Alkaloids from Catecholamines in Adrenal Tissue: Possible Role in Alcoholism

Abstract. *Epinephrine and norepinephrine condensed with acetaldehyde or with formaldehyde in dilute aqueous solution at neutral* p*H and room temperature to form 1,2,3,4-tetrahydroisoquinoline alkaloids. Similar condensation reactions occurred in cow adrenal glands perfused with dilute aldehyde solutions at 37°C. Biosynthesis of these alkaloids in vivo could play a role in altering an individual's behavior during and after the ingestion of alcohol.*

We report that when cow adrenal glands are perfused with dilute solutions of acetaldehyde, 1,2,3,4-tetrahydroisoquinoline (TIQ) alkaloids are formed. These substances are derived from a condensation reaction of acetaldehyde with the tissue catecholamines, epinephrine and norepinephrine (Fig. 1); they are related to certain plant alkaloids that exhibit pharmacologic actions on nerves and smooth muscles. Since acetaldehyde is an intermediate in the metabolism of ethanol, we suspect that TIQ alkaloids are formed in man when alcoholic beverages are imbided. These alkaloids could be involved in the development of alcohol dependence and withdrawal symptoms; they could also contribute, in general, to the pharmacologic actions of ethanol.

The impetus for our study was a recently developed fluorescence method (*1*) for the visualization of catecholamines in tissues which is based on formation of TIQ's. When freeze-dried tissue slices are exposed to moist formaldehyde vapor at 80°C, the aldehyde and the endogenous catecholamines undergo a Pictet-Spengler type of condensation (*2*, *3*) to yield TIQ alkaloids. Subsequently, these alkaloids are oxidized to 3,4-dihydroisoquinolines which tautomerize to fluorescent quinone imines (*1*). Recognizing the structural similarity between these products and the plant isoquinoline alkaloids, we sought to determine whether the Pictet-Spengler reaction would occur in intact cells under physiologic conditions when the condensing aldehyde was acetaldehyde. If so, a mechanism would be available for making TIQ alkaloids in man during alcohol intoxication and metabolism.

Initial experiments were done at room temperature with 5 to 10 m*M* catecholamine (L-epinephrine and L-norepinephrine bitartrates and hydrochlorides, Calbiochem., Los Angeles, Calif., and Sterling-Winthrop, Rensselaer, N.Y.) in aqueous buffer (1*M* acetate, *p*H 6.0, and 0.1*M* phosphate, *p*H 7.0). Acetaldehyde (Eastman Organic Chemicals, redistilled and sealed in ampules under nitrogen) or formaldehyde (Fisher Certified 40 percent solution) was added to a final concentration of 2*M*. We used formaldehyde in model experiments because its reaction with catecholamines has been well-documented (*1*) and proceeds more quickly than does that of acetaldehyde.

The reactions were monitored by removing serial aliquots and oxidizing the catecholamines with iodine to highly colored "iodochrome" products (*4*). As the condensation with aldehyde progressed, the iodochrome test became weaker, until only a yellow color formed. The condensation of catecholamines with formaldehyde was complete within 1 to 2 minutes; the reaction with acetaldehyde required 40 to 50 minutes. Acidification stopped the reactions. The reaction mixtures were analyzed by thin-layer chromatography on Adsorbosil-1 (Applied Science Labs, State College, Pa.) with a mixture of sec-butanol, formic acid, and water (15:3:2) in a nitrogen atmosphere (Fig. 2).

Epinephrine and norepinephrine each produced a major product that migrated with lower R_F than the corresponding catecholamine. In addition, there appeared one minor product with formaldehyde and two minor products with acetaldehyde, all with higher R_F than the corresponding major product (*5*). The yield of major product was about 70 to 80 percent for reactions of formaldehyde with norepinephrine and epinephrine, while for reactions of acetaldehyde it was about 70 percent with norepinephrine and about 50 percent with epinephrine. Condensation products were also observed after incubation of dopamine or dopa (dihydroxyphenylalanine) with acetaldehyde or formaldehyde.

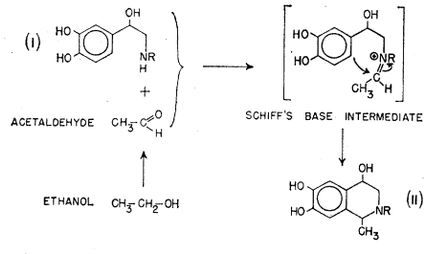

Fig. 1. Reaction scheme for the formation of tetrahydroisoquinolines from catecholamines. (I) R = H = norepinephrine, R = CH_3 = epinephrine. (II) R = H = 1-methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline; R = CH_3 = 1,2-dimethyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinoline.

The major products of the reactions are tentatively identified as the 1-methyl-4,6,7-trihydroxy analog of TIQ from norepinephrine and the 1,2-dimethyl-4,6,7-trihydroxy analog of TIQ from epinephrine. These are the expected products of Pictet-Spengler type condensations. To our knowledge, the formation of TIQ alkaloids by condensation of acetaldehyde with epinephrine or norepinephrine has not been previously described.

As judged by thin-layer chromatography, the major products of dopamine with formaldehyde and acetaldehyde were identical, respectively, to the synthetic 6,7-dihydroxy analog of TIQ (*6*) and to the commercial 1-methyl-6,7-dihydroxy analog of TIQ (salsolinol HBr, K and K Labs, Plainview, N.Y.). The major products of dopamine, norepinephrine, and epinephrine with either formaldehyde or acetaldehyde became fluorescent on heating the chromatograms at 100°C for 30 minutes. The fluorescence was quenched with 1 percent sodium borohydride. This behavior is consistent with the reversible redox reactions of tetrahydroisoquinolines and fluorescent 3,4-dihydroisoquinolines (*7*).

Alternative condensation products that might have formed are Schiff bases or oxazolidines; however, these were eliminated on the basis of infrared spectra and stability to acid (*p*H 1) and to reducing conditions (*8*). One of the minor products may be the 4,7,8-trihydroxy analog of TIQ formed by ring closure ortho to the phenolic hydroxyl (*9*). Another possibility is the presence of *cis* and *trans* isomers of the 1-methyl and 4-hydroxyl groups as was recently reported by Fourneau *et al.* (*10*) for the condensations of epinephrine and norepinephrine with glyoxalic aldehyde under neutral conditions.

Pictet-Spengler condensations had previously been carried out under strongly acidic conditions (*2*, *3*). However, in 1934, Schöpf and Bayerle (*11*) reported the isolation of TIQ alkaloids from the condensation of acetaldehyde, under physiologic conditions of *p*H and temperature, with two catecholamines, dopamine and its *N*-methyl congener (epinine). Since then investigators have reported the formation of TIQ alkaloids on condensation of catecholamine derivatives under neutral conditions with various aldehydes—namely, formaldehyde (*1*, *12*), acetaldehyde (*13*), 3,4- dihydroxyphenylacetalde-

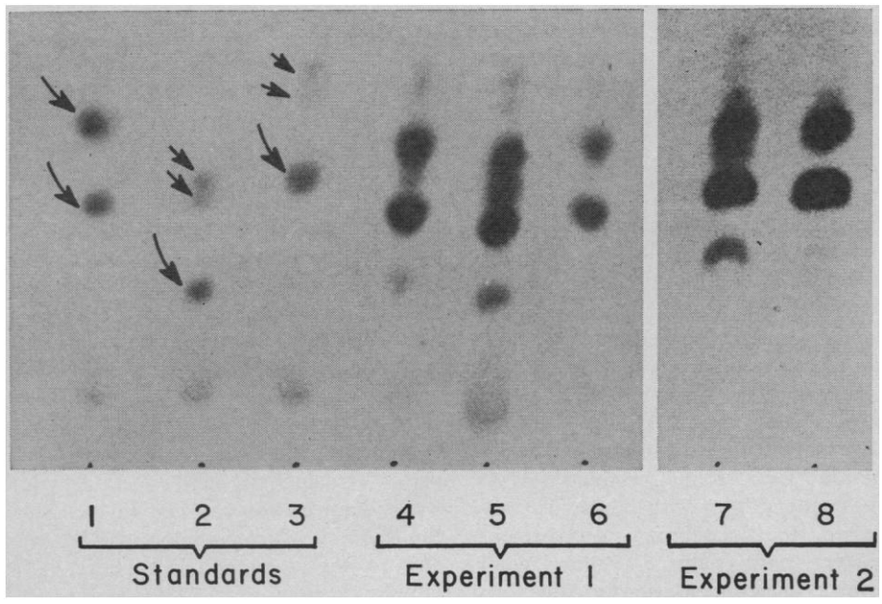

Fig. 2. Thin-layer chromatograms of two perfusion experiments with cow adrenals. The catecholamines and their condensation products with acetaldehyde were visualized by spraying first with $K_3Fe(CN)_6$ (0.25 mg/ml in 0.2*M* phosphate buffer, *p*H 8.3), drying, and then respraying with $FeCl_3$ (2.5 percent $FeCl_3$ in water, freshly mixed with 1.5 volumes of acetone); this spray combination yields Prussian-blue spots with catechols (*25*). Standards: lane 1, epinephrine (arrow, lower spot) and norepinephrine (arrow, upper spot); lane 2, major (large arrow) and minor (small arrows) reaction products of epinephrine with acetaldehyde; lane 3, major (large arrow) and minor (small arrows) reaction products of norepinephrine with acetaldehyde. Experiment 1: lane 4, gland perfused with acetaldehyde (100 μg/ml); lane 5, cochromatography of lanes 2, 3, and 4; lane 6, control gland perfused with saline. Experiment 2: gland perfused with acetaldehyde (100 μg/ml); lane 8, control gland perfused with saline.

hyde (*14*), pyridoxal phosphate (*15*), and glyoxalic aldehyde (*10*). Some of these reactions are believed to be biosynthetic pathways in plants for formation of simple TIQ alkaloids (for example, salsoline) as well as the 1-benzyl-TIQ alkaloids (for example, norlaudanosoline); the latter serve as precursors of more complex plant alkaloids such as morphine and codeine (*3*, *11*, *16*).

To determine whether the TIQ alkaloids would form in mammalian tissues under "physiologic" conditions, we performed retrograde perfusions (*17*) of cow adrenal glands with dilute solutions of aldehyde in an isotonic saline–0.01*M* sodium phosphate buffer (*p*H 7.4) at 37°C. Cow adrenals were chosen because they are rich and convenient sources of epinephrine and norepinephrine. Paired glands were stored on ice until use, generally 2 to 3 hours after removal from the cow. One gland was perfused, first with aldehyde solution and then, briefly, with saline-phosphate buffer to remove excess aldehyde. Perfusion rates were maintained with a peristaltic pump (Buchler Instruments, Fort Lee, N.J.). The second gland served as a control and was perfused simultaneously with saline-phosphate buffer which did not contain aldehyde. Then, the whole adrenal medulla or a 1-g portion was homogenized in the cold with 5 volumes of a mixture of 95 percent ethanol and concentrated HCl (99:1). Homogenates were stored overnight at 0°C, homogenized again, and centrifuged at 700*g* for 15 minutes. Thin-layer chromatography was performed directly on the supernatant fluid or on fivefold concentrates (evaporation at 40°C under a stream of nitrogen) or after the catecholamines and their acetaldehyde-condensation products had been isolated by adsorption onto, and elution from, freshly precipitated $Al(OH)_3$ (*18*).

Results of two experiments in which the glands were perfused with 100 μg of acetaldehyde per milliliter (about 0.002*M*) at 16 ml/min for 90 to 120 minutes are shown in Fig. 2. The saline-perfused control glands (lanes 6 and 8) show the expected catecholamines while the acetaldehyde-perfused glands (lanes 4 and 7) show, in addition, several new substances that are identical in R_F to the condensation products of the catecholamines with acetaldehyde. To eliminate the possibility that the new substances arose as artifacts of the extraction procedure, we homogenized fresh glands in ethanolic HCl to which formaldehyde or acetaldehyde had been added and then treated them as described above. Since no products appeared, we conclude that the TIQ alkaloids formed in the gland tissue during perfusion.

Other perfusion experiments were performed with higher concentrations of formaldehyde or acetaldehyde [1 mg/ml (0.02 to 0.03*M*)] yielding increased amounts of products. Glands were perfused at 37°C for 60 to 180 minutes with flow rates of 0.5 to 2.0 ml/min. The conversion of catecholamines to products was in the range of 25 to 100 percent; formaldehyde, in particular, produced large conversions.

Concentrations of acetaldehyde in the blood of man have been reported over a wide range. However, the specificity of earlier methods has been challenged and the most recent estimates lie below 1 μg/ml for nonalcoholic subjects ingesting "moderate" amounts of alcohol (*19*). The lowest acetaldehyde concentration with which we observed TIQ synthesis in perfused cow adrenal glands was 100 μg/ml (Fig. 2). However, we feel that the limitation was in the current technology; specifically, the streaking and merging of catecholamines on the chromatograms when larger quantities of extract were chromatographed tended to obscure smaller amounts of TIQ alkaloids that may have been present.

The TIQ alkaloids we found (Fig. 1) are similar in structure to a number of naturally occurring simple alkaloids, such as anhalamine, carnegine, and salsoline, which are found in desert cacti (*20*) and which exhibit pharmacologic actions such as excitation, narcosis, blood pressure changes, and convulsions. According to Schultes (*21*), the TIQ alkaloids found in peyote are hallucinogenic and are responsible for differences between intoxication states produced by peyote versus that produced by pure mescaline, the major active ingredient. In a survey of pharmacologic actions of synthetic, phenolic TIQ alkaloids, all lacking a 4-hydroxyl group, Hjort, DeBeer, and Fassett (*22*) observed effects on blood pressure, respiration, and smooth muscle, as well as antianesthetic properties, blockade of pressor responses to epinephrine, tremor, and convulsions. The TIQ alkaloids made in

our experiments by condensation of acetaldehyde with epinephrine or norepinephrine have not yet been studied.

We observed TIQ alkaloids by fluorescence microscopy in the adrenals of rats treated in vivo with methanol, a metabolic precursor of formaldehyde (*23*). In 1961, McIsaac (*24*) reported the presence of a 1,2,3,4-tetrahydro-β-carboline alkaloid in the urine of rats treated with ethanol, 5-methoxytryptamine, iproniazid (a monoamine oxidase inhibitor), and disulfiram (an aldehyde oxidase inhibitor). The Pictet-Spengler condensation of acetaldehyde with tryptamines to form tetrahydro-β-carbolines is analogous to the condensation with catecholamines to yield TIQ alkaloids; if formed in tissues, the tetrahydro-β-carbolines could play a role in alcoholism analogous to the one we postulate for the TIQ alkaloids.

Our experiments demonstrate the relative ease of formation of TIQ alkaloids in adrenal tissue. Other areas where these alkaloids might form are in adrenergic fibers of the sympathetic nervous system and brain. These alkaloids retain the original catecholamine structure, but ring closure prevents free rotation of the ethanolamine side chain; if the resultant conformational state were to correspond to that required for binding to smooth muscle or brain receptor sites, these substances could be unique neurotransmitter or blocking agents. Since the TIQ alkaloids were not lost during the final perfusion of the glands with saline, it appears that they may be bound in tissues, perhaps at the same loci as the catecholamines. If so, they would be in a unique anatomical location to function physiologically, although present in small amount. We suggest that the TIQ alkaloids, either actively secreted or leaked from nerve termini, contribute to the behavior changes caused by alcohol. The characteristic neurological disturbances, such as hyperexcitability, tremulousness, hallucinosis, and seizures, which occur when concentrations of alcohol in the blood are falling or absent, could be due to the persistent physiologic actions of the TIQ alkaloids. In this way, the actions of these alkaloids could underlie the process of physical dependence and addiction in alcoholism.

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9. Although ring closure ortho to the phenolic hydroxyl has not been reported (*3*), earlier work was based on isolation of a purified product and the minor products may have been lost. In our work, based on TLC analyses of unpurified reaction mixtures, minor products would be more readily observed.
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25. An advantage of this spray is that it distinguishes the catecholamines from the reaction products. Upon oxidation with ferricyanide, the catecholamines form bright rose-to-violet spots (for example, adrenochrome), while the aldehyde condensation products show as tan spots that are difficult to observe. Upon spraying with $FeCl_3$, the ferrocyanide that was produced in the redox reaction yields readily visible ferri-ferrocyanide (Prussian blue) spots for both starting catecholamines and products. The method is sensitive to about 0.1 μg of catecholamine.
26. We thank D. Dembiec, J. Marcus, E. Dicker, and F. Cabbat for technical assistance. Supported by PHS research grants MH-17071 and HE-01045 and training grant MH-10315. A preliminary report of our work has been presented (*13*).

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Physiological Effects of Transcendental Meditation

Abstract. *Oxygen consumption, heart rate, skin resistance, and electroencephalograph measurements were recorded before, during, and after subjects practiced a technique called transcendental meditation. There were significant changes between the control period and the meditation period in all measurements. During meditation, oxygen consumption and heart rate decreased, skin resistance increased, and the electroencephalogram showed specific changes in certain frequencies. These results seem to distinguish the state produced by transcendental meditation from commonly encountered states of consciousness and suggest that it may have practical applications.*

For thousands of years philosophers have held that it is possible for man to attain "higher" states of consciousness through meditation techniques. At present, scientists are investigating the physiological changes that take place during some of these practices and the practical applications that they may have.

During the practice of various techniques of meditation, expert Zen monks decreased their rate of respiration, oxygen consumption, and spontaneous Galvanic skin response (GSR), and their pulse rate and blood *p*H showed a slight increase (*1*). The electroencephalograph (EEG) record was predominantly alpha-wave activity (even with eyes half open). The alpha waves progressively increased in amplitude and decreased in frequency, and occasional theta activity