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SHORT COMMUNICATIONS

Effect of metronidazole on liver alcohol dehydrogenase

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METRONIDAZOLE [1-(2′-hydroxyethyl)-2-methyl-5-nitroimidazole] has been used in the treatment of alcoholism and reports indicate that this drug might interfere with ethanol metabolism in intact animals (for a review, see ref. 1). Based on data obtained from a spectrophotometric assay for alcohol dehydrogenase (ADH), which measures NADH formation,[2] Fried and Fried[3] reported that this drug inhibited ethanol oxidation activity of commercial horse liver alcohol dehydrogenase (LADH) *in vitro*. Similar observations have also been made by others.[4–6] However, in a later communication, Fried and Fried[7] pointed out that metronidazole strongly absorbs light around 340 nm and, when NADH and metronidazole were combined, nonenzymatically, the absorbance was not additive. The possibility of charge-transfer complex formation or of some other interaction between NADH and metronidazole was suggested. These authors, therefore, questioned the validity of the spectrophotometric method for the studies of the inhibitory effect of metronidazole on alcohol dehydrogenase. The desirability of an alternate assay method, which did not depend on spectrophotometric assay of NADH, was evident.

Recently, Gupta *et al.*[8–10] described a new assay for studies of ADH activity. The assay measures volatile acetaldehyde formed during ethanol oxidation catalyzed by alcohol dehydrogenase. Acetaldehyde is distilled from the reaction vessel by a stream of N_2 and is trapped in another vessel containing semicarbazide hydrochloride. The concentration of the acetaldehyde semicarbazone formed was measured by its absorbance at 224 nm. These authors also reported that LADH catalyzed a coupled oxidation–reduction reaction between ethanol and lactaldehyde to produce acetaldehyde and propanediol in the presence of catalytic amounts of $NAD^+(H)$. The reaction can be represented by the following equation:

$$CH_3CH_2OH + CH_3CHOH\,CHO \xrightarrow{NAD^+(H),\ LADH} CH_3CHO + CH_3CHOHCH_2OH.$$

We studied the effect of metronidazole on ethanol oxidation activity of horse liver alcohol dehydrogenase (Worthington) assayed by the acetaldehyde semicarbazone method (Table 1). Under the standard assay conditions, metronidazole has no inhibitory effect on ethanol oxidation activity of the enzyme. In the presence of lactaldehyde, the ethanol oxidation activity was increased approximately 12-fold; also under these conditions metronidazole has no effect on the rate of ethanol oxidation. The increase in acetaldehyde production upon the addition of lactaldehyde was only 12-fold in these experiments, which was considerably lower than previously reported.[9] However, the amounts of acetaldehyde produced in the presence of lactaldehyde under the two experimental conditions were comparable (0·96 μmole acetaldehyde formed in the presence of 4·5 μg enzyme in the previous experiment[9] compared to 2·4–2·5 μmoles acetaldehyde produced in the presence of 10 μg enzyme in the present experiments). The apparent difference in stimulation lies in the fact that under the conditions of the present experiments, which included phosphate buffer at pH 7·7, 0·5 mM NAD^+, and continuous removal of acetaldehyde produced, the rate of acetaldehyde production in the absence of lactaldehyde was significantly increased. Further studies describing the factors controlling ethanol oxidation in the presence and absence of lactaldehyde will be published elsewhere.*

In the coupled oxidation–reduction reaction, the rate of ethanol oxidation (with lactaldehyde) gave the usual enzyme–substrate saturation relationships with added NAD^+ in the concentration range of 10^{-6} to 10^{-4}M. If the imidazole group or nitro group of metronidazole forms an enzymatically inactive charge-transfer complex with $NAD^+(H)$, one would expect inhibition of ethanol oxidation by LADH when measured in the presence of a limiting concentration of NAD^+. In the presence of limiting concentrations of NAD^+, metronidazole markedly inhibited ethanol oxidation

* C. L. Woodley, N. K. Chatterjee and N. K. Gupta, manuscript in preparation.

TABLE 1. EFFECT OF METRONIDAZOLE ON ETHANOL OXIDATION ACTIVITY OF LADH WITH AND WITHOUT LACTALDEHYDE*

Metronidazole (M)	Acetaldehyde formed (μmoles/3 min)	
	−Lactaldehyde	+Lactaldehyde
None	0·19	2·53
$4·5 \times 10^{-4}$	0·17	2·40
$2·2 \times 10^{-3}$	0·19	2·56
$4·5 \times 10^{-3}$	0·20	2·42
$6·7 \times 10^{-3}$	0·19	2·42

* Ethanol oxidation activity of LADH was measured by the procedure described by Gupta *et al.*[9,10] The incubation mixture contained potassium phosphate buffer, pH 7·7, 500 μmoles; ethanol, 100 μmoles; NAD^+, 1·5 μmoles; and where indicated lactaldehyde, 15 μmoles; in a total volume of 3 ml. The reaction mixture containing buffer, ethanol, NAD^+, and where indicated lactaldehyde and metronidazole, was preincubated in a 37° water bath for 1 min while N_2 was bubbled through it. The reaction was started by the addition of 10 μg enzyme, and acetaldehyde formed during the reaction was measured as described previously.

activity of LADH; the inhibition was relieved as the NAD^+ concentration was raised (Fig. 1A). The nature of the inhibition is complex. Figure 1B shows the data in the form of a double reciprocal plot. It is possible that the inhibition is due to charge-transfer complex formation between NAD^+ and metronidazole. It has been observed that an aqueous solution containing NAD^+ and metronidazole becomes yellow upon freezing, which indicates interaction between these two compounds. The nature of this interaction is currently being studied. Other compounds such as tryptophan, which are known to form charge-transfer complexes with NAD^+,[11] showed similar inhibition patterns, i.e. marked inhibition was observed in the presence of a limiting concentration of NAD^+ and the inhibition was relieved as the NAD^+ concentration was raised. The possibility that the inhibition is competitive with NAD^+ for the enzyme site cannot be ruled out.

The side chain of metronidazole has a $—CH_2OH$ moiety, and the possibility was explored that metronidazole could act as substrate for LADH and that it could competitively inhibit ethanol oxidation by the enzyme. In order to test this possibility, metronidazole (5 mM) was incubated in the standard reaction mixture containing 500 μmoles of potassium phosphate buffer, pH 7·7, 1·5 μmoles NAD^+ and 25 μg of dialyzed crystalline LADH in a total volume of 3 ml; the solution was assayed for the formation of carbonyl compound by the procedure described by Böhme and Winkler.[12] During a 2-hr incubation period at 37°, no detectable formation of a carbonyl compound was observed, which indicates that under the conditions of the experiment metronidazole was not oxidized by LADH. Also, no significant inhibition of the rate of ethanol oxidation was observed when the reaction was studied with limiting concentrations (1×10^{-3}M to 5×10^{-3}M) of ethanol in the presence of 5×10^{-3}M metronidazole.

Preincubation of the enzyme with metronidazole for 10 min at 37° did not alter the enzymic activity when assayed with or without lactaldehyde.

In conclusion, this work shows that metronidazole inhibits ethanol oxidation activity of LADH when the NAD^+ concentration is limiting. It is known from the work of Gupta *et al.*[8,9] that, for efficient ethanol oxidation in the presence of another aldehyde, only catalytic amounts of NAD^+ are required; at this concentration of NAD^+, the drug exerts a profound inhibitory effect. Furthermore, this work describes an interesting application of the acetaldehyde semicarbazone assay method[9,10] in the studies of the inhibitors of LADH. We have noted* that several compounds reported in the litera-

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ture to be inhibitors of LADH also absorb light strongly around 340 nm; at the concentrations of these inhibitors studied, the absorbance increase on mixing the inhibitor and NADH was not additive. The effect of these inhibitors on LADH was studied using the NADH-spectrophotometric assay procedure.[2] Consequently, the validity of such results could be seriously questioned. This paper provides an unambiguous method of checking the effect of various substances on alcohol dehydrogenase activity.

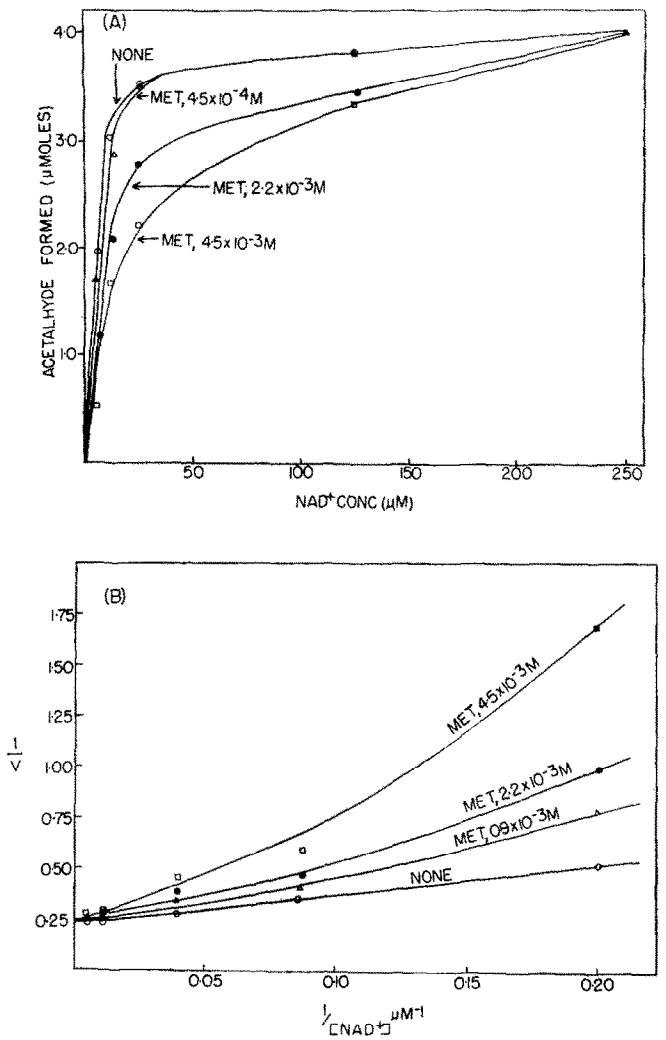

FIG. 1. Effect of metronidazole on ethanol oxidation (in the presence of lactaldehyde) by LADH in the presence of limiting concentrations of NAD^+. Standard incubation conditions as described in Table 1 were used. (A) Ethanol oxidation rate at varying concentrations of NAD^+ in the presence of different concentrations of metronidazole. (B) Lineweaver–Burk plot of NAD^+ concentration at different concentrations of metronidazole.

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Department of Chemistry,
The University of Nebraska,
Lincoln, Neb. 68508, *and*
Department of Biochemistry,
The Creighton University,
Omaha, Neb. 68131, *U. S. A.*

NABA K. GUPTA
CHARLES L. WOODLEY
RAINER FRIED

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Inhibition by aflatoxin B_1 of rat liver zoxazolamine hydroxylase induction*

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AFLATOXIN B_1 is a potent hepatocarcinogen and hepatotoxin.[1] Among several biochemical effects observed in the rat liver, rapid and extensive inhibition of precursor incorporation into nuclear and cytoplasmic RNA follows injection of the toxin.[2] Dissociation of ribosomes from hepatic rough endoplasmic reticulum resulting from aflatoxin treatment of rats has been reported.[3,4] Polysomal disaggregation in liver within 3 hr after toxin treatment has been observed.[5]

It has also been found that aflatoxin B_1 affects rat liver protein synthesis, as indicated by its suppression of amino acid incorporation into liver slices, cell-free systems, and the isolated perfused rat liver.[6–9]

In view of these responses, it seemed important to determine whether the toxin would show analogous effects on specific syntheses *in vivo* of micromolecules in rat liver. Microsomal drug-metabolizing

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