Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 520 (2005) 51 – 58

www.elsevier.com/locate/ejphar

Production of reactive oxygen species following acute ethanol or acetaldehyde and its reduction by acamprosate in chronically alcoholized rats

Abdelkader Dahchour [a,1], Frédéric Lallemand [a], Roberta J. Ward [b], Philippe De Witte [a,*]

[a] *Biologie du Comportement, Université catholique de Louvain, 1 place Croix du Sud, 1348 Louvain-la-Neuve, Belgium*
[b] *Unité de Biochimie, Université catholique de Louvain, 1 Place Louis Pasteur, 1348 Louvain-la-Neuve, Belgium*

Received 21 February 2005; received in revised form 13 July 2005; accepted 19 July 2005
Available online 29 August 2005

Abstract

The salicylate trap method, combined with microdialysis, has been used to validate whether reactive oxygen species, particularly hydroxyl radicals, ($^{\bullet}$OH), are generated in the hippocampus of male Wistar rats after acute intraperitoneal administration of either ethanol, 2 and 3 g/kg, or acetaldehyde, 200 mg, or during the initial stages of ethanol withdrawal after chronic ethanol intoxication. Salicylate (5 mM) was infused into the hippocampus via the microdialysis probe and the products of its metabolism by hydroxyl radical, particularly 2,3-dihydroxybenzoic acid (2,3-DHBA) as well as 2,5-dihydroxybenzoic acid (2,5-DHBA) assayed by HPLC (High Pressure Liquid Chromatography).

Acetaldehyde, 200 mg/kg, and the higher acute dose of ethanol, 3 g/kg, induced transitory increases in 2,3-DHBA and 2,5-DHBA microdialysate content. At the cessation of four weeks of chronic ethanol intoxication, (by the vapour inhalation method), the mean blood alcohol level was 1.90 g/l. Significant increases of microdialysate 2,3-DHBA and 2,5-DHBA levels were assayed 3 h after alcohol withdrawal which were sustained for a further 5 and 1 h 40 min respectively. Oral administration of Acamprosate, 400 mg/kg/day, during the chronic ethanol intoxication procedure prevented the increased formation of 2,3- and 2,5-DHBA by comparison to rats chronically ethanol intoxicated alone.

Keywords: Reactive oxygen species; Acute ethanol; Acetaldehyde; Acamprosate; Microdialysis

1. Introduction

There is considerable evidence that microsomal oxidation of ethanol will generate reactive oxygen species, both in vivo (Lieber, 1997) and in vitro (Sun and Sun, 2001), via the inducible cytochrome P450 2E1 (CYP2E1) both in the liver (Ekstrom and Ingelman-Sundberg, 1989; Cederbaum, 1991) and the brain (Sun and Sun, 2001). Acetaldehyde, the major metabolite of ethanol metabolism, can react with cytoskeletal protein to form adducts, (Upadhya et al., 2000) which can interact with proteins, fatty acids etc. (Peters and Ward, 1988) to cause many adverse metabolic effects. In vitro studies have also shown that acetaldehyde can react with unbound serotonin (5-HT) to form carboline metabolites and hydroxyl radicals which will decrease the reduced glutathione content, one of the major antioxidants in the brain (Han and Dryhurst, 1996). The excessive generation of reactive oxygen species in the brain, an environment where there is comparatively low antioxidant and cytoprotection protection, despite the high oxygen metabolism, may contribute to the pathogenesis of alcoholic brain disease (Pratt et al., 1990). Ethanol-induced changes in neurotransmitter concentration and receptor sensitivity, particularly elevated glutamate release during periods of alcohol withdrawal, could also contribute directly or indirectly to increased generation of reactive oxygen species.

* Corresponding author. Tel.: +32 10 474384; fax: +32 10 474094.
E-mail address: dewitte@bani.ucl.ac.be (P. De Witte).
[1] Current address: Lab. de Neuropsychopharmacologie, Université Sidi Mohammed Ben Abdellah, Av. des Almohades. B.P. : 2626, 30000, Fès, Morocco.

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.07.012

1.1. Acute ethanol injection

It remains unclear whether acute ethanol injection, at high or low doses, will be sufficient to enhance reactive oxygen species generation. Ascorbic acid is released in specific brain region in response to an acute ethanol dose, thereby enhancing antioxidant protection particularly during the initial 60 min after an ethanol dose (Huang et al., 2002). The formation of the heat shock protein, HSP70, may indicate an oxidative stress, despite the lack of changes in antioxidant status (Calabrese et al., 1998). Transient changes in various neurotransmitters, e.g. dopamine and serotonin (Ward et al., 1997) or neuromodulators, e.g. taurine, (Dahchour et al., 1996) after acute ethanol or acetaldehyde injection are unlikely to compromise antioxidant capacity.

1.2. Chronic ethanol administration

Chronic ethanol administration induces oxidative stress in the central nervous system; for example malondialdehyde content increases, as well as changes in a variety of antioxidants, reduced glutathione (Aydin et al., 2002) and cytoprotective enzyme activities, e.g superoxide dismutase (Aydin et al., 2002) and catalase (Ward et al., 2001). Despite such alterations, homeostatic adaptations by the alcoholic brain ensure that oxidative stress is not extensive. However, it is during the initial ethanol withdrawal period when excessive glutamate release occurs, due to changes in the function of the up-regulated *N*-methyl-D-aspartate (NMDA) receptors in the alcoholic brain, that a robust oxidative stress response may occur. Recurrent infusion of glutamate in specific brain regions of rats, analogous to repeated ethanol withdrawal in man, induced an excessive production of hydroxyl radicals (Cauquil-Caubere et al., 1999).

1.3. Amelioration of reactive oxygen species

Various compounds may mitigate the toxic effects of ethanol on the oxidant-antioxidant system in rat brain. *N*-acetylcysteine, a sulphydryl donor, (Aydin et al., 2002) would contribute to the regeneration of glutathione by directly scavenging hydroxyl radicals while polyphenols such as resveratrol ameliorate neuronal damage (Sun et al., 2000), although the molecular mechanisms of its action are undefined. Taurine analogues such as acamprosate reduce withdrawal symptoms, and may correct deranged sulphur amino acid metabolism (e.g. ethanol-induced hyper-homocysteine). It is reputed that homocysteine could contribute to neuronal death by stimulation of NMDA receptors and generation of free radicals (Bleich et al., 2000).

The in vivo measurement of highly reactive oxygen species such as hydroxyl radicals particularly in brain is very difficult. However the salicylate trap method, which is based on the ability of $^{\bullet}OH$ to attack the benzene ring of the aromatic molecule, has proved to be a sensitive method for the identification and quantification of hydroxyl radical formation in a variety of clinical diseases (Halliwell et al., 1991). In vivo the drug salicylate is metabolised to 2,5-DHBA (dihydroxybenzoic acid) by the microsomal CYP2E1; this is inhibited with diethyldithiocarbamate, a mechanism-based selective inhibitor of CYP2E1. Salicylate will also react with hydroxyl radicals to form 2,3-DHBA, which is not CYP2E1 dependent (Dupont et al., 1999) as well as 2,5-DHBA. The salicylate trap has been used in these present studies, combined with the microdialysis technique, to ascertain whether an acute injection of either ethanol or acetaldehyde, or chronic ethanol administration elicits hydroxyl radical formation in the hippocampus of rat brain. The drug acamprosate was orally administered to the rats during the chronic ethanol intoxication period to investigate whether the hydroxyl generation was altered, particularly during the initial ethanol withdrawal stage.

2. Materials and methods

Male Wistar rats, 200–250 g, were individually housed in standard plastic cages and maintained in a temperature (22 °C) and light controlled environment (12 light / 12 dark light cycle, light on 7.30 am). They were given free access to commercial rat chow and tap water. All animal procedures were in strict accordance with the recommendations of EEC (86/609/CEF) and with the Belgian "projet de loi" (Moniteur Belge 19.02.1992, p. 3437) on the care and use of laboratory animals.

2.1. Surgical procedure

Rats underwent surgical procedures, as described below. Under general anesthesia (chloral hydrate 400 mg/kg i.p.), the rats were fixed in a stereotaxic frame. Through a midline incision of skin and soft tissue, the skull was exposed and Bregma point was identified. A guide cannula (20 gauge stainless steel; Small Parts, Miami, FL, USA) was inserted into the hippocampus (A/P-4.3 mm; M/L 4.0 mm; D/V-3.0 mm) according to the atlas of Paxinos and Watson (1982). The guide cannula was secured to the skull with two steel screws and cranioplastic cement and kept patent with a 26 gauge stainless steel obturator (Small Parts Inc., FL, USA). The dialysis experiments commenced at the earliest 72 h post operation recovery period. The dialysis probes had a molecular cut-off of 13 kDA and an inner membrane diameter of 0.2 mm (Spectrum Laboratories, Inc., USA). Dialysis tubing extended 3 mm beyond the tip of the probe. The probe was connected to a micro-infusion pump (Infusion syringe pump 22, Harvard apparatus, South Natick, Mass, USA) and continuously perfused at a flow of 1.1 μl/min with Ringers solution containing 145 mM NaCl, 4 mM KCl, 1.3 mM $CaCl_2$ (Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and 5 mM salicylate (Merck KGaA, Darmstadt, Germany), phosphate buffered to pH 7.2. Microdialysis tubing

connected to probes was placed within a coiled metallic spring to allow the rat free movement while protecting the tubing from damage. The water used throughout the experiments had a resistively of 18.2 MΩ cm obtained from a Milli-Q water purification system (Millipore, Molsheim, France).

2.2. Acute ethanol and acetaldehyde i.p. administration

For the microdialysis experiments, rats received an intraperitoneal injection of ethanol 2 or 3 g/kg (15% ethanol solution prepared in saline) or acetaldehyde 200 mg/kg. An appropriate control group, for each injection types, was injected with saline. Microdialysis experiments were conducted on freely moving animals. After a 60 min equilibration period, fractions were collected every 20 min in vials containing 10 μl of perchloric acid 0.4 M (Janssen Chimica, Geel, Belgium) for 120 min. Fraction collection continued during the following 3 h. All fractions were immediately frozen at −20 °C until analysis.

2.3. Chronic ethanol intoxication

Three different groups of rats (9 in each group) were maintained in an isolated plastic chamber (160 × 60 × 60 cm) in an alcohol-containing atmosphere with a mixture of alcohol and air pulsed into the chamber via a mixing system, allowing the quantity of alcohol to be increased every 2 days during the experimental procedure of 4 weeks (Le Bourhis, 1975). One group of rats received an acamprosate dose, 400 mg/kg/day, in their drinking water, during the chronic ethanol intoxication procedure. The other two groups of alcoholized rats were used either for comparison with the acamprosate treated group or for comparison with a non-alcoholized group. This latter control group, (n = 9), received only air for the same period while in an isolated plastic chamber. During the third week, the rats were removed from the chamber for surgery as described in the surgical procedure. The rats were allowed to recover from the anesthetic and then returned to the appropriate environment within the chambers for a further week.

2.4. HPLC analysis

The fractions, 20 μl, were analyzed by HPLC with two channel electrochemical detector (Coulochem II, ESA, Bedford, Mass. USA) which consisted of a LDC Consta-Metric 3200 pump (LDC Analytical, Riviera Beach, FL, USA) delivering 1 ml/min, at a pressure of 5300 psi, of the mobile phase (100 mM NaH_2PO_4, 15% methanol, pH 2.8 adjusted with Phosphoric Acid). The mobile phase had been filtered through a 0.2-μm cellulose nitrate filter (Gelman Sciences, Ann Arbor, MI, USA) and degassed under vacuum before use in the HPLC system. Separation of compounds was achieved by reversed-phase column chromatography, (125 × 3 mm, ODS Hypersil 3 μm) (VDS Optilab, Chromatographie Technik, GmbH) and detected coulometrically using three electrodes, a guard (0.4 V) (guard cell ESA 5020) placed between the pump and the autosampler (HPLC Autosampler 465, Bio-TeK Instruments, Milano, Italy), a preoxidation (E2: 0.7 V, Range: 100 μA))and a working (E1: 0.225 V, Range: 200 nA) electrode (analytical cell ESA5011).

The position and height of DHBA peaks within the dialysates was compared to an internal standard, 3,4-DHBA (Merck-Schuchardt, Hohenbrunn, Germany) which was added prior to the HPLC analyses, and the DHBA's area was quantified by a PC Integration pack (Kontron Instruments Spa, Milano, Italy). The means of the 3 fractions before the injection was used as the baseline level. The variation of concentrations in each perfusate was then expressed as a percentage of the baseline value.

2.5. Histochemical analysis

On completion of the experiments, rats were sacrificed and the brain fixed with 10% formalin. Coronal sections through the extent of the cannula tracks were cut (100 μm) with a vibratome (Polaron H 1200, Biorad, Cambridge, MA, USA). Dialysis probe placement was localized according to the atlas of Paxinos and Watson (1982). The data were discarded if the probe was incorrectly positioned.

2.6. Blood ethanol concentration

Blood samples were collected from the caudal portion of each rat's tail at 30, 60, 180, 240 min after acute ethanol injections, at the end of the chronic ethanol alcoholization period and at 12 and 24 h during withdrawal. The blood was placed in microcentrifuge tubes containing sodium fluoride (S-1504, Sigma Aldrich Chemie, Gmbh, Steinheim, Germany) as the anticoagulant. The concentration of ethanol in the blood samples was assayed by an alcohol-dehydrogenase-based method (Boerhinger-Mannheim, Germany).

2.7. Statistical evaluation

The results are presented as mean ± standard error of the mean (S.E.M.). Data were analyzed by two-way analysis of variance (ANOVA) with repeated measures on time for each treatment group vs. control to assess the significance of difference in 2,3- and 2,5-DHBA level after acute ethanol i.p., 2 or 3 g/kg, or acute acetaldehyde i.p. injection, 200 mg/kg, or after chronic ethanol administration with or without concomitant acamprosate p.o. administration, 400 mg/kg/day. Where appropriate, post-hoc pairwise comparisons were analyzed by Fisher protected least significant difference test (GB-Stat 5.3 for Windows, Dynamic Microsystems, MD, USA). Criterion for significance was set at $P < 0.05$ for all tests.

3. Results

3.1. Ethanol concentrations

Plasma concentration for the rats acutely administered 2 or 3 g/kg ethanol, assayed at 30, 60, 90, 180 and 240 min, showed a maximum alcohol concentration at 30 min, 1.90 ±0.14 g/l and 2.70±0.18 g/l respectively. A mean blood alcohol level of 1.90±0.13 g/l was assayed after 4 weeks of chronic ethanol treatment in the chronically alcoholized rats, which received acamprosate or not. 12 h after cessation of alcoholization, the plasma alcohol levels had declined to 0.96±0.12 g/l while at 24 h there was no measurable alcohol content.

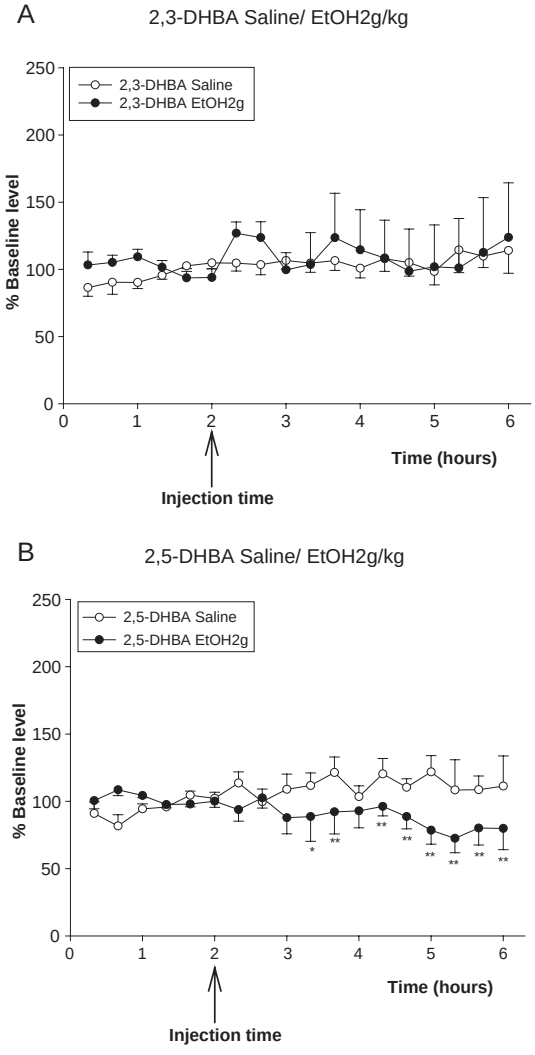

Fig. 1. A) Top. Effect of ethanol 2 g/kg i.p. injection on mean 2,3-DHBA levels compared to saline. B) Bottom. Effect of ethanol 2 g/kg i.p. injection on mean 2,5-DHBA levels compared to saline. Open circles represent the saline group and black circles represent ethanol treated group. Significant time points between ethanol and saline are represented by *$P<0.05$, **$P<0.01$. Results are presented as mean±S.E.M.

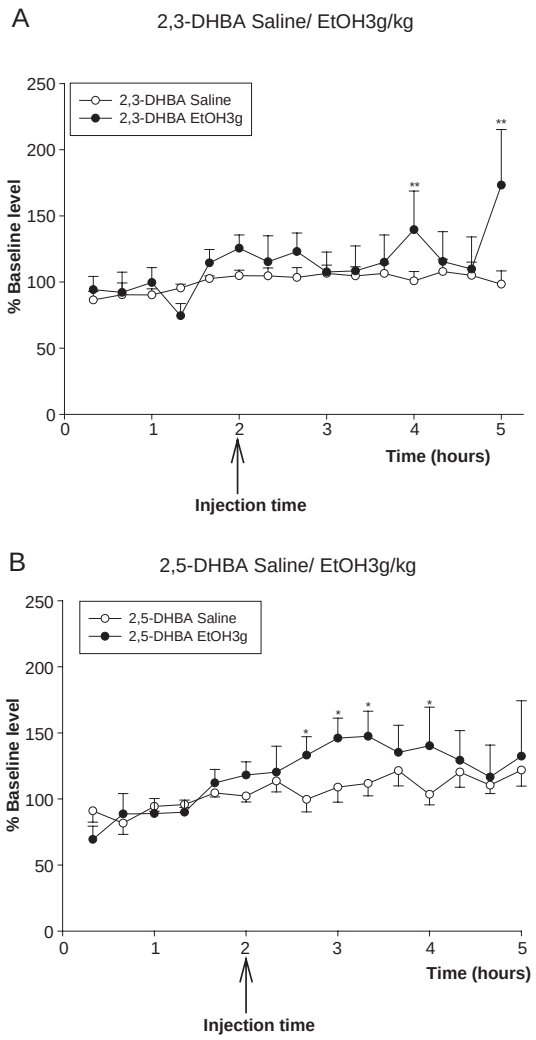

Fig. 2. A) Top. Effect of ethanol 3 g/kg i.p. injection on mean 2,3-DHBA levels compared to saline. B) Bottom. Effect of ethanol 3 g/kg i.p. injection on mean 2,5-DHBA levels compared to saline. Open circles represent the saline group and black circles represent ethanol treated group. Significant time points between ethanol and saline are represented by *$P<0.05$, **$P<0.01$. Results are presented as mean±S.E.M.

3.2. Acute doses of ethanol and acetaldehyde

After an i.p. injection of ethanol, 2 g/kg, 2,3-DHBA level were comparable to the saline group [$F(1,344)=0.34$; $P=0.56$], and there was no interaction between ethanol treatment and time [$F(14,344)=0.83$; $P=0.62$] (Fig. 1A). Significant changes in 2,5-DHBA levels were evident 80 min after ethanol injection that continued for the remainder of the experimental time, (Fig. 1B), statistical analysis showing a significant interaction between ethanol treatment and time [$F(14,389)=3.44$; $P<0.0001$]. However, 3 g/kg of ethanol, showed a significant interaction between ethanol treatment and time for 2,3-DHBA level [$F(14,329)=2.43$; $P=0.003$](Fig. 2A). The post hoc test provided two significant time points with higher 2,3-DHBA level at 2 and 3 h after the ethanol injection by comparison to saline

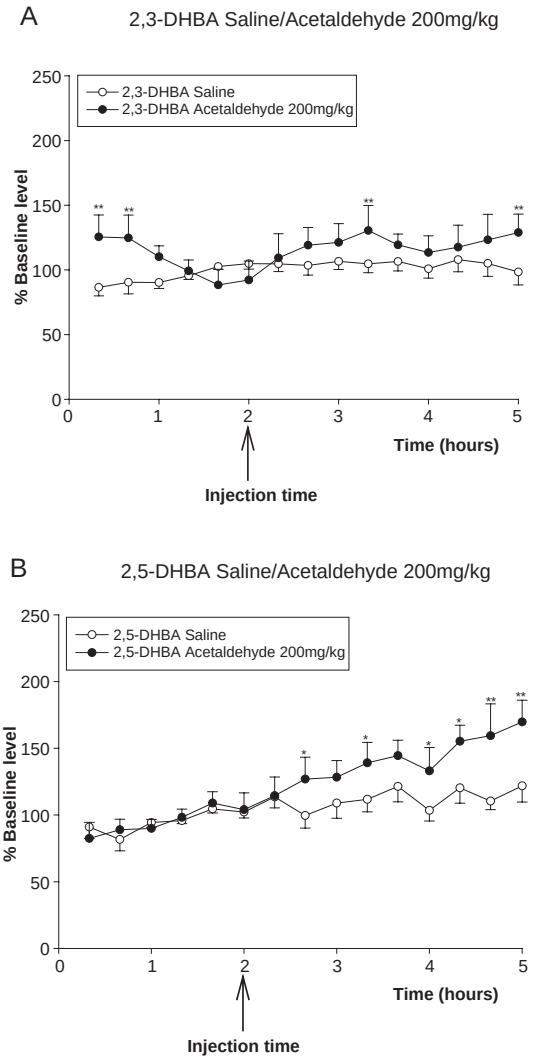

Fig. 3. A) Top. Effect of acetaldehyde 200 mg/kg i.p. injection on mean 2,3-DHBA levels compared to saline. B) Bottom. Effect of acetaldehyde 200 mg/kg i.p. injection on mean 2,5-DHBA levels compared to saline. Open circles represent the saline group and black circles represent acetaldehyde treated group. Significant time points between acetaldehyde and saline are represented by $*P<0.05$, $**P<0.01$. Results are presented as mean±S.E.M.

control values. In this group of rats, 2,5-DHBA level was not significantly different to saline [$F(1,359)=1.42$; $P=0.245$], and there was no interaction between ethanol treatment and time [$F(14,359)=1.42$; $P=0.14$](Fig. 2B). However there was a highly significant interaction between 2,5-DHBA level and time [$F(14,359)=5.317$; $P<0.0001$]. The post hoc test provided four significant time points at 40, 60, 80, 120 min for which 2,5-DHBA levels were significantly higher than the saline control.

Intraperitoneal administration of acetaldehyde, 200 mg/kg, induced significant changes in 2,3-DHBA level by comparison to saline control rats [$F(1,329)=5.15$; $P=0.03$] (Fig. 3A). There was also a significant interaction between acetaldehyde treatment and time [$F(14,329)=2.26$; $P=0.0062$]. Post hoc test provided two significant time points at 80 and 180 min where 2,3-DHBA levels were higher in acetaldehyde treated rats than in saline rats. The mean 2,5-DHBA levels in acetaldehyde treated group were significantly different by comparison to saline group [$F(1,359)=5.56$; $P=0.27$], as well as for the interaction between acetaldehyde treatment and time [$F(14,359)=2.398$; $P=0.0034$] and for time alone [$F(14,359)=10.13$; $P<0.0001$](Fig. 3B). The post hoc test showed that 2,5-DHBA levels were significantly higher at 40, 80, 120, 140, 160 and 180 min after acetaldehyde i.p. administration than in saline controls.

3.3. Chronic ethanol intoxication

Although there was no significant differences between the 2,3-DHBA production between the non-alcoholized

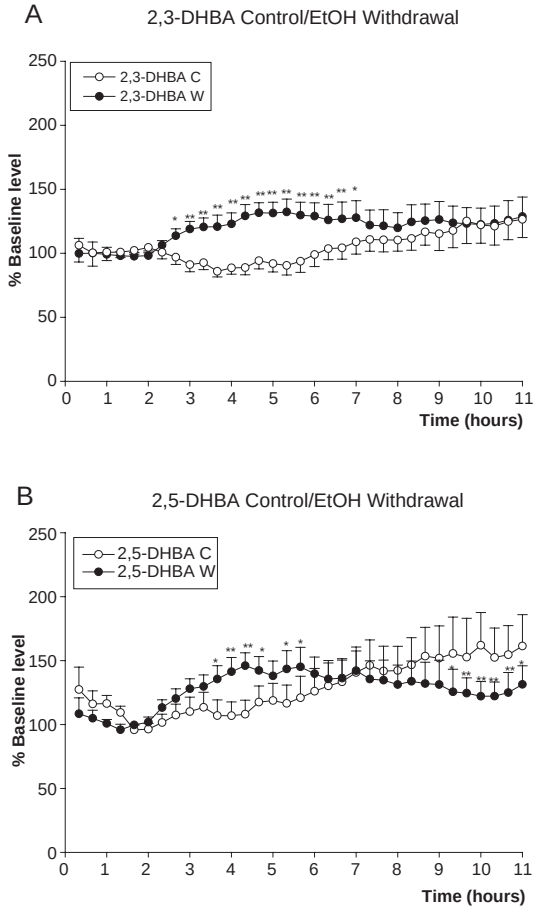

Fig. 4. A) Top. Effect of ethanol withdrawal on mean 2,3-DHBA levels in chronically alcoholized rats compared to non-alcoholized rats under same experimental conditions except the ethanol was not present. B) Bottom. Effect of ethanol withdrawal on mean 2,5-DHBA levels in chronically alcoholized rats compared to non-alcoholized rats under same experimental conditions except the ethanol was not present. Open circles represent the control group (C) and black circles represent withdrawal in alcoholized group (W). Significant time points between ethanol withdrawal and saline are represented by $*P<0.05$, $**P<0.01$. Results are presented as mean±S.E.M.

controls and chronically alcoholized rats for the 18 h after cessation of the chronic alcoholization [$F(1,645)=1.53$; $P=0.23$], there was a highly significant effect for the time alone [$F(33,645)=4.21$; $P<0.0001$] and for the interaction between treatment and time [$F(33,645)=3.35$; $P<0.0001$]. There were significant increases in 2,3-DHBA levels 3 h after the end of chronic ethanol intoxication period which were maintained for a further 5 h (Fig. 4A). The 2,5-DHBA content did not alter significantly in either the chronically alcoholized rats or controls but significant differences for the time [$F(33,645)=6.12$; $P<0.0001$] and interaction between treatment and time were found [$F(33,645)=4.10$; $P<0.0001$]. Post hoc test showed that there is a significant increase of 2,5-DHBA levels after 4 h that lasted for a further 2 h. 10 h after the cessation of alcohol intoxication the levels of 2,5-DHBA significantly decreased until the end of the experiment (Fig. 4B).

When acamprosate was administered during the chronic ethanol intoxication period, the mean levels of microdialysate 2,3-DHBA remained steady throughout the initial stages of the alcohol withdrawal period by comparison to the chronically alcoholized rats [$F(1,647)=5.88$; $P=0.027$]. There were significant differences for time [$F(35,647)=3.12$; $P<0.0001$] and interaction between treatment and time [$F(35,647)=1.53$; $P=0.027$] (Fig. 5A). The levels of 2,5-DHBA increased significantly in both of these groups, although there were significant differences between the acamprosate treated group and the chronically alcoholized group [$F(1,647)=5.09$; $P=0.038$]. There was also significance for time [$F(35,647)=14.618$; $P<0.0001$] but no significant interaction between treatment and time [$F(35,647)=1.00$; $P=0.47$] (Fig. 5B).

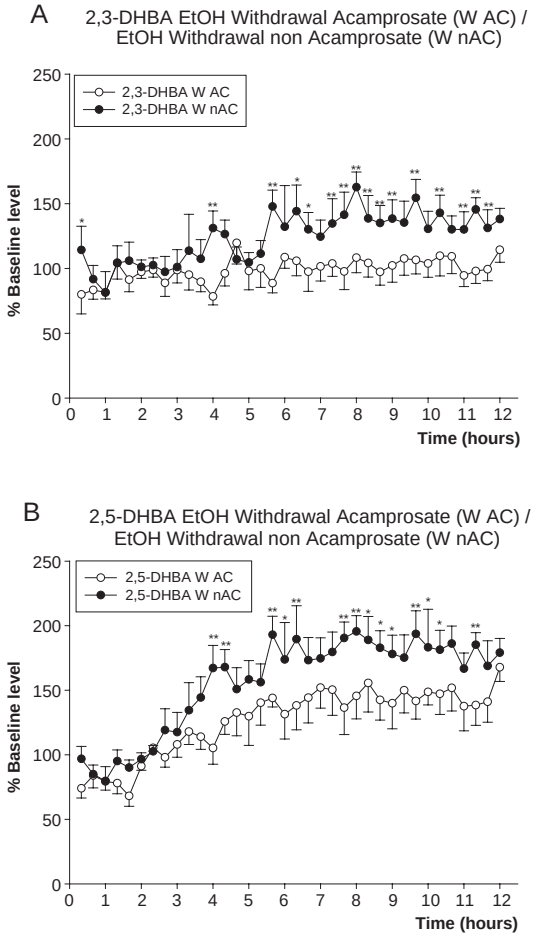

Fig. 5. A) Top. Effect of ethanol withdrawal on mean 2,3-DHBA levels in chronically alcoholized rats treated with acamprosate 400 mg/kg/day or not. B) Bottom. Effect of ethanol withdrawal on 2,5-DHBA level in chronically alcoholized rats treated with acamprosate 400 mg/kg/day or not. Black circles represent the ethanol withdrawal in non-acamprosate treated group (W nAC) and open circles represent the ethanol withdrawal in acamprosate treated group (W AC). These two groups of rats, acamprosate treated or not, were alcoholized in the same vapor chamber. Significant time points between ethanol withdrawal in non-acamprosate and acamprosate treated groups and are represented by $*P<0.05$, $**P<0.01$. Results are presented as mean±S.E.M.

4. Discussion

In these present studies the salicylate trap method has been used to assess whether hydroxyl radicals are generated in a specific brain region, the hippocampus, after administration of either an acute dose of ethanol or acetaldehyde or during the initial stages of ethanol withdrawal after the administration of acamprosate, during the alcohol intoxication regime. A high acute dose of ethanol, 3 g/kg, elicited hydroxyl radicals formation to a limited extent, the time delay in the formation of 2,3-DHBA, 2–3 h after the ethanol injection, could relate to ethanol-induced ascorbic acid release (Huang et al., 2002). Evidence for an increased oxidative stress after this dose of ethanol was also identified in our previous studies (Ward et al., 1996) when enhanced NFkappaB activation was identified in brain nuclear fraction at 15, 30 and 45 min after cessation of alcoholization. This transcription factor is activated by a disbalance of the redox environment, i.e. increasing oxidative stress (Schoonbroodt and Piette, 2000). Although there were no changes in 2,3-DHBA microdialysate content in rats administered the lower dose of ethanol, 2 g/kg, this may be attributed to the presence of other endogenous antioxidants in the brain such as glutathione, as well as dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) which could scavenge oxygen derived free radicals in the brain (Spencer et al., 1996) thereby minimizing the formation of hydroxyl radicals. The 2,5-DHBA metabolite is formed from metabolism of salicylate by enzymes of the endoplasmic reticulum (Ingelman-Sundberg et al., 1991) as well its reaction with the hydroxyl free radical (Floyd et al., 1984). Since a constant level of salicylate was present in the perfusion media, increases in the 2,5-DHBA metabolite content between 40 and 120 min after 3 g/kg, might indicate increased protein synthesis of the cytochrome CYP2E1 or hydroxyl radical formation. In a previous study, where a lower acute dose of ethanol, 0.8 g/kg was administered, this

cytochrome was detectable by Western blotting only after 16 h (Warner and Gustafsson, 1994).

Acute acetaldehyde administration generated increased levels of 2,3-DHBA only at 80 and 180 min. Although brain acetaldehyde levels were not assayed in this present study, similar levels to that of blood would be expected since acetaldehyde rapidly traverses the blood brain barrier (Heap et al., 1995). Acetaldehyde-induced changes in neurotransmitter content of microdialysate from hippocampus at this dose were observed in our previous studies (Ward et al., 1997). 2,5-DHBA microdialysate content increased progressively between 40 and 180 min, although whether such a dose of acetaldehyde will enhance protein synthesis in such a short time period is unknown. This result may therefore indicate the production of hydroxyl radicals by acetaldehyde.

The alcohol withdrawal syndrome has been well documented in human and animal models and is generally characterized by autonomic nervous system excitability, central nervous system excitability, physiological discomfort e.g. anxiety agitation, while in its most severe form hallucinations and delirium occur (Becker, 2000).

Chronic ethanol intoxication initiates adaptation by the brain to prevent ethanol-induced oxidative stress which is exemplified by changes in the brain's cytoprotection e.g. diminished glutathione peroxidase (Rouach et al., 1997), increased catalase activity (Heap et al., 1995) while no activation of the redox sensitive transcription factor NFkappaB was apparent immediately at the end of a four week period of chronic alcoholization prior to detoxification (Ward et al., 1996).

In these present studies an enhanced oxidative stress occurred during the ethanol withdrawal stages that was exemplified by the elevated 2,3-DHBA in the hippocampus microdialysate commencing approximately 3 h after alcohol cessation and maintained for a further 5 h. In a previous study, where 2,3- and 2,5-DHBA were assayed in brain homogenates after chronic ethanol withdrawal, a peak level of 2,3-DHBA was assayed at 2 h, which correlated with tremor (Vallett et al., 1997). In our previous studies (Dahchour et al., 1998; Dahchour and De Witte, 2003) an increased glutamate release occurred during this time period in this brain region. Moderate to high levels of glutamate promote the production of hydroxyl radicals (Ferger et al., 1998). It has been reported that reactive oxygen species generated during this time may prevent astrocytic glutamate uptake mechanisms through a direct action on the transporter protein (Berman and Hastings, 1997) and thereby perpetuate oxidative damage.

2,5-DHBA microdialysate content increased 4–6 h after ethanol withdrawal in this present study; in a previous study of 2,5-DHBA levels in whole brain homogenate, the peak level occurred 10h after the cessation of chronic alcoholization and correlated with the occurrence of spontaneous seizures (Vallett et al., 1997). In the latter experiments salicylate was administered intraperitoneally 1h prior to the sacrifice of the animals while in these present studies salicylate was continually perfused through the microdialysate probe such that only the region of the brain under investigation i.e. the hippocampus, contributed to the 2,5-DHBA content. It was noteworthy that when acamprosate was co-administered during the alcoholization period there was a total absence of the 2,3-DHBA metabolite in the microdialysate. In addition the levels of 2,5-DHBA were also reduced by comparison to the chronically alcoholized rats not receiving acamprosate. This may be related to the fact that such treatment is known to reduce glutamate release during this time period (Dahchour and De Witte, 1999; Dahchour and De Witte, 2000) which may be caused by a decrease in calcium dependent release of excitatory amino acids. The chemical structures of taurine and its analogue acamprosate would preclude them acting as antioxidants, although our previous studies have indicated that taurine and its analogues have anti-inflammatory properties (Della Corte et al., 2002) which could be of benefit in the brain where reactive oxygen species are being generated excessively. When acamprosate was administered to humans during the detoxification process, i.e. 10 days, no evidence of a reduction in withdrawal symptoms were observed (Ward et al., 2000), indicating that this taurine analogue might need to be administered prior to the ethanol detoxification stage to reduce withdrawal symptoms. Other drugs which interact with the voltage dependent NMDA receptor, e.g. Ensaculin or dizocilpine (MK801), similarly reduced glutamate-induced hydroxyl free radicals formation in vivo, decreasing 2,3-DHBA microdialysate content (Teismann and Ferger, 2000).

In conclusion these studies have shown that acute, high non-physiological doses of either ethanol or acetaldehyde induce only minor oxidative stress in the brain hippocampus region over the experimental time period. Chronic ethanol intoxication alone did not induce oxidative stress in the brain, the brain being able to adapt to the metabolic disturbances induced by this neurotoxin and prevent gross brain damage. However during the initial ethanol withdrawal stage, excessive damage occurs in the brain, primarily due to the increased fluxes of calcium, causing increased glutamate release and oxidative damage as exemplified by enhanced 2,3-DHBA in the hippocampus microdialysate. Acamprosate, when administered during the alcoholization period prior to alcohol withdrawal modified the response by the NMDA receptors, such that excessive quantities of hydroxyl radicals were not released during the alcohol withdrawal stage. Clearly the use of acamprosate, during the alcohol withdrawal regime in man, may be part of a therapeutic regime to prevent the associated brain damage.

Acknowledgment

This work has been supported by the Fonds de la Recherche Scientifique Médicale (FNRS ref. 3.4589.02).

References

Aydin, S., Ozaras, R., Uzun, H., Belce, A., Uslu, E., Tahan, V., Altug, T., Dumen, E., Senturk, H., 2002. *N*-acetylcysteine reduced the effect of ethanol on antioxidant system in rat plasma and brain tissue. Tohoku J. Exp. Med. 198, 71–77.

Becker, H.C., 2000. Animal models of alcohol withdrawal. Alcohol Res. Health 24, 105–113.

Berman, S.B., Hastings, T.G., 1997. Inhibition of glutamate transport in synaptosomes by dopamine oxidation and reactive oxygen species. J. Neurochem. 69, 1185–1195.

Bleich, S., Degner, D., Wiltfang, J., Maler, J.M., Niedmann, P., Cohrs, S., Mangholz, A., Porzig, J., Sprung, R., Ruther, E., Kornhuber, J., 2000. Elevated homocysteine levels in alcohol withdrawal. Alcohol Alcohol. 35, 351–354.

Calabrese, V., Renis, M., Calderone, A., Russo, A., Reale, S., Barcellona, M. L., Rizza, V., 1998. Stress proteins and SH-groups in oxidant-induced cellular injury after chronic ethanol administration in rat. Free Radic. Bio. Med. 24, 1159–1167.

Cauquil-Caubere, I., Oxhamre, C., Kamenka, J.M., Barbanel, G., 1999. Recurrent glutamate stimulations potentiate the hydroxyl radicals response to glutamate. J. Neurosci. Res. 56, 160–165.

Cederbaum, A.I., 1991. Microsomal generation of rective oxygen species and their possible role in alcohol hepatotoxicity. Alcohol Alcohol. 1, 291–296.

Dahchour, A., De Witte, P., 1999. Acamprosate decreases the hypermotility during repeated ethanol withdrawal. Alcohol 18, 77–81.

Dahchour, A., De Witte, P., 2000. Taurine blocks the glutamate increase in the nucleus accumbens microdialysate of ethanol-dependent rats. Pharmacol. Biochem. Behav. 65, 345–350.

Dahchour, A., De Witte, P., 2003. Effects of Acamprosate on excitatory amino acids during repeated ethanol withdrawal: an in vivo microdialysis study. Alcohol Clin. Exp. Res. 27, 465–470.

Dahchour, A., Quertemont, E., De Witte, P., 1996. Taurine increases in the nucleus accumbens microdialysate after acute ethanol administration to naive and to chronically alcoholised rats. Brain Res. 735, 9–19.

Dahchour, A., Bolo, N., Nedelec, J.F., De Witte, P., Durbin, P., Macher, J.P., 1998. Central effect of Acamprosate: Part 1. Acamprosate blocks the glutamate increase in the nucleus accumbens microdialysate during ethanol withdrawn rats. Psychiatr. Res.-Neuroimag. 82, 107–114.

Della Corte, L., Crichton, R.R., Duburs, G., Nolan, K., Tipton, K.F., Tirzitis, G., Ward, R.J., 2002. The use of taurine analogues to investigate taurine functions and their potential therapeutic applications. Amino Acids 23, 367–379.

Dupont, I., Berthou, F., Bedenez, P., Bardou, L., Guirriec, C., Stephan, N., Dreano, Y., Lucas, D., 1999. Involvement of cytochroms P-450 2E1 and 3A4 in the 5-hydroxylation of salicylate in humans. Drug Metab. Dispos. 27, 322–326.

Ekstrom, G., Ingelman-Sundberg, M., 1989. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol inducible cytochrome P-450 (P-450IIE1). Biochem. Pharmacol. 38, 1313–1319.

Ferger, B., van Amsterdam, C., Seyfried, C., Kuschinsky, K., 1998. Effects of α-phenyl-tert-butylnitrone and selegiline on hydroxyl free radicals in rat striatum by local application of glutamate. J. Neurochem. 70, 276–280.

Floyd, R.A., Watson, J.J., Wong, P.K., 1984. Sensitive assay of hydroxyl free radical formation utilizing high pressure liquid chromatography with electrochemical detection of phenol and salicylate products. J. Biochem. Biophys. Methods 10, 221–235.

Halliwell, B., Kaur, H., Ingelman-Sunberg, M., 1991. Hydroxylation of salicylate as an assay for hydroxyl radicals: a cautionary note. Free Radic. Biol. Med. 10, 439–441.

Han, Q.P., Dryhurst, G., 1996. Influence of glutathione on the oxidation of 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-beta-carboline: chemistry of potential relevance to the addictive and neurodegenerative consequences of ethanol use. J. Med. Chem. 39, 1494–1508.

Heap, L., Ward, R.J., Abiaka, C., Dexter, D., Lawlor, M., Pratt, O., Shaw, K., Peters, T.J., 1995. The influence of brain acetaldehyde on oxidative status, dopamine metabolism and visual discrimination task. Biochem. Pharmacol. 50, 263–270.

Huang, M., Liu, W., Li, Q., Wu, C.F., 2002. Endogenous released ascorbic acid suppresses ethanol-induced hydroxyl radical production in rat striatum. Brain Res. 944, 90–96.

Ingelman-Sundberg, M., Kaur, H., Terelius, Y., Halliwell, B., 1991. Hydroxylation of salicylate by microsomal fractions and cytochrome P-450: lack of production of 2,3-dihydroxybenzoate unless hydroxyl radical is permitted. Biochem. J. 276, 753–757.

Le Bourhis, B., 1975. Alcoolisation du rat par voie pulmonaire. C. R. Soc. Biol. 169, 898–904.

Lieber, C.S., 1997. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. Adv. Pharmacol. 38, 601–628.

Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Peters, T.J., Ward, R.J., 1988. Role of acetaldehyde in the pathogenesis of alcoholic liver disease. Mol. Aspects Med. 10, 179–190.

Pratt, O.E., Rooprai, H.K., Shaw, G.K., Thomson, A.D., 1990. The genesis of alcoholic brain tissue injury. Alcohol Alcohol. 25, 217–230.

Rouach, H., Fataccioli, V., Gentil, M., French, S.W., Morimoto, M., Nordmann, R., 1997. Effect of chronic ethanol feeding on lipid peroxidation and protein oxidation in relation to liver pathology. Hepatology 25, 351–355.

Schoonbroodt, S., Piette, J., 2000. Oxidative stress interference with the nuclear factor-kappa B activation pathways. Biochem. Pharmacol. 60, 1075–1083.

Spencer, J.P.E., Jenner, A., Butler, J., Aruoma, O.I., Dexter, D.T., Jenner, P., Halliwell, B., 1996. Evaluation of the pro-oxidant and antioxidant actions of L-DOPA and dopamine in vitro: implications for Parkinson's disease. Free Radic. Res. 24, 95–105.

Sun, A.Y., Sun, G.Y., 2001. Ethanol and oxydative mechanism in the brain. J. Biomed. Sci. 8, 37–43.

Sun, G.Y., Xia, J., Draczynska-Lusiak, B., Simoneyi, A., Sun, A.Y., 2000. Grape polyphenols protect neurodegenerative changes induced by chronic ethanol administration. Neuroreport 10, 93–96.

Teismann, P., Ferger, B., 2000. Comparison of the novel drug Ensaculin with MK-801 on the reduction of hydroxyl radical production in rat striatum after local application of glutamate. Brain Res. 857, 165–171.

Upadhya, S.C., Tirumalai, P.S., Boyd, M.R., Mori, T., Ravindranath, V., 2000. Cytochrome P4502E (CYP2E) in brain: constitutive expression, induction by ethanol and localization by fluorescence in situ hybridization. Arch. Biochem. Biophys. 373, 23–34.

Vallett, M., Tabatabaie, T., Briscoe, R.J., Baird, T.J., Beatty, W.W., Floyd, R. A., Gauvin, D.V., 1997. Free radical production during ethanol intoxication, dependence, and withdrawal. Alcohol Clin. Exp. Res. 21, 275–285.

Ward, R.J., Zhang, Y., Crichton, R.R., Piret, B., Piette, J., De Witte, P., 1996. Identification of the nuclear transcription factor NFkappaB in rat after in vivo ethanol administration. FEBS Lett. 389, 119–122.

Ward, R.J., Colantuoni, C., Dahchour, A., Quertemont, E., De Witte, P., 1997. Acetaldehyde-induced changes in momoamine and amino acid extracellular microdialysate content of the nucleus accumbens. Neuropharmacology 36, 225–232.

Ward, R.J., Martinez, J., Ball, D., Marshall, E.J., De Witte, P., 2000. Investigation of the therapeutic efficacy of a taurine analogue during the initial stages of ethanol detoxification: preliminary studies in chronic alcohol abusers. Adv. Exp. Med. Biol. 483, 375–381.

Ward, R.J., Kest, W., Bruyeer, P., Lallemand, F., De Witte, P., 2001. Taurine modulates catalase, aldehyde dehydrogenase and ethanol elimination rates in rat brain. Alcohol Alcohol. 36, 39–43.

Warner, M., Gustafsson, J.A., 1994. Effect of ethanol on cytochrome P450 in the rat brain. Proc. Natl. Acad. Sci. U. S. A. 91, 1019–1023.