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Effect of Chronic Ethanol or Acetaldehyde on Hepatic Alcohol and Aldehyde Dehydrogenases, Aminotransferases and Glutamate Dehydrogenase

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Ethanol or acetaldehyde orally administered (15 % and 2 % respectively in drinking water) to male Wistar rats for three months induced alterations in the main liver enzymes responsible for ethanol metabolism, aspartate and alanine aminotransferases and NAD glutamate dehydrogenase. Ethanol produced a significant decrease in the activity of soluble alcohol dehydrogenase, while acetaldehyde induced alterations both in soluble and mitochondrial aldehyde dehydrogenases: soluble activity was significantly higher than in the control and ethanol-treated groups, and mitochondrial activity was significantly diminished. Both soluble aspartate and alanine aminotransferases showed pronounced increases by the chronic effect of acetaldehyde, while mitochondrial activities were practically unchanged by the effect of ethanol or acetaldehyde. Mitochondrial NAD glutamate dehydrogenase showed a rise in its activity both by the effect of chronic ethanol and acetaldehyde consumption. The level of metabolites assayed in liver extracts showed marked differences between ethanol and acetaldehyde treatment which indicates that ethanol produced a remarkable increase in glutamate, aspartate and free ammonia together with marked decrease in pyruvate and 2-oxoglutarate concentrations. Acetaldehyde consumption induced a significant decrease in 2-oxoglutarate and pyruvate concentrations. These observations suggest that ethanol has an important effect on the urea cycle enzymes, while the effect of acetaldehyde contributes to the impairment of the citric acid cycle.

Key words: Acetaldehyde, Ethanol, Alcohol and aldehyde dehydrogenases, Aminotransferases, Glutamate dehydrogenase, Liver metabolism.

The liver is the organ in which more than 90 % of ethanol is oxidized and also the primary site for the oxidation of the acetaldehyde produced. Although the enzymes that metabolize acetaldehyde are found throughout the organism (13, 21), only a small part of the extrahepatic capacity is effective. Acetaldehyde is considered to be 10-20 times more toxic than ethanol and its toxicity derives from

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its chemical reactive aldehyde group (1). Acetaldehyde inhibits mitochondrial respiration, and oxidative phosphorilation at the rate of ^{32}P-ATP exchange (11). The low levels in mitochondrial metabolites acetoacetate and β-OH$^-$-butyrate due to acetaldehyde (10) are a proof of the decrease in mitochondrial fatty acid oxidation.

ERIKSSON and SIPPEL (14) reported the existence of a parallelism between the concentration of ethanol and acetaldehyde in the liver. This correlation can be due either to a slightly faster rate of ethanol oxidation at higher ethanol concentrations or to a reflection of the tendency of the ethanol/acetaldehyde pair to establish equilibrium with NADH/NAD$^+$ ratio in the liver cytosol.

Ethanol oxidation to acetaldehyde as well as the oxidation of acetaldehyde to acetate, generate NADH. The ethanol metabolism thus becomes related to the increased NADH/NAD$^+$ ratio. As the ADH * is located in the cytosol of the hepatocyte, at least one NADH equivalent, produced in the ethanol oxidation, is generated outside the mitochondria. AlDH is located both in the cytosol and in the mitochondria. MARJANEN (22) was the first to show that at 0.5 mM acetaldehyde concentration about 80 % of the aldehyde dehydrogenase activity was confined to the mitochondria. Mitochondrial AlDH exhibits a higher affinity for acetaldehyde than that located in the cytosol (29). Observations from our laboratory (26) on the alterations in the mitochondrial AlDH in ethanol induced fatty liver, have led to the present comparative study between the effect of ethanol and acetaldehyde consumption. The aminotransferases GOT and GPT and GluDH are involved in metabolic pathways which interchange intermediary metabolites such as glutamate, aspartate and alanine with 2-oxoglutarate, oxaloacetate and pyruvate. They function at the cross-point between carbohydrate and protein metabolism. These enzymes may act as a source of ketoacids for the citrate cycle and gluconeogenesis and as a final stage in the process of nitrogen elimination from aminoacids through the urea cycle. Previous reports (8) pointed out that the activities of the urea cycle enzymes are diminished in the liver of long-term ethanol fed rats.

The purpose of this paper is to study the alterations of cytosolic alcohol dehydrogenase, cytosolic and mitochondrial aldehyde dehydrogenases, alanine and aspartate aminotransferases and mitochondrial NAD$^+$ glutamate dehydrogenase, after chronic liver damage produced by both ethanol and acetaldehyde.

Enzymes and abbreviations

Alcohol dehydrogenase (ADH); Alcohol: NAD oxidoreductase (EC 1.1.1.1).
Aldehyde dehydrogenase (AlDH); Aldehyde: NAD$^+$ oxidoreductase (EC 1.2.1.3).
Aspartate aminotransferase (GOT); L-aspartate: 2-oxoglutarate aminotransferase (EC 2.6.1.1).
Alanine aminotransferase (GPT); L-alanine: 2-oxoglutarate aminotransferase (EC 2.6.1.2).
Glutamate dehydrogenase (GluDH); L-glutamate: NAD$^+$ oxidoreductase (EC 1.4.1.2).

Materials and methods

Animals and treatment. Male Wistar rats (180-220 g) three months old, were divided into four groups. Each received the same standard diet (60 % carbohydrates, 3.5 % fat, 19 % proteins, 1 % vitamins and 7 % minerals), but differed in their drinking fluid as follows: I, plus water as control group; II, plus 15 % ethanol; III, plus 2 % acetaldehyde; and IV, plus 15 % ethanol + 2 % acetaldehyde.

The rats were maintained *ad libitum* on these conditions for three months and grown curves were carried throughout the treatment.

For enzyme determination rats were anaesthetized with Nembutal (50 mg/Kg

body weight); its abdomen was opened by a midline abdominal incision and the portal vein was cannulated. The aorta and inferior vena cava were severed and 0.15 M NaCl solution was infused until the liver was essentially free of blood, thus preventing contamination of the hepatic soluble fraction with enzymes from red cells. The liver was immediately chilled in ice-cold buffer, and a biopsy specimen was taken for homogenization.

The rats intended for metabolite measurements were cervically dislocated and liver samples were immediately frozen in liquid nitrogen by the procedure of freeze-clamping.

Preparation of homogenates. Liver homogenates for enzyme determination were prepared in a medium containing 0.25 M sucrose, 0.02 M Tris-HCl buffer at pH 7.4 and 0.1 mM dithiothreitol (4 volumes medium: 1 part liver) with a Potter-Elvehjem homogenizer fitted with a teflon pestle. The homogenate obtained was centrifuged at 800 *g* for 10 min to remove nuclei and cell debris. The supernatant obtained was centrifuged at 10,000 *g* for 20 min to obtain the mitochondrial pellet. To prevent the contamination with the soluble fraction the mitochondrial pellet was washed twice by suspension in 10 ml of the medium and centrifuged. The 10,000 *g* supernatant was centrifuged at 105,000 *g* for 45 min and the supernatant obtained was the soluble fraction. Both the mitochondrial and the soluble fraction were dialyzed against the medium for 1 h at 4° C. The mitochondrial fraction was lysed with Triton X-100 (0.1 % final concentration).

For the assay of intermediary metabolites, perchloric acid extracts of liver samples, neutralized with KOH, were used. The detailed procedure was previously described by WILLIAMSON *et al.* (31).

Measurements of enzyme activities. Enzyme activities were determined spectrophotometrically by the methods cited as follows: Alcohol dehydrogenase (EC 1.1.1.1) was assayed in the soluble fraction by the method of BERGMEYER *et al.* (5), using tetraethyl thiouram disulfide (1 mM) as inhibitor of aldehyde dehydrogenase. Aldehyde dehydrogenase (EC 1.2.1.3) was determined both in the soluble and mitochondrial fractions as described by MARSELOS and HÄNNINEN (23), using pyrazol as inhibitor of alcohol dehydrogenase. Soluble and mitochondrial aspartate aminotransferase (EC 2.6.1.1) were assayed following the technique described by BERGMEYER and BERNT (2). Soluble and mitochondrial alanine aminotransferases (EC 2.6.1.2) were assayed as described by BERGMEYER and BERNT (3). Mitochondrial NAD-glutamate dehydrogenase (EC 1.4.1.3) were determined by the technique of SCHMIDT (27).

Enzyme activities are expressed in Units/gram of fresh liver. A unit is defined as the amount of extract that transforms 1 μmol of substrate per min at 37° C.

Measurements of metabolite concentrations. The hepatic concentrations of the following metabolites were spectrophotometrically determined: Glutamate (7), aspartate (6), alanine (16), 2-oxoglutarate (4), pyruvate (12) and free ammonia (15).

Results are expressed as nmols/g of fresh liver. All values, enzyme activities and metabolite concentrations, were given as mean ± S.E.M. of six experimental observations. Paired *t* test analysis was used to evaluate the significance of the differences versus control.

Reagents. Enzymes used were obtained from Boehringer Manheim Corporation, and substrates and coenzymes were purchased from Sigma Chemical Co. All other biochemicals, obtained from Merck, were of the highest purity available commercially.

Results and Discussion

The increase in body weight of the animals was determined along the three-month treatment and the differences were calculated as means ± S.E.M. of 6 to 10 rats. The results obtained were 209 ± 13 g for control group and 189 ± 19 g (90 %); 150 ± 17 g (71 %) and 133 ± 15 g (63 %) for ethanol, acetaldehyde and ethanol + acetaldehyde groups, respectively. These results indicate that acetaldehyde toxicity, even when it was administered at very low concentrations, was significantly higher than ethanol.

In order to evaluate the effects of chronic ethanol or acetaldehyde intake, the activities of the main enzymes responsible for ethanol and acetaldehyde oxidation were assayed in liver of rats. The soluble alcohol dehydrogenase activity was significantly decreased to 59 % by the long-term ethanol consumption and showed a slight and non-significant decrease, to 89 %, with the administration of acetaldehyde (table I). However, soluble and mitochondrial aldehyde dehydrogenase remained practically unchanged by the effect of ethanol (103 % and 104 %, respectively). The significant increase of soluble aldehyde dehydrogenase to 194 % due to the effect of acetaldehyde was accompanied by a significant decrease in the mitochondrial aldehyde dehydrogenase (81 %). These results demonstrate that ethanol and acetaldehyde are reciprocally antagonistic at the level of enzymes responsible for their metabolism. This lack of parallelism is reflected mainly in the mitochondria.

Acetaldehyde, the primary metabolite of ethanol oxidation, has numerous toxic effects on mitochondrial functions. Acetaldehyde depressed CO_2 production from citric acid cycle intermediates (oxaloacetate, succinate and malate) at concentrations in which acetate had no effect (11). Chronic exposure of low levels of acetaldehyde, as in our experiments, could contribute to the impairment of the citric acid cycle. This impairment can be found in mitochondria from ethanol fed rats. Moreover, acetaldehyde oxidation by mitochondrial aldehyde dehydrogenase did not change after chronic ethanol consumption (104 %), while it decreased (81 %) when acetaldehyde was directly administered.

These results are in agreement with those of MATSUZAKI and LIEBER (24), who demonstrate that acetaldehyde toxicity decreases the mitochondrial capacity to metabolize acetaldehyde.

On the other hand, there is controversy on whether or not ethanol consumption affects the activity of soluble alcohol dehydrogenase and it was reported (20)

Table I. *Alcohol and aldehyde dehydrogenases in liver of rats chronically treated with ethanol or acetaldehyde.*

Results, expressed as percentage of control group activities are the mean ± S.E.M. of six experimental observations. Mean of the control group values for sADH, sAlDH and mAlDH were: 2.20 ± 22, 0.35 ± 0.024, 0.52 ± 0.068 U/g fresh liver, respectively. Statistical significance was calculated versus control. sADH = soluble alcohol dehydrogenase, sAlDH = soluble aldehyde dehydrogenase, mAlDH = mitochondrial aldehyde dehydrogenase.

Enzyme	Ethanol	Acetaldehyde	Ethanol + Acetaldehyde
sADH	59 ± 7***	89 ± 10	64 ± 14**
sAlDH	103 ± 12	194 ± 21***	194 ± 18***
mAlDH	104 ± 8	81 ± 7**	85 ± 11

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

Table II. *Aspartate and alanine aminotransferases and glutamate dehydrogenase in liver of rats chronically treated with ethanol or acetaldehyde.*
Results, expressed as percentage of control group activities are the mean ± S.E.M. of six experimental observations. Mean of the control group values for sGOT, mGOT, sGPT, mGPT and mGluDH, were: 64 ± 9, 10 ± 1.2, 41 ± 5, 1.65 ± 0.14 and 11.40 ± 1.33 Units per gram of fresh liver, respectively. Statistical significance was calculated versus control. sGOT = soluble aspartate aminotransferase, mGOT = mitochondrial aspartate aminotransferase, sGPT = soluble alanine aminotransferase, mGPT = mitochondrial alanine aminotransferase, mGluDH = mitochondrial glutamate dehydrogenase.

Enzyme	Ethanol	Acetaldehyde	Ethanol + Acetaldehyde
sGOT	112 ± 13	163 ± 12***	165 ± 13***
mGOT	108 ± 8	109 ± 13	111 ± 12
sGPT	86 ± 12*	122 ± 12*	83 ± 7**
mGPT	95 ± 13	85 ± 8*	111 ± 14
mGluDH	125 ± 11*	145 ± 16***	167 ± 15***

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

that this activity increases, decreases or remains unchanged by the effect of ethanol. These contradictory results can be explained establishing that in the long-term ethanol feeding, two processes are involved in altering the enzyme activity: one, an adaptative response which originates a specific induction of the enzyme activity; the other, an unspecific response which induces a decrease in the enzyme protein synthesis as a result of liver failure. Thus, HORTON and BARRET (18) observed the rapid increase in the specific activities of mitochondrial aldehyde dehydrogenase due to the effect of acute ethanol administration as a specific reply to the increased concentrations of acetaldehyde resulting from the oxidation of ethanol. This rapid increase represents a protective mechanism against the toxicity of acetaldehyde.

Table II shows the activities of soluble and mitochondrial GOT and GPT and NAD-mitochondrial GluDH. The decreased value observed in the activity of soluble GPT by the effect of ethanol to 86 % are in contrast to the increase due to the effect of acetaldehyde (122 %, $p < 0.01$). Conversely, the concentrations of alanine (Table III) showed alterations depending on ethanol and acetaldehyde administration (122 and 88 %, respectively), in an opposite way to that of GPT soluble activities. It may be concluded that in this aspect ethanol and acetaldehyde act antagonistically when they are administered in chronic form to rats. This different actuation was also made evident above (table I), according to the results obtained on alcohol and aldehyde dehydrogenases. Changes observed in the activities of soluble GOT and GPT were not significant (112 and 86 %, respectively) by the effect of ethanol, when compared to control group. Soluble GOT increased to 163 %, when acetaldehyde was administered. In the mitochondrial fraction, GOT and GPT did not show significant variations by the effect of ethanol (108 and 95 %). A slight but significant increase in GluDH activity to 125 % was also due to ethanol administration. Acetaldehyde produced a significant decrease in mitochondrial GPT to 85 % and a remarkable increase in GluDH activity to 145 %.

Table III shows the hepatic concentration of the intermediary metabolites involved in the enzyme activities related to glutamate metabolism, that appeared

in Table II. The increased concentration in glutamate (164 %) and aspartate (220 %), due to chronic ethanol intake, have no parallel results when acetaldehyde was administered. In this case, the results were 112 and 102 % for glutamate and aspartate respectively. These observations plus the higher levels in free ammonia (184 %) due to ethanol, are in agreement with the results of CASCALES *et al.* (8), who demonstrate that ethanol inhibits to a great extent the activity of the urea cycle enzymes. The accumulation of aspartate has also been observed by STUBBS and KREBS (30), using isolated hepatocytes, as a result of ethanol metabolism and was related to a decrease in the cytosolic levels of 2-oxoglutarate and to an increase in the mitochondrial concentration of glutamate. The present data suggest that 2-oxoglutarate formed in the mitochondria by mitochondrial GOT (unchanged in both cases: ethanol and acetaldehyde), is rapidly converted to glutamate via GluDH (increased to 125 % by ethanol and to 145 % by acetaldehyde), in a NAD-generating process. Aspartate formed is then translocated into cytosol and it will be accumulated if there is a lack of 2-oxoglutarate. On the other hand, the increased level of glutamate (164 %) and aspartate (220 %) by the effect of chronic ethanol intake, also reported by other authors (17, 30) and the inhibition of the urea cycle enzymes (8) provide additional evidence that the formation of glutamate and aspartate represents a regulatory mechanism involved in he control of ammonia in order to remove this toxic compound in the liver under pathological conditions. As 2-oxoglutarate is an indispensable intermediary to glutamate formation, the elimination of ammonia from the medium will be subjected to 2-oxoglutarate concentration. In our experiments 2-oxoglutarate concentrations were low both in ethanol (80 %) and in acetaldehyde (55 %) treated rats. In liver perfusion studies (31) the major site of inhibiting the citric acid cycle was reported to be the citrate to 2-oxoglutarate pathway, whereas in isolated liver cells the site of ethanol inhibition was situated at the level of 2-oxoglutarate dehydrogenase (25). These findings may explain the low levels in 2-oxoglutarate originated by the chronic ethanol or acetaldehyde intake. As discussed before by several groups of investigators (11) it seems not logical to attribute the alterations originated by the effect of ethanol meabolism only to the changes of the redox stage of the cell, since in this case the levels of 2-oxoglu-

Table III. *Levels of glutamate, aspartate, alanine, 2-oxoglutarate, pyruvate and free ammonia in liver of rats chronically treated with ethanol or acetaldehyde.*

Results, expressed as percentage of control group activities are the mean ± S.E.M. of six experimental observations. Mean of the control group values for glutamate, aspartate, alanine, 2-oxoglutarate, pyruvate and ammonia were: 2478 ± 599, 814 ± 116, 525 ± 66, 76 ± 12, 101 ± 11, 214 ± 10 nmol/g fresh liver, respectively. Statistical significance was calculated versus control.

Metabolite	Ethanol	Acetaldehyde	Ethanol + Acetaldehyde
Glutamate	164 ± 18***	112 ± 9	172 ± 20***
Aspartate	220 ± 31***	102 ± 15	180 ± 21***
Alanine	122 ± 16*	88 ± 12	101 ± 9
2-oxoglutarate	80 ± 11**	55 ± 7***	65 ± 9**
Pyruvate	52 ± 6***	75 ± 9**	30 ± 6***
Ammonia	184 ± 30**	92 ± 10	160 ± 20**

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; NS $p > 0.05$.

tarate in acetaldehyde-treated rats are lower than in the ethanol-treated ones, the amount of acetaldehyde administered being 7.5 times lower and whose oxidation would originate a reducing power 15 times lower.

It is well known that ammonia increases under certain circumstances such as high protein diet, diabetes, etc., and the urea cycle functions as a regulatory mechanism to maintain the intracellular ammonia at a normal level. There are two main mechanisms for the elimination of ammonia: the urea cycle and the formation of glutamate via GluDH. As the urea cycle is inhibited by the effect of ethanol (8), ammonia increases to 184 % and this increase inhibits 2-oxoglutarate formation (19). In spite of the fact that glutamate dehydrogenase activity is enhanced (table II), both in the case of ethanol or acetaldehyde, glutamate formation is limited by the low concentration of 2-oxoglutarate.

Therefore, in the case of ethanol, as ammonia increases (184 %), 2-oxoglutarate decreases (80 %) and the glutamate dehydrogenase (125 %) is unable to remove the excess of ammonia by the reductive biosynthesis of glutamate. In the case of acetaldehyde, although glutamate dehydrogenase activity is higher (164 %) the low levels of 2-oxoglutarate (55 %) and the unchanged concentrations of NH_4^+ does not permit the increase in the reductive synthesis of glutamate.

GOT and GPT are in close connection to the urea cycle. The conversion of glutamate to aspartate provides half of the nitrogen that enters the urea cycle. When soluble GOT increases (163 %) as in the case of acetaldehyde, the formation of aspartate from glutamate would increase if the «pool» of oxaloacetate remained high. The involvement of GOT, GPT and GluDH in the urea synthesis depends not only on the level of urea precursors but also on the citric acid cycle intermediates. In spite of the high GluDH activity (145 %) by the effect of acetaldehyde, due to the low levels of the precursors of glutamate (2-oxoglutarate and free ammonia), the reductive synthesis of glutamate is limited and the intramitochondrial formation of aspartate would not be as high as in the case of ethanol. However, as soluble GOT is also increased (162 %) the low levels of 2-oxoglutarate will be lowered attempting to generate oxaloacetate. The nonaccumulation of glutamate and aspartate by acetaldehyde suggests a normal functioning of the urea cycle.

Ethanol alters aminoacid metabolism in different aspects: a) The increased generation of reducing equivalents causes a metabolic deviation towards the formation of reduced aminoacids. b) Acetaldehyde and acetate, compete with aminoacids for binding sites of enzymes causing competitive inhibition, and c) Damage of the liver, due to ethanol or to any hepatotoxic compound, originates a decreased metabolism of aminoacids (8, 9).

Alterations in the metabolism of aminoacids are of special interest because of their relationship to collagen synthesis, neurotransmitter formation, protein synthesis, etc. Acute ethanol toxicity increases the conversion of glutamate to proline within the liver (17). Following gastric intubation with ethanol in rats, these authors have observed increased levels of glutamate relative to 2-oxoglutarate in liver. As a precursor of proline, glutamate accumulation in liver by the effect of ethanol may play an important role in the pathogenesis of cirrhosis.

Considering especially the rise in aminoacids and ammonia, together with the low levels in ketoacids, by the effect of ethanol, in contrast to the normal levels in aminoacids and ammonia and lower ketoacids due to acetaldehyde, these findings indicate that chronic intake of acetaldehyde, does not affect the urea cycle enzymes. However, the low levels in

2-oxoglutarate and pyruvate suggests the existence of an impairment in the citric acid cycle.

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Resumen

El etanol (15 %) o el acetaldehído (2 %), administrados oralmente en el agua de bebida a ratas Wistar macho, durante tres meses, induce alteraciones en los enzimas hepáticos principalmente encargados del metabolismo del etanol, las aspartato y alanina aminotransferasas y la glutamato deshidrogenasa NAD. El etanol produce un descenso significativo en la alcohol deshidrogenasa mientras que el acetaldehído provoca alteraciones tanto en la aldehído deshidrogenasa soluble como en la mitocondrial, presentando la soluble una actividad significativamente elevada, frente al grupo control o al tratado con etanol, y la mitocondrial significativamente disminuida. Las aspartato y alanina aminotransferasas solubles presentan pronunciados incrementos por efecto del acetaldehído permaneciendo las mitocondriales prácticamente inalteradas, tanto por efecto del etanol como del acetaldehído. La glutamato deshidrogenasa muestra incremento en su actividad por efecto del etanol, siendo más acusado por efecto del acetaldehído. Los niveles de metabolitos ensayados en extractos hepáticos muestran diferencias notables entre el etanol y el acetaldehído. El etanol origina incrementos muy notorios en el glutamato, aspartato y amonio, junto con marcados descensos en los de piruvato y 2-oxoglutarato. El consumo de acetaldehído induce descensos significativos en las concentraciones de 2-oxoglutarato y piruvato. Estos resultados hacen pensar que el efecto del etanol se refleja principalmente sobre los enzimas del ciclo de la urea, mientras que el acetaldehído contribuye más bien a un deterioro en el ciclo cítrico.

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