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INFLUENCE OF ETHANOL AND BUTYRALDOXIME ON LIVER METABOLISM

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Abstract—*n*-Butyraldoxime at a concentration of 10^{-5} M inhibited the oxidation rate of ethanol by 71 per cent in rat liver slices. The inhibition was competitive with respect to ethanol, with an apparent inhibition constant of 3×10^{-7} M. Butyraldoxime did not cause a rise in the acetaldehyde level of the intact rat liver during ethanol oxidation. No influence on the oxygen consumption of liver slices was observed. At a concentration of 10^{-7} M the oxime counteracted the inhibiting effect of ethanol on the carbon dioxide production of liver slices and normalized the disturbances in the redox state of the liver cytosol. The unpleasant effects caused by butyraldoxime during ethanol metabolism seem not to be produced by disturbances of liver metabolism.

INTRODUCTION

n-BUTYRALDOXIME has been shown to produce unpleasant reactions in persons who have consumed alcoholic beverages.[1] These effects were assumed to result from an increase in the acetaldehyde level of the blood. Koe and Tenen[2] found that the acetaldehyde level likewise increased in mice given butyraldoxime and ethanol. These authors found that the oxime decreased the aldehyde dehydrogenase activity *in vivo* but not *in vitro*. The latter observation was also noted by Deitrich and Helleman.[3] In our laboratory we have observed that rats refused to drink a 10% (v/v) alcohol solution when it contained small amounts of butyraldoxime.

The present investigation was performed in order to study whether butyraldoxime and ethanol exert a combined influence on the metabolism of the liver.

MATERIALS AND METHODS

Animals

The animals used were albino rats of Wistar origin, weighing 250–350 g, and fed on a standard laboratory diet and tap water. They were fasted for 18–20 hr before the experiments.

Experimental procedure

In the experiments with intact rats the animals were given 1·2 g of alcohol/kg body wt. intraperitoneally as a 10% (v/v) saline solution. In the experiments with butyraldoxime the solution contained 0·1% (w/v) of this compound. The animals were anesthetized with Nembutal (40 mg/kg body wt.) given intraperitoneally as a 1% (w/v) solution 10 min before liver sampling. The liver was quickly frozen *in situ* by means of

aluminium tongs precooled in liquid nitrogen, as described by Wollenberger *et al.*,[4] and the metabolites were extracted in ice cold 0·6 M perchloric acid.

Experiments with rat liver slices were performed as described by Forsander.[5] A 10% homogenate of the livers was prepared in Krebs–Ringer bicarbonate buffer containing 1% Triton x-100 (obtained from Rohm & Haas Co., Philadelphia, USA). It has been shown by Räihä and Koskinen[6] that this procedure gives a maximal ADH activity in liver homogenates. After centrifugation of the homogenate for 60 min at 75,000 *g* the supernatant was decanted and used for alcohol dehydrogenase assays.

Analyses

The O_2 uptake and CO_2 production of the liver slices were measured in a standard Warburg apparatus by the method of Dickens and Simer, as described by Dixon.[7] The respiratory quotient and the acids formed were calculated from the results of the experiments. The lactate, pyruvate and ethanol in the medium were determined enzymatically with the test kits supplied by C. F. Boehringer und Soehne G.m.b.H., Mannheim, W. Germany. The acetaldehyde was measured gas chromatographically as described by Forsander *et al.*[8] The ADH activity was estimated in accordance with the methods of Bonnichsen and Brink.[9]

The *n*-butyraldoxime was obtained from Fluka A.G. Buchs, Switzerland.

RESULTS

The rat liver slices were incubated for 30 min before addition of ethanol to the medium, to allow the system to equilibrate. The rate of ethanol oxidation was 0·82 μmoles/g wet wt./min without additions, but was strongly inhibited by butyraldoxime at a concentration as low as 10^{-7} M (Table 1). In experiments with supernatant of rat

TABLE 1. INFLUENCE OF BUTYRALDOXIME ON THE RATE OF ETHANOL OXIDATION*

Butyraldoxime (M)	Ethanol oxidation (μmole/g wet wt./min)	Inhibition (%)
0	0·94 ± 0·26	0
10^{-5}	0·27 ± 0·09	− 71
10^{-7}	0·68 ± 0·27	− 28
10^{-9}	1·05 ± 0·09	+ 12

* Rat liver slices, 0·5 g, were incubated at 37° in 5 ml of Krebs–Ringer bicarbonate buffer containing 11·1 mM of glucose and 17·4 mM of ethanol. Ethanol was added after a preincubation period of 30 min and the reaction stopped 60 min later. Mean ± S.E.M. of six experiments are given.

liver homogenate it was found, when the reciprocal of the reaction velocity was plotted against the inhibitor concentrations according to Dixon's[10] method, that the butyraldoxime was competitive with ethanol with an apparent inhibitor constant of 3×10^{-7} M (Fig. 1) and uncompetitive in respect to NAD (Fig. 2).

In the experiments with liver slices the acetaldehyde level was very low. The level in the liver in intact animals rose from 12·8 to 60·3 nmoles/g after treatment with ethanol (Table 2). Butyraldoxime had no influence on this level, in either the absence or presence of ethanol. For comparison, the acetaldehyde level of the liver was

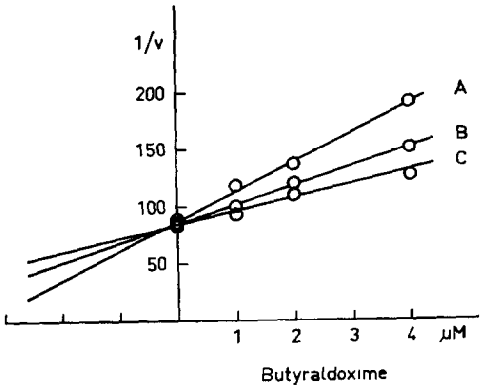

FIG. 1. The reciprocal of the velocity plotted against the butyraldoxime concentrations. The ethanol concentrations used were (A) $0{\cdot}6 \times 10^{-3}$ M, (B) $0{\cdot}9 \times 10^{-3}$ M and (C) $1{\cdot}2 \times 10^{-3}$ M. 25 μl of the supernatant of a 10% rat liver homogenate and 100 μl of a 27 mM NAD solution were added to 3·0 ml of the test mixture containing the butyraldoxime.

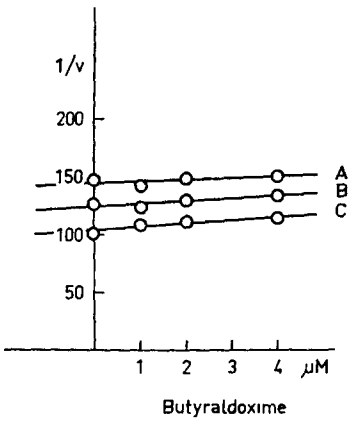

FIG. 2. The reciprocal of the velocity plotted against the butyraldoxime concentrations. The NAD concentrations used were (A) 9×10^{-5} M, (B) 13×10^{-4} M and $2{\cdot}2 \times 10^{-4}$ M. 25 μl of the supernatant of a 10% rat liver homogenate and 100 μl of a 0·12 M ethanol solution were added to 3·0 ml of the test mixture containing the butyraldoxime.

studied in rats pretreated with tetraethylthiuramdisulphide. This compound increased the aldehyde level drastically in animals which had been given ethanol.

Ethanol had no effect on the oxygen consumption of the liver slices, in either the presence or absence of butyraldoxime (Table 3). Without addition of butyraldoxime ethanol totally depressed carbon dioxide formation, but in the presence of the oxime its CO_2 formation was not greatly influenced. The formation of organic acids was not much affected by addition of both ethanol and butyraldoxime to the medium, but increased strongly when ethanol was added alone.

Butyraldoxime had no influence on the lactate level of rat liver slices but the pyruvate

TABLE 2. INFLUENCE OF ETHANOL AND BUTYRALDOXIME ON THE ACETALDEHYDE LEVEL OF THE LIVER OF INTACT RATS

Treatment	Acetaldehyde (nmoles/g wet wt.)
Control	
No ethanol	12·8 ± 5·1
Ethanol	60·3 ± 11·4
Butyraldoxime	
No ethanol	12.8 ± 0·6
Ethanol	71·9 ± 5·0
Thiuram disulphide	
No ethanol	17·4 ± 5·0
Ethanol	160·1 ± 36·5

The rats received ethanol and butyraldoxime intraperitoneally 60 min before liver sampling as described in the text. Means ± S.E.M. of ten experiments are given.

TABLE 3. INFLUENCE OF BUTYRALDOXIME AND ETHANOL ON OXYGEN CONSUMPTION, CARBON DIOXIDE PRODUCTION, RESPIRATORY QUOTIENT AND ACID PRODUCTION OF RAT LIVER SLICES

	Oxygen	Carbon dioxide (μmoles/g wet wt./min)	Acids	R.Q.
Control	1·46 ± 0·60	0·78 ± 0·52	0·67 ± 0·19	0·53
Control + ethanol	1·36 ± 0·63	− 0·07 ± 0·40	1·37 ± 0·22	0
Butyraldoxime	1·66 ± 0·58	1·07 ± 0·68	0·61 ± 0·39	0·64
Butyraldoxime + ethanol	1·71 ± 0·60	0·76 ± 0·59	1·02 ± 0·22	0·45

Liver slices, 150 mg, were incubated in Warburg flasks in 1 ml of Krebs–Henselein's bicarbonate buffer containing 11·1 mM of glucose. The ethanol concentration was 17·4 mM the butyraldoxime concentration 10 μM. A mixture of O_2 and CO_2 (95:5) was used as gas phase. The incubation time was 60 min and the temperature 37°. Means ± S.E.M. of seven experiments are given.

TABLE 4. INFLUENCE OF BUTYRALDOXIME ON THE LACTATE AND PYRUVATE LEVELS OF RAT LIVER SLICES

Butyraldoxime (M)	Lactate (mM)	Pyruvate (mM)	L/P	L + P (mM)
0	5·43 ± 0·68	0·128 ± 0·046	42·5	5·56
$4·6 \times 10^{-5}$	5·84 ± 0·76	1·159 ± 0·072	5·0	6·00
$4·6 \times 10^{-7}$	5·68 ± 0·66	0·194 ± 0·070	29·2	5·87
$4·6 \times 10^{-9}$	5·66 ± 0·98	0·150 ± 0·024	37·8	5·81

Rat liver slices, 0·5 g, were incubated at 37° in 5 ml of Krebs–Ringer bicarbonate buffer containing 11·1 mM of glucose. After preincubation for 30 min, ethanol (17·4 mM) was added and various amounts of butyraldoxime as indicated. Means ± S.E.M. of six experiments are given.

level increased when larger amounts of the oxime were present in the medium (Table 4). With $4{\cdot}6 \times 10^{-5}$ M butyraldoxime ethanol had no effect at all on the lactate/pyruvate ratio, but with decreasing amounts of the oxime the ratio increased.

In intact rats butyraldoxime had no influence on the blood glucose level in either the presence or absence of ethanol.

DISCUSSION

The acetaldehyde level after ethanol administration was studied in the intact rat liver, since it has been shown that acetaldehyde disappears very rapidly from the blood after it has been transported from the liver. It can therefore be concluded that, in contrast to tetraethylthiuramdisulfide, butyraldoxime does not bring about an accumulation of acetaldehyde in the rat (Table 2). Lewis and Schwartz[1] report that butyraldoxime increases the blood acetaldehyde level in man, and Koe and Tenen[2] have reported that the same phenomenon occurs in mice. The authors assume that the unpleasant effect of the drug is produced by the increased acetaldehyde concentration. However, this explanation does not hold for the rat, and the aversion to alcohol must depend on other factors. There are many compounds which, in combination with alcohol, produce unpleasant effects without having any effect on acetaldehyde metabolism.[11]

In experiments using mouse liver tissues, Koe and Tenen[2] found that butyraldoxime was competitive with ethanol for alcohol dehydrogenase with an inhibition constant of 10^{-6} M. In experiments *in vivo* they found no inhibition of alcohol dehydrogenase, whereas the aldehyde dehydrogenase was strongly depressed. Deitrich and Hellerman,[3] on the other hand, observed an influence of the oxime on partially purified bovine liver aldehyde dehydrogenase. In our experiments with unpurified rat liver homogenate the inhibition constant was lower and it was also competitive in respect of ethanol and uncompetitive with NAD.

Butyraldoxime counteracts the effect of ethanol on the redox level of the liver cytosol (Table 3). At an oxime concentration of 10^{-5} M, ethanol had no influence on the lactate/pyruvate ratio. At this concentration the rate of ethanol oxidation was inhibited by 71 per cent and the production of NADH in the liver cytosol can be assumed to decrease proportionally. For this reason ethanol has no influence on the redox systems of the liver. Consequently, when ethanol is present in conjunction with butyraldoxime, no change can be expected in the balance between the substrate pairs catalyzed by NAD-dependent enzymes.

A correlation has earlier been shown to exist between the influence of alcohols on the redox level of the liver cytosol and the inhibition of CO_2 production.[5] Since butyraldoxime counteracts the effect of ethanol on the lactate/pyruvate ratio, it can be understood that it also decreases the effect on CO_2 production. Since the oxidation of ethanol is inhibited, it is also understandable that the production or organic acids, *i.e.* acetic acid, is decreased by the oxime.

The metabolism of the liver is much more normal in the combined presence of ethanol and butyraldoxime than when ethanol is present alone. Consequently it is hard to understand how the unpleasant effects produced by ethanol and the oxime could be due to the metabolic processes of the liver. They might be connected, for instance, with peripheral sensitivity to acetaldehyde.

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