EFFECT OF ACUTE ETHANOL INGESTION ON LIPOPEROXIDATION AND ON THE ACTIVITY OF THE ENZYMES RELATED TO PEROXIDE METABOLISM IN RAT LIVER

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Received 11 December 1979

1. Introduction

Data presented in [1] show that acute ethanol administration to rats stimulates hepatic lipoperoxidative processes in conditions of maximal depletion of the content of reduced glutathione (GSH). Apart from the content of tissue GSH, the levels of vitamin E as well as the activity of the enzymes related to peroxide metabolism have been suggested to contribute to the maintenance of the antioxygenic capacity of the liver cell [2–4].

This work deals with the influence of acute ethanol ingestion on liver lipoperoxidation in relation to the activity of:

(i) Superoxide dismutase and catalase as an antioxidant system preventing lipoperoxide formation by free radicals;
(ii) Glutathione (GSH)-peroxidase and glutathione (GSSG)-reductase as a detoxifying system decomposing hydroperoxides and hydrogen peroxide (H_2O_2) to inactive metabolites [4].

2. Experimental

2.1. *Enzyme activity assays and measurement of diene conjugation*

Determinations were performed in male Wistar rats (Facultad de Medicina Occidente, Universidad de Chile) (150–200 g) fasted overnight (16 h) and after 6 h oral administration of 5 g ethanol/kg as a 40% (w/v) solution in saline. Saline and isocaloric glucose controls were carried out as in [1]. For enzyme activity assays, the livers were washed and homogenized in cold 0.15 M KCl (25%, w/v). GSH-peroxidase was assayed in diluted homogenates (1:40) by measuring decreases in A_{340} due to NADPH oxidation by added glutathione reductase [5]. The activities of superoxide dismutase [6], catalase [6] and GSSG-reductase [7] were determined in supernatants after centrifugation of original homogenates at 30 000 × *g* for 10 min. Superoxide dismutase was estimated by the inhibition of the reduction of nitroblue tetrazolium (NBT) by superoxide (O_2^-) generated by photoreduction of riboflavin [6]. One unit of activity is defined as the amount of enzyme producing 50% inhibition of the reduction of NBT to formazan observed in the blank [6]. Catalase activity was measured by determining residual H_2O_2 by light emitted from oxidation of luminol by horse radish peroxidase [6] and results are expressed as Sigma units/mg protein (one unit will decompose 1.0 μmol H_2O_2/min at pH 7.0 at 25°C, while the H_2O_2 concentration falls from 10.3–9.2 μmol/ml reaction mixture). Microsomal NADPH oxidase was measured as in [8]. Formation of diene conjugates was determined at 233 nm in the original liver homogenates and results are expressed as μmol hydroperoxide/mg protein by using the ϵ_M 2.52×10^4 M^{-1} [9]. Proteins were measured as in [10]. All the reagents used were purchased from Sigma (St Louis). Results are expressed as means ± SEM, and significance of the effect of alcohol treatment was assessed by the Student's *t*-test for unpaired data.

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3. Results and discussion

The administration of a single dose of 5 g ethanol/kg to rats fasted overnight produced a 26–34% increase in liver lipoperoxidation when compared to saline or glucose control animals, measured as formation of diene conjugates (table 1A). This is in agreement with previous studies in which the effect of ethanol treatment on the lipoperoxidative capacity of the liver cell was estimated by either the same procedure [3], malondialdehyde production [1,11], chemiluminescence [12] or in vivo ethane [13] or pentane [14] exhalation in the rat.

The alcohol-induced enhancement of liver lipoperoxidation was found to occur concomitantly with a 40% increase in the activity of superoxide dismutase (table 1B), a finding that could conceivably represent an adaptive change to an elevated supply of superoxide anions (O_2^-). In fact, the activity of the microsomal NADPH oxidase, an enzyme known to generate O_2^- [15], was found to increase by 39–45% in this condition (table 1B). Furthermore, the microsomal NADPH-cytochrome *c* reductase and cytochome P_{450} may produce significant amounts of O_2^- as well as H_2O_2 [16–18] and that they are involved in the activity of the intact and reconstituted microsomal ethanol-oxidizing system [19]. Apart from these microsomal enzymes, xanthine oxidase and aldehyde oxidase, cytosolic enzymes secondarily involved in the oxidation of acetaldehyde arising from ethanol metabolism, are responsible for a minor fraction of cellular O_2^- generation [4,20]. This evidence would suggest a greater production of O_2^- in the rats intoxicated with ethanol for 6 h, which exhibit blood alcohol levels of 378 mg/100 ml (82.2 mM) [1], than the corresponding control animals given saline or isocaloric glucose.

The increase in the activity of liver superoxide dismutase found after acute ethanol treatment (table 1B) would protect the liver against the lipoperoxidative action of O_2^-, but, at the same time, it will generate H_2O_2. Although no changes in the activity of catalase and the glutathione peroxidase–reductase couple were found in vitro in these conditions (table 1B), handling of H_2O_2 could be deficient because catalase is primarily concerned with the disposal of peroxisomal H_2O_2 [4] and the glutathione peroxidase–reductase couple could become ineffective in situ due to the drastic GSH depletion induced by ethanol intoxication [1]. In fact, liver GSH levels are decreased to ~2 mM in this condition [1], a value that is below the K_m of GSH-peroxidase for GSH (3 mM) [21]. Hydrogen peroxide, in turn, can produce hydroxyl radicals ($HO^\cdot$) by the Haber-Weiss and/or the Fenton reactions [4], a free radical that seems to be more active than O_2^- in determining lipoperoxidation [22]. In line with these suggestions are the findings that hydroxyl radical scavengers effectively reduce the oxidation of ethanol by intact microsomes [23] or in a reconstituted microsomal ethanol-oxidizing system [24],

Table 1
Effect of acute ethanol ingestion on the formation of diene conjugates and on the activity of the enzymes related to peroxide metabolism in rat liver

Treatment	Saline	Glucose	Ethanol	Effect (%)	*P*
A Diene conjugation (6) (μmol hydroperoxide/mg protein)	0.19 ± 0.008	0.18 ± 0.017	0.24 ± 0.02	+26–34	0.005
B. Enzyme activities					
Superoxide dismutase (8) (units/mg protein)	53.4 ± 7.9	52.4 ± 9.2	74.0 ± 8.4	+39–41	0.02
Catalase (6) (Sigma units/mg protein)	1342 ± 309	1358 ± 438	1358 ± 337	+ 0– 1	N.S.
Glutathione peroxidase (8) (μmol NADPH/mg protein/min)	5.4 ± 1.8	5.7 ± 1.8	5.3 ± 1.3	– 2– 7	N.S.
Glutathione reductase (8) (μmol NADPH/mg protein/min)	4.6 ± 0.7	4.8 ± 1.1	4.1 ± 0.6	–11–14	N.S.
NADPH oxidase (7) (μmol NADPH/mg protein/min)	6.9 ± 0.6	6.6 ± 0.4	9.6 ± 1.0	+39–45	0.05

Animals fasted overnight (16 h) were given a single dose of 5 g ethanol/kg and were sacrificed 6 h later. Control rats received isovolumetric amounts of saline or isocaloric amounts of glucose. The number of animals used is shown in parentheses. *P* values correspond to the significance of the effect of ethanol treatment compared to saline or glucose control groups

while the addition of superoxide dismutase, which scavenges O_2^-, has no effect [24].

In conclusion, stimulation of hepatic lipoperoxidation by acute ethanol ingestion could be the result of an enhanced $HO^\cdot$ generation, which, in turn, could derive from H_2O_2 produced by an increased metabolism of O_2^-. GSH depletion [1] would contribute to the effect by limiting the disposal of H_2O_2 and hydroperoxides through GSH-peroxidase.

Acknowledgements

This work was supported in part by grants M 308-792 and B 691-802 from the Servicio de Desarrollo Científico, Artístico y de Cooperación Internacional, Universidad de Chile. The secretarial assistance of Miss V. Penjean is gratefully acknowledged.

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