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Formaldehyde TBHQ

Alcohol, Amines, and Alkaloids: A Possible Biochemical Basis for Alcohol Addiction

Abstract. *Tetrahydropapaveroline is a benzyltetrahydroisoquinoline alkaloid derivative of the biogenic amine, dopamine. Alcohol, by way of its primary metabolite, acetaldehyde, competitively inhibits nicotinamide-adenine dinucleotide-linked aldehyde dehydrogenase and augments the formation of tetrahydropapaveroline in vitro. The limited capacity of brain to oxidize aldehydes may be of pharmacological importance because it facilitates the production of tetrahydropapaveroline in the presence of drugs which inhibit this enzyme.*

No form of physical dependence is more prevalent than that produced by the drug alcohol. Furthermore, no substances are known to have a greater capability of producing physical dependence than the narcotic alkaloids related to morphine. The temporal and proportional differences involved in the development of alcohol addiction, on the one hand, and narcotic addiction, on the other, have greatly contributed to concealing the possible identity of these phenomena. Because of the resemblance of symptoms occurring on withdrawal of either alcohol or the opiates (*1*), it seems possible that the addictions may be similar and that the real distinctions between the two drugs could be only the length of time and dosage required for development of dependence. The differences, therefore, may be purely quantitative rather than qualitative.

Accordingly, we offer a hypothesis and supporting data for a biochemical explanation of the similarities that are characteristic of the addictions produced by these two distinct molecular entities (Fig. 1). This hypothesis delineates the relation of alcohol-evoked modification in the metabolic disposition of the biogenic amine, dopamine, with the resultant formation of morphine-like alkaloids as a basis for the addiction liability of alcohol. Biotransformation of a drug (alcohol) to its active metabolite (acetaldehyde) induces alterations in the metabolism of a neuroamine (dopamine) and produces aberrant metabolites having unique pharmacological activity (alkaloids). The metabolism of ethanol is essential for the activation of a pathway available to this neurotransmitter which may be normally inoperative. This metabolic aberration could then lead to the endogenous production of addictive compounds.

The foundation for this concept stands on several known and diverse experimental findings. (i) The effect of ethanol in altering the metabolism of biogenic amines is well known (*2*). Ethanol ingestion by man inhibits the normal oxidative metabolism of the intermediate aldehyde produced by deamination of the corresponding biogenic amines serotonin and norepinephrine (*3*). (ii) The mechanism of ethanol-induced alterations in neuroamine metabolism involves competitive inhibition of aldehyde dehydrogenase by acetaldehyde, the primary metabolite of ethanol, both in vitro and in vivo (*4*). The presence of acetaldehyde blocks the normal conversion of the amine-derived aldehyde to the corresponding acid. (iii) The inhibition of the nicotinamide-adenine dinucleotide (NAD)-linked aldehyde dehydrogenase after ethanol ingestion may result in localized elevated concentrations of aromatic aldehydes in tissues rich in biogenic amines. These intermediate aldehydes are highly reactive compounds. A possible alternate route for the metabolism of these aldehydes is condensation with the parent amine. In the case of condensation of dopamine and its aldehyde (3,4-dihydroxyphenylacetaldehyde) the product is a benzyltetrahydroisoquinoline alkaloid, tetrahydropapaveroline (THP) (*5*). (iv) Tetrahydropapaveroline (norlaudanosoline) is the requisite intermediate in the biosynthesis of morphine in the opium poppy *Papaver somniferum* (*6*). (v) Marked differences in the captivity of various tissues to oxidize aldehydes may have an important effect on the localized disposition of the aldehydes arising from biogenic amines (*7*). These differences may be of pharmacological importance. Tissues with a limited capacity for aldehyde oxidation may be inordinately sensitive to the presence of drugs which inhibit the oxidation of aromatic aldehydes by NAD-linked aldehyde dehydrogenase. The relatively high activity of aldehyde dehydrogenase from liver may be adequate to cope with the aromatic aldehydes and readily convert them to the acid derivatives. However, in tissue, such as brain, with comparatively low aldehyde-oxidizing activity, the aldehyde might readily condense with the amine leading to enhanced formation of alkaloid metabolites.

To test this hypothesis in an in vitro system, we incubated rat brainstem homogenates with dopamine (0.4 μc of C^{14}-dopamine plus 5.0 mg of dopamine hydrochloride) and 100 mg of tissue. Reaction mixtures consisted of 1 ml of rat brainstem homogenate, 1 ml of dopamine substrate, and 2 ml of 0.067*M* phosphate buffer containing 0.5 mg of ascorbic acid per milliliter, *p*H 7.4. Additions of NAD (16 m*M*), ethanol (100 m*M*), or acetaldehyde (0.5 to 2.0 m*M*) were made in a volume of 1 ml at the expense of the phosphate buffer. The total reaction volume was 4.0 ml.

Incubations were carried out in open Erlenmeyer flasks (25 ml) at 37°C for 30 minutes and stopped with 45 percent perchloric acid. After tissue protein and perchlorate salts were removed, the extracts were adjusted to *p*H 8.4 and applied to alumina columns (1 g of aluminum oxide, Wohlm, neutral). Catechol compounds (includ-

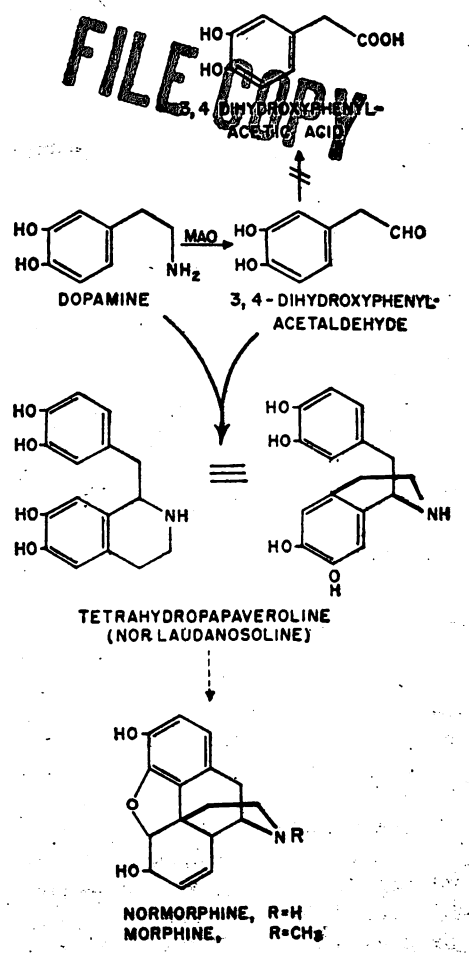

Fig. 1. A representation of a hypothesis depicting the relation of alcohol-induced alteration of the metabolic disposition of the biogenic amine, dopamine, with the postulated resultant formation of morphine-like alkaloids as a biochemical basis for the addiction liability of alcohol. *MAO*, monoamine oxidase.

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Table. 1. Effect of alcohol and acetaldehyde on the metabolic disposition of dopamine-C^{14} by rat brainstem homogenates in the presence of NAD. The percent of dopamine deaminated represents the nanomoles of dopamine disappearing minus the nanomoles of THP formed because one molecule of intact undeaminated dopamine is incorporated into each THP molecule. The values represent the mean ± S.D. of five determinations. Dopet, 3,4-dihydroxyphenylethanol; dopac, 3,4-dihydroxyphenylacetic acid.

Condition	Dopamine deaminated (%)			
	Dopet	Aldehyde	Dopac	THP
Control	< 0.1	4.00 ± 1.14	16.78 ± 0.40	46.65 ± 3.32
Ethanol (100 m*M*)	< 0.1	4.00 ± 0.64	10.10 ± 0.93*	55.98 ± 2.86†
Acetaldehyde (0.5 m*M*)	< 0.1	3.73 ± 1.11	11.76 ± 0.54*	58.44 ± 2.63*
Acetaldehyde (1.0 m*M*)	< 0.1	3.98 ± 0.98	9.28 ± 0.85*	63.67 ± 1.90*
Acetaldehyde (2.0 m*M*)	< 0.1	5.39 ± 1.53	9.93 ± 0.35*	65.00 ± 2.57*

*$P < .001$. †$P < .01$.

ing THP) were eluted from alumina with 7 ml of 0.2*N* HCl. The eluates were adjusted to *p*H 2 and quantitatively transferred to columns (4 by 100 mm) of a strongly acidic cation-exchange resin (Dowex AG-50W × 4, 100 to 200 mesh, hydrogen form). The catechol bases (dopamine and THP) were adsorbed on this resin and were completely separated from the deaminated catechols which passed through in the effluent.

The effluents (containing deaminated catechols) were passed over columns (4 by 110 mm) of a weakly basic anion-exchange resin (Amberlite CG-4B, 100 to 200 mesh, acetate form). The aldehyde (3,4-dihydroxyphenylacetaldehyde) reacts with the polyamine functional groups of the resin (*8*); the neutral alcohol (3,4-dihydroxyphenylethanol) passes through in the effluent; and the acid (3,4-dihydroxyphenylacetic acid) can be easily eluted from the resin with 20 ml of 3*N* NH_4OH.

The catechol bases (dopamine and THP, adsorbed on the cation-exchange resin) were eluted in two separate fractions. Dopamine was eluted from the resin with 80 ml of 0.5*N* HCl. The THP was then eluted with 10 ml of 3*N* HCl in 50 percent ethanol. Dopamine and THP were completely separated from each other. The identity of THP was verified by infrared spectrophotometry, gas chromatography, and thin-layer chromatography. The radioactivity attributable to each compound was determined by liquid scintillation spectrometry.

The effect of cofactor (NAD), ethanol, and acetaldehyde on the metabolism of dopamine and on the relative formation of THP was determined. The experimental results support the hypothesis outlined in Fig 1. (i) In the absence of exogenous cofactor, THP represents the major catabolite of dopamine in both liver and brain in vitro (*9*). When NAD is added to incubation mixtures, THP formation in liver homogenates is virtually abolished. Although THP production is significantly decreased in the presence of NAD, this alkaloid still remains the predominant metabolite of dopamine in brainstem homogenates, most probably because of the limited aldehyde-oxidizing capacity of brain (Table 1). (ii) Ethanol and its metabolite, acetaldehyde, significantly enhance the synthesis of THP in brainstem (Table 1). Acetaldehyde in concentrations ranging from 0.5 to 2.0 mmole/liter produced incremental increases in the formation of this tetrahydroisoquinoline alkaloid. Enhanced formation of THP was caused by inhibition of dihydroxyphenylacetic acid formation and diversion of the aldehyde to the condensation (THP) pathway. (iii) The effect of ethanol in inhibiting formation of the acid implies the presence of brain alcohol dehydrogenase (*10*) for its conversion to acetaldehyde, the active inhibitor. (iv) Results of initial experiments indicate that the latter step in the hypothesis (THP conversion to morphine-like alkaloids) may likewise occur. Intravenous administration of THP-C^{14} to rats resulted in biotransformation of this compound. Of the radioactivity excreted by these animals, 50 percent of it was isolated as intact bases (adsorb on AG-50W resin) from the fraction (alumina effluent) which would contain such alkaloids as normorphine, morphine, norcodeine, and codeine.

These data support the concept that alcoholism is a true addiction which may involve specific biochemical events leading to the formation of the morphine-type alkaloids. Several types of experimental evidence have implied this relation. (i) In mice habituated to alcohol, the mortality dose-response curve for morphine was displaced to the left even in the absence of alcohol in the blood (*11*). (ii) Opiate addicts will take drugs of the hypno-sedative type if they cannot obtain morphine (*12*). (iii) Many opiate addicts are known to substitute alcohol (a quart or more per day) during periods of abstinence from a particular narcotic drug (*13*). (iv) Conversely, many opiate addicts have had previous alcoholic histories, suggesting there may exist a cross-dependence between these agents (*13*).

It is feasible that THP may have intrinsic addiction liability of its own because alkaloids of this type have been shown to produce analgesia and dependence (*14*). However, the possibility that dopamine metabolism may transverse pathways leading to morphine-like compounds in the alcoholic patient as it does in the opium poppy is an even more intriguing concept. In addition, the addiction liability of other nonspecific hypnotic drugs such as chloral hydrate and paraldehyde which inhibit aldehyde dehydrogenase and also potentiate THP synthesis could involve a similar mechanism (*15*).

This hypothesis is offered as a possible biochemical explanation for the role of catecholamines in the genesis of alcohol dependence. Investigation of alcoholism should surely include the role that alcohol as a drug plays in the development and maintenance of this condition. Physical dependence on alcohol might be envisioned as a persistent inhibition of the oxidation of 3,4-dihydroxyphenylacetaldehyde sending it along a normally untraveled metabolic pathway for the biosynthesis of tetrahydropapaveroline as a consequence of heavy and prolonged alcohol consumption. Preeminence of the THP pathway evoked by alcohol may then make this alkaloid available for subsequent conversion to addictive alkaloids.

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Cretinism in Rats: Enduring Behavioral Deficit Induced by Tricyanoaminopropene

Abstract. *Rats reared on diets containing tricyanoaminopropene, the antithyroid compound that stimulates RNA synthesis, showed a deficit in performance on automated closed-field maze tests many weeks after discontinuation of the drug. The rats were also tested while still receiving the drug, and performance deficits were indicated in tests of Y-maze reversal and manual closed-field maze performance; rats treated with the drug and with thiouracil behaved in a highly similar fashion on several tasks. No evidence of facilitation by tricyanoaminopropene appeared in any of the eight learning situations used. Exposure to tricyanoaminopropene before and after birth, at doses sufficient to produce anatomical cretinism, apparently induces an enduring behavioral deficit which is similar to that of neonatal thyroidectomy-induced cretinism in rats and which parallels the mental retardation associated with human cretinism.*

The compound 1,1,3-tricyano-2-amino-1-propene (TCAP) is of interest to those studying biochemical processes in learning and memory primarily because of its reported stimulation of brain RNA and protein synthesis (*1*, *2*). Several investigators (*2*–*4*) have reported facilitative effects of TCAP in learning situations, but conflicting reports (*5*, *6*) have also appeared, and thus the status of TCAP as a "learning facilitator" is in doubt.

The antithyroid properties of TCAP are more firmly established than its RNA- and protein-stimulating effects. Ingbar (*7*) has shown that in rats TCAP inhibits the organic binding of iodine, the formation of thyroxine, the conversion of monoiodotyrosine to diiodotyrosine, and the action of the thyroidal iodide-concentrating mechanism; others (*8*) have reported that, in rats treated with TCAP, concentrations of protein-bound iodine are reduced and ultrastructural changes in thyroid tissue occur which are similar to those produced by other antithyroid compounds. When the drug is fed over a long period to immature rats, beginning before or after birth, the typical anatomical signs of cretinism, including dwarfism, altered skeletal development, and persistence of immature hair and skin features, appear.

Better than normal performance has been reported (*4*) in rats with cretinism induced by TCAP in both the original learning and reversal of an automated Y-maze discrimination, in contrast to apparent learning deficits (*9*) for rats treated with thiouracil and those with thyroidectomy-induced cretinism. The Y-maze testing was done when the rats treated with TCAP were receiving the drug and were partially deprived of food, conditions which could have generated motivational confounding. Up until now no tests of the effects of early long-term TCAP administration have been conducted in a learning situation after discontinuation of the drug.

In two experiments, rats were made cretinoid by diets of TCAP given before and after birth and were tested on a variety of learning tasks, some of which (on-drug tests) were given during early months when physical growth was arrested by maintenance of the drug, and others (post-drug tests) were given after the drug was discontinued. In experiment 1, the behavior of rats reared on a TCAP dose (1.5 g per kilogram of mash) (*4*) was compared with that of control rats reared on plain mash. In experiment 2, the effects of a lower (1.0 g per kilogram of mash) dose of TCAP, a dose of thiouracil (1.0 g per kilogram of mash), and a control diet were compared.

In both experiments, pregnant albino rats (Holtzman Co.) were started on the experimental diets on day 5 of gestation. The rats were given free access to experimental and control regimens (*10*) throughout the gestation and lactation periods and in the offspring's maintenance after weaning until the ages of 130 (experiment 1) or 212 (experiment 2) days (*11*). Physical growth of the offspring fed TCAP and thiouracil, in terms of body weight, leveled off within the first 80 days. Weights in the female offspring treated with the drugs at 60 days of age averaged 73 g for the group fed 0.15 percent TCAP, 78 g for the group fed 0.1 percent TCAP, and 56 g for the group fed 0.1 percent thiouracil; weights of female controls at that age were 185 g. More pronounced dwarfism occurred in the male groups given TCAP.

When the drugs were discontinued, all animals were then given free access to a Purina chow diet. Regrowth in the cretinoid rats took place on the latter diet and, in terms of nearly asymptotic body weights, amounted to 83 percent (experiment 1) and 73 percent (experiment 2) of control weights in the females fed TCAP and 71 percent of normal in the females fed thiouracil. During the tests after discontinuation of the drug, the animals were maintained at 85 percent of terminal regrowth weights.

In experiment 1, the on-drug tests consisted of discrimination and seven reversals in a Y-maze followed by tests in a Rabinovitch-Rosvold closed-field maze (*12*). The post-drug tests were (in order): bar-pressing acquisition, extinction, and reacquisition; multiple-choice performance in four-lever operant chambers; four additional Y-maze reversals; running wheel activity; runway discrimination; and automated symmetrical maze tests (*13*). The rats in experiment 2 received on-drug tests of running wheel activity, Y-maze discrimination and reversal, and runway discrimination and post-drug tests of symmetrical maze performance and