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Biochemical and ultrastructural alterations in the rat ventral prostate due to repetitive alcohol drinking

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ABSTRACT: Previous studies showed that cytosolic and microsomal fractions from rat ventral prostate are able to biotransform ethanol to acetaldehyde and 1-hydroxyethyl radicals via xanthine oxidase and a non P450 dependent pathway respectively. Sprague Dawley male rats were fed with a Lieber and De Carli diet containing ethanol for 28 days and compared against adequately pair-fed controls. Prostate microsomal fractions were found to exhibit CYP2E1-mediated hydroxylase activity significantly lower than in the liver and it was induced by repetitive ethanol drinking. Ethanol drinking led to an increased susceptibility of prostatic lipids to oxidation, as detected by t-butylhydroperoxide-promoted chemiluminiscence emission and increased levels of lipid hydroperoxides (xylenol orange method). Ultrastructural alterations in the epithelial cells were observed. They consisted of marked condensation of chromatin around the perinuclear membrane, moderate dilatation of the endoplasmic reticulum and an increased number of epithelial cells undergoing apoptosis.

The prostatic alcohol dehydrogenase activity of the stock rats was 4.84 times lower than that in the liver and aldehyde dehydrogenase activity in their microsomal, cytosolic and mitochondrial fractions was either not detectable or significantly less intense than in the liver. A single dose of ethanol led to significant acetaldehyde accumulation in the prostate. The results suggest that acetaldehyde accumulation in prostate tissue might result from both acetaldehyde produced *in situ* but also because of its low aldehyde dehydrogenase activity and its poor ability to metabolize acetaldehyde arriving via the blood. Acetaldehyde, 1-hydroxyethyl radical and the oxidative stress produced may lead to epithelial cell injury. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: alcohol; prostate; acetaldehyde; free radicals; ethanol

Introduction

The available past and recent literature show that prostate epithelial cells (from human and experimental animals) suffer deleterious effects after repetitive alcohol administration (Castro and Castro, 2005). The mechanism for the observed alterations, however, remains unknown despite the fact that alcohol consumption has been related to the two most important health problems of the aging male; benign prostatic hyperplasia (BPH) and prostate cancer. In effect, extensive reviews on the subject showed that alcohol consumption in humans was inversely related to the total BPH incidence (Platz *et al.*, 1999; Gass, 2002). Further, intraprostatic injection of pure alcohol has been successfully employed to treat urinary retention in men with BPH (Plante *et al.*, 2004).

The analysis of a potential correlation between alcohol consumption and prostate cancer, in contrast, led to equivocal or even conflicting findings (Breslow and Weed, 1998; Dennis and Hayes, 2001; De Stefani *et al.*, 1995; Putnam *et al.*, 1998; Schuurman *et al.*, 1999; Tonnesen *et al.*, 1994). In view of the existing contradictory observations, Breslow and Weed (1998) proposed that for continuing research in this field, considerations concerning the biological plausibility and the related criteria of analogy, should be made. That is, they considered that it would be critical to shed some light on the existing disparate observations, in order to learn whether prostate tissue, upon interaction with ethanol, leads to some alterations that could be responsible for the alcohol promoted liver cell injury or cancer (Garro and Lieber, 1990; Nagy, 2004). In line with that approach, our laboratory recently reported on the ability of the rat ventral prostate cytosol and microsomal fractions to metabolize ethanol (EtOH) to acetaldehyde (AC) and 1-hydroxyethyl free radicals (1HEt) (Castro *et al.*, 2001, 2002). Further, in preliminary observations our laboratory

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anticipated the presence of ultrastructural alterations in the prostate epithelial cells of animals repetitively receiving a standard Lieber and De Carli liquid diet containing EtOH, for 28 days (Castro and Castro, 2005). That observation confirms and extends previous studies of others which employed other non-standardized repetitive forms of exposure to EtOH (Cagnon *et al.*, 1998, 2001).

The present study reports the existence of additional potential cytosolic and microsomal pathways of EtOH activation to AC as well as its detoxication and the increased oxidability of prostate tissue upon exposure to the EtOH-promoted stressful oxidative conditions. The resulting cell injury is also described.

Materials and Methods

Chemicals

Ethanol (EtOH) (analytical grade) and methanol (HPLC-grade) were from Sintorgan (Argentina). $NADP^+$, p-nitrophenol, p-nitrocatechol, D-glucose-6-phosphate, glucose-6-phosphate dehydrogenase, deferoxamine mesylate and xylenol orange were from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of the best quality available.

Animals

Random-bred Sprague-Dawley male rats were used. The starting breeding colony was from Charles River (Massachusetts, USA). The procedures used for breeding, housing and handling our stock animals were those established by the Food, Drug and Medical Technology National Administration (ANMAT; Buenos Aires, Argentina).

The rats were bred in our laboratory. They were maintained in a controlled room on a 12 h light-dark cycle (light phase 06:00–18:00). The temperature in the animal room was 23 ± 2 °C and the relative humidity was between 35% and 65%.

Single Dose EtOH Administration

Non-inbred Sprague-Dawley male rats (280–300 g) were used in these experiments. Food was withdrawn 12–14 h before ethanol was given, but the animals had free access to water. Groups of six animals were used in each experiment. Ethanol was administered p.o. in water at a dose of 3.8 g kg^{-1} (5 ml 200 g^{-1} bw). In the control group, the calories provided by the alcohol were replaced by sucrose. After different times, the animals were killed by decapitation and prostate tissue was rapidly excised and frozen. Blood samples were obtained by bleeding and the plasma separated by centrifugation.

Repetitive Treatment with EtOH Liquid Diet

In the experiments employing liquid diets, rats (130.9 ± 5.0 g body weight, approximately 5 weeks of age) were fed with a nutritionally adequate liquid diet (Lieber and De Carli standard rat diet, purchased from Dyets. Inc., Pennsylvania, USA). The rats were housed in individual cages and separated into two dietary groups: EtOH and control group (control). Both groups were pair fed with the same diet except that in the control group, EtOH was isocalorically replaced with carbohydrate (dextrin-maltose, included in the diet formula provided by Dyets). The liquid diet used provided 1 kcal ml^{-1} where 35% of the total calories derived from fat, 47% from carbohydrate and 18% from protein. In the EtOH-treated animals, EtOH provided 36% of the calories replacing isocalorically carbohydrate. Feeding with the control and EtOH diet was continued for 28 days. The amount of EtOH started at 30 g l^{-1} of the liquid diet for the first 2 days, 40 g l^{-1} for the subsequent 2 days followed by the final formula containing 50 g l^{-1} (Lieber and De Carli, 1989).

Isolation of Prostate Cytosolic, Microsomal and Mitochondrial Fractions

Animals were killed by decapitation and their ventral prostates were rapidly excised and processed. Cytosolic and microsomal fractions were obtained by cellular fractionation procedures via ultracentrifugation at 4 °C of the 9000 *g* supernatant of tissue homogenates as previously described (Castro *et al.*, 1989). Mitochondrial fractions employed to determine aldehyde dehydrogenase (AldDh) activity were prepared according to (Koivula and Koivusalo, 1975).

p-Nitrophenol Hydroxylase Activity in Liver and Prostate Microsomes from Rats receiving and EtOH Liquid Diet

p-Nitrophenol hydroxylase activity was determined essentially as described by Mishin *et al.* (1996) with minor modifications. Liver microsomes (~0.75 mg protein ml^{-1}) or prostate microsomes (~2.10 mg protein ml^{-1}) were incubated for 30 min and 1 h respectively, at 37 °C in 50 mM phosphate buffer, pH 7.4, containing 100 μM p-nitrophenol and NADPH generating system (26 mM $NADP^+$, 66 mM D-glucose-6-phosphate, 66 mM $MgCl_2$, 40 U ml^{-1} glucose-6-phosphate dehydrogenase), final volume 0.5 ml. Blanks were also run by omitting NADPH generating system from the incubation mixture. The reaction was terminated by the addition of 100 μl trichloroacetic acid (TCA) and centrifuged for 10 min at 10 000 *g*. The supernatant was used for the HPLC determination. Analysis of the metabolite, 4-nitrocatechol,

was performed by HPLC (Hewlett Packard 1090) on a reversed phase C18 column (HP ODS Hypersil 5 μm, 200 × 2.1 mm). An ESA Coulochem II electrochemical detector was used, equipped with a 5011A analytical cell, set at 700 mV and ultraviolet absorbance was also monitored at 345 nm. Elution was isocratical with a mobile phase consisting of 25% acetonitrile in 0.1% TCA.

Determination of tert-Butylhydroperoxide induced Chemiluminiscence in Prostate Tissue from Rats receiving an EtOH containing Liquid Diet

Chemiluminiscence was measured in a Wallac-Rack Beta 1214 liquid scintillation counter at room temperature in an out of coincidence mode (Boveris *et al.*, 1983). Prostate tissue from rats receiving the alcohol containing liquid diet was homogenized (9.4 mg protein ml^{-1}) in 0.25 M sucrose, 50 μM deferoxamine in TKM buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM KCl), pH 7.5. The 600 *g* supernatant was kept at 37 °C for 10 min in a Dubnoff shaker. Chemiluminiscence measurement was started by the addition of 3 mM tert-butylhydroperoxide.

Measurement of Lipid Hydroperoxides by the Xylenol Orange Method in Prostate Tissue from Rats receiving an EtOH containing Liquid Diet

Rat prostate tissue was homogenized (1/6, w/v) in cold HPLC-grade methanol with an Ultra-Turrax (Jank & Kunkel, IKA-Werk, Stanfen, Germany), then centrifuged for 10 min at 1000 *g* and the supernatants were used for hydroperoxide determination. The ferrous oxidation-xylenol orange (FOX) reagent (100 μM xylenol orange; 0.25 mM ammonium ferrous sulphate hexahydrate; 25 mM H_2SO_4) was prepared just before use (Nourooz-Zadeh *et al.*, 1994). For the determination of the hydroperoxides, aliquots of prostate tissue extracts (270 μl) were pipetted into screw cap tubes. A blank was run using methanol instead of the tissue extract. In order to discern color development due to authentic lipid hydroperoxide from that due to H_2O_2 or other interfering components, triphenylphosphine (TPP), a specific hydroperoxide reducing agent that has no effect on H_2O_2, was added to a set of vials to reduce lipid hydroperoxides (0.1 mM final concentration) and methanol was added to the remaining set of vials. All vials were then vortexed and incubated at room temperature for 30 min, prior to the addition of the FOX reagent (2.7 ml). After mixing, the samples were incubated again, in the darkness at room temperature, until the reaction was complete (180 min). Absorbance of the xylenol complex was measured at 560 nm every 30 min. Levels of hydroperoxides were determined as the difference in vials with and without TPP. A standard curve was developed with different concentrations of tert-butylhydroperoxide (TBHP) in methanol. The levels of hydroperoxides were expressed as nmol of TBHP equivalents g^{-1} of prostate tissue.

Protein Concentration Determination

Protein concentrations were determined by the method of Lowry *et al.* (1951), using bovine serum albumin as standard.

Electron Microscopy

Male Sprague-Dawley rats were anesthetized after 28 days of Lieber and De Carli liquid diet. The prostate gland complex was excised and further dissected into individual lobes (ventral, lateral and dorsal prostate). Immediately, ventral prostate lobes were trimmed into small pieces (1 mm^3) and fixed in 2.0% glutaraldehyde, 2% formaldehyde and 0.02% $CaCl_2$ in 0.1 M sodium cacodylate buffer, pH 7.4 for 24 h. After washing in veronal buffer, the tissues were post fixed in 1% osmium tetroxide for 1 h. Then the tissues were stained with uranyl acetate, dehydrated in ethanol solutions and embedded in Epon resin. Thick sections stained with toluidine blue were used to select specific tissue areas for further TEM observations. Ultrathin sections were counter stained with uranyl acetate and lead citrate and examined with a Philips EM300 (Rodríguez de Castro *et al.*, 1984).

Acetaldehyde Levels in Rat Prostate Tissue after Single Dose EtOH Administration

Frozen tissue samples (1 g) were rapidly chipped and placed in vials. For blood samples, 1 ml plasma was used. After adding 1 ml of saturated $ZnSO_4$ solution, 1 ml saturated NaCl solution and 1 ml $NaH_2PO_4.H_2O$ 50 mM (pH 7.4), samples were kept at 37 °C for half hour with shaking and then an aliquot (100 μl) of the headspace was analysed by GC-FID (Castro *et al.*, 2001).

Determination of Alcohol Dehydrogenase and Aldehyde Dehydrogenase Activities in Rat Liver and Prostate Tissue

Alcohol dehydrogenase (Adh) was measured in the cytosolic fraction of prostate tissue by the detection of the NADH formed, at 340 nm. Under an excess of alcohol, the rate of NADH formation is proportional to enzyme concentration (Racker, 1950).

Aldehyde dehydrogenase activity in prostate tissue was measured by the method described by Koivula and

Koivusalo (1975), with minor modifications. Cytosolic, microsomal or mitochondrial fractions were resuspended in pyrophosphate buffer pH 8, 1.67 mM pyrazol was added and the mixture (3 ml) was incubated at 37 °C for 30 min. Immediately after adding 6 mM propanal and 0.67 mM NAD, absorbance at 340 nm was measured at 15 s intervals in quartz cuvettes with a thermostat at 37 °C. Values obtained for prostate tissue were compared with those in the liver of the same animals.

Statistics

The significance of the difference between mean values was assessed by unpaired Student's *t*-test (Gad, 2001). Calculations were performed using GraphPad Software. Differences were considered significant when $P < 0.05$.

Results

Nitrophenol Hydroxylase Activity in Liver and Prostate Microsomes From Rats Receiving an EtOH Containing Liquid Diet

Prostate microsomal fractions exhibited p-nitrophenol hydroxylase activity. That activity was approximately nine times lower than that measured in liver. That metabolism was significantly induced by repetitive alcohol drinking in the microsomal fractions of both tissues. However, the inductive effect was significantly more intense in the liver than in the prostate (Table 1).

Tert-Butylhydroperoxide Induced Chemiluminiscence in Prostate Tissue from Rats Receiving an EtOH Containing Liquid Diet

The hydroperoxide-induced chemiluminiscence in ventral prostate homogenates from EtOH treated rats was significantly more intense than in controls. Results (expressed as area $\times 10^6$ mg^{-1} protein) were: control: 0.918 ± 0.131; EtOH treated: 1.356 ± 0.031 ($P < 0.05$) (Fig. 1).

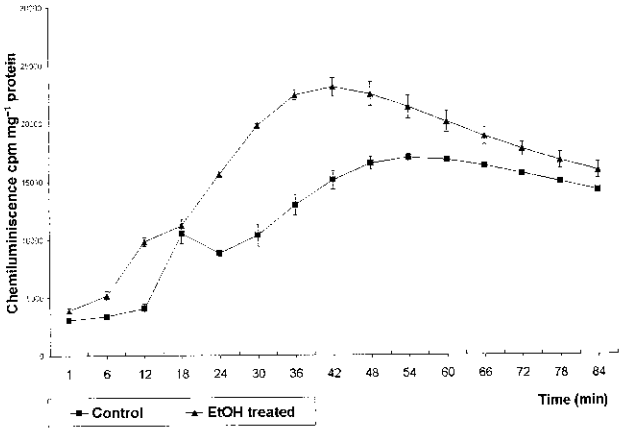

Figure 1. Tert-butylhydroperoxide induced chemiluminiscence in prostate tissue from rats receiving an alcohol containing liquid diet. Rat prostate was homogenized (9.4 mg protein ml^{-1}) in 0.25 M sucrose–50 µM deferoxamine–TKM buffer, pH 7.5. The 600 *g* supernatant was kept at 37 °C for 10 min in a Dubnoff shaker. Chemiluminiscence measurement was started by addition of 3 mM tert-butylhydroperoxide. Values are the mean ± SD. Three separate samples per group were run, each consisting of a homogenate from a separate lot of pooled prostate tissue (five animals each)

Formation of Lipid Hydroperoxides Measured by the Xylenol Orange Method, in Prostate Tissue from Rats Receiving an EtOH Liquid Diet

The levels of hydroperoxides expressed as TBHP equivalents were measured in the controls as well as in rats treated with the EtOH liquid diet. After incubating with the reagent at different times and subtracting the possible formation of hydroperoxides due to H_2O_2, a significant

Table 1. p-Nitrophenol hydroxylase activity in liver and prostate microsomes from rats receiving an EtOH liquid diet

	Formation of 4-nitrocatechol (pmol min^{-1} mg^{-1} protein $\times 10^{-2}$)[b]	
Organ[a]	Control	Ethanol treated
Prostate microsomes	2.51 ± 0.17	4.34 ± 0.38[c]
Liver microsomes	21.65 ± 2.14	69.82 ± 7.61[c]

[a] Liver microsomes (~0.75 mg prot ml^{-1}) or prostate microsomes (~2.1 mg prot ml^{-1}) were incubated in 50 mM phosphate, pH 7.4, NADPH generating system and 100 µM p-nitrophenol at 37 °C for 30 min and 1 h respectively. Then they were processed to determine enzymatic activity by HPLC with electrochemical detection as described in Methods.

[b] Results are the mean ± SD of three separate determinations from three different livers and for prostate from pools of eight organs each.

[c] $P < 0.05$ (ethanol vs control).

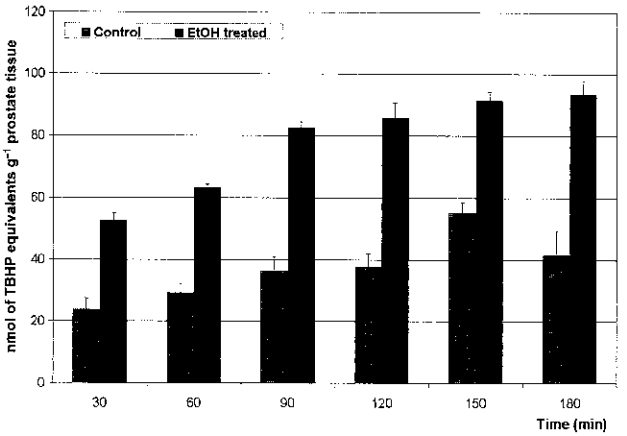

Figure 2. Levels of lipid hydroperoxides determined by the xylenol orange complex formation in ventral prostate from rats receiving an EtOH liquid diet. Rat prostate tissue extracts were incubated with the xylenol orange reagent at room temperature for different times, as described in Methods. Values are the mean ± SD. Four separate samples per group were run, each consisting of a homogenate from a separate lot of pooled prostate tissue (five animals each)

higher level of hydroperoxides in the EtOH treated rats was observed compared with controls (Fig. 2).

Ultrastructural Alterations in The Ventral Prostate Lobe of Rats Receiving an EtOH Liquid Diet

The ultrastructural features of epithelial cells in the ventral prostate lobe of control and EtOH treated rats were observed. In the control rats the glandular elements of the prostate gland were surrounded by varying amounts of stroma, composed of smooth muscle and fibroblast. The secretory acini were lined by a typical pseudostratified columnar epithelium with secretory granules in the apical zone. Cells were attached to each other by a tripartite junctional complex. The nuclei were located close to the base and had their euchromatin uniformly dispersed and a small amount of heterochromatin was condensed along the nuclear envelope. In the cytoplasm, the granular endoplasmic reticulum showed numerous flattened and parallel cisternae. Rounded secretory granules of floccular appearance were observed in the apical cytoplasm. The surface of the cell facing the lumen was covered with microvilli (Fig. 3a–c).

Apoptosis was seen in some cells in the secretory epithelium of EtOH treated rats. The heterochromatin and euchromatin were arranged in a crescent or moon-shaped pattern. The autolytic vacuoles formed by autolysis of the cytoplasm in epithelial cells contain degraded organelles and membranes. The nuclear envelope was wrinkled, forming deep indentations. The dilated rough endoplasmic reticulum cisternae with a few secretory vacuoles and the periphery of the epithelial cells were covered with broken and a scarce number of broken microvilli (Fig. 4a–d).

Acetaldehyde Levels in Rat Prostate Tissue After Single Dose EtOH Administration

The results obtained show that after a single dose of alcohol, acetaldehyde accumulated in prostate tissue to reach concentrations higher than in blood. Acetaldehyde concentrations in prostate tissue remained significantly higher than in plasma during the 24 h following EtOH administration (Fig. 5).

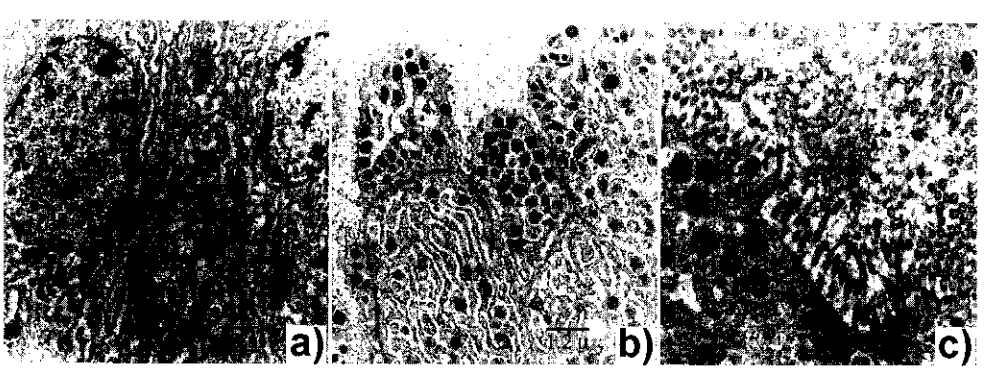

Figure 3. Electron micrographs of epithelial cells from the ventral lobe of the rat prostate: Animals treated with the Lieber and De Carli liquid diet (control) for 28 days. (a) High cuboideal epithelial cells have slightly elongated basal nuclei. They are surrounded by coarse clumps of chromatin. Few mitochondria, and well developed rough endoplasmic reticulum × 7216. (b) The apical and supranuclear portions of the cells contain many densely packed round secretory granules. Flattened and parallel cisternae of granular endoplasmic reticulum × 6100. The apical plasma membrane facing the lumen is studded with numerous elongated microvilli × 14 080

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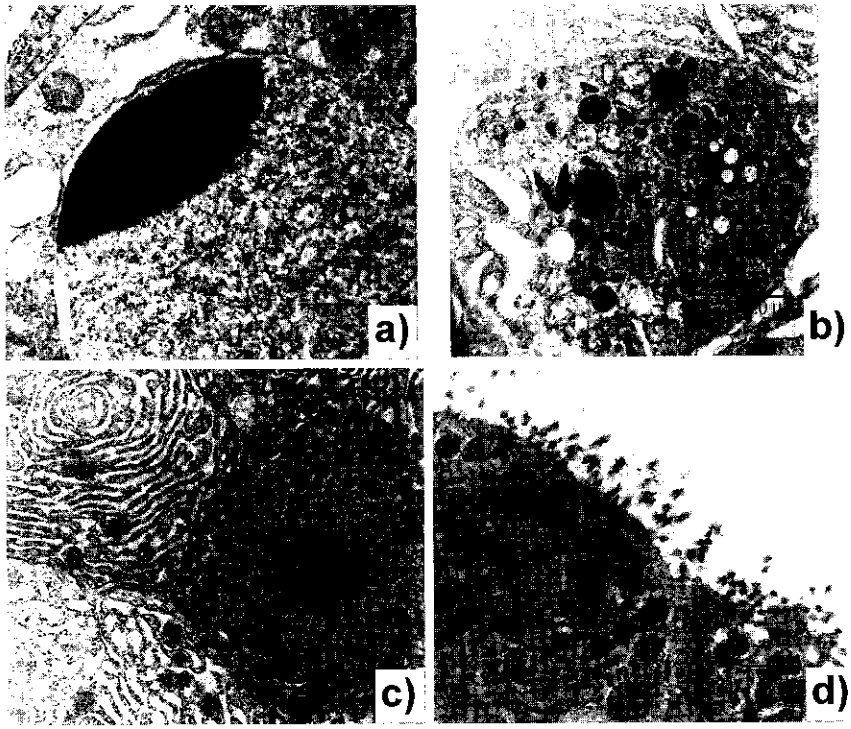

Figure 4. Electron micrographs of epithelial cells from the ventral lobe of the rat prostate: Animals treated with the Lieber and De Carli liquid diet (ethanol) for 28 days.
(a) Apoptotic cells are present, showing the condensed heterochromatin in nucleous × 14 080.
(b) Large vacuoles containing altered cell organelles in apoptotic cells × 8800.
(c) Dilated cisternae of the granular endoplasmic reticulum organized in a concentric manner and nucleus with a clearly visible nucleolus and deep infolding × 8800.
(d) Decreased number of microvilli and broken microvilli on the cell surface × 14 080

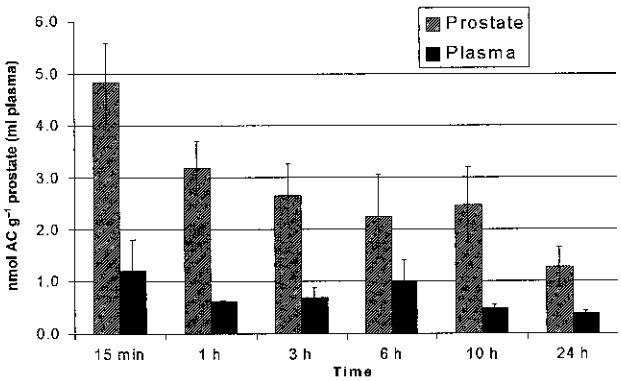

Figure 5. Time course of acetaldehyde levels in prostate tissue and plasma, after a single dose of alcohol (3.8 g kg^{-1}). Animals (six per group) received alcohol p.o. as a solution in water (5 ml 200 g^{-1} bw). In control groups, alcohol was isocalorically replaced with sucrose. $P < 0.05$ (prostate vs plasma)

Alcohol Dehydrogenase and Aldehyde Dehydrogenase Activities in Rat Liver and Prostate Tissue

Alcohol dehydrogenase and aldehyde dehydrogenase activities in rat prostate tissue were lower than those found in the liver of the same animals (see Table 2).

Discussion

The present studies give evidence that after a single dose of EtOH some degree of AC accumulation in prostate tissue can be observed. The increased AC levels observed may derive from AC produced *in situ* through our previously described cytosolic and non CYP2E1 microsomal pathway (Castro *et al.*, 2001, 2002) as well as from the presence of a prostatic ADh activity evidenced here. However, the contribution of AC arriving via blood from, for example, liver to prostate tissue, might be relevant since the present observations show that prostatic AldDh activities are very low. An additional minor source of AC could also be possible. In effect, the present studies showed that rat prostate microsomes are able to

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Table 2. Alcohol dehydrogenase and aldehyde dehydrogenase activities in rat liver and prostate tissue

Organ	ADh activity (mU mg^{-1} protein)[a]	AldDh activity (mU mg^{-1} protein)[b]		
		Microsomes	Cytosol	Mitochondria
Prostate	5.10 ± 2.30	n.d.	0.13 ± 0.01	0.07 ± 0.01
Liver	24.68 ± 0.67	38.43 ± 0.93	2.74 ± 0.10	9.21 ± 0.09

[a] The ADh activity was determined in the rat prostate cytosolic fraction by measuring NADH formation at 340 nm. In the presence of an excess of ethanol the NAD reduction is proportional to the enzyme concentration. Each value is the mean from three separate samples. One enzymatic unit is defined by the change of 0.001 optical density per minute under these experimental conditions (Racker, 1950).
[b] The AldDh activity was determined in the rat prostate cytosolic, microsomal and mitochondrial fraction by measuring NADH formation at 340 nm. Each value is the mean from three separate samples. One enzymatic unit is defined by the enzyme concentration necessary to catalyse the formation of 1 μmol NADH min^{-1} under these experimental conditions.
n.d., not detected.

metabolize p-nitrophenol to p-nitrocatechol. Previous studies from other laboratories failed to detect that activity (Jian *et al.*, 1998). Our possibility for detection rested in the highly sensitive methodology employed in our studies involving a coulometric detector rather than an UV/visible procedure (Jian *et al.*, 1998). The microsomal activity detected in control animals was about 9 times smaller than that in the liver and far less responsive to induction after repetitive EtOH drinking than that of the liver. In effect, in the latter case the ratio liver to prostate changed from 9 to 16 times higher in the liver. The p-nitrophenol hydroxylase activity has been considered a valuable marker of CYP2E1 activity (Mishin *et al.*, 1996). CYP2E1 is known to be involved in the EtOH metabolism to AC and 1-HEt radicals (Lieber, 2005). This CYP2E1 microsomal mediated pathway of EtOH activation to reactive metabolism might be partially involved in our previously reported studies on the microsomal metabolism of EtOH in prostate tissue (Castro *et al.*, 2002). Both AC and 1HEt are able to covalently bind to macromolecules. AC is a mutagenic, carcinogenic and toxic chemical able to react with DNA, proteins and lipids and other relevant molecular components (Lieber, 1992). In addition, the 1HEt free radicals have the possibility to give adducts but also to become involved in hydrogen abstraction reactions upon interaction with DNA, proteins, lipids and other cellular components and promote their further oxidation and become involved in chain reactions (Marnett, 2000). Our laboratory previously reported that both the cytosolic and the microsomal fraction of this tissue also generate those deleterious EtOH metabolites in the presence of adequate cofactors (Castro *et al.*, 2001, 2002). The present studies also provide some initial working hypothesis to address the question concerning the potential harmful consequences of rat ventral prostate tissue exposure to EtOH. In rats exposed repetitively to EtOH, the increased susceptibility of prostate tissue to oxidizing conditions was shown by our experiments on the tert-butylhydroperoxide induced chemiluminiscence in the rat ventral homogenates. It is known that the intensity of the hydroperoxide induced chemiluminiscence can be considered as an expression of ROS in biological samples and of the depending capacity of living systems against oxidative stress (Török, 2004). In our experiments it was observed that chronic EtOH ingestion increased the susceptibility to oxidation of rat ventral prostate homogenates when challenged with tert-butylperoxide. Further, when determination of lipid hydroperoxides by the xylenol orange method was performed in those prostate homogenates an increased production of lipid hydroperoxides was observed. This might be of interest, since it has become apparent that oxidative stress may be an important etiological factor in the development and progression of prostate cancer (Pathak *et al.*, 2005) and it is a well known process involved in chemically induced cell injury and chemical carcinogenesis (Fariss *et al.*, 2005; Hussain *et al.*, 2003).

A key reason for these increased risks includes DNA damage, protein and lipid modification encompassing the free radical generation overload as well as the changes in the transcriptional activation and/or repression of genes that are responsible for cellular homeostasis (Fariss *et al.*, 2005; Hussain *et al.*, 2003).

In our observations of the rat ventral tissue ultrastructure from the chronically exposed rats to the Lieber and De Carli diet, in the prostate epithelial cells a markedly dilated endoplasmic reticulum and a significant condensation of chromatin around the perinuclear membrane accompanied of very irregularly shaped nuclei with deep infoldings were observed. An increased presence of apoptotic cells was also observed. These observations confirmed previous results from others using brandy as the source of EtOH as well as preliminary work from our laboratory (Cagnon *et al.*, 2001; Castro and Castro, 2005).

Our past and present studies on AC and free radicals generation from EtOH metabolism at cytosolic and microsomal level might be related to the ultrastructural alterations observed. In the particular case of the promoted apoptotic cell formation by EtOH consumption, the pathway of generation of both AC and free radicals might have a special contribution. In effect, the *in situ*

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metabolism of EtOH to reactive moieties such as AC but more importantly of very short lived 1HEt, has more chance to target components by either adduct formation or oxidative stress.

An additional indirect contribution to the observed proapoptotic effects of repetitive alcohol drinking might arise from the well known toxicological effects on testes of chronic EtOH consumption (Emanuele and Emanuele, 1998; Quintans *et al.*, 2005). The balance between proapoptotic effects and tumor promoting actions of chronic alcohol drinking might partially explain the many conflicting results observed among the epidemiological studies available.

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