EFFECT OF ETHANOL ON THE OXIDATIVE METABOLISM OF TRYPTAMINE BY RAT LIVER HOMOGENATE

M.M. ASAAD[1], H. BARRY, III, D.E. CLARKE[1] & B.N. DIXIT
Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261, USA

1 The effect of a wide range of ethanol concentrations (v/v) on indoleacetic acid (IAA) formation from the oxidative deamination of tryptamine was studied *in vitro*, in rat whole liver homogenate.

2 IAA production was inhibited progressively by ethanol in concentrations between 0.01% to 0.2%, but the inhibition declined when the ethanol concentration was increased further to 6%.

3 Ethanol-induced inhibition of IAA formation was only partially reversed by excess aldehyde dehydrogenase, whereas reductions in IAA formation were completely prevented by pyrazole or ethanol (6% and 10%) itself.

4 Excess nicotinamide adenine dinucleotide failed to alter the inhibitory effect of ethanol and no evidence was obtained for inhibition of monoamine oxidase by ethanol or its metabolite, acetaldehyde.

5 We conclude that ethanol indirectly inhibits IAA production as a result of oxidation of ethanol by alcohol dehydrogenase, during which the oxidative metabolism of tryptamine is shifted towards the reductive pathway, thus favouring the formation of tryptophol in place of IAA.

Introduction

Ethyl alcohol (ethanol), during its two-stage oxidation through acetaldehyde to acetate by the liver, produces a change in the redox state of the hepatocyte by rapidly converting oxidized nicotinamide adenine dinucleotide (NAD) to its reduced form (NADH). Such a change has been consistently observed after both acute and chronic intake of ethanol (Isselbacher & Greenberger, 1964a, 1964b; Forsander, Räihä, Salaspuro & Mäenpää, 1965; Williamson, Browning, Thurman & Scholz, 1969; Hawkins & Kalant, 1972). It has been shown that at least some of the NAD-dependent oxidative metabolic pathways are adversely affected. For instance, ethanol decreases the production of 5-hydroxyindoleacetic acid from 5-hydroxytryptamine with a concomitant increase in formation of 5-hydroxytryptophol (Davis, Brown, Huff & Cashaw, 1967; Feldstein, Hoagland, Freeman & Williamson, 1967; Feldstein & Sidel, 1969). Other experiments have shown that the oxidative metabolism of catecholamines is likewise modified (Lahti & Majchrowicz, 1969; Walsh, Truitt & Davis, 1970; Bonham Carter, Karoum, Sandler & Youdim, 1970). Thus ethanol promotes the formation of 4-hydroxy-3-methoxyphenylglycol at the expense of 4-hydroxy-3-methoxymandelic acid (Lahti & Majchrowicz, 1969; Bonham Carter *et al.*, 1970). Dopamine and tyramine metabolism are similarly affected and an increase in urinary excretion of their neutral metabolites, 4-hydroxy-3-methoxyphenylethanol and tyrosol, has been noted in man (Bonham Carter *et al.*, 1970).

The present paper reports on what to our knowledge are the first experiments concerning the influence of ethanol on the oxidative metabolism of tryptamine, a potentially important endogenous monoamine in the mammalian central nervous system (Saavedra & Axelrod, 1972). From previous reports it was predicted that ethanol would promote the formation of tryptophol at the expense of the acid metabolite, indoleacetic acid (IAA). Thus experiments were designed to study this. Furthermore, since little attention has been directed towards the influence of various concentrations of ethanol on monoamine metabolism, this particular aspect was emphasized. Some of these results were reported at the 1973 meeting of the Federation of American Societies in Experimental Biology.

[1] Present address: Department of Pharmacology, School of Pharmacy, University of Houston, Houston, Texas 77004, U.S.A.

Methods

All experiments were conducted *in vitro*. A 5% whole liver homogenate in 0.25 M sucrose was the source of the monoamine oxidase (MAO) system and was obtained from male albino Wistar rats weighing between 250 and 300 grams. In a few experiments the liver was homogenized either in distilled water or in a 0.2% aqueous solution of Triton-X100. Throughout these experiments, concentrations of ethanol and acetaldehyde are expressed as a percentage, v/v.

Oxidative metabolism of tryptamine and kynuramine

The oxidative metabolism of tryptamine was studied by measuring the rate of IAA formation as described by Lovenberg, Levine & Sjoerdsma (1962). The IAA was measured spectrofluorometrically (ex: 286 nm, em: 365 nm; uncorrected) and the values were corrected for extraction losses by running a set of standards through the entire procedure. Unless otherwise stated, the incubation medium contained 50 mg of liver tissue (1 ml of the homogenate), 14 μmol NAD (0.7 ml), 60 μmol nicotinamide (0.5 ml), aldehyde dehydrogenase (0.4 ml), and 1 M phosphate buffer, pH 7.4 (0.4 ml). The incubations were carried out under air in a Dubnoff metabolic shaker at 37°C for 20 minutes. In all experiments ethanol or an equivalent volume of distilled water was added to ice-cold incubation mixtures. The final volume was adjusted to 5.0 ml by adding suitable amounts of distilled water. The reaction was started at the end of a 10 min equilibration period by adding 14 μmol of tryptamine (0.4 ml). The source of aldehyde dehydrogenase was the 78,000 x *g* supernatant of 1 : 4 sucrose (0.25 M) homogenate of guinea-pig kidney obtained from animals killed 18 to 24 h after receiving tranylcypromine sulphate, 20 mg/kg, i.p., a long-acting MAO inhibitor. In a few earlier experiments pheniprazine hydrochloride was also used. The aldehyde dehydrogenase preparation exhibited no inherent MAO activity.

In other experiments, the effect of various concentrations of ethanol on the oxidative metabolism of kynuramine was investigated by measuring the rate of 4-hydroxyquinoline formation as described by Kraml (1965). This method is based on the observation (Weissbach, Smith, Daly, Witkop & Udenfriend, 1960) that kynuramine is deaminated by MAO to an unstable aminoaldehyde which, by intramolecular condensation (nonenzymic), is rapidly and quantitatively converted to a highly fluorescent product, 4-hydroxyquinoline (ex: 315 nm, em: 380 nm, pH 11.0-12.0). Because the product formation involves no enzyme except MAO, this method allows a study of the effect of ethanol on MAO itself. In preliminary experiments it was shown that ethanol influenced neither the spontaneous formation nor the fluorescence of 4-hydroxyquinoline. Incubations were carried out in test tubes under air for 30 min at 37°C in a total volume of 3.0 ml containing liver homogenate (10 mg), kynuramine (100 μg), and 0.5 ml of phosphate buffer (0.5 M, pH 7.4).

Pargyline (60 μg/ml) completely inhibited the *in vitro* oxidative deamination of tryptamine and kynuramine, demonstrating that the assay systems used were indeed measuring MAO activity.

Isolation and identification of tryptophol

In experiments with tryptamine, incubation mixtures were deproteinized with 0.5 M HCl, the supernatant fractions from four incubation flasks were pooled, made alkaline to pH 8.0 by solid bicarbonate, and extracted with two volumes of diethyl ether. The remaining aqueous phase was rendered acidic with 5 M HCl and again extracted with two volumes of diethyl ether. Both ethereal layers were pooled, evaporated to dryness with a Rotary Film Evaporator, and the residue was dissolved in a small volume of distilled water. Tryptophol, tryptamine and IAA contained in this aqueous phase were separated by descending paper chromatography (Whatman paper no. 1). The solvent was the toluene layer of a two phase system containing toluene, acetic acid and water in a proportion of 4 : 1 : 5, the aqueous layer serving as the stationary phase. After 2.5 h, the chromatograms were dried and the indole compounds were identified by spraying with Ehrlich's reagent. With this system it was possible to separate and localize tryptamine, tryptophol and IAA.

Chemicals

Sources of chemicals were as follows: all chemical reagents (A.R. grade) from Fisher Scientific Co., Pittsburgh, PA 15238; kynuramine dihydrobromide, tryptophol, nicotinamide adenine dinucleotide (NAD) (ethanol free) and nicotinamide from ICN Nutritional Biochemicals, Cleveland, Ohio 44128; 4-hydroxyquinoline trihydrate, tryptamine hydrochloride and indoleacetic acid from Aldrich Chemical Co. Inc., Milwaukee, Wis. 53233; pyrazole from Eastman Kodak Co., Rochester, N.Y. 14650; tranylcypromine sulphate from S.K.F. Laboratories, Philadelphia, PA 19101.

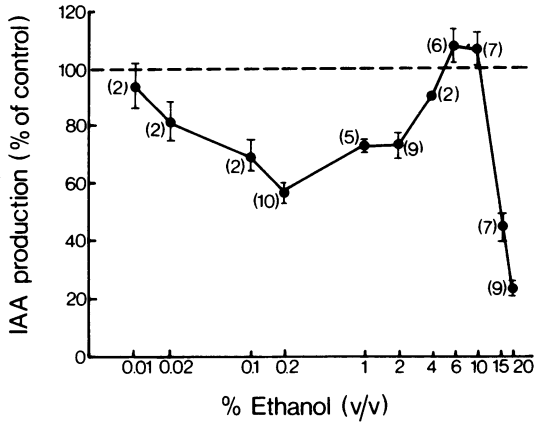

Fig. 1 Effect of various concentrations of ethanol on indoleacetic acid (IAA) production from tryptamine by rat liver homogenate. Results are means, expressed as % control (100%), ± s.e. mean. The numbers in parentheses indicate the number of determinations for each point. All points except 0.01%, 0.02%, 6.0% and 10.0% are significantly different from control, $P < 0.01$. The mean control value ± s.e. mean was 21.0 ± 1.2 μmol IAA g^{-1} h^{-1} (n = 19).

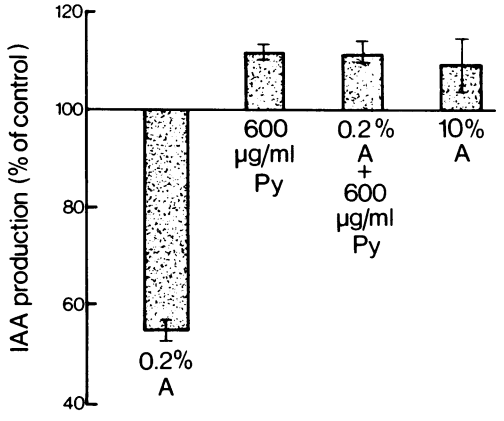

Fig. 2 Effects of ethanol (A), pyrazole, (Py) and ethanol plus pyrazole on indoleacetic acid (IAA) production from tryptamine by rat liver homogenate. Results are means, expressed as % control (100%), ± s.e. mean for six determinations or more under each condition. Differences from control: A (0.2%; v/v), Py and A plus Py, $P < 0.01$; Py, A plus Py and A (10%; v/v), are significantly different from A (0.2%; v/v), $P < 0.001$. The mean control value ± s.e. mean was 21.0 ± 1.2 μmol IAA g^{-1} h^{-1} (n = 19).

Statistical Analysis

The statistical significance of the data was determined by Student's t test.

Results

Effect of ethanol on indoleacetic acid formation

As shown in Fig. 1, a complex effect of ethanol was observed when its concentration in the incubation medium was increased from 0.01% to 20% (v/v). Low concentrations of ethanol inhibited IAA production and this reached a maximum of 42% at the 0.2% concentration of ethanol. These results were obtained on liver homogenized in isotonic sucrose. The inhibitory action was not attributable to interference by ethanol with the transmembrane movement of tryptamine onto mitochondrial MAO, since identical results were obtained with liver homogenates prepared in more disruptive media, such as distilled water or a 0.2% aqueous solution of Triton-X100. The addition of excess NAD failed to alter the ethanol-mediated inhibition of IAA formation showing that this inhibitory effect was not due to a lack of this cofactor.

Figure 1 also shows that the inhibitory effect of ethanol decreased with increasing concentrations until no inhibition was obtained at concentrations of 6% to 10%. A further increase in the ethanol concentration beyond 10% produced a precipitous inhibition of IAA production that reached a level of 75% with an ethanol concentration of 20%. The mechanism of this latter effect was not investigated.

Effect of pyrazole

Addition of 600 μg/ml of pyrazole to the incubation medium completely removed the inhibitory effect produced by 0.2% ethanol (Figure 2). In the absence of ethanol, the same concentration of pyrazole increased IAA formation by about 10%. Similar results were obtained with 10% ethanol. The concentration of pyrazole used in these experiments is well above that necessary for complete inhibition of alcohol dehydrogenase (Theorell & Yonetani, 1969; Krebs, Freedland, Hems & Stubs, 1969).

Effect of acetaldehyde and aldehyde dehydrogenase

Previous studies have shown that acetaldehyde, the initial metabolite of ethanol, can compete with the intermediate aldehyde metabolite of monoamines such as 5-hydroxytryptamine, thereby reducing

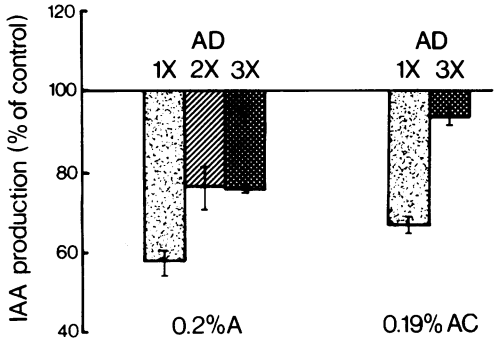

Fig. 3 Effects of ethanol (A), acetaldehyde (AC) and various aldehydedehydrogenase (AD) concentrations (1X = 0.4 ml; see methods section) on indoleacetic acid (IAA) production from tryptamine by rat liver homogenate. Results are means, expressed as % control (100%), ±s.e. mean for five determinations or more in each column. All AD (1X) are significantly different from AD (2X) and AD (3X), $P < 0.05$. With the exception of AD (3X) for 0.19% AC, all results are significantly different from control, $P < 0.01$. The mean control value ± s.e. mean was 21.0 ± 1.2 μmol IAA g^{-1} h^{-1} ($n = 19$).

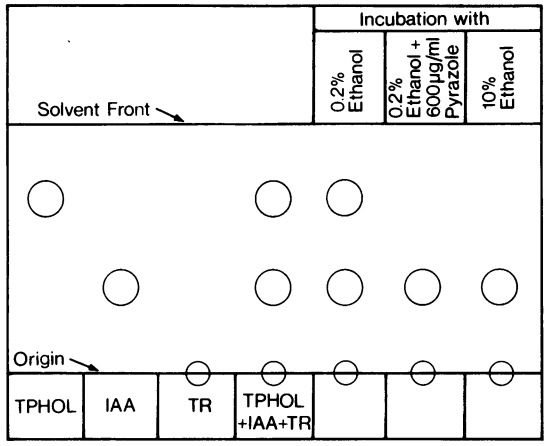

Fig. 4 Identification and separation of tryptophol (TPHOL), indoleacetic acid (IAA) and tryptamine (TR) by paper chromatography (see methods section). The authentic standards are shown on the left-hand side of the figure, both separately and in combination. In rat liver homogenate, tryptophol was identified in the presence of 0.2% ethanol but this metabolite was absent after incubation with pyrazole or 10% ethanol.

the amount of 5-hydroxyindoleacetic acid formation (Lahti & Majchrowicz, 1969). When acetaldehyde was added to the incubation medium in a final concentration of 0.19%, IAA production was inhibited by about 25% (Figure 3). This concentration of acetaldehyde is equivalent to that which would have been produced if all of the 0.2% ethanol was converted to acetaldehyde during the incubation period. Although the inhibition due to acetaldehyde was almost completely prevented by excess aldehyde dehydrogenase, only 40% of the inhibition produced by 0.2% ethanol was removed by this means. The NAD levels were not rate limiting in experiments with added aldehyde dehydrogenase. Therefore at least 60% of the inhibition produced by 0.2% ethanol was not related to acetaldehyde itself.

Formation and identification of tryptophol

The inability of excess aldehyde dehydrogenase to remove completely the inhibition produced by 0.2% ethanol suggested that some of the indoleacetaldehyde formed from tryptamine by MAO may be reduced to tryptophol instead of being converted to IAA. In chromatographic studies, it was possible to demonstrate that in the presence of 0.2% ethanol significant amounts of tryptophol were formed, in addition to IAA (Figure 4). When incubations were carried out either in the presence of 0.2% ethanol plus 600 μg/ml of pyrazole or with a high ethanol concentration (10%), only IAA was formed. This indicates that alcohol dehydrogenase is involved in the production of tryptophol.

Effect of ethanol on kynuramine oxidation by monoamine oxidase

Kynuramine deamination by liver homogenate, as measured by the rate of 4-hydroxyquinoline formation, was not affected by 0.2% ethanol. The rates (mean ± s. e. mean) of 4-hydroxyquinoline formation (nmol g^{-1} h^{-1}) were 38.1 ± 0.36 ($n = 5$) for controls and 37.2 ± 0.31 ($n = 5$) in presence of 0.2% ethanol.

Discussion

The present data indicate that ethanol interferes with the oxidative metabolism of tryptamine, not by inhibiting MAO directly but by shifting the substrate for the oxidative reaction catalyzed by aldehyde dehydrogenase into a reductive pathway. The net result of this shift is to decrease the amount of IAA formed and simultaneously to increase the production of tryptophol. The results also indicate that the conversion of indoleacetaldehyde to tryptophol is probably catalyzed by alcohol dehydrogenase itself, since both the oxidation of ethanol to acetaldehyde and the

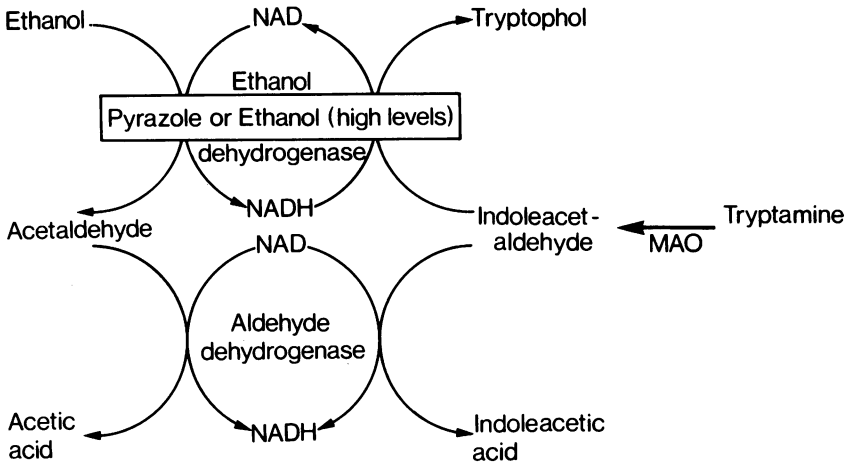

Fig. 5 Schematic representation of the suggested mechanism of the inhibitory effect of ethanol on the oxidative metabolism of tryptamine by rat liver monoamine oxidase (MAO). See discussion section for details. NAD and NADH refer to oxidized and reduced forms of nicotinic acid adenine dinucleotide, respectively.

production of tryptophol were completely inhibited by pyrazole and by high concentrations of ethanol. The present study thus establishes a parallelism between the apparent inhibitory effect of ethanol on IAA production and the activity of liver alcohol dehydrogenase. Inhibition of IAA production becomes progressively less with increasing ethanol concentrations above 0.2%, thus correlating with a reduction in liver alcohol dehydrogenase activity at high ethanol concentrations (von Wartburg, Bethune & Vallee, 1964; Dalziel & Dickinson, 1966). Inactivation of alcohol dehydrogenase by pyrazole also removes the inhibition produced by ethanol. This parallelism is conclusive proof that it is not the presence of ethanol, but its interaction with alcohol dehydrogenase, that results in the inhibition of IAA production. During the conversion of ethanol to acetaldehyde, NAD is converted to NADH and is immediately available for mediating the reduction of indoleacetaldehyde to tryptophol. Alcohol dehydrogenase has an unusually low substrate specificity and it can catalyze the oxidation of ethanol, including tryptophol (von Wartburg & Schenker, 1968), as well as the reduction of aldehydes and ketones (Sund & Theorell, 1963). Thus, alcohol dehydrogenase can establish an efficient coupled oxido-reduction reaction, on the one hand oxidizing ethanol to acetaldehyde and on the other hand reducing indoleacetaldehyde to tryptophol (Figure 5). This reaction is responsible for at least 60% of the observed inhibitory effect on IAA production. The effects of pyrazole and 10% ethanol on the tryptophol production were evaluated qualitatively by paper chromatographic technique and, since tryptophol concentrations were not determined, the conclusions should be considered tentative. A significant portion of the inhibition is also attributable to the possible competitive antagonism of aldehyde dehydrogenase by acetaldehyde, thus resulting in a decreased conversion of indoleacetaldehyde to IAA. In fact, Lahti & Majchrowicz (1969), from their *in vitro* experiments on brain aldehyde dehydrogenase, have shown that acetaldehyde, in concentrations ranging from 1 to 7.5 μM, competitively inhibited 5-hydroxyindoleacetaldehyde oxidation to 5-hydroxyindoleacetic acid. The lack of a significant inhibitory effect of 0.2% ethanol on the MAO catalyzed oxidation of kynuramine further substantiates the suggestion that ethanol produces its inhibitory effect on IAA production not by directly inhibiting MAO but by interfering with the metabolism of tryptamine at a site beyond the deamination step.

It is not difficult to speculate on the *in vivo* implications of this *in vitro* effect of 0.2% ethanol on tryptamine metabolism. Blood levels of ethanol in the order of 0.15 to 0.2% are not infrequently obtained in man (Wallgren & Barry, 1970; Goth, 1972). Furthermore, tryptamine is a known constituent of the mammalian central nervous system (Martin, Sloan & Christian, 1971; Saavedra & Axelrod, 1972; Horn & Snodgrass, 1973) and is also present in peripheral tissues (Saavedra & Axelrod, 1972) and in human urine (Rodnight, 1956). Formation of tryptophol is therefore a possible factor contributing to some of the pharmacological effects of alcohol. This neutral

metabolite of tryptamine could easily pass the blood-brain barrier and affect the central nervous system or it may be produced in the central nervous system itself. Although little information is available with regard to the pharmacological activity of tryptophol, it is pertinent that tryptophol, 5-hydroxytryptophol and 5-methoxytryptophol, in high doses, induce sleep in mice, rats and cats (Feldstein, Chang & Kucharski, 1970; Feldstein & Kucharski, 1971).

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