concentrations often reached an unusual level of 0.2 percent (*20*) when samples were collected at 8:00 a.m. after a night of drinking. We detected enhanced proneness to audiogenic seizures (*16, 20*) if tests were done during the daytime hours when the animal was normally asleep and not drinking. Although four of five rats reacted to the sound by darting around the test chamber and jumping in an uncoordinated manner, actual seizures were not induced until days 8 to 12 of the THP infusion sequence. In one animal, audiogenic seizures occurred from day 4 onward. Also of interest are the results obtained with two other rats, not included in these analyses. They were given long-term infusions with the 2.0 μg/μl dose rate of THP and subsequently rejected alcohol at nearly every concentration offered. However, within 3 to 4 days after the infusion sequence began, they exhibited tail extension, rearing, intermittent forelimb myoclonus, hyperactivity, chewing, whisker-twitching, jerking movements, and characteristic "wet-dog shakes" (*16*).

To consider the issue of stereospecificity, we infused the S−(−)−THP isomer into the ventricle of rats prepared in the same way as those in the previous experiments. Following doses of 1.0 μg/μl ($N = 2$), 10 ng/μl ($N = 2$), or 100 pg/μl ($N = 2$) given in 4 μl every 30 minutes, the mean proportional intakes of alcohol over the 12-day preference sequence were 0.83, 0.60, and 0.43, respectively; over the same interval, the mean intakes of alcohol (per kilogram of body weight) were 5.7, 4.3, and 3.3g, respectively. All of these values again were significantly greater than those of the controls ($P < .01$), and thus this particular isomer of THP approaches the same range of efficacy as the racemic mixture of THP.

These results demonstrate that a condensation product of a biogenic amine induces the drinking of alcohol in concentrations always rejected by this strain of rats when water is concurrently available. Moreover, the graph of intake measured as grams per kilogram of body weight surprisingly shows an increasing consumption of alcohol which is not typically seen as the concentration offered is increased daily (*17*). The enormous dilution factor in CSF (*11*) following each THP infusion in the effective nanomolar dose suggests that perhaps only picomoles of the metabolite are required within critical brainstem structures to trigger aberrant drinking.

Table 1. Average values calculated for the first 6 days (3 to 9 percent alcohol offered) and the second 6 days (11 to 30 percent alcohol offered) in terms of the proportion of alcohol consumed to the total fluid intake, and the amount consumed when measured as grams per kilogram of body weight. The alcohol preference threshold (*18*) and overall total fluid intakes are also presented. The THP nonventricular controls were those which, on postmortem examination, revealed that the infusion solution failed to enter the cerebral ventricle.

Group	N	Alcohol intake				Preference threshold (%)	Total fluid intake (ml/day)
		Proportion to total fluid intake		Amount (g/kg)			
		3 to 9	11 to 30	3 to 9	11 to 30		
Controls prior to infusion	20	0.29	0.08	0.83	0.72	4.1	29.1
Infusion controls							
THP nonventricular	7	0.27	0.03	0.69	0.44	4.0	30.0
CSF	4	0.15	0.06	0.34	0.96	3.7	55.6
Infused with THP							
Dose rate 2.0 μg/μl	5	0.85	0.55	3.48	4.95	24.6	26.1
Dose rate 0.02 μg/μl	4	0.94	0.76	4.89	8.41	27.0	38.3

Caution is required in interpreting the direct action of THP per se for the following reasons: (i) A metabolic degradation product of THP such as a protoberberine (*21*) may be sequestered in the brain and may underlie the alcohol drinking. (ii) Other normally occurring condensation products, such as a β-carboline or salsolinol may be of equal etiological significance (*3*); for example, we find that similar infusions of these substances produce the same sort of addictive-like intake of alcohol (*22*). (iii) Although monoamine-rich areas of the brain would presumably subserve the metabolite's formation, the neuroanatomical locus of the powerful effect of THP is unknown. (iv) The critical configuration, stereospecificity in the central nervous system, and the exact central actions of the THP molecule are indeed uncertain (*23*). But how then could THP act?

Synaptic activity of neurons in the brain can be altered by the presence of abnormal metabolites, and they could operate as "false" transmitters (*23*). Alternatively, tetrahydroisoquinolines could interfere with the enzymatic mechanism for the normal metabolism of the biogenic amines or displace them from their vesicles (*24*). A vital issue in this regard is whether the formation of endogenous metabolites is enhanced in the brain of a living, alcohol-drinking animal. Recently, it was reported that a fivefold elevation in dopaldehyde levels in either liver or brain tissue of the rat fails to augment the formation of THP in vitro (*25*). Thus, the rat may not possess the inherent biochemical mechanism by which the endogenous presence of alcohol in excess would lead autocatalytically to an increased production of THP in vivo. In fact, this could be precisely the reason why the rat and most other species do not select and drink alcohol voluntarily to the point of physical dependence even though alcohol is previously forced upon the animal by feeding or by other means (*20, 26*). What we have done here in artificially providing an excess of THP intracerebrally is to simulate a neurochemical condition as if the condensation product were actually synthesized in an aberrant amount in the brain.

Should one or more of this family of metabolites (*21, 27*) actually be formed in the presence of alcohol and in a sufficient concentration within a crucial structure of the brain, its local presence could stimulate the abnormal imbibition of alcohol. In spite of the noxious taste of the fluid and its deleterious aftereffects, the pathological sequelae would thus be underway. Clearly, further study of the role of amine metabolites in the brain now seems to offer a most promising approach to the etiology of human alcoholism.

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References and Notes

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14. At the end of the experiment each animal was anesthetized, and 2.0 μl of a solution of India ink was infused into the ventricle through the same injector needle. To verify the extent of dispersion, the ventricular cavities were exposed by dissection and tracings of the structures reached by the infusate were drawn [R. D. Myers, in *Methods in Psychobiology*, R. D. Myers, Ed. (Academic Press, New York, 1976), vol. 3., in press]. In animals presenting no evidence of intraventricular infusion, the anatomical verification revealed that the dye either sequestered partially in a glial mass surrounding the shaft of the guide tube or penetrated into the subarachnoid spaces.
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18. Preference threshold is defined as that alcohol concentration at which the two curves depicting the volume of water and volume of alcohol solution consumed intersect (*17*).
19. Tests of alcohol preference repeated 2 weeks to 6 months after the infusion sequence, again with the same 3 to 30 percent sequence, revealed a similar range of high intakes of alcohol in terms of grams per kilogram of body weight. In still other experiments in which a palatable solution of saccharin was offered in the third drinking tube, other rats being infused with THP nevertheless drank significantly more alcohol than under control conditions (C. L. Melchior and R. D. Myers, in preparation). This shows that THP simply does not cause a loss of taste discrimination.
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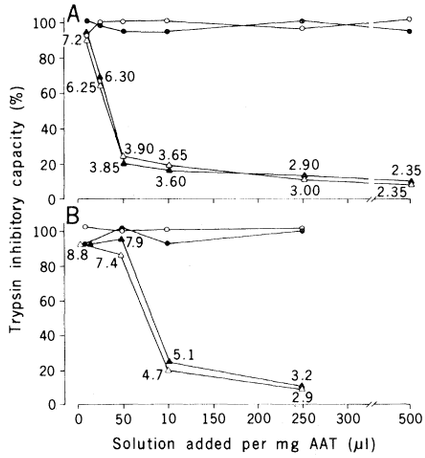
Influence of Cadmium on Human Alpha-1-Antitrypsin: A Reexamination

An inherited deficiency in the major proteinase inhibitor in human plasma, alpha-1-antitrypsin (AAT), is associated with chronic obstructive lung disease (*1*). This may result from the unregulated action of proteolytic enzymes released in the lung by leukocytes and alveolar macrophage cells (*2*). An increased incidence of emphysema is found in industrial workers exposed to cadmium over long periods of time (*3*). In addition, the cadmium concentrations in emphysematous lungs are increased as compared to that of normal lungs (*4*); also cadmium accumulates in the human body as a consequence of cigarette smoking (*5*).

Chowdhury and Louria found a progressive decrease in both the trypsin inhibitory capacity (TIC) and the AAT levels as assayed by radial immunodiffusion (RID) when increasing levels of cadmium were added to either plasma or partially purified AAT (*6*). Other heavy metals (Pb, Hg, Ni, Fe, and Zn) had no effect. Chowdhury and Louria used a cadmium reference solution in dilute nitric acid, as provided by Fisher Scientific Company (*7*). They suggested that the toxic effects of cadmium on the human lung might be a consequence of its specific interaction with AAT. However, from our own studies, we conclude that the



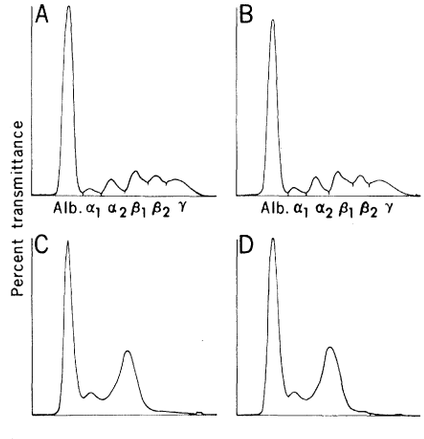


Fig. 1 (left). Trypsin inhibitory capacity (TIC) after incubation of (a) alpha-1-antitrypsin in 0.2*M* tris-HCl buffer, *p*H 8.0, and (b) normal human plasma (diluted 1:1 with saline) with different media at 37°C for 1 hour; o——o cadmium nitrate or •——• cadmium acetate in the tris buffer, ▲——▲ cadmium reference solution (Fisher), ▲——▲ 0.29*N* nitric acid. The AAT concentration was 1 mg/ml in (a) and (b). The cadmium concentration of all solutions was 1 mg/ml. The TIC values are relative to either a control solution of AAT or normal human plasma; *p*H values are indicated on the graph for those experiments where acidic media were used. The AAT concentrations as measured by RID decreased in parallel with the TIC data reported. Fig. 2 (right). Cellulose acetate electrophoretic patterns of (A) normal human plasma; the same after incubation of 1 ml with 250 μl of (B) cadmium nitrate in 0.02*M* tris-HCl buffer, *p*H 8.0; (C) 0.29*N* nitric acid; (D) cadmium reference solution (Fisher). In experiments (C) and (D), more than 80 percent of immunologically active AAT was lost, as indicated by RID.