Alcoholism & Drug Dependence

Stefanini et al., J Alcohol Drug Depend 2014, 2:2
http://dx.doi.org/10.4172/2329-6488.1000150

Research Article Open Access

Ethane-β-Sultam Modifies the Activation of the Innate Immune System Induced by Intermittent Ethanol Administration in Female Adolescent Rats

Stefanini C[1], Colivicchi MA[1], Della Corte L[1], Ward RJ[1,2,4]*, De Witte P[2], Lallemand F[2], Hemmings K[3], Pitard A[3], Page MI[3], Nayak K[4] and Dexter DT[4]

[1]*Dipartimento di Farmacologia Preclinica e Clinica M. Aiazzi Mancini, Università degli Studi di Firenze, Viale G. Pieraccini 6, 50139 Firenze, Italia*
[2]*Biologie du Comportement, Université catholique de Louvain, 1 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium*
[3]*Chemistry Department, Huddersfield, UK*
[4]*Centre for Neuroinflammation & Neurodegeneration, Division of Brain Sciences, Faculty of Medicine, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London, W12 0NN, UK*

Abstract

Intermittent ethanol abuse or 'binge drinking' during adolescence induces neuronal damage, which may be associated with cognitive dysfunction. To investigate the neurochemical processes involved, rats were administered either 1 g/kg or 2 g/kg ethanol in a 'binge drinking' regime. After only 3 weeks, significant activation of phagocytic cells in the peripheral (alveolar macrophages) and the hippocampal brain region (microglia cells) was present, as exemplified by increases in the release of pro-inflammatory cytokines in the macrophages and of iNOS in the microglia. This was associated with neuronal loss in the hippocampus CA1 region. Daily supplementation with a taurine prodrug, ethane-β-sultam, 0.028 g/kg, during the intermittent ethanol loading regime, supressed the release of the pro-inflammatory cytokines and of reactive nitrogen species, as well as neuronal loss, particularly in the rats administered the lower dose of ethanol, 1 g/kg. Plasma, macrophage and hippocampal taurine levels increased marginally after ethane-β-sultam supplementation. The 'binge drinking' ethanol rats administered 1 g/kg ethanol showed increased latencies to those of the control rats in their acquisition of spacial navigation in the Morris Water Maze, which was normalised to that of the controls values after ethane-β-sultam administration.

Such results confirm that the administration of ethane-β-sultam to binge drinking rats reduces neuroinflammation in both the periphery and the brain, suppresses neuronal loss, and improved working memory of rats in a water maze study.

Keywords: Binge drinking; Innate immune system; Microglia; Alveolar macrophages; Microdialysis; Glutamate; Hippocampus

Introduction

'Binge drinking' is becoming an increasing medical and social problem, particularly amongst adolescents. Intermittent alcohol abuse or 'binge drinking', is defined as a period of excessive drinking, (5 or more alcoholic drinks consecutively over a 4-6 h period), which is then followed by a period of abstinence. The corticolimbic brain regions appear to be susceptible to binge-induced degeneration and induced relearning deficits, [1], particularly during adolescence when neurogenesis is occurring. Over the past few years some of the underlying biochemical and neurochemical processes involved in the cognitive [2] and electrophysiological abnormalities [3] have been identified in various animal models of binge drinking.

Clinical studies have clearly shown that chronic alcohol abuse is associated with increases in infections [4,5], which are caused by alcohol-induced changes in the innate and adaptive immune systems. There is an inability of white cells to migrate to the site of infection or inflammation, as well as functional changes in lymphocytes, [6] natural killer cells, monocytes and macrophages [7] . In addition, alcohol will increase the permeability of the gut; such that gut derived endotoxins will be transported to the liver via the portal vein to stimulate toll-like receptors to induce inflammation and the release of damaging pro-inflammatory cytokines [8]. The release of pro-inflammatory cytokines will activate phagocytic cells, such as macrophages and microglia and induce inflammation in both the liver [9] and specific brain regions [10]. It is noteworthy that as the excessive alcohol intake continues, there is adaptation by these cells, particularly in the brain, to reduce the inflammatory response by altering the gene expression of various transcription factors, e.g. NFkappaB, [11] which is referred to as neuro-adaptation. Over the period of chronic alcohol abuse, >10 years, there will be progressive loss of behavioural control, caused by decreased frontal cortical regulation of attention and cognitive flexibility, combined with increased limbic negative feelings [12]. In addition the hippocampus will be adversely affected by chronic ethanol abuse, and shows decreased hippocampal volume as well as deficits in hippocampal-dependent learning and memory [13].

In contrast, binge drinking will rapidly induce inflammation in the periphery [14] and hippocampus and prefrontal cortex brain regions in a rat model of binge drinking [14,15], as well as altering the ratio of pro-inflammatory cytokines to anti-inflammatory cytokines in the blood of University binge drinkers [16]. The explanation for such

***Corresponding author:** Roberta J Ward, Biologie du Comportement, Université catholique de Louvain, 1 Place Croix du Sud, 1348 Louvain-la-Neuve, Belgium, Tel: 003-210-456-004; E-mail: Roberta.Ward@uclouvain.be

Received November 26, 2013; **Accepted** February 15, 2014; **Published** February 20, 2014

Citation: Stefanini C, Colivicchi MA, Della Corte L, Ward RJ, de Witte P, et al. (2014) Ethane-β-Sultam Modifies the Activation of the Innate Immune System Induced by Intermittent Ethanol Administration in Female Adolescent Rats. J Alcohol Drug Depend 2: 150. doi:10.4172/2329-6488.1000150

vulnerability in young binge drinkers is related to the fact that there is fine-tuning of specific neuronal connections, via synaptic pruning, during this adolescent period [17]. Two brain regions show particularly marked ontogenetic alterations during adolescence, the prefrontal cortex, where considerable remodeling occurs within regions which form an interconnecting network of circuitry and the hippocampus, where hippocampal stem cells are present in the subgranular zone, inside the dentate gyrus granule cell layer. These neural stem cells are linked to hippocampal function, which include learning, memory and mood [18]. It is in these two regions where binge drinking has its most profound neurotoxic effect, inducing adverse changes in structural integrity which could result in a variety of cognitive deficits [3]. Furthermore such vulnerability occurs within a relatively short time, e.g. approximately 2 years, of commencing a binge drinking regime in susceptible adolescents.

The excitatory amino acid glutamate plays an important role in alcoholism. Glutamate mediates approximately 70% of synaptic transmission, reaching concentration in the low millimolar range. Once released into the synaptic cleft it can bind to one of the three types of ionotrophic glutamate receptors, the N-methyl-D-aspartate receptor, NMDA, the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor and the kainite receptor. In addition glutamate can bind to metabotrophic glutamate receptors in the perisynaptic regions or on the presynaptic terminal. Glutamate is cleared from the extracellular environment by sodium dependent excitatory amino acids, EAAT, which include GLT1, EAAT2 and EAAT5. In addition, EAAT1 and EAAT2 are expressed in glial cells and can remove excess glutamate [19]. Chronic alcohol abuse will inhibit neuronal NMDA receptor function, the NR2B containing NMDA receptors being particular sensitive to inhibition by ethanol. Microdialysis studies have shown that there are no changes in extracellular glutamate in various brain regions in an animal model of chronic alcohol abuse, although during detoxification there is a rapid increase in glutamate release [20]. In contrast, microdialysis studies in a rat model of a binge drinking revealed significantly increased extracellular glutamate in the hippocampus after only 3 weeks of a binge drinking regime [14]. The precise mechanisms as to how ethanol alters extracellular glutamate are unknown, although it may be due to ethanol-induced changes in glutamate uptake by glial cells [21-23].

The sulphonated amino acid taurine is widely distributed in human tissue, being present at high concentrations, 50mM in leucocytes, microglia and macrophages, where it plays an important anti-inflammatory role [24]. In our recent studies [25], we clearly showed that the anti-inflammatory property of taurine was mediated via stabilisation of IkaBa, thus preventing activation of NFkappaB. Although taurine can be synthesised intracellularly from cysteine and methionine, the diet is the main source for human nutrition. Taurine uptake from the plasma is tightly controlled by the taurine transporter, TauT, such that supplementation with taurine will only transiently increase taurine levels within the liver and to a lesser extent in the brain [25].

Since TauT activity is decreased by inflammation [26-28], this might indicate that the ability of such cells to protect themselves from inflammation will be decreased. Therefore taurine analogues which are able to traverse cellular membranes independently of TauT may enhance intracellular taurine levels and promote anti-inflammatory pathways

Beta sultam is an analogue of β-lactams, a group of compounds which are able to inactivate serine enzymes, i,e, elastase, which is released in response to inflammatory stimuli and plays a major role in protein digestion following phagocytosis [29]. The parent substituted β -sultam does not inhibit serine enzymes but is slowly hydrolysed to taurine. It therefore has the potential to diffuse across cellular membranes independent of TauT and increase intracellular taurine content. In addition, it has also been shown that ceftriaxone, an FDA approved β -lactam antibiotic, reduced ethanol consumption in alcohol preferring rats [30] which in part is due to the up regulation of glutamate transporter 1 [31]. Therefore ethane-β-sultam may also diminish extracellular glutamate levels by a comparable mechanism, and diminish the neurotoxicity of binge drinking.

Since we had shown the anti-inflammatory action of ethane-β-sultam in vitro in macrophages and microglia in our earlier cell culture studies [25], it was of interest to ascertain whether it would have an anti-inflammatory action in an animal model of binge drinking. In addition, since β-lactams antibiotics alter glutamate transporter 1, it was of interest to ascertain whether extracellular glutamate content might be influenced by ethane- β-sultam. Lastly, since pro-inflammatory cytokines play an important role in the modulation of learning, memory, neural plasticity and neurogenesis [32], the binge drinking rats were assessed in a water maze trial to investigate whether their learning, possibly impaired by binge drinking, could be rectified by ethane- β-sultam administration.

Methods

Animals

Adolescent Wistar female rats (Harlan-Nossan, Milan, Italy) at puberty (6 weeks of age), with average body weights of approximately 125-155 g, were housed under controlled humidity and temperature with 12h dark/light cycles and a free supply of food and water within a polypropylene cage. All animals were treated in accordance with the Italian Guidelines for Animal Care (D. L. 116/92) and European Communities Council Directives (86/609/ECC).

Animal treatment and binge drinking regime

The rats were randomly assigned to various binge treatment groups, each with n=4, repeating the binge treatment on at least two different occasions (total number of rats in each experimental group=8). A minimum number of 3 rats in each group completed the various analysis/tests at the end of each binge treatment. Animals were administered by gavage 1 g/kg ethanol or 2 g/kg ethanol +/- ethane-β -sultam. Ethanol doses (20%) were administered 3x /day with 3 h intervals on 2 consecutive days by gavage, followed by 5 days abstinence. This was repeated for a total of three weeks. The control rats were administered either ethane- β-sultam or water alone at the same time points as the binge drinking ethanol rats (Figure 1).

The synthesis of ethane- β-sultam has been previously described [25]. Ethane- β-sultam was freshly prepared before each administration (2.86 mg/ml) and given by gavage at a dose of 28 mg/kg. The administration of ethane-β-sultam was initiated one week before commencing the binge drinking regime, this compound being administered by gavage, each morning at 0900 h. It was then continued daily for the subsequent 3 weeks of the binge drinking regime, at 09.00 h or 30 min before the first daily dose of ethanol.

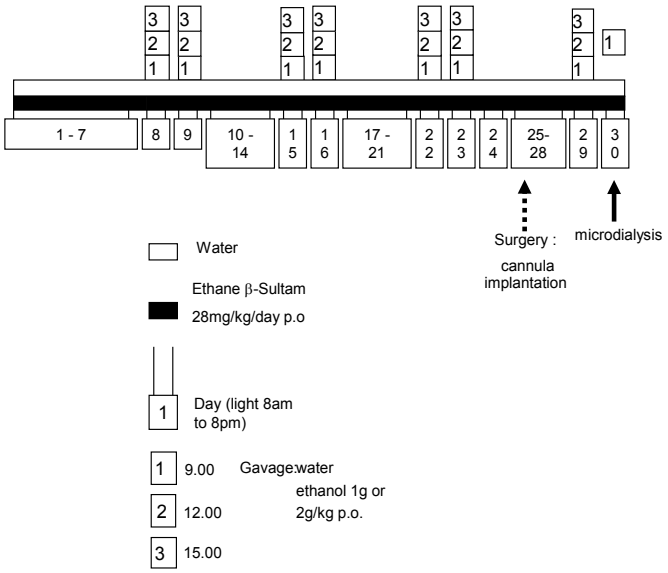

Figure 1: Diagrammatic scheme of ethane β Sultam and ethanol administration during the experimental procedures.

Microdialysis

The rats underwent surgical procedures at the end of the second week of the binge drinking regime, five days before the administration of the last ethanol dose, as previously described [14]. Rats were anaesthetised with chloral hydrate (400 mg/kg i.p), mounted onto a stereotaxic frame (Stellar, Stoelting Co., Wood Dale, IL, USA) and a guide cannula (concentric design, CMA Microdialysis AB, Stockholm, Sweden) was implanted vertically into the right ventral hippocampus, using the following coordinates, relative to Bregma and skull surface: AP -4.8, L -5.2, V-4.0 at the ventral extent of the guide cannula. The rats were allowed to recover for 5 days after the surgery, after which time microdialysis was commenced on the last day of the three weeks binge drinking regime, when a vertical microdialysis probe (2 mm exposed surface, CMA 12, CMA Microdialysis AB, Stockholm, Sweden) was inserted into the guide cannula. The inlet of the probe was connected to a microdialysis pump (CMA/100, CMA Microdialysis AB, Stockholm, Sweden) and the ventral hippocampus perfused with artificial cerebrospinal fluid (aCSF) consisting of, 3.0 mM KCl, 1.0 mM $MgCl_2$, 140 mM NaCl, 1.2 mM $CaCl_2$, 0.27 mM NaH_2PO_4, 7.2 mM glucose and 1.2 mM Na_2HPO4 at pH 7.4 at a rate of 2µl/min [14]. After a stabilisation period of 1 h, the perfusion fractions were collected every 30 minutes. The-fractions collected at -90, -60 and -30 min were taken as representative of the basal extracellular concentrations for glutamate and taurine. After the collection of the -30 min microdialysis fraction, rats were administered ethanol or water, i.e. during the first 30 min collection of the stimulated period, which lasted a further 5 h duration (10 fractions of 30 min. The correct positioning of the probe was confirmed at a later time (see below).

To measure the extracellular concentrations of glutamate and taurine, the microdialysis samples were treated with OPA-reagent for pre-column derivatisation, which consisted of mercaptoethanol and O-phthalaldehyde (OPA). The amino acid derivatives were separated with 5µm reverse-phase Nucleosil C18 column (250 × 4 mm; Machery-Nagel, Duren, Germany), maintained at room temperature. The mobile phase consisted of 0.1 M potassium acetate (pH adjusted to 5.48 with glacial acetic acid) and methanol with a 3 linear step gradient from 25% to 90% methanol (flow rate of 1.0 ml/min). Column effluent was analysed using a High-Performance Liquid Chromatography (HPLC) reverse-phase Shimadzu spectrofluorimeter system (Shimadzu Italia S.r.l., Milan, Italy) set to an excitation wavelength of 340nm and emission wavelength of 455nm (controlled by Class-VPTM 7.2.1 SP1 Client/Server Chromatography Data System) [14].

Alveolar macrophage isolation and blood collection

Alveolar macrophages were isolated from rats within 24 h of completing microdialysis. Rats were anaesthetised with Nembutal, prior to a small incision in the trachea, to allow a small tube to be inserted into the lungs. A phosphate buffer solution (pH 7.4), approximately 40 ml, was used to lavage the alveolar macrophages from the lungs, which were recovered after centrifugation at 1,500 rpm for 10 min. Cells at densities of 1×10^5 or 2×10^5 were pipetted into wells (Corning Inc. USA) containing culture medium Dulbecco media, 10% foetal calf serum, streptomycin (100 µg/ml) and penicillin (100 µg/ml). The alveolar macrophages were left for 24 h to adhere to the wells. The supernatant was then removed and the cells resuspended in culture medium and stimulated with LPS (1 µg/ml) for 24 h. The supernatants were removed and stored at –20°C prior to further analysis for NO and the two cytokines, IL6 and TNFα. The rats were then decapitated, the brains removed, and blood collected. The blood was spun at 3000 rpm for 15 minutes to collect serum, which was then stored at -20°C prior to taurine analysis.

In a separate experiment, blood was removed from the tail vein of adolescent binge drinking rats +/- ethane- β-sultam rats, n=8, at timed intervals after the first, second and third ethanol administration. The blood alcohol concentrations were estimated by an enzymatic method, where the conversion of NAD^+ to NADH results in an increase in absorbance at 340 nm that is proportional to the ethanol concentration

Nitrite analysis

The levels of nitrites in the cell supernatants were evaluated by combining 100 µl aliquots with an equal volume of Greiss reagent (2.5% phosphoric acid, 1% sulphanilamide and 0.1% naphthalene diamine dihydrochloride). The mixture was incubated for 10 min at room temperature and optical density measured at 540 nm. Standards were prepared in the range 1-50 µM.

Cytokine analyses

IL6 and TNFα were assayed in the supernatants by ELISA (R & D System, Inc. UK)

Serum taurine analysis

Trichloroacetic acid (2%) was added to the serum to precipitate proteins and the supernatant recovered after centrifugation at 3000 r.p.m. for 15 minutes. The supernatant was diluted 1:500, after which its taurine content was assayed by HPLC with fluorescence detection of the o-phthalaldehyde derivative.

Brain preparation for histological investigation

The brains were removed from each rat at the completion of the pulmonary lavage. The brains were initially preserved in 4% formaledehyde in buffer solution and then cryopreserved in 30% sucrose solution prior to being frozen in isopentane at -80°C prior to analysis. For cutting, the frozen brains were mounted in the cryostat (Bright Instruments, UK) and coronal sections, 20 microns, cut

through the hippocampus, approximately 40 slides/rat, and transferred onto slides (2 per slide). Slides were kept at -80°C after cutting to avoid degradation and only defrosted when required for staining.

Cresyl fast violet staining (CFV)

CFV staining was used to stain the neurons in the hippocampus, 8-10 sections for each rat. The neurons are substantially larger than microglia cells and are clearly distinguishable from these phagocytic cells by their morphology. The correct positioning of the probe in the CA1 hippocampal region was confirmed by this stain.

Immunohistochemisty

OX-6 and iNOS immunohistochemical staining: Every 7th slide was stained for presence of MHC-II [14] and iNOS, which constituted approximately 8-10 brain sections for each rat. Slides were rehydrated in changes of ethanol, circled with a pap pen (Daido Sangyo Co. Ltd., Tokyo, Japan) and then left in phosphate buffer saline (PBS; 16 g NaCl, 2.3 g Na_2PO_4, 0.4 g KH_2PO_4, in 400 ml adjusted to pH 7.4). Endogenous peroxide activity was blocked by 1% H_2O_2 in 100% methanol (45 min). Slides were washed and incubated (1 h) first in 5% normal horse serum (Vector Laboratories, UK) with PBS/Triton X (PBS, 0.1% Triton X-100; Sigma-Aldrich, UK) then in the same solution with the OX-6 antibody (Serotec Ltd., Oxford, UK), 1:500 dilution or the iNOS antibody 1:200 dilution, and refrigerated for 20 h. The slides were washed in PBS/TX prior to incubation with 5% normal horse serum and 0.5% anti-mouse IgG (2nd antibody) in PBS/TX (90min). The slides were washed and the ABC mix applied (Vector stain Elite Kit, Vector Laboratories, UK) and slides covered (1 h). ABC mix was washed off with PBS and the chromogen, 3,3'-diaminobenzidine (DAB; 5 ml H_2O, 2 drops buffer, 4 drops DAB, 2 drops H_2O_2; Vector Laboratories, UK) added and left (5-15 min) until the brain sections had turned pale brown. Slides were then dehydrated and mounted, as described earlier.

Stereological cell quantification

Neuronal cell counts were made on the CFV stained slides (approximately 8 slides per brain) from within the dentate gyrus regions region from -4.3mm to -4.52mm bregma (Figure 2a). The hippocampal regions were maintained in the same position in both hemispheres, within the "fork" of the hippocampus, encompassing the polymorph layer of the dentate gyrus and CA1 neurons. Microglia counts encompassed the entire region of the CA1 hippocampal region from -4.30mm to -4.5mm bregma (number of rats =8) (Figure 2a). A computer based stereology software system (Image Pro, Media Cybernetics, PA, USA) attached to a Nikon Eclipse E8—microscope (Nikon Instruments, Surrey, UK) and JVC (London, UK) 3CCD camera was used. Briefly, for each section, an area of interest was delineated manually with relation to previously published boundaries, to create an Area of Interest (AOI) (Figure 2b). The software system then created counting frames (100 × 60μm) which fell within the AOI using the uniform random sampling method. The total area of the counting frames relative to the area of the AOI gives the Area Sampling Fraction (ASF). The height of the optical dissector, which was measured by taking an average of 3 random points across the section using a Heidenhain microcator (Hedenhain, Traunreut, Germany), relative to the section thickness gives the Height Sampling Fraction (HSF). The Section Sampling Fraction (SSF) was 1/7 as every 7th section in either the dendate gyrus or total hippocampus was analysed. To avoid edge effects, when counting microglia or neurons, within the counting frames, "acceptance" and "forbidden" lines were used (Figure 2c). Total cell estimates were calculated as follows, where n equal the number of cells counted:

$$N = n\ (1/SSF)\ (1/ASF)\ (1/HSF)$$

Cognitive function

In order to minimise stress, the rats were handled daily throughout their period in the animal house and during the treatment phase preceding the water maze experiment. Twenty four hours after the conclusion of the binge drinking regime +/- ethane-β-sultam, the rats commenced training in the spatial version of the Morris water maze test. The water maze apparatus consisted of a circular pool (169 cm width, and 50 cm depth) made of white plastic. The pool was filled to a depth of 30-35 cm with water maintained at room temperature (20 + 1°C) and made opaque by the addition of a non-toxic black ink. A hidden platform (30 cm high, 12 cm diameter) was placed under the water in the centre of one quadrant of the pool. Extra-maze visual cues (i.e. coloured paper in different forms was placed on the edge of the wall) and two lights as well as an auditory cue, (a radio) remained in fixed positions throughout the experiments. Training in the Morris Water Maze Task consisted of 4 trials/day for 4 days during which the rat learned to navigate to a submerged platform located in a constant spatial position. The rat was released into the water in one quadrant of the tank and the latency (time in seconds) to climb onto the platform was recorded. The starting point for each trial was in a different quadrant for three trial runs while the fourth trial run reverted to the original quadrant. If the rat had not found the platform after 60 seconds it was placed on the platform by the experimenter, and left there for 20 seconds to collect visual spatial information.

Five hours after the last training test, a probe test was carried out in which each rat was placed in the water in the 1st quadrant, and given 30

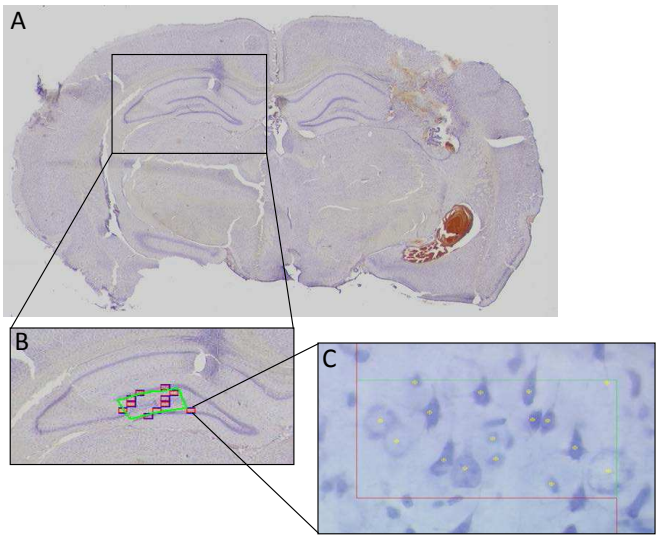

Figure 2a: Serological counting. Left hand side Neuronal cell counts. The boxes show the areas where the neurons were counted. **Figure 2b** shows counting area for the microglia. Only regions in the left hemisphere were counted, due to potential damage induced by probe insertion in the right hemisphere. (Adapted from Paxinos & Watson, 1998). **Figure 2c: The counting frames used for stereology.** A randomising algorithm was used to locate counting frames within the areas of interest. Neurons marked with a crosshair are those, which have been counted. Neurons within the box were counted except those, which were touching the red lines. Cells, which make up capillaries and blood vessels, were not included, such as the two cells shown in the centre.

seconds to find the position of the platform in the pool, which had been removed. The time that the rat spent in the 1st quadrant, (dial entry), was recorded as well as the time in the quadrant where the platform had been, (target dial). After 30 seconds, the rat was removed from the tank.

Statistical evaluation

The data are presented as mean ± standard error, SEM or mean ± standard deviation, SD. Statistical analysis was carried out using one-way or two-way ANOVA as appropriate, followed by the post hoc Fisher LSD (protected t test), when GB-Stat 5.3 for windows (Dynamic Microsystems, MD USA) was the software used, or Bonferroni multiple comparison test, when Prism 5.0d for Max (Graph Pad Software Inc., San Diego CA) was used. Differences were considered significant at a p value, <0.05. Morris water maze data was first analysed by General Linear Model ANOVA (SPAA for Windows, SPSS Inc, Chiago, IL). Details of the statistical analyses are shown in the Figure legends.

Results

Body weight of rats

The body weight of all rats, measured at the beginning and the end of the binge drinking regime, indicated comparable weight gains in all of the treatment groups, when administered water or ethanol +/- ethane-β-sultam (Figure 3).

Blood ethanol levels

Blood ethanol levels increased during the period of the binge drinking regime. After the 2 g/kg dose, administered 3x during one day at 3 hourly intervals, the peak blood ethanol level was 0.5 g/l at 30 minutes after the first binge, while the blood ethanol peak concentration was at 1h, 0.88 g/l and 1.32 g/l after the second and third dose, respectively. However the rate of clearance was similar for each dose. The dose of 1 g/kg ethanol induced blood ethanol levels 50% lower. The administration of ethane- β-sultam did not significantly alter either the blood ethanol concentrations or the ethanol clearance rates.

Serum and macrophage taurine levels

Serum taurine levels assayed in each treatment group are shown in (Figure 4) Ethanol treatment induced a statistically significant decrease

Figure 3: Body weight gain (g) of the different treatment groups at the conclusion of the 3 weeks of "Binge Drinking" regime. Columns represent mean s.e.m. obtained from a total of 6-15 rats. No statistically significant differences in weight gain were apparent.

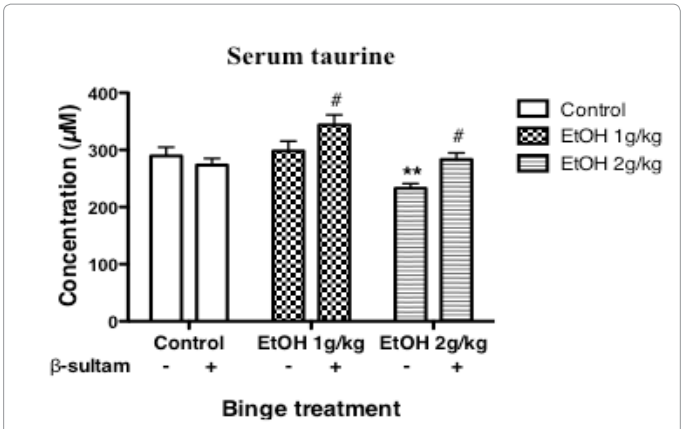

Figure 4: Plasma concentration of taurine in the different experimental groups at the conclusion of the "Binge Drinking" regime. Columns represent mean ± s.e.m. (μM) obtained from 3-6 rats per group. Data analysed by a two way ANOVA, where the two factors were Binge regime and β-sultam supplementation, respectively, followed by the *post hoc* Fisher's LSD (Protected t-Test) for multiple comparisons. Binge, $F_{2,24}$=9.557, p<001; ethane-β-sultam $F_{1,24}$=5.054, p<0.034; Fisher's LSD test: *p<0.05, **p<0.01 *versus* control, #p<0.5, ##p<0.01 *versus* Binge alone.

in the concentration of serum taurine in the rats administered 2 g/kg EtOH (two way Anova, post hoc test: p<0.01 versus control), but not at the lower dose of 1 g/kg. When ethanol treatment was associated with ethane-β-sultam, the serum concentration of taurine showed a statistically significant increase, in both, 1 and 2 mg/kg ethanol-administered groups, as compared to those not administered ethane-β-sultam (p<0.05, 1 and 2 g/kg EtOH + ethane-β-sultam, versus EtOH alone). The treatment with ethane-β-sultam alone, however, did not modify the serum levels of taurine in the control group.

The mean concentration of taurine assayed in the macrophages isolated from the ethanol-treated rats +/- ethane- β-sultam supplementation did not show any statistically significant difference.

Pro-inflammatory markers released from alveolar macrophages before and after stimulation with lipopolysaccharide, LPS

Parallel release of each of these pro-inflammatory markers from the alveolar macrophages in different treatment conditions can be observed in (Figure 5). The LPS-induced release of TNFα is shown in (Figure 5a) and the release of NO and IL-6, before and 24 h after LPS stimulation, in (Figure 5b) and (Figure 5c), respectively. Following LPS stimulation, there were statistically significant increases in each of these markers in the binge drinking rats administered either 1 g/kg or 2 g/kg, by comparison to the control group administered water alone (p<0.05 or p<0.01 by two way Anova followed by post hoc test, see (Figure 5) legend for details). In both 'binge drinking' EtOH groups, where ethane-β-sultam was co-administered during the binge drinking regime, there were significant decreases in the release of the pro-inflammatory markers from the alveolar macrophages, to almost control values.

Hippocampal taurine and glutamate microdialysate content after binge drinking +/- ethane-β-sultam supplementation

The extracellular concentrations of taurine and glutamate were measured by microdialysis in the ventral hippocampus, under basal conditions and following the last EtOH dose of the binge drinking

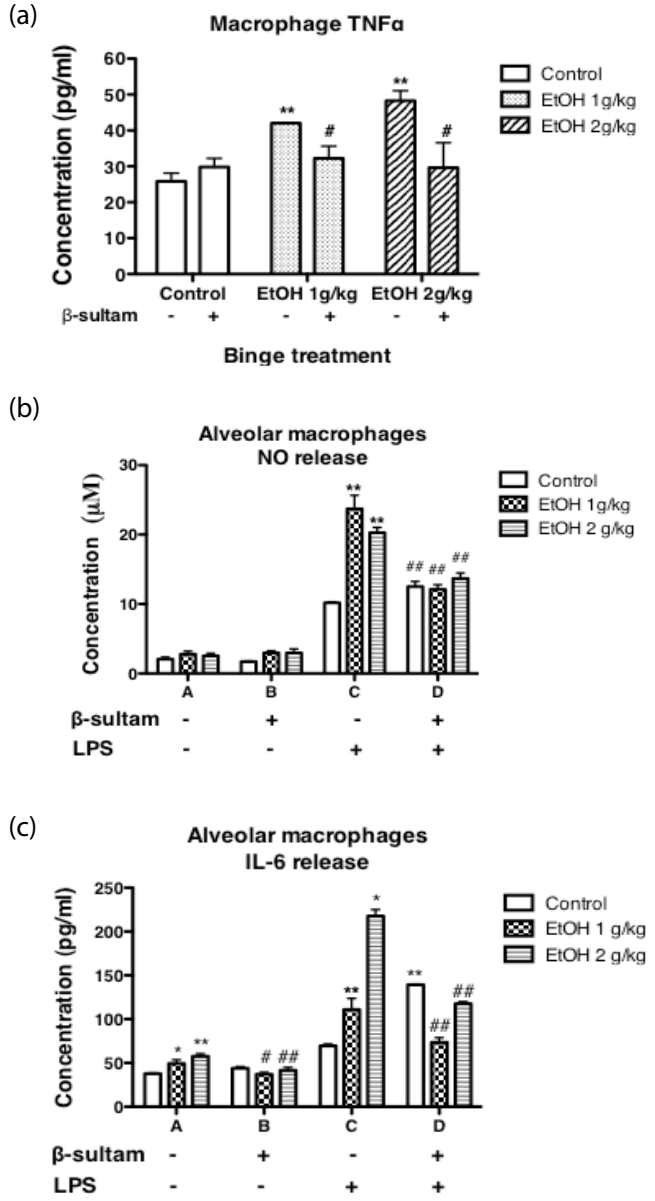

Figure 5: Release of pro-inflammatory cytokines and NO from alveolar macrophages before and after stimulation with LPS, 1mg/ml, for 24h. **Panel a,** shows stimulated TNFα release; panel **b,** NO release without stimulation (left columns) and after LPS stimulation (right columns); **panel c,** IL-6 release without stimulation (left columns) and after LPS stimulation (right columns). Columns represent mean ± s.e.m. (μM) obtained from 3-10 rats per group. Data analysed by a two way ANOVA, where the two factors were Binge regime and β-sultam supplementation, respectively, followed by the *post hoc* Fisher's LSD (Protected t-Test) for multiple comparisons. TNFα + LPS: Binge $F_{2,20}$=6.5, p<0.0067; ethane-β-sultam $F_{1,20}$=9.5, p<0.0064; Interaction Binge X ethane-β-sultam, $F_{2,20}$=9.5, p<0.01; NO basal, NS; NO + LPS: Binge, $F_{2,27}$=7.22, p<0.031;, ethane-β-sultam $F_{1,27}$=14.74, p<0.0007; Interaction Binge X ethane-β-sultam, $F_{2,27}$=26.52, p<0.0001; IL-6 basal: Binge, $F_{2,11}$=4.31, p<0.041;ethaneβ-sultam, $F_{1,11}$=8.91, p<0.012; Interaction Binge X ethane-β-sultam $F_{2,11}$=7.42, p<0.0091; IL-6 + LPS: Binge, $F_{2,10}$=60.73, p<0.0001; ethane-β-sultam, $F_{1,10}$=14.18, p<0.0037; Interaction Binge X ethane-β-sultam, $F_{2,10}$=68.21, p<0.0001; *post hoc* Fisher's LSD test: *p<0.05, **p<0.01 *versus* control, #p<0.5, ##p<0.01 *versus* Binge alone.

regime. The basal extracellular levels of taurine were not affected by ethanol treatment, either 1 g/kg or 2 g/kg. Overall, ethane-β-sultam administration induced a statistically significant increase in the basal extracellular concentration of taurine in the hippocampal microdialysate, as compared to the non-supplemented rats (two way ANOVA, +/- ethane-β-sultam $F_{1,37}$=7.29, p<0.01) (Figure 6a). In the post hoc group comparison, however, presence versus absence of ethane-β-sultam was statistically significant only for the control group. The basal extracellular concentration of glutamate showed a statistically significant increase in the rats administered 2 g/kg ethanol as compared to the control group (two way ANOVA post hoc comparison p<0.05). Such increases were abolished in rats administered 2 g/kg ethanol +ethane-β-sultam, (two way Anova post hoc comparison versus 2 g/kg, p<0.05), (Figure 6b). No further significant changes in taurine or glutamate extracellular concentrations were evident, after the last ethanol dose, during the 5 h of microdialysis, in any of the animal groups.

Activation of microglia in the hippocampus after binge drinking +/- ethane-β-sultam

Representative microphotographs of the region within the hippocampus where OX-6-immunopositive microglia were present, in binge drinking +/- ethane-β-sultam supplemented rats, are shown in the two upper panels of (Figure 7a). Stereological cell counts of the activated microglia in the CA1 region of the hippocampus, showed

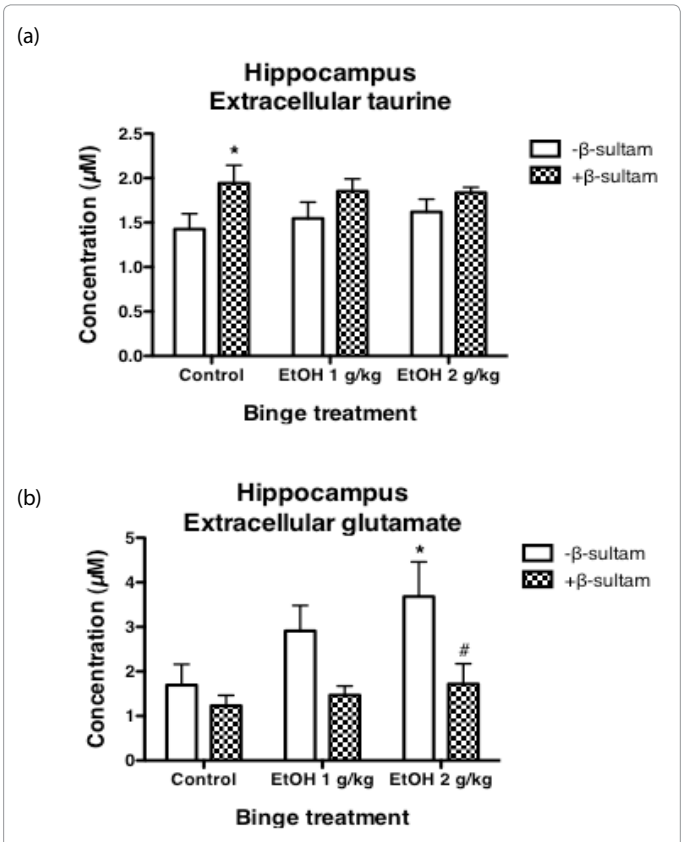

Figure 6: Basal extracellular concentrations of hippocampal taurine and glutamate in binge-treated rats +/- β-sultam supplementation. Columns represent mean concentration (μM) ± s.e.m. from 5-9 rats per group. Data analysed by a two way ANOVA, where the two factors were Binge regime and β-sultam supplementation, respectively, followed by the *post hoc* Fisher's LSD (Protected t-Test) for multiple comparisons. Ethane-β-sultam: $F_{1,34}$=8.60, p<0.006, *post hoc* comparisons *p<0.05, **p<0.01 *versus* control, #p<0.05, ##p<0.01 *versus* Binge treatment alone.

that, contrary to the absence of OX-6 positive cells in the control group +/- ethane-β-sultam, there was a highly significant increase in these phagocytic cells in the binge drinking rats which received either 1 g/kg or 2 g/kg ethanol (Two way ANOVA, post hoc p<0.0001 versus controls). Supplementation with ethane-β-sultam significantly reduced the number of activated microglia in the 1g/kg ethanol administered rats, by approximately 50% (Two way ANOVA, post hoc p>0.01 versus control), but did not reduce the numbers of these inflammatory cells in the 2 g/kg ethanol administered rats (Figure 7b).

Further immunohistochemical studies with then iNOS antibody confirmed that there was co- localisation of iNOS with the activated microglia, (Figure 7a), thereby confirming that such microglia were releasing pro-inflammatory cytokines as well as NO.

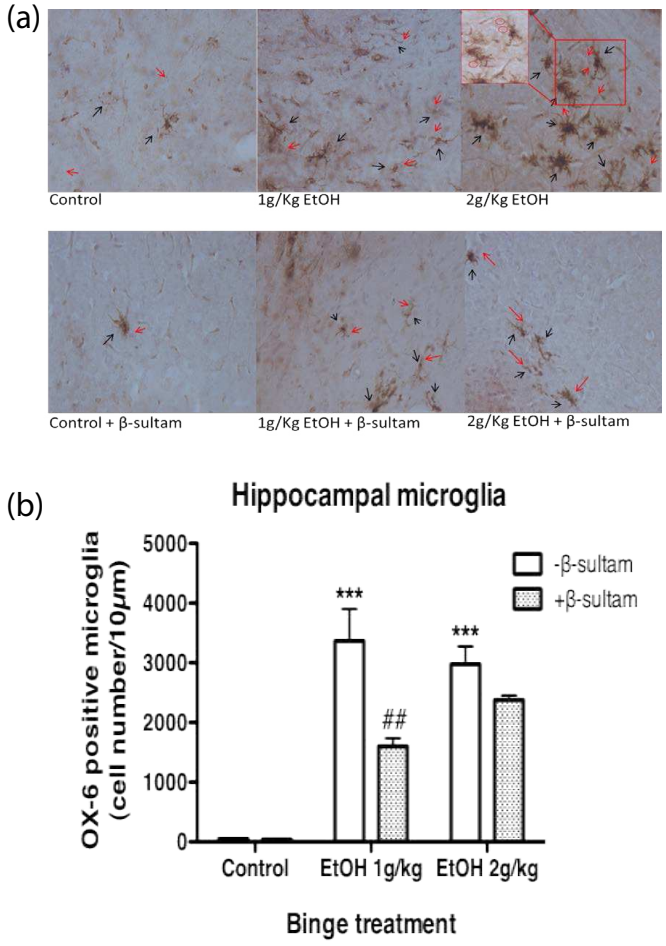

Figure 7: a: Microglial activation in response to the "Binge Drinking" regime and β-sultam supplementation. Representative microphotographs of OX-6 positive microglia in the hippocampus of binge-treated rats without (upper panels) and with β-sultam supplementation (lower panels). The micrograph shows co-localisation of OX-6 and iNOS immunoreactivities. Black arrows indicate iNOS, red arrows indicate activated microglia. **b:** Quantitation of microglial activation is presented. Number of OX-6 immunopositive cells (3-4 rats per group) analysed by two way ANOVA, where the two factors were Binge regime and β-sultam supplementation, followed by the *post hoc* Fisher's LSD (Protected t-Test) for multiple comparisons. Binge $F_{2,14}$=66.24, p<0.0001; ethane-β-sultam: $F_{1,14}$=14.50, p<0.0019; Interaction binge x ethane-β-sultam: $F_{2,14}$=6.12, p<0.0123; *post hoc* Bonferroni multiple comparison test: *p<0.05, **p<0.01, ***p<0.001 *versus* control, #p<0.5, ##p<0.01, ###p<0.001 *versus* Binge alone.

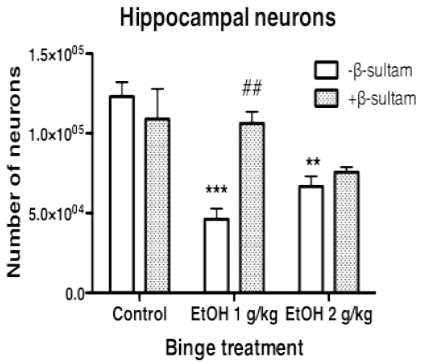

Figure 8: Neuronal counts at the hippocampal fork in rats after "Binge Drinking" regime +/- ethane-β-sultam supplementation. Columns represent mean value of neuronal counts +/- s.e.m. from 3 rats per group. Data analysed by a two way ANOVA, where the two factors were Binge regime and β-sultam supplementation, respectively, followed by the *post hoc* Bonferroni multiple correction test; Binge: $F_{2,12}$=12.53, p<0.0012; ethane-β̃sultam: $F_{1,12}$= 5.23, p<0.0412; Interaction Binge x ethane-β-sultam $F_{2,12}$=7.46.p<0.0078; *post hoc* comparisons *p<0.05, **p<0.01 *** p<0.001 *versus* control, #p<0.05, ##p<0.01 *versus* Binge treatment alone.

Cell counts of neurons in the dendate gyrus brain region after binge drinking +/- ethane-β-sultam

Stereological cell counts of the neurons in the dendate gyrus region identified statistically significant losses of neurons in the areas where the activated microglia had been observed, (Figure 8), in both 1 and 2 g/kg EtOH-treated groups (two way ANOVA, post hoc group comparisons versus control, p<0.01 and p<0.05, respectively) which were not significantly different from each other. Ethane-β-sultam restored the neuronal number to almost that of the control group in the binge drinking rats which had been administered 1 g/kg ethanol (post hoc group comparison, p<0.01). However, no protection against neuronal loss was evident in the rats administered 2 g/kg + ethane-β-sultam.

Morris water maze studies

Spatial learning and memory was assessed in the rats at the conclusion of the binge drinking regime. It was of interest to note that the rats administered ethanol showed no fear of the water and immediately started to swim when placed in the pool. In contrast, control rats as well as the rats, which had received ethanol + ethane-β-sultam were timid and slow to commence the task. Task acquisition along the 4 days is shown in Figure 9, where mean values of the escape latency time (seconds) are plotted against number of days. Global statistical analysis (Figure legend for details) was performed using the General Linear Model (GLM) for repeated measures, which indicated a difference of the escape latency for the factor binge treatment only at the end of the acquisition period, day 4. Thus for multiple comparisons, a two way ANOVA applied to escape latency values obtained on day 4, showed that the ethanol-fed adolescent female rats, both 1 and 2 g/kg, had performed with significantly higher latencies to find the hidden platform than the control group (post hoc comparison versus control, p<0.05), (Figure 9). In the 1 g/kg rats administered ethane-β-sultam, there were significantly lower latencies (post hoc comparison versus ethanol alone, p<0.05), similar to those of the controls, (Figure 9). In contrast, the 2 g/kg rats administered the pro-taurine drug showed identical higher latencies as compared to those administered ethanol

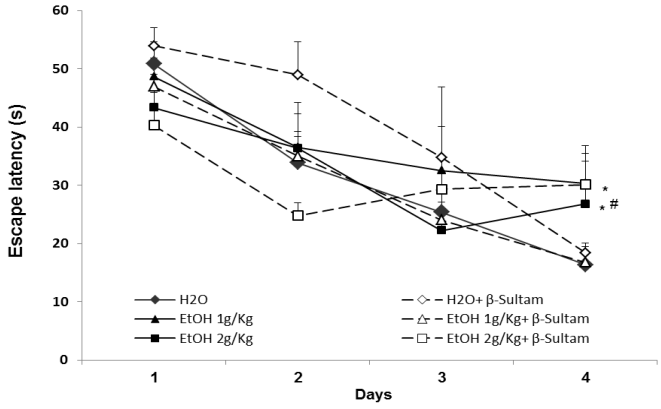

Figure 9: *Morris Water Maze*. Spatial learning assessed 24 h after the conclusion of the binge drinking regime. Latency times (s), mean ± s.e.m., from 3-8 rats per group, are plotted as a function of the 4 successive days of training (mean of 4 trials/day). Data analysed across the 4 days of testing, using the commercial software SAS (version 9.2 for Windows, SAS Institute Inc., Cary, NC) with General Linear Model (GLM) ANOVA. When factors represented treatment, Days, Rats, with Model with significant $F_{29,90}$, p<0.0013 and p<0.0001 for day 2 and day 3,4, respectively A significant treatment factor ($F_{4,90}$=2.5. p<0.0001 was found only for Day 4. Day 4 escape latency times were then analysed by two ways ANOVA, where the two factors were binge regime and ethane-β-supplementation, respectively, followed by post hoc Fisher's LSD (Protected t-test) for multiple comparisons. Binge: $F_{2,29}$=3.39, p<0.047, *post hoc* comparisons *p<0.05, **p,0.01 *versus* control, #P<0.05, ##P<0.01, *versus* Binge treatment alone.

alone. There were no significant differences between the controls or the binge drinking rats +/- ethane-β-sultam in the dial entry or probe trials.

Discussion

In these present studies the activation of the innate immune system by intermittent alcohol administration was modified in both the periphery and the brain hippocampal region after the administration of ethane-β-sultam, as exemplified by decreases in both the activation of phagocytic cells, (macrophages and microglia) and the associated release of pro-inflammatory markers. It was predicted that ethane-β-sultam would increase cellular levels of taurine, as a result of its slow hydrolysis to taurine; however only marginal changes in plasma and macrophage taurine levels were analysed after ethane-β-sultam in these present studies. Increasing taurine status (by taurine supplementation) influences ethanol metabolism [25], as exemplified by increased ethanol clearance from the blood, which was associated with altered brain activities of aldehyde dehydrogenase and catalase. Beta-lactam antibiotics, such as ceftriaxone also influence ethanol elimination rates, [33] as a result of inhibition of liver ALDH activity by the N-methyltetrazolethiomethyl group on the 3-position of the cephem nucleus [33]. However since ethane-β-sultam has no methyltetrazolethiomethyl group on the 3-position of the ethane-β-sultam molecule it would not be expected to alter enzymes involved in ethanol metabolism. No changes in ethanol metabolism occurred in these present studies, which might have been a factor in the induction of a pro-inflammatory state in the brain.

The pro-inflammatory state induced by a binge drinking regime has been previously reported by many investigators, significant damage being reported in limbic association regions, including the cortex and the hippocampus, in particular the ventral dentate gyrus. The extent of such inflammatory changes is possibly associated with the ethanol dose, duration of ethanol intoxication and the number of periods of ethanol cessation. The biochemical and neurochemical changes induced remain unclear. It is reported that binge drinking does not induce changes in N-methyl-D-aspartate (NMDA) sensitivity or in the brain density of voltage-gated calcium channels [1]. Furthermore the levels of blood ethanol achieved in these present studies after 3 successive ethanol administration was not excessively high. The increased glutamate concentrations may therefore be an important factor in the neurotoxicity observed in the binge drinking model.

The binge drinking regime induced a significant increase in hippocampal glutamate after 1 g/kg and 2 g/kg ethanol, which could be due to the multiple withdrawal episodes occurring between the ethanol drinking periods [20]. In our previous studies of chronic alcohol loading in experimental animals, there was a cumulative effect of several withdrawal episodes where basal glutamate content increased in specific brain regions [20]. Glutamate can stimulate glial cells towards an inflammatory phenotype. Supplementation with ethane-β-sultam reduced hippocampal glutamate levels, which returned towards control values. The mode of action involved in this diminution is unclear although it is reported that β-lactam antibiotics are potent stimulators of GLT1 expression and protein content in the hippocampal CA1 astrocytes and mixed neuron/glial cortical cultures [34]. Up regulation of these specific glutamate transporters may decrease extracellular glutamate content. Interestingly, when administered to animal models of motor neuron degeneration, there was a delayed loss of neurons and muscle cells as well as a reduction of hypercellular gliosis, [34] which was associated with decreased extracellular glutamate levels [31,35]. The pathway involved in the activation of the promoter region of the GLT1 gene is unknown. It was of note that there was a reduction in extracellular glutamate content in the hippocampus region after ethane- β-sultam supplementation, in the controls as well as the binge drinking rats possibly indicating an increased GLT1 expression. Clearly this would need to be analysed in future studies.

The release of cytokines by peripheral cells, as identified in the alveolar macrophages, may readily compromise the endothelial function and permeability of the blood brain barrier [36], thereby facilitating the migration of inflammatory cells into the brain to further promote neuro-inflammation [37]. In addition it is of interest that the hippocampus highly expresses the pro-inflammatory cytokine receptors, e.g TNFα receptors, which may account for its vulnerability to systemic proinflammatory cytokines [38]. Glial cells play important roles in the nurturing of the neurons, as well as important roles in the immune and inflammatory response. In preliminary studies, acute ethanol doses (20mM-100mM) were shown to induce limited activation of an immortilised cell line, N9, (Unpublished data Ward and Nayak). Similarly, primary microglia, incubated between 7 and 24h with ethanol, 50mM, also showed only a marginal activation with increased NO release [39]. However in another study of murine macrophages, low to moderate levels of ethanol, (10-50mM) did stimulate TLR4, which triggered MAPKs pathways, translocation of NFkB to the nucleus and the release of pro-inflammatory cytokines and NO. However higher doses, 100mM were inhibitory [39]. An ethanol-induced priming stimulus of the microglia may certainly be an initial event in binge drinkers, when high circulating levels of ethanol may be achieved. Other factors may further potentiate the pro-inflammatory phenotype.

In these present studies OX-6 was used to stain and measure activated microglia in the dentate gyrus region of the hippocampus

after 3 weeks, which induced the release of pro-inflammatory cytokines as well as NO, the latter being confirmed by immunohistochemical staining. Higher ethanol doses, 5 g/kg, administered in a single 4-day binge study, elicited a more widespread microglia infiltration in all regions of the hippocampus [40], which were associated with a range of central mediators of inflammation, e.g. pro-inflammatory cytokines, as well as COX2 and iNOS. However, this model of binge drinking had no prolonged period of abstinence from ethanol. In another study when 5 g/kg, was administered intra-gastrically for a shorter time period, every 8 h for 4 days, [41] there was no evidence of increased cytokine release in various brain regions. However there are no reported studies of the effect of β-lactam antibiotics on pro- and anti-inflammatory cytokines in the brain. A decrease in the number of inflammatory microglia was evident after ethane-β-sultam supplementation, as well as a decrease in NO expression (as assayed by immunohistochemical techniques), which might be related to the decrease in glutamate content or some other unknown biochemical effect of β-lactams.

The neuro-inflammatory changes induced by binge drinking in this present study were paralleled by hippocampal neuronal loss. Neuronal loss has also been reported in other studies [1,42] although the ethanol concentrations used were much higher, 4-9 g/kg. The dentate gyrus region is particularly vulnerable since it contains neural progenitor cells, which will proliferate throughout life, but particularly during adolescence, to form neurons, astrocytes and oligodendrocytes. High doses of ethanol were shown to decrease the survival of these neural progenitor cells [43]. In another study of marque monkeys, where a binge type regime was administered for varying time periods, there were significantly decreased numbers of actively dividing type 1, 2a, and 2b cell types without significantly altering the early neuronal type 3 cells. Such results, as concluded by the authors, were caused by alcohol interfering with the division and migration of hippocampal pre-neuronal progenitors [44].

Pharmaceutical agents may prevent the neurotoxicity of binge drinking. For example, the anti-oxidant butylated hydroxytoluene reversed binge induced brain damage, possibly via NFκb inhibition, and blocked ethanol inhibition of neurogenesis in several brain regions after the administration of very high doses of ethanol (8-12g/kg/day) 3 x /day for 4 days with no abstinence period [45]. Administration of indomethacin to adolescent rats, exposed to ethanol 3g/kg for 2 consecutive days at 48h intervals, abolished both COX-2 and iNOS expression, as well as cell death and behavioural deficits [46].

Another approach would be to prevent the activation of transcription factors, which mediate inflammation, i.e. NFκB. Taurine a sulphonated amino acid will prevent NFκB activation by stabilising IκBα and preventing its phosphorylation [25]. Although ethane-β-sultam diminished the activation of the innate immune system in both the alveolar macrophages in the periphery and the glial cells in the hippocampus, its exact mode of action awaits detailed investigation since its administration was not associated with significant increases in taurine cellular levels.

The immune system plays an important role in both brain function and behavioural processes [32]. Peripheral inflammation can profoundly affect the functioning of the brain with respect to memory and cognition. In early studies, ethanol was shown to disrupt acquisition of a spatial task in adolescent rats in the Morris Water Maze [47] although Rajendran and Spear [48] indicated that this was a stressful technique which was not substantiated in the less stressful test- the sand box maze. Chronic binge-type ethanol exposure, 5 g/kg every 48 h for 20 days, showed evidence of tolerance to ethanol-induced spatial deficits, when tested immediately. In mice, which were chronically alcoholised by administration of 10% alcohol for 5 months followed by withdrawal, there were glia cell activations in frontal cortex and striatum, which were associated with cognitive and anxiety-related behavioural impairment [49]. In our present study it was shown that spatial learning and memory was impaired in rats administered intermittent alcohol for 3 days, which was corrected by the pro-drug in the 1g/kg ethanol administered rats.

These present studies have shown that administration of ethane-β-sultam significantly reduced the inflammatory response both in the periphery and in the brain [50,51]. Although increased taurine levels were not discernible in some of the tissues, possibly due to homeostatic controls, a reduction in neuro-inflammation and neuronal cell loss occurred as well as an improvement in ethanol-associated cognitive impairment. These studies have identified the importance of the innate immune system in the toxicity of binge drinking.

Acknowledgements

The financial support of ERAB, Università degli Studi di Firenze and COST CM1103 for our international cooperation are gratefully acknowledged. We should also like to thank Patrick J Brennan, Katy Pollard and Ross Munroe for their excellent technical assistance during a work experience programme.

References

1. Crews FT, Nixon K (2009) Mechanisms of neurodegeneration and regeneration in alcoholism. Alcohol Alcohol 44: 115-127.
2. Scaife JC, Duka T (2009) Behavioural measures of frontal lobe function in a population of young social drinkers with binge drinking pattern. Pharmacol Biochem Behav 93: 354-362.
3. Crego A, Holguín SR, Parada M, Mota N, Corral M, et al. (2009) Binge drinking affects attentional and visual working memory processing in young university students. Alcohol Clin Exp Res 33: 1870-1879.
4. Ahmed FE (1995) Toxicological effects of ethanol on human health. Crit Rev Toxicol 25: 347-367.
5. Szabo G (1998) Monocytes, alcohol use, and altered immunity. Alcohol Clin Exp Res 22: 216S-219S.
6. Curtis BJ, Zahs A, Kovacs EJ (2013) Epigenetic targets for reversing immune defects caused by alcohol exposure. Alcohol Res 35: 97-113.
7. Romeo J, Wärnberg J, Nova E, Díaz LE, Gómez-Martinez S, et al. (2007) Moderate alcohol consumption and the immune system: a review. Br J Nutr 98 Suppl 1: S111-115.
8. Kirpich IA, Feng W, Wang Y, Liu Y, Barker DF, et al. (2012) The type of dietary fat modulates intestinal tight junction integrity, gut permeability, and hepatic toll-like receptor expression in a mouse model of alcoholic liver disease. Alcohol Clin Exp Res 36: 835-846.
9. Wang HJ, Gao B, Zakhari S, Nagy LE (2012) Inflammation in alcoholic liver disease. Annu Rev Nutr 32: 343-368.
10. He J, Crews FT (2008) Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol 210: 349-358.
11. Ward RJ, Zhang Y, Crichton RR, Piret B, Piette J, et al. (1996) Identification of the nuclear transcription factor NFkappaB in rat after in vivo ethanol administration. FEBS Lett 389: 119-122.
12. Crews FT, Zou J, Qin L (2011) Induction of innate immune genes in brain create the neurobiology of addiction. Brain Behav Immun 25 Suppl 1: S4-4S12.
13. Mandyam CD (2013) The Interplay between the Hippocampus and Amygdala in Regulating Aberrant Hippocampal Neurogenesis during Protracted Abstinence from Alcohol Dependence. Front Psychiatry 4: 61.
14. Ward RJ, Colivicchi MA, Allen R, Schol F, Lallemand F, et al. (2009) Neuro-inflammation induced in the hippocampus of 'binge drinking' rats may be mediated by elevated extracellular glutamate content. J Neurochem 111: 1119-1128.

15. Vetreno RP, Crews FT (2012) Adolescent binge drinking increases expression of the danger signal receptor agonist HMGB1 and Toll-like receptors in the adult prefrontal cortex. Neuroscience 226: 475-488.

16. Lallemand F, Ward RJ, De Witte P, Petit G, Saeremans M, et al. (2013) Changes in the innate immune responses by intermittent ethanol consumption may influence cognition in susceptible adolescent binge drinkers. J Alcoholism Drug Depend 1: 114.

17. Spear LP (2013) Adolescents and alcohol: Acute sensitivities, enhanced intake, and later consequences. Neurotoxicol Teratol 41C: 51-59.

18. Balu DT, Lucki I (2009) Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev 33: 232-252.

19. Gass JT, Olive MF (2008) Glutamatergic substrates of drug addiction and alcoholism. Biochem Pharmacol 75: 218-265.

20. Dahchour A, De Witte P (2003) Effects of acamprosate on excitatory amino acids during multiple ethanol withdrawal periods. Alcohol Clin Exp Res 27: 465-470.

21. Smith TL (1997) Regulation of glutamate uptake in astrocytes continuously exposed to ethanol. Life Sci 61: 2499-2505.

22. Othman T, Sinclair CJ, Haughey N, Geiger JD, Parkinson FE (2002) Ethanol alters glutamate but not adenosine uptake in rat astrocytes: evidence for protein kinase C involvement. Neurochem Res 27: 289-296.

23. Melendez RI, Hicks MP, Cagle SS, Kalivas PW (2005) Ethanol exposure decreases glutamate uptake in the nucleus accumbens. Alcohol Clin Exp Res 29: 326-333.

24. Schuller-Levis GB, Park E (2004) Taurine and its chloramine: modulators of immunity. Neurochem Res 29: 117-126.

25. Ward RJ, Lallemand F, De Witte P, Crichton RR, Piette J, et al. (2011) Anti-inflammatory actions of a taurine analogue, ethane β-sultam, in phagocytic cells, in vivo and in vitro. Biochem Pharmacol 81: 743-751.

26. Kim HW, Lee EJ, Kim WB, Kim BK (2000) Ionomycin restores taurine transporter activity in cyclosporin-A treated macrophages. Adv Exp Med Biol 483: 127-135.

27. Romio L, Zegarra-Moran, Varesio L, Galietta LJV (2001) Regulation of taurine transport in murine macrophages. Amino Acids 21: 151-160.

28. Kim HW, Kim JH, An HS, Park KK, Kim BK, et al. (2003) Myo-inositol restores the inflammation-induced down-regulation of taurine transport by the murine macrophage cell line, RAW 264.7. Life Sci 73: 2477-2489.

29. Page MI (2004) Beta-sultams-mechanism of reactions and use as inhibitors of serine proteases. Acc Chem Res 37: 297-303.

30. Sari Y, Sakai M, Weedman JM, Rebec GV, Bell RL (2011) Ceftriaxone, a beta-lactam antibiotic, reduces ethanol consumption in alcohol-preferring rats. Alcohol Alcohol 46: 239-246.

31. Knackstedt LA, Melendez RI, Kalivas PW (2010) Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. Biol Psychiatry 67: 81-84.

32. Yirmiya R, Goshen I (2011) Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun 25: 181-213.

33. Shimada J, Miyahara T, Otsubo S, Yoshimatsu N, Oguma T, et al. (1987) Effects of alcohol-metabolizing enzyme inhibitors and beta-lactam antibiotics on ethanol elimination in rats. Jpn J Pharmacol 45: 533-544.

34. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, et al. (2005) Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433: 73-77.

35. Qrunfleh AM, Alazizi A, Sari Y (2013) Ceftriaxone, a beta-lactam antibiotic, attenuates relapse-like ethanol-drinking behavior in alcohol-preferring rats. J Psychopharmacol 27: 541-549.

36. Rachal Pugh C, Fleshner M, Watkins LR, Maier SF, Rudy JW (2001) The immune system and memory consolidation: a role for the cytokine IL-1beta. Neurosci Biobehav Rev 25: 29-41.

37. Terrando N, Eriksson LI, Ryu JK, Yang T, Monaco C, et al. (2011) Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 70: 986-995.

38. Chowdhury N, Quinn JJ, Fanselow MS (2005) Dorsal hippocampus involvement in trace fear conditioning with long, but not short, trace intervals in mice. Behav Neurosci 119: 1396-1402.

39. Fernandez-Lizarbe S, Pascual M, Guerri C (2009) Critical role of TLR4 response in the activation of microglia induced by ethanol. J Immunol 183: 4733-4744.

40. McClain JA, Morris SA, Deeny MA, Marshall SA, Hayes DM, et al. (2011) Adolescent binge alcohol exposure induces long-lasting partial activation of microglia. Brain Behav Immun 25 Suppl 1: S120-128.

41. Zahr NM, Luong R, Sullivan EV, Pfefferbaum A (2010) Measurement of serum, liver, and brain cytokine induction, thiamine levels, and hepatopathology in rats exposed to a 4-day alcohol binge protocol. Alcohol Clin Exp Res 34: 1858-1870.

42. Crews FT, Collins MA, Dlugos C, Littleton J, Wilkins L, et al. (2004) Alcohol-induced neurodegeneration: when, where and why? Alcohol Clin Exp Res 28: 350-364.

43. Nixon K, Crews FT (2002) Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem 83: 1087-1093.

44. Taffe MA, Kotzebue RW, Crean RD, Crawford EF, Edwards S, et al. (2010) Long-lasting reduction in hippocampal neurogenesis by alcohol consumption in adolescent nonhuman primates. Proc Natl Acad Sci U S A 107: 11104-11109.

45. Crews F, Nixon K, Kim D, Joseph J, Shukitt-Hale B, et al. (2006) BHT blocks NF-kappaB activation and ethanol-induced brain damage. Alcohol Clin Exp Res 30: 1938-1949.

46. Pascual M, Blanco AM, Cauli O, Miñarro J, Guerri C (2007) Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. Eur J Neurosci 25: 541-550.

47. Markwiese BJ, Acheson SK, Levin ED, Wilson WA, Swartzwelder HS (1998) Differential effects of ethanol on memory in adolescent and adult rats. Alcohol Clin Exp Res 22: 416-421.

48. Rajendran P, Spear LP (2004) The effects of ethanol on spatial and nonspatial memory in adolescent and adult rats studied using an appetitive paradigm. Ann N Y Acad Sci 1021: 441-444.

49. Pascual M, Baliño P, Alfonso-Loeches S, Aragón CM, Guerri C (2011) Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. Brain Behav Immun 25 Suppl 1: S80-91.

50. Ward RJ, 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.

51. West MJ, Slomianka L, Gundersen HJ (1991) Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. Anat Rec 231: 482-497.

Citation: Stefanini C, Colivicchi MA, Della Corte L, Ward RJ, de Witte P, et al. (2014) Ethane-β-Sultam Modifies the Activation of the Innate Immune System Induced by Intermittent Ethanol Administration in Female Adolescent Rats. J Alcohol Drug Depend 2: 150. doi:10.4172/2329-6488.1000150