EFFECT OF ACUTE ETHANOL INTOXICATION ON THE CONTENT OF REDUCED GLUTATHIONE OF THE LIVER IN RELATION TO ITS LIPOPEROXIDATIVE CAPACITY IN THE RAT

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1. Introduction

Glutathione is considered to be the most abundant and important intracellular sulfhydryl compound [1,2]. Both reduced (GSH) and oxidized (GSSG) glutathione are related to several structural and functional processes of the cell, and are involved in the protective mechanisms against the deleterious effects of several agents and/or their metabolites [2]. The later function of glutathione has been proposed to be accomplished by either the formation of excretable conjugates [3] or by its participation in the metabolism of peroxides arising from the enhancement of lipoperoxidative processes [4].

Lipoperoxidation, the oxidative alteration of polyunsaturated fatty acids that seems to be of importance in the production of liver injury by some hepatotoxins [5,6], has been shown to be increased in the liver following acute [7–10] and chronic [8,11–14] alcohol ingestion. However, this finding has not been confirmed in the acute model [15–17]. This report describes the influences of the sex, nutritional status, dosage and the period of intoxication of animals given alcohol acutely on the content of GSH of the liver in relation to its lipoperoxidative capacity. The effect of alcohol on the activity of the enzymes of peroxide metabolism, the other main contributors to the maintenance of the antioxygenic capacity of the hepatocyte [1,4,18], is dealt with in [19].

2. Experimental

2.1. *Measurements of malondialdehyde production and GSH content*

Male and female Wistar rats (Facultad de Medicina Occidente, Universidad de Chile) (150–200 g) were kept on a pellet diet (Alimentos Balanceados SA, Santiago). Experiments were done in animals fed ad libitum or fasted overnight (16 h), intubated with 5 g ethanol/kg body wt as a 40% (w/v) solution in saline. The corresponding control groups received either isovolumetric amounts of saline or isocaloric amounts of glucose as a 50% (w/v) solution. Studies were carried out in rats intoxicated for 6 h kept in a warm environment (20–25°C). The time course study was performed in fasted rats given 5 g ethanol/kg, sacrificed after 1,2,3,4,5,6,9 and 12 h treatment. Blood alcohol levels during this period were determined enzymatically as in [20]. The dose relationship study was carried out in fasted rats given 2,3,4 or 5 g ethanol/kg and sacrificed 6 h later. For estimation of lipoperoxidation, the livers were homogenized (30% w/v) in 0.15 M KCl and centrifuged at 2500 rev./min for 10 min. Thiobarbituric acid assay for malondialdehyde (MDA) production [21] was performed in 2 ml supernatant supplemented with 1 ml 0.15 M potassium phosphate buffer (pH 7.4) and 0.1 ml 0.16 M glucose, incubated for 30 min at 37°C and stopped by addition of 2 ml 30% (w/v) trichloroacetic acid. GSH concentration was determined with 5,5′-dithiobis-(2-nitrobenzoate) at 412 nm according to [22] in the same liver preparations. Protein was assayed as in [23]. All the chemicals

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and biochemicals used were obtained from Sigma (St Louis). Results are expressed as means ± SEM. The significance of the differences between mean values was assessed by the Student's *t*-test for unpaired results.

3. Results and discussion

3.1. *Liver reduced glutathione content*

The administration of a single dose of 5 g ethanol/kg produced a 21–31% reduction in liver GSH levels after 6 h intoxication, both in male and female rats fed ad libitum as compared to saline and glucose control groups (table 1). Fasting produced a similar decrease in liver GSH in control animals as in [24]. In this condition, the ingestion of 5 g ethanol/kg induced a greater reduction in liver GSH content (47–55%) than in fed animals after 6 h treatment, reaching levels of ~2 mM (table 1). Decreases of 22% [25] and 35% [7] in GSH have been reported in fed mice and fasted rats given 4.1 or 6 g ethanol/kg, respectively. The study of the time course of alcohol-induced hepatic depletion of GSH reveals a significant effect after 3 h intoxication (21%; $P < 0.005$; fig.1A), time at which the peak value of blood alcohol concentration is achieved (fig.1B), the maximum response being produced at 6 h. GSH levels returned to the

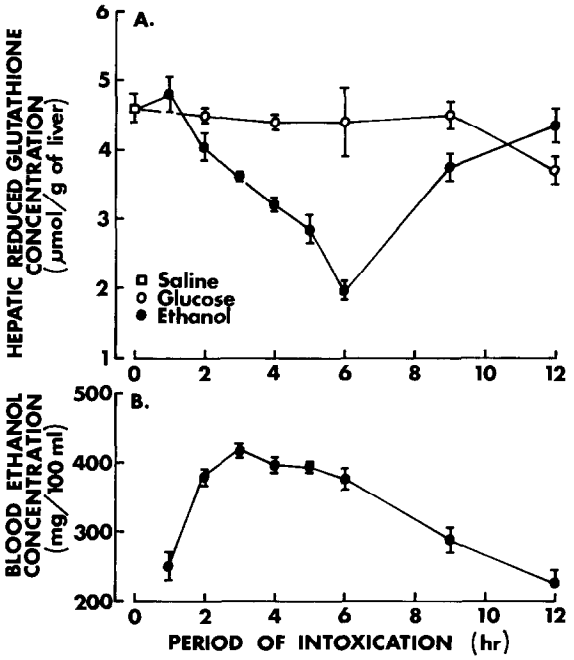

Fig.1.(A) Time course of alcohol-induced hepatic depletion of reduced glutathione in the rat. Groups of 4–5 rats fasted overnight (16 h) were given either 5 g ethanol/kg, isovolumetric amounts of saline or isocaloric amounts of glucose, and were sacrificed at the selected times. Blood alcohol levels in the same ethanol-treated group were determined in samples obtained from the cut tails of the animals, prior to their sacrifice for measurement of liver GSH as in section 2.1.

Table 1
Influence of the nutritional state and sex in the changes in liver reduced glutathione levels and malondialdehyde production induced by acute ethanol administration in rats

Sex	Nutritional state	Treatment	GSH content (μmol/g wet wt liver)	Malondialdehyde production (nmol . mg protein^{-1} . 30 min^{-1})
Male rats	Fed (6)	Saline	6.76 ± 0.15	0.20 ± 0.03
		Glucose	5.88 ± 0.24	0.13 ± 0.01
		Alcohol	4.64 ± 0.22[a]	0.15 ± 0.03
	Fasted (5)	Saline	4.25 ± 0.25	0.40 ± 0.04
		Glucose	4.04 ± 0.41	0.40 ± 0.03
		Alcohol	1.93 ± 0.17[a]	0.67 ± 0.06[a]
Female rats	Fed (4)	Saline	7.08 ± 0.21	0.17 ± 0.02
		Glucose	6.51 ± 0.35	0.20 ± 0.02
		Alcohol	4.86 ± 0.17[a]	0.20 ± 0.02
	Fasted (5)	Saline	4.74 ± 0.13	0.17 ± 0.02
		Glucose	4.76 ± 0.30	0.23 ± 0.01
		Alcohol	2.52 ± 0.25[a]	0.45 ± 0.03[a]

[a] $P < 0.01$ when compared to saline or glucose control groups

Animals fed ad libitum or 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

initial control values 9–12 h after ethanol ingestion, a finding that is at variance with [26] showing a constant depletion up to 24 h in mice fasted overnight and given 6 g ethanol/kg. Apart from the rather high dose of alcohol used, no control animals were carried out in [26] which are essential to assess the decreasing effect of fasting by itself on the concentration of GSH of the liver ([24], table 1). This is most critical after 24 h treatment since it actually represents ~40 h food deprivation [26]. In fact, a 19% decrease ($P < 0.05$) in liver GSH content is already observed when control animals given glucose and studied at 12 h are compared to rats receiving saline at zero time (fig.1A). In agreement with the data presented here, recovery of liver GSH levels decreased by the administration of chloroform [27] and acetaminophen [28], has been shown to occur 12–19 h after treatment.

Although the mechanisms of hepatic depletion of GSH induced by acute alcohol ingestion are not known, it is likely that they continue to operate at 9 and 12 h after the treatment (fig.1A), since blood alcohol levels are still high (fig.1B). Recovery to basal levels of GSH at these experimental times of intoxication could conceivably be the result of an increased synthesis, considering that the first enzyme involved in this process, γ-glutamyl-cysteine synthetase, is markedly inhibited by concentrations of GSH similar to those found in normal conditions [29]. Since after 6 h alcohol ingestion the content of liver GSH is reduced to 2 mM as compared to 4 mM (fasted controls) or 6.8 mM (fed controls) (table 1), an activation of γ-glutamyl-cysteine synthetase by de-inhibition could take place, provided that there is no limitation in the availability of the corresponding substrates. No changes in the hepatic concentration of cysteine have been found in these experimental conditions [30], the rate limiting substrate in the synthesis of GSH [31].

The alcohol-induced GSH depletion in the liver was found to be dependant on ethanol dosage (fig.2A), being significant with 3 g/kg (26%; $P < 0.05$) and maximal with 5 g/kg (55%; $P < 0.001$). Higher doses of alcohol were not included in this study due to high animal mortality [26] and to the irrelevance of comparative alcohol consumption in man. The effect of acute alcohol intoxication on sulfhydryl compounds of the liver seems to be a rather general action since apart from the GSH depletion found (table 1, fig.1,2; [7,25,26]), a marked reduction in

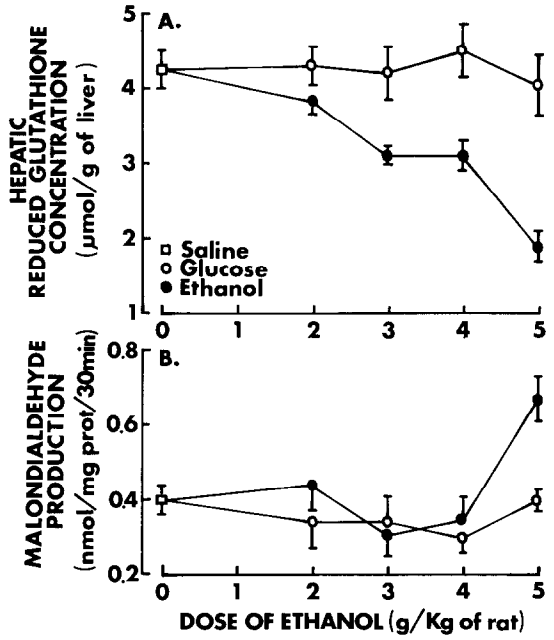

Fig.2.(A) Relationship between alcohol dosage and liver GSH levels in the rat. Groups of 5–6 animals fasted overnight (16 h) were given either 2,3,4 or 5 g ethanol/kg, isovolumetric saline or isocaloric glucose, and were sacrificed 6 h later. (B) Relationship of alcohol dosage and hepatic malondialdehyde production carried out in the same experimental groups as in section 2.1.

coenzyme-A levels has also been reported in liver [32] and brain [32,33]. The mechanism(s) of alcohol-induced depletion of liver GSH is currently under study in our laboratory.

3.2. *Liver lipoperoxidative capacity*

The administration of 5 g ethanol/kg produced increases of 65–165% in MDA production in liver homogenates from male and female rats only in the fasted state (table 1). This is in agreement with earlier studies using higher doses of alcohol, except that the values obtained are lower than those reported by [7,8]. This is probably due to differences in the time of incubation of liver homogenates. Experiments reported here were carried out for 30 min since after 60 or 120 min incubation [7,8] there is no detectable GSH in the preparations (unpublished data), a factor that could potentiate lipoperoxidation [34]. A similar effect of acute ethanol ingestion has been found when lipoperoxidation is assessed as formation of diene conjugates [19]. The alcohol-induced

increase in MDA production by the liver is observed in conditions of maximal depletion in its GSH content (table 1). This is in accord with the observation that no effect in MDA production is elicited in fasted animals given doses of 2–4 g ethanol/kg after which the decrease in liver GSH is less pronounced (fig.2). When liver GSH levels are correlated with MDA production in all the conditions studied (table 1, fig.2), an inversed relationship is obtained ($r = -0.82$; $P < 0.001$), supporting the contention that hepatic depletion of GSH, due to its antioxidant properties in the cell [1,4], may be one of the major factors responsible for the stimulation of lipoperoxidation by acute ethanol ingestion. In addition, it has been suggested that this effect could also be mediated through a diminution in the tissue lipid soluble antioxidant capacity [8].

Discrepancies in the effect of acute ethanol ingestion on liver lipoperoxidation could be possibly due to the period of intoxication studied [7–10,15–17]. Assays measuring lipoperoxidation were performed 15 min [17], 3 or 12 h [16] and 16 h [15] after de-administration of alcohol, times at which no major changes in liver GSH are found (fig.1), a condition that seems to determine the extent of the process.

The following conclusions can be drawn from this study:

1. Acute ethanol ingestion induces a drastic depletion of hepatic GSH, regardless of the sex and dependant on the nutritional status of the animals;

2. This effect was found to be dose-dependant, progressive in time and reversible at 12 h;

3. Liver lipoperoxidation, as measured by MDA formation, increases only in conditions of maximal GSH reduction.

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