Ethanol-Induced Alterations of the Antioxidant Defense System in Rat Kidney

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ABSTRACT: We report here the effects of chronic ethanol consumption on the antioxidant defense system in rat kidney. Thirty-two male Wistar rats were randomly divided in two identical groups and were treated as follows: control group (water for fluid) and the ethanol-fed group (2 g/kg body weight/24 h). The animals were sacrificed after 10 weeks, and respectively 30 weeks of ethanol consumption, and the renal tissue was isolated and analyzed. Results revealed that kidney alcohol dehydrogenase activities increased significantly after ethanol administration, but the electrophoretic pattern of alcohol dehydrogenase isoforms was unmodified. The SDS polyacrylamide-gel electrophoretic study of kidney proteins has revealed the appearance of two new protein bands after long-term ethanol consumption. The kidney reduced glutathione/oxidized glutathione ratio decreased, indicating an oxidative stress response due to ethanol ingestion. The malondialdehyde contents and xanthine oxidase activities were unchanged. The antioxidant enzymatic defense system showed a different response during the two periods of ethanol administration. After 10 weeks, catalase, glutathione peroxidase, glutathione reductase, and glucose-6-phosphate dehydrogenase were activated, while superoxide dismutase, glutathione transferase, and γ-glutamyltranspeptidase levels were stationary. After 30 weeks, superoxide dismutase and glutathione peroxidase activities were unmodified, but catalase, glutathione transferase, γ-glutamyltranspeptidase, glutathione reductase, and glucose-6-phosphate dehydrogenase activities were significantly increased. Remarkable changes have been registered after 30 weeks of ethanol administration for glutathione reductase and glucose-6-phosphate dehydrogenase activities, including an increase by 106 and 216% of control values, respectively. These results showed specific changes in rat kidney antioxidant system and glutathione status as a consequence of long-term ethanol administration.
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KEYWORDS: **Ethanol; Alcohol Dehydrogenase; Antioxidant Enzymes; Glutathione; Oxidative Stress; Kidney**

INTRODUCTION

It is generally accepted that excessive alcohol consumption can induce dramatic changes in the physiological and biochemical processes of the whole organism and in the cells [1–5]. Ethanol, the only form of alcohol that we considered in this study, is absorbed mainly in the intestine, where it is channeled through the portal vein directly toward the liver before passing through the circulatory system and the rest of the body. Three enzymatic systems are able to carry out the ethanol oxidation: alcohol dehydrogenase (ADH), the microsomal ethanol-oxidizing system (MEOS), and catalase (CAT) [6]. Ethanol is metabolized mostly by ADH, an enzyme which couples the oxidation of ethanol into acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD^+). The MEOS system connects ethanol and nicotinamide adenine dinucleotide phosphate (NADPH) oxidation to the reduction of molecular oxygen to hydrogen peroxide, and requires the participation of the P-450 cytochrome [7]. In the third system, the oxidation of an ethanol molecule into acetaldehyde is linked to the simultaneous decomposition of a hydrogen peroxide in a reaction catalyzed by the CAT enzyme. The contribution of CAT to ethanol metabolism has been long questioned

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because the ethanol elimination was not affected by the treatment with aminotriazole, a CAT inhibitor [8].

Because about 80% of ingested ethanol is metabolized in the liver, the structural and functional alterations of liver due to alcohol abuse have been extensively studied [9–12]. The exact pathogenesis of alcoholic liver injury is well understood, while immunomediated and free radical hepatic injury are thought to be important [13]. Chronic ethanol ingestion leads to the formation of reactive oxygen species (ROS) [14], and can induce a decrease of antioxidant defense [15]. Thus, chronic and excessive alcohol consumption may accelerate oxidative mechanism directly or indirectly, which eventually produces cell death and tissue damage [16].

The liver is the primary organ responsible for the oxidation of ingested ethanol, but other tissues, including the kidney, may contribute to ethanol metabolism as well [17]. The biochemical changes induced by ethanol consumption on the kidney are not well understood. Some clinical and experimental studies have been focused on the effects of ethanol feeding on renal function, gross and microscopic morphology of the kidney [18–20]. These studies suggest that chronic exposure to ethanol leads to kidney abnormalities. Experimental studies on animal models have also indicated that ethanol enhanced the fatty acid oxidation by kidney microsomes and peroxisomes [21], and affected the activities of some kidney lysosomal hydrolases [22]. Oxidative stress and ROS-mediated toxicity have been considered the primary routes to alcohol-induced kidney injury [21,23,24]. Oxidative stress is the term used to describe an imbalance favoring prooxidants and/or disfavoring antioxidants, potentially leading to damage [25].

In this paper, we report the consequences of long-term ethanol administration in rat kidney on the level of the free radical scavenger gluthatione and antioxidant enzymes activities, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GST), and glutathione reductase (GR). In addition, the content of lipid peroxidation product, malondialdehyde (MDA) and the activity of xanthine oxidase (XOD) were examined. Glucose-6-phosphate dehydrogenase (G6PD), the enzyme that regenerates the NADPH consumed in the GR-catalyzed reaction, and γ-glutamyltranspeptidase (GGT), the enzyme involved in tissue-reduced glutathione (GSH) uptake, were also investigated. Taking into account that extrahepatic ADH may also contribute to ethanol metabolism, we have been also interested in exploring the kidney ADH activity.

MATERIALS AND METHODS

Chemicals

Ethanol 95% (v/v), perchloric acid, phenanthroline, NAD^+, $NADP^+$, NADPH, hydrogen peroxide 30% (w/w), pyrogallol, 1-chloro-2,4-dinitrobenzene (CDNB), GSH, oxidized glutathione (GSSG), glucose-6-phosphate, xanthine, 2-thiobarbituric acid, 1,1,3,3-tetramethoxy propane, 5,5′-dithiobis-(2-nitro)-benzoic acid, glutathione reductase, and the kit for protein determination were purchased from Sigma–Aldrich (St. Louis, MO, USA). SDS-polyacrylamide and native gel electrophoresis reagents were obtained from Bio-Rad (Hercules, CA, USA). All other reagents and chemicals were of the highest available analytical grade.

Animals

Thirty-two healthy male Wistar rats weighing 140–160 g were housed two per cage under controlled conditions of a 12 h light/dark cycle, 50% humidity and 24°C. Before the experiments, rats were monitored daily and had free access to water and standard pellet diet (10 g/100 g body weight/day). After 1 week of adaptation, the animals were randomly divided into two groups of 16 each. Group 1, the control group, continued to receive water for fluid. Group 2, the ethanol-fed group, was treated daily with 1.0 mL of 35% (w/w) ethanol, equivalent to 2 g/kg body weight, as an aqueous solution, using an intragastric tube. After 10 weeks, eight rats of each group were killed by cervical decapitation under light ether anesthesia. The remained rats of each group were sacrificed in the same conditions after 30 weeks.

Tissue Preparation

Both kidneys of each rat were quickly excised, cleared of adhering fat, rinsed with a cold 0.9% sodium chloride solution, and weighted. One kidney of each rat was immediately submerged in 7% perchloric acid and 2 mM phenanthroline and homogenized with a Potter–Elvejhem homogenizer at 0°C until a uniform suspension was obtained. All deproteinized tissue homogenates were stored at −80°C for assay of GSH. The other kidney was homogenized in demineralized water and stored at −80°C until the analysis of enzymatic activities.

Enzyme Activity Assays

The ADH (EC 1.1.1.1) activity was assayed using the method of Boleda et al. [26] with a minor modification. The assay mixture contained 4.5 mM NAD^+,

33 mM ethanol, and 50 mM phosphate buffer, pH 7.4. ADH activity was measured by recording the changes in absorption intensity at 340 nm for 5 min after enzyme addition, following the conversion of NAD^+ to NADH. The results were calculated as ADH units (U), with one unit expressed as 1 nmol of NAD^+ consumed per minute.

The activities of the antioxidant enzymes were measured using the following methods. The CAT (EC 1.11.1.6) activity was assayed by monitoring the disappearance of H_2O_2 at 240 nm, according to the method of Aebi [27]. One unit of CAT activity represents the decrease of 1 μmol of H_2O_2 per minute. The SOD (EC 1.15.1.1) activity was estimated according to the method of Marklund and Marklund [28], which uses the color change due to the auto-oxidation of pyrogallol. The addition of 1 N HCl stopped the reaction, and one unit of enzyme activity was calculated as the protein content inhibiting 50% of the auto-oxidation of 15 mM pyrogallol without enzyme. The total GPX (EC 1.11.1.9) activity was assayed by Paglia and Valentine's method [29], using H_2O_2 and NADPH as substrates. The conversion of NADPH to $NADP^+$ was followed by recording the changes in absorption intensity at 340 nm, and one unit was expressed as 1 nmol of NADPH consumed per minute. GST (EC 2.5.1.18) was assayed spectrophotometrically at 340 nm by measuring the rate of CDNB conjugation with GSH, according to the method of Habig et al. [30]. One unit of GST activity was defined as the formation of 1 nmol of conjugated product per minute. The extinction coefficient 9.6 mM^{-1} cm^{-1} of CDNB was used for the calculation [30]. GR (EC 1.6.4.2) was determined according to the method of Carlberg and Mannervich [31], in 0.1 M phosphate buffer, pH 7.4 with 0.66 mM GSSG, and 0.1 mM NADPH. One unit of GR activity was calculated as 1 nmol of NADPH consumed per minute. G6PD (EC 1.1.1.49) activity was assayed as described by Lee [32], in 100 mM Tris–HCl buffer, pH 8.0, containing 1 mM glucose-6-phosphate, 1 mM $NADP^+$, and a suitable amount of diluted protein. One unit of G6PD activity was expressed as 1 nmol of $NADP^+$ converted in NADPH per minute. GGT (EC 2.3.2.2) was measured spectrophotometrically at 37°C according to Meister et al. [33]. One unit of GGT activity was defined as 1 μmol of *p*-nitroaniline released from L-γ-glutamyl-*p*-niroanilide per minute at pH 8.5 and 37°C.

XOD (EC 1.1.3.22) activity was determined by using xanthine as substrate, and by following the rate of reduction of nitroblue tetrazolium at 560 nm [34]. One unit of XOD was defined as the amount of enzyme that produces 1 nmol of uric acid per minute. All the enzymatic activities were expressed as units per mg of protein. The protein content was determined using the method of Lowry et al. [35].

Biochemical Analysis

The levels of lipid peroxidation were measured via the thiobarbituric acid color reaction for MDA according to the method of Ohkawa et al. [36]. An MDA solution freshly made by the hydrolysis of 1,1,3,3-tetramethoxy propane was used as a standard. The results were expressed as nmol of MDA per mg protein.

GSH was determined by its reaction with 5,5′-dithiobis-(2-nitro)-benzoic acid) (Ellman's reagent) to yield a yellow chromophore which was measured spectrophotometrically at 412 nm [37]. The GSH level was expressed as nmol per mg protein. GSSG was determined according to Klotzsch and Bergmeyer [38] using 0.1 mM NADPH and 0.4 U/mL GR in 0.2 mM phosphate buffer, pH 7.4. The GSSG content was calculated as nmol per mg protein.

Electrophoresis

The protein fractions from kidney homogenates were examined by electrophoresis under denaturing conditions in 7.5% (w/v) polyacrylamide slab gel as described by Laemmli [39]. To locate protein bands, gels were fixed by the immersion in 12% trichloroacetate solution, and then stained with 0.2% (w/v) Coomassie brilliant blue R250 in water/methanol/acetic acid (6:3:1, by volume). Gels were destained in water/methanol/acetic acid (6:3:1, by volume). The fractions presenting alcohol activities were separated by native electrophoresis, in 7.5% polyacrylamide gel, and detected using tetrazolium systems [40]. Gels were analyzed by densitometry assay using a Bio-Profil system (Vilber Lourmat, Marne-la-Vallée, France) with Bio-1D image analysis software.

Statistical Analysis

All values were expressed as means ± SEM. The differences between control and ethanol-treated groups were compared by Student's *t*-test using standard statistical packages. The results were considered significant only if the P value was less than 0.05.

RESULTS

By comparison to the control group, the ethanol-treated animals exhibited an increase in the kidney weight. Thus, the kidneys weight of the 30 weeks-ethanol-treated rats was 40% greater ($P < 0.05$) than the corresponding control group (Table 1). However, the increase in the kidney weight cannot have a pathological meaning, as the body weight of the animals

TABLE 1. Effect of Chronic Ethanol Consumption on the Weight of Rat Kidneys and Their Protein Content

	10 Weeks Experiment		*30 Weeks Experiment*	
	Control	*Ethanol*	*Control*	*Ethanol*
Kidneys' weight (g)	0.73 ± 0.01	0.79 ± 0.06	0.80 ± 0.05	1.12 ± 0.09*
Protein (mg/g wet tissue)	38.38 ± 1.32	38.26 ± 1.31	37.24 ± 1.58	37.19 ± 1.32
Relative kidney weight (g/100 g body weight)	0.35 ± 0.01	0.31 ± 0.02	0.32 ± 0.02	0.36 ± 0.03

The relative kidney weight is derived as the ratio between the kidney weight and body weight. Values are expressed as mean ± SEM.
* $P < 0.05$ vs. controls.

expanded during the experiment, whereas the relative kidney weight did not undergo significant modification (Table 1). The relative kidney weight is derived as the ratio between the kidney weight and body weight (Table 1). The protein content of the kidney, expressed as mg/g wet tissue, remained unmodified after exposure of the rats to ethanol (Table 1).

There were no changes observed in the electrophoretical protein patterns after 10 weeks of ethanol consumption (Figure 1). However, for prolonged treatment certain changes were observed. As revealed by the densitometric analysis, two new protein bands appeared only in lane 4, which corresponds to the 30 weeks-ethanol consumption (Figure 1). One of these new protein bands corresponds to a lower molecular weight, while the other corresponds to a higher molecular weight.

The activity of kidney ADH showed a significant increase after ethanol treatment, with increases of 31.5 and 70.6% after 10 and 30 weeks of ethanol treatment, respectively (Figure 2). The three isoforms of ADH emphasized by electrophoresis were not significantly affected following the ethanol treatment (Figure 3).

The effects of ethanol treatments on XOD activity, a pro-oxidant enzyme involved in the generation of oxidative stress, and the consequences of a possible redox imbalance, i.e. lipid peroxidation process, were also investigated (Figure 4). The results, which are illustrated in Figure 4a, indicate that XOD activity remained unaltered in kidney after ethanol consumption compared to controls ($P > 0.05$). Figure 4b shows the MDA levels, a measure of lipid peroxidation, in controls and ethanol-treated rats. No important change in MDA level was observed in the rat kidney exposed to ethanol

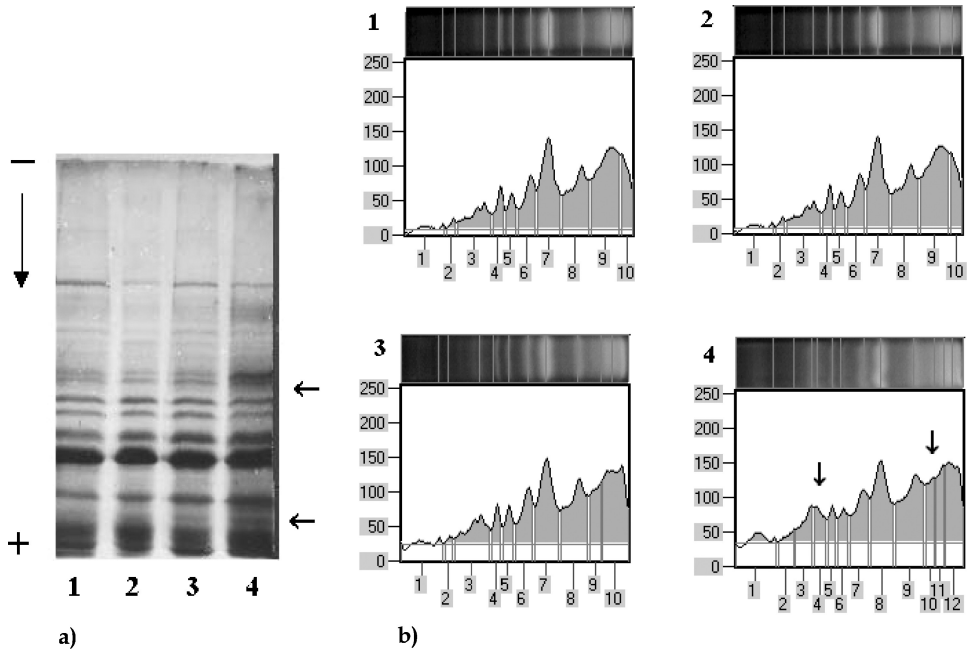

FIGURE 1. SDS polyacrylamide gel analysis of kidney proteins. (a) Electrophoretic pattern of proteins in ethanol-treated rats and their control groups: lane 1—control for the 10 weeks treatment; lane 2—10 weeks ethanol treatment; lane 3—control for the 30 weeks treatment; and lane 4 — 30 weeks of ethanol treatment. (b) Densitometry assay. Small arrows indicate new protein bands for the long-term ethanol exposure. Fifty microgram proteins were applied in each well. The protein content was determined using the method of Lowry et al. [35].

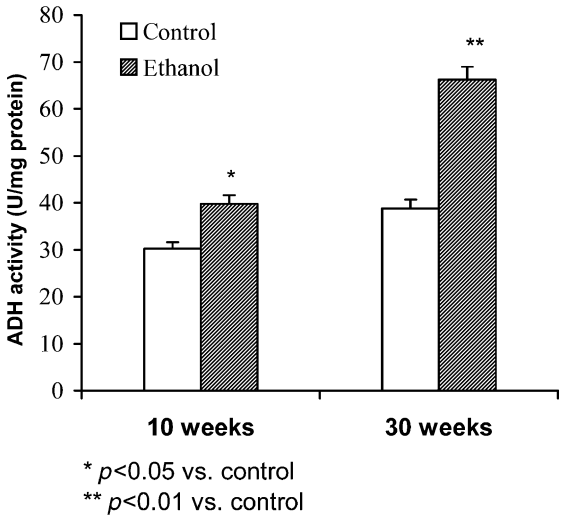

FIGURE 2. Effect of chronic ethanol consumption for 10 and 30 weeks on the activity of ADH in rat kidney. Values are mean ± SEM.

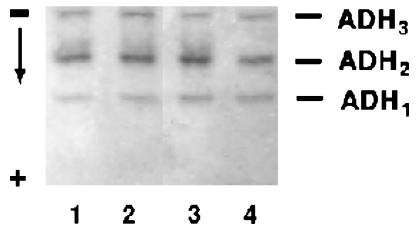

FIGURE 3. Isoforms of ADH in the kidney of rats after chronic ethanol treatment for 10 and 30 weeks. Lane 1—control for the 10 weeks treatment; lane 2—10 weeks ethanol treatment; lane 3—control for the 30 weeks treatment; and lane 4—30 weeks ethanol treatment.

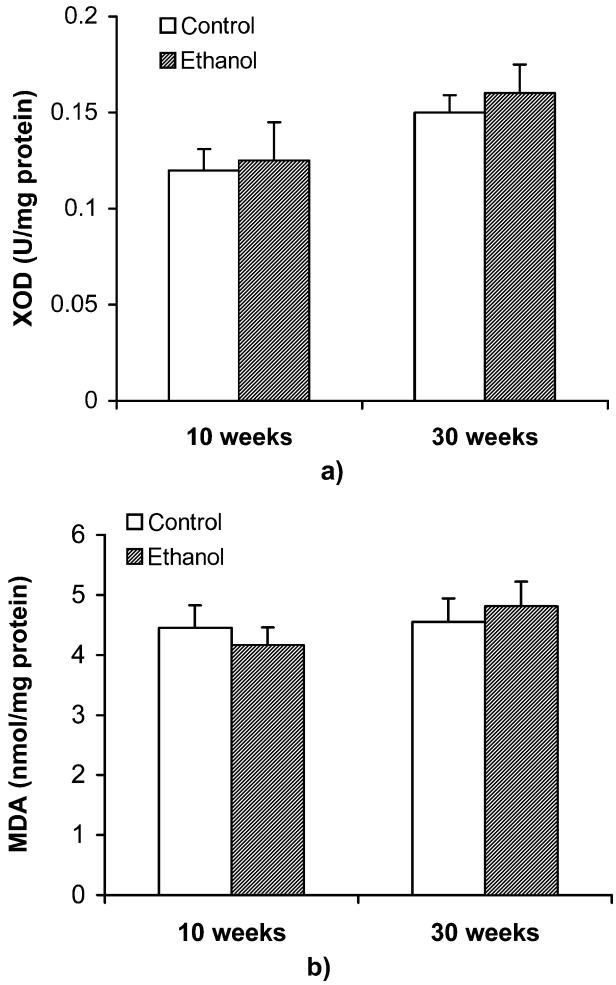

FIGURE 4. Effects of chronic ethanol consumption on the XOD activity (a), and lipid peroxidation level (b) in rat kidney. Values are mean ± SEM.

for 10 and 30 weeks, respectively versus controls ($P > 0.05$).

The effect of chronic ethanol feeding on the content of GSH and GSSG, and on the GSH/GSSG ratio is shown in Table 2. The kidneys of ethanol-treated rats exhibited decreased levels of GSH and a lower GSH/GSSG ratio as compared to the control groups of animals. GSH was diminished by 32.7% after 10 weeks treatment, and by 10.6% after 30 weeks treatment. The GSH/GSSG ratio underwent a substantial decrease, by 47.6 and 17.4%, respectively. The dramatic fall of the GSH/GSSG ratio recorded after 10 weeks of treatment may be correlated both with the decrease of the GSH level and the increase of the GSSG level (Table 2).

Table 3 shows the effects of chronic ethanol administration on the activities of antioxidant enzymes in rat kidney. The CAT activity increased by 11.8 and 32.4% after 10 and 30 weeks of treatment, respectively, whereas SOD activity showed no change. The activity of GPX was up-regulated by 26% after 10 weeks, and GST was increased by 31.6% after 30 weeks of ethanol consumption. GGT activity remained unmodified after 10 weeks of ethanol treatment, but a 41.7% increase in the activity was recorded after 30 weeks of ethanol treatment.

The activities of GR and G6PD increased substantially in the kidney of ethanol-treated rats, and this modification is more evident after the administration of ethanol for a long period of time (Figure 5). Thus, after 10 weeks of ethanol consumption, the GR activity has increased by 46.7%, and after 30 weeks of treatment the GR activity has increased by more than 2-fold. Similar results were noticed for G6PD. Its activity level increased by 43.5% after 10 weeks of ethanol treatment, and the increase was 3-fold higher after 30 weeks of treatment, as compared to the control group of animals.

It is well known that most antioxidant enzymes display an age-dependent decline in their catalytic activities in different tissues [41,42]. This observation has determined us to observe the differences between the level of the antioxidant enzyme activities of control

TABLE 2. Glutathione Concentrations in the Rat Kidney after Chronic Ethanol Treatment for 10 and 30 Weeks

	10 Weeks Experiment		*30 Weeks Experiment*	
	Control	*Ethanol*	*Control*	*Ethanol*
GSH (nmol/mg protein)	90.32 ± 8.31	$60.79 \pm 5.98^{**}$	76.32 ± 4.35	$68.23 \pm 7.31^{*}$
GSSG (nmol/mg protein)	16.23 ± 0.98	$20.31 \pm 1.93^{*}$	23.25 ± 2.41	25.18 ± 2.12
GSH/GSSG ratio	5.52 ± 0.43	$2.89 \pm 0.16^{*}$	3.21 ± 0.29	$2.65 \pm 0.18^{*}$

Values are mean ± SEM.
$^{*}P < 0.05$ vs. control.
$^{**}P < 0.01$ vs. control.

TABLE 3. Effects of Chronic Ethanol Consumption for 10 and 30 Weeks on the Activities of Antioxidant Enzymes in Rat Kidney[a]

	10 Weeks Experiment		*30 Weeks Experiment*	
Enzyme	*Control*	*Ethanol*	*Control*	*Ethanol*
SOD	2.71 ± 0.11	2.66 ± 0.24	2.52 ± 0.13	2.55 ± 0.17
CAT	210.30 ± 7.27	$235.18 \pm 17.79^{*}$	$184.03 \pm 5.61^{\Delta}$	$243.60 \pm 7.38^{**}$
GPX	33.41 ± 1.69	$42.11 \pm 6.47^{*}$	40.25 ± 1.75	44.36 ± 1.94
GST	133.12 ± 4.51	125.3 ± 3.31	123.21 ± 4.12	$162.10 \pm 14.10^{*}$
GGT	1.43 ± 0.37	1.39 ± 0.28	$0.84 \pm 0.03^{\Delta}$	$1.19 \pm 0.10^{**}$

Values are mean ± SEM.
[a] Activities are expressed as U/mg protein.
$^{*}P < 0.05$, ethanol-treated vs. control.
$^{**}P < 0.01$, ethanol-treated vs. control.
$^{\Delta}P < 0.05$, 30 weeks control vs. 10 weeks control.

animals for 10 and 30 weeks. Thus, with the exception of GPX, it can be stated that the antioxidant enzyme activities remained unchange or decreased in older-control group (Table 3). The most striking modification was recorded for CAT and GGT, for which we found only 87.5 and 58.7% in the older-control groups, as compared to younger-control groups.

DISCUSSION

The liver is the main organ involved in the metabolism of ethanol, but other extrahepatic tissues, i.e. the kidney, may also contribute to the ethanol metabolism. It has been reported that acetaldehyde is a highly toxic metabolite of ethanol and protein–acetaldehyde adducts are formed in vivo during chronic alcohol ingestion [43]. Adduct formation may lead to several adverse consequences, such as interference with protein function, stimulation of fibrogenesis, and induction of immune responses. These are key events in the pathogenesis of alcoholic liver disease [44]. In the present study, the electrophoretic patterns of proteins suggest that the covalent binding of acetaldehyde to the proteins may also occur to some extent in the kidney. Thus, we have noticed the appearance of a new protein band with high molecular weight corresponding to the 30 weeks-ethanol treatment that are absent from controls or 10 weeks-ethanol treatment (Figure 1). Protein modification in kidney induced by ethanol treatment was previously reported by Rodrigo et al. [19], suggesting a pathologic role for acetaldehyde in the nephrotoxic effect of ethanol ingestion. Furthermore, we have noticed that a prolonged duration of treatment induces the appearance of a new protein band with a low molecular weight (Figure 1). We think that this new protein may be a stress response component. This observation is consistent with the observation of Unno et al. [45] regarding the increased levels of heat shock protein 70 in old rat brain caused by chronic alcohol intake.

In this study, we report an increase in kidney weight following ethanol intake, while the protein content undergoes no change (Table 1). Ethanol consumption seems to favor a weight gain, but the relative kidney weight is not altered (Table 1). Other studies have confirmed that alcohol ingestion plays an important role in energy balance regulation and could result in body weight gain [46,47].

The chronic ethanol consumption resulted in an increased ADH activity in rat kidney (Figure 2), and a similar observation has been reported previously by Orellana et al. [21]. This up-regulation of ADH may be due to an adaptation of the kidney to the ethanol consumption. Electrophoretical analysis shows that rat kidney exhibits three ADH isoforms (Figure 3). These ADH isoenzymes, with very different capacities of ethanol oxidation and characteristic distribution, have also been detected in other rat tissues [26,48]. ADH-1

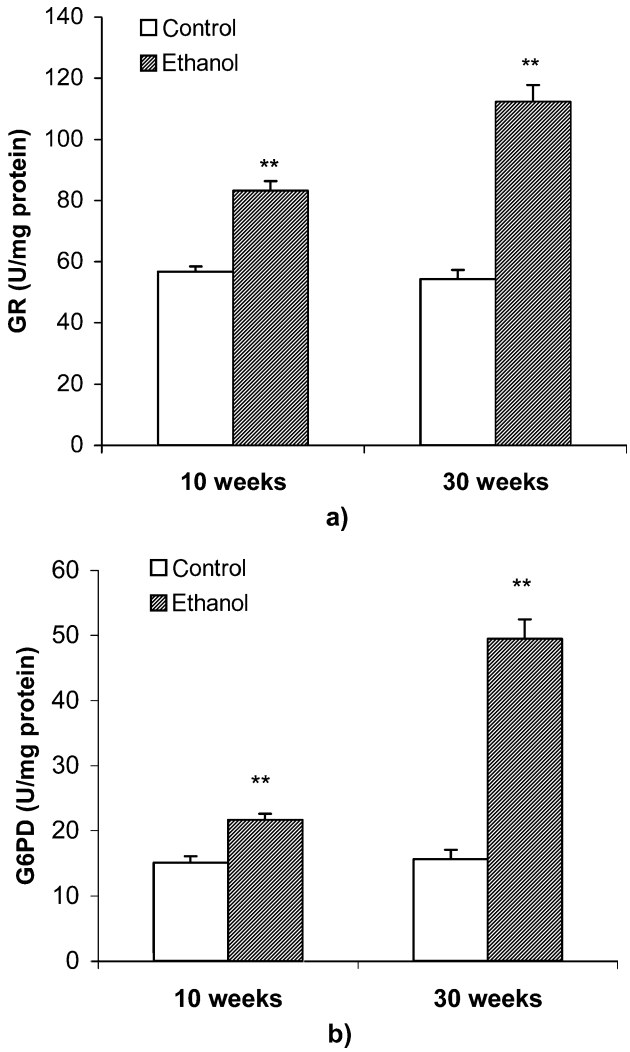

FIGURE 5. GR (a) and G6PD (b) activities in kidneys of control and ethanol-fed rats for 10 and 30 weeks. Values are mean ± SEM. ** $P < 0.01$ vs. control.

and ADH-2 are anodic isoenzymes, while ADH-3 represents the group of cathodic forms. The specific localization and the kinetic properties of rat ADH isoenzymes suggest that ADH-1 and ADH-3 may act as metabolic barriers to external alcohols, whereas ADH-2 may have a vital function in the metabolism of the endogenous long-chain alcohols and aldehydes [48]. Despite an increased ADH activity in long-term ethanol administration, the distribution and the migration of the kidney ADH isoforms are apparently not altered (Figure 3).

The oxidative stress biomarkers, MDA and XOD, were also studied in the kidney of rats subjected to chronic ethanol exposure. No significant changes in MDA level were observed in the kidney after 10 and 30 weeks of ethanol ingestion (Figure 4a). Previous results concerning lipid peroxidation induced by ethanol feeding in rat kidney are controversial. No significant modification in the MDA concentration has been previously reported in the rat kidney subjected to chronic ethanol exposure [49,50] or in acute ethanol intoxication [22], which is in agreement with our data. In contrast, other studies have shown a significant increase of the MDA level in kidney after chronic ethanol ingestion [51,52]. Regarding the peroxidative phenomenon in the liver, there is a consent, the chronic alcohol administration is correlated with increased MDA levels in the hepatocytes [16,50,53]. We think that the kidney is less susceptible to peroxidative damage than the liver because the peroxidative process is facilitated by the accumulation of iron in the liver of ethanol-treated rats [54].

To investigate a possible role of XOD in free radicals production involved in ethanol-induced pathogenesis, the activity of this enzyme was examined. XOD activities were unmodified after exposure to ethanol (Figure 4b). The data suggest that this enzyme is not a major source of oxidative stress in rat kidney under long-term ethanol exposure. A different mechanism has been observed in the liver by Kato et al. [55]. They have reported increased levels of XOD that might have contributed to enhance free radical production and lipid peroxidation in the liver following ethanol administration.

Although it has been suggested that SOD activity is induced by an increase in ROS and lipid peroxide level [56], we have not observed changes in the activity of SOD as a response to ethanol treatment (Table 3). Previous studies are controversial. Thus, a significantly decrease in SOD activity in kidney, to 83% of control values, after 6.5 weeks of ethanol given orally, has been reported [51]. In contrast, a 4-fold increase in SOD activity in kidney, after 12 weeks of intragastric ethanol administration, has been reported as well [50]. Therefore, it seems that the capacity of SOD induction in response to ethanol treatment is largely brought about by various conditions, including the dose, the mode of administration, the duration of treatment, and the age of animals.

Ethanol metabolism by cytochromes P-450 leads to the formation of hydrogen peroxide, a precursor of a more potent ROS, such as the hydroxyl radical. Hydrogen peroxide is decomposed by CAT at high concentration, and by GPX at low concentration. The relative contribution of CAT and GPX in decomposition of endogenous hydrogen peroxide is dictated by tissue specificity. GPX has a more important contribution in the liver [57], whereas CAT predominates in the renal tissue [58]. In this study, the increase in kidney CAT activity after ethanol treatment was observed (Table 3). The CAT enzyme is responsible for the catabolism of hydrogen peroxide and the metabolism of ethanol at high concentration [8,21]. Therefore, an increase in CAT

activity would indicate an enhanced ethanol toleration of the kidney. The CAT/SOD ratio is an indicator of the tissue ability to cope with oxidative stress [59]. This ratio increased from 78.29 ± 2.76 to 90.99 ± 1.34 after 10 weeks, and from 77.90 ± 2.58 to 93.61 ± 4.58 after 30 weeks of ethanol administration. This enhancement in CAT/SOD ratio may suggest an increase in kidney resistance to oxidative damage.

The enzymes that reduce the levels of peroxides protecting the cell from peroxidative damages, GPX, and GST, showed different responses during ethanol administration. GPX was up-regulated after 10 weeks of treatment, whereas GST activity was increased after a longer period of treatment (Table 3). We interpret that under long-term alcohol consumption, GPX exhibits the maximum catalytic activity. Accordingly, the kidney antioxidant defense system of the rat reacts positively to the ethanol toxicity by increasing the GST activity. The increase of the GPX and GST activities may be correlated with the observed decrease in GSH content of the kidney as a response to the ethanol consumption (Table 2). Thus, the decrease of the GSH level after 10 weeks of ethanol treatment may be explained by its use as a GPX substrate. The increased GSH participation in conjugation reactions mediated by increased GST activity seems to be a plausible model for the reduced GSH content due to the long-term ethanol exposure.

Our study also reveals that GGT, a key enzyme in the metabolism of glutathione, was significantly increased in kidney after 30 weeks of ethanol consumption (Table 3). This up-regulation presumably enhances the renal GSH uptake from the blood stream, and this mechanism may contribute to the decrease of the GSH concentration in plasma (data not shown). The kidney is characterized by the highest GGT activity, nearly 900 times higher than in the liver [60]. Furthermore, the very rapid turnover of cellular GSH has been demonstrated in the kidney and it is considered fundamental for this organ [61]. Thus, the kidney plays a pivotal role in the cleavage and interorgan transport of GSH. This means that the availability of cysteine, necessary for the biosynthesis of cellular GSH, depends upon the GGT activity. Our results also show that GGT activity in the kidneys of the control group corresponding to 30 weeks was less than 59% of that at 10 weeks (Table 3). This finding reveals age-dependent changes in GGT activity. Other authors also reported a decline in GGT activity in rat kidney with aging [62].

Striking changes concerning the enzymatic antioxidant response to ethanol-linked oxidative stress were recorded in the activities of GR and G6PD (Figure 5). The GR activity showed a substantial increase after ethanol consumption. This response is likely to be triggered by the relatively high GSSG levels in the kidney, and probably plays an important role in preventing the deterioration of the GSH status after ethanol administration. Increased levels of GSSG also activated G6PD, the enzyme that provides the reducing power of NADPH, which is the co-substrate required for GR activity in the recycling of GSSG [63]. Nevertheless, these enzymatic adaptations were insufficient to prevent the kidney GSH/GSSG ratio from a significant reduction (Table 2) that suggested an oxidative stress condition.

Ethanol metabolism has been shown to produce a free radical metabolite, the 1-hydroxyethyl radical (HER), which may interact to various cellular antioxidants. Thus, it has been demonstrated the GSH consumption in the presence of the HER-generating system [64]. The observed decrease in GSH levels in this study may be also explained by this mechanism, not only by the usage of GSH as a reductant in the enzymatic antioxidant reactions mediated by GPX and GST. As shown in Table 2, the content of GSH significantly decreased by 32.7 and 10.6% after ethanol administration for 10 and 30 weeks, respectively. Our results have also shown that GSH levels were by 15.5% lower in the case of older-animal control groups than in the case of the younger ones, which suggests that the process of growing or aging is accompanied by a decreased GSH level in the kidneys. Similarly, other studies reported an age-dependent lowering of the GSH levels in various rat tissues, including kidney and liver [65,66].

This study demonstrates that chronic exposure to ethanol exerts an oxidative stress and alters the GSH homeostasis. The long-term ethanol exposure increases enzyme activities related to the recycling and utilization of glutathione in the kidney. The enhancement in the activities of the kidney antioxidative defense system represents one of the protective mechanisms of the body against oxidative tissue damage caused by excessive ethanol consumption. The antioxidant enzymatic profile of the kidney resembles that of the liver, but some differences can be noticed in the case of the direct or indirect enzymatic activities involved in the GSH cycle. These differences may be correlated with the specific metabolism of these two organs. It is well known that in kidney cells the concentration of GSH is lower than in hepatocytes [67]. Furthermore, the kidney in contrast to the liver can use methionine as a cysteine precursor for GSH biosynthesis to a small extent only [68]. We conclude that the mechanisms of antioxidant protection against the long-term alcohol consumption present peculiarities linked to the organ type, but they are also altered by several factors, including the treatment duration and the age of the subjects.

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