



HAL
open science

Anti-inflammatory actions of a taurine analogue; ethane β -sultam; in phagocytic cells; in vivo and in vitro

Roberta J Ward, Frederic Lallemand, Philippe de Witte, Robert R Crichton, Jacques Piette, Keith Tipton, Karl Hemmings, Arnaud Pitard, Mike Page, Laura Della Corte, et al.

► To cite this version:

Roberta J Ward, Frederic Lallemand, Philippe de Witte, Robert R Crichton, Jacques Piette, et al.. Anti-inflammatory actions of a taurine analogue; ethane β -sultam; in phagocytic cells; in vivo and in vitro. *Biochemical Pharmacology*, 2011, 81 (6), pp.743. 10.1016/j.bcp.2010.12.030 . hal-00672299

HAL Id: hal-00672299

<https://hal.science/hal-00672299>

Submitted on 21 Feb 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Anti-inflammatory actions of a taurine analogue; ethane β -sultam; in phagocytic cells; in vivo and in vitro

Authors: Roberta J Ward, Frederic Lallemand, Philippe de Witte, Robert R Crichton, Jacques Piette, Keith Tipton, Karl Hemmings, Arnaud Pitard, Mike Page, Laura Della Corte, Deanna Taylor, David Dexter



PII: S0006-2952(11)00017-7
DOI: doi:10.1016/j.bcp.2010.12.030
Reference: BCP 10798

To appear in: *BCP*

Received date: 15-9-2010
Revised date: 16-12-2010
Accepted date: 20-12-2010

Please cite this article as: Ward RJ, Lallemand F, de Witte P, Crichton RR, Piette J, Tipton K, Hemmings K, Pitard A, Page M, Corte LD, Taylor D, Dexter D, Anti-inflammatory actions of a taurine analogue; ethane β -sultam; in phagocytic cells; in vivo and in vitro, *Biochemical Pharmacology* (2010), doi:10.1016/j.bcp.2010.12.030

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

MASTER

1
2
3
4
5
6
7
8
9
10 **Anti-inflammatory actions of a taurine analogue, ethane β -sultam, in**
11 **phagocytic cells, in vivo and in vitro**
12
13
14
15
16
17
18
19
20

21 ^{1,6,7} **Roberta J Ward, ¹Frederic Lallemand, ¹Philippe de Witte,**
22 **²Robert R Crichton, ³Jacques Piette ⁴Keith Tipton,**
23 **⁵Karl Hemmings, ⁵Arnaud Pitard, ⁵Mike Page,**
24 **⁶Laura Della Corte ⁷Deanna Taylor, ⁷David Dexter**
25
26
27
28
29

30 ¹*Biologie du Comportement, ²Unite de Biochimie, Université Catholique de Louvain,*
31 ³*University of Liege Belgium, ⁴Trinity College Dublin Ireland, ⁵University of*
32 *Huddersfield, UK, ⁶University of Florence, Italy and ⁷Imperial College, London, UK*
33
34
35
36
37
38
39
40
41

42 Communicating Author

43
44 Roberta J Ward
45 Biologie du Comportement,
46 University of Louvain
47 1 Croix de Sud
48 Louvain-la-Neuve
49 1348 BELGIUM
50 Phone. 0032 10456004
51 Fax 003210456004
52 roberta.ward@uclouvain.be
53
54
55
56
57
58
59
60
61
62
63
64
65

Abstract

1
2 The ability of a taurine prodrug, ethane β -sultam, to reduce cellular inflammation has
3
4 been investigated, *in vitro*, in primary cultures of alveolar macrophages and an
5
6 immortalised N9 microglial cell line and *in vivo* in an animal model of inflammation
7
8 and control rats. Ethane β -sultam showed enhanced ability to reduce the
9
10 inflammatory response in alveolar macrophages, as assayed by the
11
12 lipopolysaccharide-stimulated nitric oxide release, (LPS stimulated-NO), in
13
14 comparison to taurine both *in vitro* (10 nM, 50 nM) and *in vivo* (0.15mmol/kg/day by
15
16 gavage). In addition, ethane β -sultam, (50, 100 and 1000nM) significantly reduced
17
18 LPS-stimulated glutamate release from N9 microglial cells to a greater extent than
19
20 taurine. The anti-inflammatory response of taurine was shown to be mediated via
21
22 stabilisation of I κ B α . The use of a taurine prodrug as therapeutic agents, for the
23
24 treatment of neurological conditions, such as Parkinson's and Alzheimer's disease
25
26 and alcoholic brain damage, where activated phagocytic cells contribute to the
27
28 pathogenesis, may be of great potential.
29
30
31
32
33
34
35
36

37 Key Words: taurine: anti-inflammatory action: ethane- β sultam: nitric oxide:
38 glutamate
39

40 Abbreviations: NO-nitric oxide: TauT-taurine transporter: LPS-lipopolysaccharide:
41 ROS- reactive oxygen species: I κ B-IkappaB: I κ B α -IkappaBalpha: NF κ B-nuclear
42 factor kappa B: TNF α -tumor necrosis factor alpha:
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1. Introduction

1
2 The sulphonated amino acid taurine is widely distributed in mammalian
3 tissues, and is present at high concentrations, ~ 50 mM, in leucocytes, microglia and
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

The sulphonated amino acid taurine is widely distributed in mammalian tissues, and is present at high concentrations, ~ 50 mM, in leucocytes, microglia and macrophages, [1], where it plays an important anti-inflammatory role. The mechanisms underlying the cytoprotective actions of taurine appear to depend, to a large extent, on the cellular type. The anti-inflammatory actions of taurine are manifested in a variety of forms, including its reaction with hypochlorous acid, in the presence of myeloperoxidase, to form the more stable and less toxic, taurine chloramine (Tau-Cl) in activated neutrophils, as well as modulation of calcium ion homeostasis [2]. The mode of taurine's anti-inflammatory action remains undefined since most studies have investigated the effects of Tau-Cl in immortalised macrophage cell lines, such as RAW 264 and NR8383 (for example see [3]). Since activated phagocytic cells, namely microglia, play an important role in many neurodegenerative conditions, such as Parkinson's disease [4] and alcohol-induced brain damage [5], compounds with anti-inflammatory actions, which are able to traverse the blood brain barrier may have therapeutic actions in the retardation of the disease processes.

Although taurine can be synthesised intracellularly from cysteine and methionine, the diet is the main source. Taurine uptake from the plasma into cells is tightly controlled by the taurine transporter (TauT). TauT controls the influx of taurine within very narrow limits, such that exogenously administered taurine only transiently increases levels within a number of cellular types, including liver [6] and, to a lesser extent, brain [7] [8] [6]. TauT activity can be modified by a number of factors including inflammation, where it is decreased [9] [10] [11], calcium ions and nitric oxide [9], glucocorticoids [12], protein kinase C activation in rat astroglial cells

1 [13] and human glioma GL15 cells [14]. Therefore taurine analogues, which are able
2 to traverse membranes independently of TauT, may enhance intracellular taurine
3 levels thereby promoting anti-inflammatory pathways.
4
5

6
7 It remains unclear as to how modifications to the taurine molecule might
8 enhance its uptake across the lipid membrane bi-layer into the cell or passage across
9 the blood brain barrier, BBB, since it would be against a concentration gradient. The
10 ability of the taurine analogue, acamprosate, calcium acetyl homotaurinate, to reduce
11 alcohol craving in detoxified alcohol abusers, is attributed to the presence of the
12 calcium moiety and the *N*-acetyl homotaurine, which is reputed to enhance its passage
13 across the blood brain barrier to bind with the metabotropic glutamate 5 receptors
14 [15].
15
16
17
18
19
20
21
22
23
24
25

26
27 Macrophages play an integral role in the development of innate and adaptive
28 immune responses against bacterial pathogens. NFkappaB, one of the most
29 ubiquitous transcription factor, plays a pivotal role when macrophages are activated,
30 partly via increased IL-6 and iNOS expression, [16] [17] [18]. Inducible nitric oxide
31 synthase (iNOS) is responsible for generating high levels of nitric oxide (NO) in
32 activated macrophages. Under conditions favouring the production of high
33 concentrations of both NO and superoxide anion, the highly reactive peroxynitrite
34 anion is also generated. Among the many inflammatory mediators produced by
35 activated phagocytic cells, i.e. macrophages and microglia, NO production has been
36 widely regarded as representative of inflammatory activation [19] [20]. Therefore
37 inhibitors which, preferentially target molecules that are involved in NFkappaB
38 activation may be of therapeutic value.
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54

55
56 In the present studies, a new taurine prodrug has been synthesised, ethane
57 β -sultam. Its mode of passage across the cellular membrane remains unknown.
58
59
60
61
62
63
64
65

1 Intracellularly ethane β -sultam will hydrolysed to taurine, **Figure 1**. It's anti-
2 inflammatory action has been studied both *in vivo* and *in vitro*, in alveolar
3
4
5 macrophages isolated from rats, supplemented or not with ethane β -sultam as well as
6
7
8 *in vitro* in an immortalised microglial cell line, N9.
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2. Material and Methods.

All tissue culture media and chemicals were purchased from Sigma (Belgium) unless stated otherwise.

2.1. Synthesis of ethane β -sultam

All chemicals were purchased from Aldrich and were used without further purification. Taurine sulfonyl chloride (30.4g, 169mmol) was added to finely ground sodium carbonate (35.9g, 339mmol) in ethyl acetate (950ml) and stirred at ambient temperature for 48 hours. The reaction mixture was filtered through celite and the solvent removed by reduced pressure rotary evaporation at 30°C, giving a fine white powder (15.9g, 89%). Melting points were determined on a Gallenkamp melting point apparatus, and were 50-51°C [lit. 51-52°C (Page, 2004)]. 400 MHz ^1H and 67 MHz ^{13}C NMR spectra were determined on a Bruker Advance 400MHz spectrometer, while for the 500Mhz ^1H and 100Mhz ^{13}C NMR spectra a Bruker AMX 500 spectrometer was utilized. The results were; ^1H NMR: δ (CDCl_3) 3.39 (2H, dt, J 4 and 7, CH_2N); 4.32 (2H, dt, J 2 and 7, CH_2SO_2); 5.53 (1H, bs, NH). ^{13}C NMR: δ (CDCl_3) 60.6, 26.8. Infra-red measurements were determined on a Gallenkamp melting point apparatus and were: ν_{max} (cm^{-1}): 3307, 3048, 3022, 2991, 2918, 1416, 1336, 1299, 1249, 1212, 1171, 1156, 1107, 966, 803, 760, 668, 615. GC-MS were determined on a Varian GC-MS with a Finnigan MAT ion trap detector. For mass spectrometry a Fisons Quatro VG quadrupole mass spectrometer was utilised; m/z (GC-MS) (M^+H):108, 77, 54, 42 . The pK_a of ethane β -sultam was determined by titration using the reversible chromophoric change at 230nm, while hydrolysis of ethane β -sultam to the β -amino acid taurine as a function of pH was followed at 300nm.

2.2.Cell culture studies

Isolation and cultures of alveolar macrophages

1
2 Alveolar macrophages were isolated from the rats by pulmonary lavage. In brief, the
3
4 rats were anaesthetised with Nembutal, a catheter inserted into the trachea, and the
5
6 lungs lavaged gently with phosphate buffered saline, pH 7.4, approximately 50 ml.
7
8 The alveolar macrophages were recovered after centrifugation at 1,200rpm for 10
9
10 min, and cell viability measured by trypan blue uptake extrusion (>98%). The cells
11
12 at densities of 1×10^5 or 2×10^5 cells, were plated in wells in Dubecco media
13
14 supplemented with 10% foetal calf serum, containing penicillin (100 μg /ml) and
15
16 streptomycin (100 μg /ml). The cells were left to adhere overnight, washed, and re-
17
18 suspended in culture media to which lipopolysaccharides, LPS, (1 μg /ml) or LPS
19
20 (1 μg /ml) + interferon-gamma, 100 U/ml were added; and the cells were left for a
21
22 further 24 h. The supernatants were removed and stored at -20°C prior to analyses.
23
24
25
26
27
28

Preparation of immortalised N9 glial cells

29
30 The N9 microglial cell line was donated by Dr Paola Ricciardi Castagnoli (CNR
31
32 Cellular and Molecular Pharmacology Centre, Milan, Italy). The cell line was
33
34 originally derived from embryonic day 13 mouse microglial cultures [21]. The
35
36 responses from these cells are very similar to those from primary rat microglia [22]:
37
38 [23]. N9 cells were maintained in DMEM supplemented with 5% fetal bovine serum,
39
40 50 μM β -mercaptoethanol, 50 U/l penicillin and 50 μg /ml streptomycin at 37°C in 5%
41
42 CO_2 . The immortalised glial cells, N9 were grown to confluence and cells recovered
43
44 after centrifugation at 1,200 rpm for 10 minutes. Cell viability was measured by
45
46 trypan blue uptake extrusion (>98%). The glial cells were then plated at densities of 1
47
48 $\times 10^5$ or 2×10^5 cells, in Dubecco media supplemented with 10% foetal calf serum,
49
50 containing penicillin (100 μg /ml) and streptomycin (100 μg /ml).
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2.3. Cellular studies of the anti-inflammatory action of taurine and the pro-drug ethane β -sultam

In vitro administration of taurine and ethane β -sultam with alveolar macrophages

Isolated alveolar macrophages were incubated with taurine or ethane β -sultam, 10 and 50 mM for 24h. The cells were then stimulated with LPS, 1 μ g/ml, for a further 24 h. The cell supernatants were then removed and both nitrite and glutamate content assayed.

In vitro studies of taurine and ethane β -sultam with immortalised N9 glial cells

N9 glial cells were incubated with taurine or ethane β -sultam, for 24h. The cells were then incubated with LPS, (1 μ g/ml) for 24h. The supernatants were then removed and assayed for nitrite and glutamate content.

Nitrite assay

Aliquots, 100 μ l, of the cell culture supernatant were mixed with an equal volume of Griess reagent (1% sulphanilamide, 0.1% naphthalene diamine dihydrochloride, and 2.5% phosphoric acid) to determine nitrite concentration. After incubation at room temperature for 10 minutes, the optical density was measured at 560 nm. Sodium nitrite standards, in the range of 1-50 μ M, were prepared

Glutamate assay

Aliquots, 250 μ l, of the cell culture supernatant, + 640 μ l water, were mixed with Tris (0.1M)-EDTA (0.002M)-hydrazine (64%) to which β -nicotinamide adenine dinucleotide (30mM) and adenosine 5'-diphosphate (100mM) had been added. The absorbance was read at 340nm after which glutamic dehydrogenase, 24 U, was added. The absorbance was re-read after 40 minutes incubation. L-glutamate standards in the range 100-500mM were prepared.

IL-6 quantification

IL-6 ELISA kit (R&D Systems, Abingdon, UK) was used for the quantitative measurement of this cytokine in the supernatants.

2.4. Animal experiments

Male Wistar rats 200-250g, were housed in polypropylene cages (*Iffa Credo, Belgium*) and allowed *ad libitum* access to normal diet (*Credo, Belgium*) and water.

All animal procedures were in strict accordance with the recommendations of EEC (86/609/CEC) and with the Belgian 'projet de loi' (Moniteur Belge 19.02.1992, p. 3437) on the care and use of laboratory animals.

In vivo administration of taurine and ethane β -sultam to control rats

Groups of rats, 200-220g, n=6 in each group, were administered, taurine or ethane β -sultam, 0.15 mmol/kg/day, by gavage, volume = 1.5ml, at 10.00 am each day for 7 days. After this time, the alveolar macrophages were isolated by pulmonary lavage from each group of rats as well as from control rats that had received water, 1.5 ml, by gavage. The cells were stimulated with LPS, 1 μ g/ml, for a further 24 h. The cell supernatants were then removed and nitrite and glutamate content assayed.

In vivo administration of ethane- β -sultam to a rat model of inflammation

A rat model of neuroinflammation has recently been described where ethanol is administered 3x/day for 2 days which is followed by 5 days of abstinence, for a period of 3 weeks [24]. Rats, 100-150g, n=6 in each group were administered the 1g ethanol /kg regime, supplemented or not with one dose/day of ethane- β -sultam, 0.15 mmol/kg/day, which was given by gavage, 30 minutes before the first ethanol administration. Ethane- β -sultam was administered daily for 3 weeks. Alveolar macrophages were isolated, plated at densities of 1×10^5 , and then stimulated with LPS for 24h. The supernatants were then removed and both NO and IL-6 assayed.

2.5.Mechanism of taurine's anti-inflammatory action

NFKappaB analysis in alveolar macrophages isolated from taurine supplemented rats

Rats received taurine orally, 12.5g/l, in the drinking water for 7 days, daily intake = 500 mg/day. = 15mmol/kg/day. The alveolar macrophages were isolated by pulmonary lavage and stimulated with LPS 1 µg/ml and TNFα, 4 U/ml, for 15, 30 and 60 minutes. Nuclear proteins were then isolated from the alveolar macrophages. In brief, the cells were suspended in ice-cold buffer A (10 mM HEPES/KOH, 2 mM MgCl₂, 0.1 mM EDTA, 10 mM KCl, 1 mM DTT, 0.5 mM phenylmethylsulphonyl fluoride (PMSF), pH 7.9) and left on ice for 20 min before being vortex-mixed and centrifuged at 15,000 x g for 30 s. The nuclear pellets were gently suspended in cold buffer B (50 mM HEPES/KOH, 50 mM KCl; 300 mM NaCl, 0.1 mM EDTA, 10% (by volume) glycerol, 1 mM DTT, 0.5 mM PMSF, pH 7.9) and left on ice for 20min. After centrifugation at 15,000x g for 5 min at 4°C, aliquots of the supernatant containing the nuclear fraction were rapidly frozen in two aliquots in liquid nitrogen and stored at -80°C. The protein concentrations were assayed, by the BioRad method, in one of the aliquots prior to the electrophoretic mobility shift assay.

The nuclear fraction containing 20 µg protein was incubated for 30 min at room temperature with 0.2 ng ³²P-labelled oligonucleotide probe,

5' GATCAGGGACTTTCGCTGGGGACTTTCAG-3', 1 mg BSA and 1.25 mg poly(dI-dC), poly(dI-dC) (Pharmacia Biotech Benelux) in buffer (20 mM HEPES/KOH, 75 mM NaCl, 1 mM EDTA, 5% (by volume) glycerol, 0.5 mM MgCl₂, 1 mM DTT, pH 7.9) in a final volume 20 µl. DNA-protein complexes were resolved on a non-denaturing 6% (w/v) polyacrylamide gel, run for 4 h at 180 V in buffer (2.5 mM Tris, 2.5 mM H₃BO₃, 2 mM EDTA, pH 8.5). The gel was then dried and autoradiographed on Fuji X-ray film (General Electrics, Antwerp, Belgium). For

1 competition experiments, unlabelled probe, either wild type 5'
2 GATCAGGGACTTTCCGCTGGGGACTTTCCAG-3' or mutated.
3

4 5'-GATCACTCACTTTCCGCTGCTCACTTTCCAG-3' was added in excess
5 (50x) in buffer. In each experiment the band of the DNA-protein complex from the
6 unstimulated and stimulated macrophages was verified by comparison to the band
7 obtained after stimulation of U937 with LPS, 10 µg/ml. The intensity of each of the
8 NFkappaB complex was quantitated.
9

10 *Ikbα studies in alveolar macrophages isolated from taurine supplemented rats*

11 Rats received taurine in their drinking water at three different concentrations,
12 6.25, 12.5 and 25 g/l for 8 days. Alveolar macrophages were isolated from each
13 treatment group, pooled, and then activated with LPS, (1 µg/ml) and TNFα (4 U/ml)
14 for 60 minutes. IκBα was detected by Western blot analysis using a specific anti-
15 human full length IκBα polyclonal antibody (Euromedex, Souffel Weyersheim,
16 France). Cytoplasmic extracts, each containing 10 µg protein, isolated from the
17 activated macrophages, were mixed with the loading buffer (10mM Tris-HCl, pH6.8,
18 1% SDS, 25% glycerol, 0.1 mM 2-ME and 0.03% bromophenol blue), prior to their
19 loading onto 10% polyacrylamide-SDS. After electrophoresis, the resultant gels were
20 electro-transferred onto Immobilon-P membranes (Millipore, Bedford, MA). Filters
21 were incubated in a primary antibody for 120 minutes, (1/10000 dilution) and in
22 peroxidase-conjugated rabbit anti-body IgG (1/1000 dilution:DAKP, Copenhagen,
23 Denmark) for 60 minutes at room temperature and finally analysed by Amersham's
24 enhanced chemiluminescence system (Amersham, Aylesbury, UK) with Fuji Xray
25 film. Coomassie blue staining was used to confirm that equal amounts of protein had
26 been applied to each lane. The density of each of the IκBα band was quantitated.
27

28 *Statistical analysis.*

1 All of the results are presented as mean \pm standard deviation. Statistical
2 analysis was by ANOVA 1. Significance was calculated by GB Stat programme and
3
4 set at $P < 0.05$ by Fischer test.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Accepted Manuscript

3. Results

3.1. Hydrolysis of ethane- β -sultam to taurine.

The hydrolysis of the ethane-1,2- β -sultam occurs with exclusive S-N fission and there is no NMR evidence of any reactions involving either C-S or C-N bond breaking, **Figure 2**. The pH rate profile for the unsubstituted β -sultam shows only reactions that are either first order in hydronium-ion or hydroxide-ion concentration, but in aqueous sodium hydroxide solutions the rate becomes pH independent.

The pK_a of ethane β -sultam was found to be 12.1 by means of a reversible chromophoric change at 230nm. In aqueous sodium hydroxide (NaOH) solutions a slow exponential decay of the chromophore of ethane β -sultam was observed at 300 nm and 30°C. The absorbance change had a very slow rate, which was found to be independent of hydroxide-ion concentration and gave a first order rate constant of $1.00 \times 10^{-5} \text{ s}^{-1}$. Above pH 7 ethane 1,2- β -sultam undergoes attack by hydroxide ion at the sulfonyl centre resulting in ring opening *via* cleavage of the S-N bond and formation of the β -amino sulfonic acid, taurine. As a result, in solutions of pH greater than the pK_a of ethane β -sultam the rate of hydrolysis becomes pH independent as it depends on the concentration of the minor species, the neutral β -sultam, which decreases as the pH is increased. The second order rate constant, k_{OH} , for the alkaline hydrolysis of the β -sultam is $5.16 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ which is 20-fold lower than the second-order rate constants for the hydroxide ion hydrolysis, k_{OH} , of simple *N*-alkyl- β -sultams e.g. *N*-methyl ($1.41 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$). At pH 7 ethane-1,2- β -sultam is relatively stable and only very slowly hydrolysed to taurine. Below pH 7, ethane-1,2- β -sultam undergoes an acid catalysed hydrolysis to taurine.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

3.2. *In vitro studies of the anti-inflammatory action of taurine or ethane β -sultam in alveolar macrophages isolated from control rat and immortalised N9 microglial cells.*

The release of NO and glutamate from isolated alveolar macrophages after LPS or LPS + IFN γ stimulation is shown in **Figure 3a**. A threefold increase in NO release was evident after LPS stimulation which increased to 4 fold with LPS + IFN γ stimulation. No changes in glutamate release were apparent after stimulation with either LPS or LPS + IFN γ , **Figure 3a**. In these initial studies it was important to ascertain whether ethane- β -sultam showed superior anti-inflammatory effects to that of taurine. **Figure 3b** clearly showed that both taurine and ethane- β -sultam reduced nitrite release in LPS-stimulated alveolar macrophages, the latter to a greater extent than taurine. Levels of glutamate present in the supernatants from un-stimulated and stimulated alveolar macrophages did not alter after LPS stimulation (data not shown). **Figure 4a** shows the release of NO and glutamate from N9 cells after stimulation with LPS or LPS + IFN γ . As can be observed there was a dramatic increase in both of these mediators of inflammation, particularly after LPS + IFN γ . Supernatant glutamate levels in un-stimulated microglial cells were approximately 90 μ M \pm 9, and increased significantly after LPS stimulation, **Figure 4a**. Both ethane- β -and taurine showed an enhanced anti-inflammatory action, as assessed by nitrite release, in LPS-stimulated N9 glial cells, **Figure 4b**. The levels of glutamate released after LPS stimulation were reduced by all doses of ethane- β -sultam, 50 nM, 100 nM and 1000nM by comparison to stimulated controls, but only with the highest taurine dose, **Figure 4c**.

3.3. *In vivo studies of the anti-inflammatory action of taurine and ethane β -sultam in alveolar macrophages isolated from control rats.*

1 The alveolar macrophages were isolated from rats which had received daily
2 doses of either ethane β -sultam or taurine, 0.15 mmol/kg/day, by gavage or water for
3
4
5 7 days. These cells were cultured and then stimulated with LPS for 24h. Nitrite
6
7 release was significantly decreased by both taurine and ethane- β -sultam, the latter to
8
9 a greater extent than taurine, **Figure 5**.

10
11
12 *3.4 In vivo studies of the anti-inflammatory action of ethane β -sultam in alveolar*
13
14 *macrophages isolated from rats with alcohol-induced inflammation.*

15
16
17 Binge drinking over a 3 week period activates the innate immune system which is
18
19 verified by activated macrophages in the periphery and neuro-inflammation in the
20
21 brain (Ward et al., 2009). The administration of ethane- β -sultam during the binge
22
23 drinking regime reduced the activation of the innate immune system which was
24
25 confirmed by decreases in the release of both NO and IL-6, **Figure 6a and Figure**
26
27 **6b**.

31 **Investigation of taurine's anti-inflammatory action**

32
33
34 *3.5. NF κ B activation in macrophages.*

35
36
37 **Figure 7a** shows the electrophoretic mobility-shift assay for the activation of
38
39 NF κ B by LPS and TNF α in alveolar macrophages isolated from either taurine
40
41 supplemented, stimulated at 15, 30 and 60 minutes, or control rats, stimulated at 15,
42
43 30, 60 and 120 minutes. The density band values identified the higher NF κ B
44
45 activation in the controls at each time interval by comparison to the taurine
46
47 supplemented rats, **Figure 7b**. It was noteworthy that NF κ B activation was reduced
48
49 at time 0 and at each of the subsequent time points assayed in the taurine
50
51 supplemented alveolar macrophages by comparison to the controls.
52
53
54
55
56

57 *3.6. IkappaB α stabilisation.*
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 8a shows a Western blot for the macrophage cytosolic extracts, which had been immobilised on the membrane and then probed with I κ B α protein. The levels of I κ B α increased in the macrophage cytosolic fraction as the concentrations of taurine administered to the rats increased. The density band intensities showed that there was a significant increase with increasing taurine concentrations, **Figure 8b**.

4. Discussion

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

In these present studies we have shown that the pro-drug ethane β -sultam showed an enhanced ability to diminish the inflammatory response after stimulation with LPS in comparison to taurine. Variable results for the anti-inflammatory action of taurine *in vitro*, have previously been presented. For example, its protective effect in co-cultures was reported in intestinal Caco-2 cell monolayers from the damage by macrophage-like THP-1 cells,[25] while no effect was reported, in peritoneal neutrophils [26], RAW 264.7 cells [27],or peripheral blood mononuclear cells [28]. In contrast the anti-inflammatory actions of its chlorinated form, taurine chloramine, *in vitro*, inhibits pro-inflammatory mediators, such as nitric oxide and $\text{TNF}\alpha$, in a variety of activated cell lines, including rodent macrophages [29] NR8383 cells, [3] and peripheral blood mononuclear cells [28]. The lack of an effect of taurine alone in such systems may be attributable to its limited ability to enter cells, due to TauT down-regulation, as well as to the specific cell line employed, [30].

The transcription of TauT is up-regulated by a number of compounds by virtue of the presence of various recognition sites on its promoter region. These sites include the TonE hypertonicity site (part of the TonE (tonicity-responsive element)/TonEBP (TonE-binding protein) system [31] the TPA responsive element (TGAGTCAG), which is responsible for gene regulation by the protein kinase C (PKC)-mediated signal transduction pathway[32] the glucose tumour suppressor gene, p53 [33], $\text{TNF}\alpha$ and an NF κ B consensus-like sequence [34].

It was our intention in these studies to overcome such restrictions on its regulation by the use of taurine analogues and prodrugs that could circumnavigate the homeostatic control exerted by TauT. Previously other taurine pro-drugs have been investigated for their anti-inflammatory action, e.g. 5-aminosalicyltaurine, which

1 liberates taurine and 5-aminosalicylic acid, and was reported to ameliorate chemically
2 induced colitis after rectal administration [35]. In our recent studies of chronically
3 alcoholised rats, oral administration of Acamprosate (400 mg/kg/day), calcium acetyl
4 homotaurinate, during the chronic ethanol intoxication procedure exhibited an anti-
5 inflammatory effect by decreasing the formation of reactive oxygen radical species
6 during the initial detoxification period, in comparison to rats chronically ethanol
7 intoxicated alone [36]. The exact mode of action remains unknown, but the presence
8 of the acetyl and calcium moiety, as well as homotaurine contributed to its protective
9 properties, mediated by metabotropic GluR5 receptors in various brain regions [15].
10 In the present studies, we clearly showed that in vitro and in vivo, ethane β -sultam
11 exhibited greater anti-inflammatory actions than taurine in both alveolar macrophages
12 and microglial cells after ex vivo LPS stimulation. Furthermore, it was shown that
13 there was an increase in taurine levels in specific brain regions after three weeks of
14 ethane- β -sultam (Della Corte, Dexter, Ward unpublished data) thereby confirming
15 that the prodrug had indeed traversed cellular membranes. In contrast it is extremely
16 difficult to increase brain taurine levels unless extremely high doses of taurine are
17 administered.

18 Microglia are the resident immune cells of the central nervous system and play
19 an important role in preserving the neurons. However when microglia are activated,
20 by pro-inflammatory stimuli, substantial levels of glutamate are released [37]. This is
21 due to the activation of NADPHoxidase which generate superoxide, leading to the
22 formation of hydroxyl radical via Fenton chemistry, resulting in oxidative stress.
23 Depletion of reduced glutathione will occur, which will be remedied by the synthesis
24 of more of this antioxidant by the influx of cystine via the Xc exchange system which
25 releases glutamate [37]. In these present studies increases in glutamate release were

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
apparent in microglia, but not macrophages, after bacterial stimulation. Expression of
inducible nitric oxide synthase will also be enhanced after microglial activation as
was evident in these present studies. Nitric oxide will deplete ATP production, and
inhibit cytochrome oxidase in competition with oxygen, thereby inducing hypoxia and
activating the hypoxia-inducible factors, which will result in the release of glutamate
and excitotoxicity [38]. However such glutamate release may also stimulate microglia
to produce neurotrophic factors which will support neuronal survival and growth.
This is mediated via increases in intracellular calcium mediated partly by the
stimulation of NMDA receptors and group III metabotropic glutamate receptors
which induces intracellular calcium release from the endoplasmic reticulum, as well
as stimulation of glutamate transporters to increase influxes of extracellular calcium
[39]. Such increases in intracellular calcium will lead to activation of the protein
kinase C pathway which induces microglial neurotrophic expression and production.

31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
Beta sultams are sulfonyl analogues of β -lactams, and activated derivatives are
able to inactivate serine enzymes, such as elastase, which is released in response to
inflammatory stimuli and plays a major role in protein digestion following
phagocytosis, and β -lactamase, (which is the main cause of bacterial resistance to
antibiotics) by sulfonation of the active site serine [40]. The parent unsubstituted β -
sultam does not inhibit serine enzymes but is slowly hydrolysed to taurine. The
potential therapeutic use of ethane β -sultam as a prodrug has several advantages
compared to taurine. These include an increased lipophilicity, which may facilitate its
uptake by the cell prior to transformation to taurine [41]. However it should be
emphasised that simple diffusion of taurine or its pro-drugs across a lipid bi-layer
against a concentration gradient, (i.e. the high concentration of taurine within the
cell), might indicate that such prodrugs have an alternative passage across the

1 membrane, possibly via a protein transporter. In the present studies ethane β -sultam
2 showed superior anti-inflammatory properties, both *in vivo* and *in vitro* to those of
3 taurine.
4
5

6
7 NFKappaB is an important transcription factor that regulates genes involved in
8 immunity and inflammation [42]. Under normal conditions NFKappaB is present in
9 the cytoplasm in an inactive state bound to the inhibitory protein I kappaB (I κ B).
10 Stimulation with pro-inflammatory cytokines such as TNF α initiates an intracellular
11 signalling cascade, resulting in the phosphorylation and subsequent degradation of
12 I κ B by the 26S-proteasome. The degradation of I κ B α releases NFKappaB allowing
13 its translocation to the nucleus, resulting in the activation of cyclooxygenase-2 (COX-
14 2) cytokines, chemokines, cell surface receptors and adhesion molecules that are
15 pivotal mediators of the immune and inflammatory responses. Therefore, therapeutic
16 intervention against NFKappaB activation might be advantageous in preventing the
17 progression of inflammation-related diseases. Previous studies have focused
18 primarily on the action of taurine chloramine on this transcription factor. Taurine
19 chloramine was shown to depress the migration of NFKappaB to the nucleus of
20 activated NR8383 cells, [3] and murine peritoneal macrophages and RAW 264.7 cells
21 [43]. Attenuation of ERK $^{1/2}$ activation was also reported in the latter study.
22 Treatment with taurine chloramine will result in decreased phosphorylation [44] [3]
23 and oxidation of I κ B α [1] and a lower activity of I κ B kinase, [3]. The present *in vivo*
24 studies demonstrated a clear interaction between taurine and I κ B α , with taurine
25 showing a dose dependent stabilisation of this inhibitory protein. Although these
26 studies were not undertaken with ethane- β -sultam, the corresponding changes in both
27 NO and IL-6 after the incubation with this taurine pro-drug would confirm indirectly
28 that these changes were occurring via changes in NFKappaB activation. Such changes
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

in IL-6 release are also important in decreasing the iron loading of the tissues, since decreases in IL-6 will diminish hepcidin secretion thereby preventing the internalisation of iron via the interaction of hepcidin with ferroportin [45].

It has been reported that elderly patients (61-81 years) have significantly lower blood taurine concentrations, compared with younger individuals (20-38 years) [46]. Such a decline could exacerbate the oxidative damage that occurs during the ageing process as well as the modulation of various neurotransmitter systems and immune responsiveness. Taurine supplementation might reverse these effects [47]. In this respect, the taurine prodrug, studied here, which may exert their beneficial effect independently of TauT, might be particularly valuable. With an ever-increasing older population, palliative measures to retard the effects of ageing and neurodegenerative processes could be of enormous financial and social advantages.

Acknowledgements

Financial support from IREB, (RJW), ERAB (RJW, DTD and LDC) and COST D34
are gratefully acknowledged

Accepted Manuscript

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- [1]. Schuller-Levis GB, Park E Taurine and its chloramines: modulators of immunity. *Neurochem Res* 2004; 29: 117-126.
- [2]. Della Corte L, Crichton RR, Duburs G, Nolan K, Tipton KF, Tirzitis G, Ward RJ. The use of taurine analogues to investigate taurine functions and their potential therapeutic applications. *Amino Acids* 2002; **23**: 367-379.
- [3]. Barua M, Liu Y, Quinn MR, Taurine chloramine inhibits inducible nitric oxide synthase and TNF-alpha gene expression in activated alveolar macrophages: decreased NFkappaB activation and IkappaB kinase activity. *J Immunol* 2001; 167: 2275-2281.
- [4]. Wilms H, Zecca L, Rosenstiel P, Sievers J, Deuschl G, Lucius R. Inflammation in Parkinson's diseases and other neurodegenerative diseases: cause and therapeutic implications. *Curr Pharm Des* **13**: 1925-1928.
- [5]. Suk K (2007). Microglial signal transduction as a target of alcohol action in the brain *Curr Neurovasc Res* 2007; 4: 131-142.
- [6]. Ward RJ, Cirkovic-Velichovia T, Ledequé F, Tirizitis G, Dubars G, Datla K, Dexter D, Heuschling P, Crichton R. Neuroprotection by taurine and taurine analogues *Adv Exp Med Biol* 2006; 583:299-306.
- [7]. Shimada M, Shimono R, Watanabe M, Imahayash, T, Ozaki HS, Kihara T *et al.* Distribution of ³⁵S-taurine in rat neonates and adults. A whole-body autoradiographic study. *Histochemistry* 1984; 80: 225-230.
- [8]. Kim C, Chung JK, Jeong JM, Chang YS, Lee YJ, Kim *et al.* Uptake of taurine and taurine chloramines in murine macrophages and their distribution in mice with experimental inflammation. *Adv Exp Med Biol* 1998; 442: 169-176.
- [9]. Kim HW, Lee EJ, Kim WB, Kim BK. Ionomycin restores taurine transporter activity in cyclosporine-A treated macrophages. *Adv Exp Med Biol* 2000; 483:127-135.
- [10]. Romio L, Zegarra-Moran L, Varesio L, Galietta LJV. Regulation of taurine transport in murine macrophages. *Amino Acids* 2001;21: 151-160.
- [11]. Kim HW, Kim JH, An HS, Park KK, Park T. Myo-inositol restores the inflammation-induced down regulation of taurine transport by the murine macrophage cell line, RAW 264.7. *Life Sci* 2003; 73:2477-2489.
- [12]. Kim H.W., Kim, M.J., Shim, W.B., Kim, W.B., Kim B.K. Regulation of taurine transporter activity by glucocorticoid hormone *J Biochem Mol Biol* 1995; 28: 527-532.

- 1 [13]. Tchoumkeu-Nzouessa GC, Rebel G. Activation of protein kinase C
2 downregulates glial but not neuronal taurine uptake *Neurosci Lett* 1996a; 206: 61-64.
3
- 4 [14]. Tchoumkeu-Nzouessa GC, Rebel G. Characterisation of taurine transport in
5 human glioma GL 15 cell line: regulation by protein kinase C. *Neuropharmacol*
6 1996b; 35: 37-44.
7
8
- 9 [15]. De Witte P, Littleton J, Parot P, Koob G. Neuroprotective and abstinence-
10 promoting effects of acamprosate elucidating the mechanism of action. *CNS Drugs*
11 2005; 19: 517-537.
12
13
- 14 [16]. Miggin SM, O'Neill LA. New insights into the regulation of TLR signalling. *J*
15 *Leukoc Biol* 2006; 220-226
16
17
- 18 [17]. Vazquez-Torres A, Fang FC. Oxygen-dependent anti-Salmonella activity of
19 macrophages. *Trends Microbiol* 2001; 9: 29-33.
20
21
- 22 [18]. Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and
23 controversies. *Nat Rev Microbiol* 2004; 2: 820-832.
24
25
- 26 [19] Jones E, Adcock IM, Ahmed BY, Panchard NA. Modulation of LPS stimulated
27 NF-kappaB mediated nitric oxide production by PKC ϵ and JAK2 in RAW
28 macrophages. 2007. *J Inflamm* 4;23
29
- 30 [20] Ward RJ, Wilmet S, Legssyer R, Deroy D, Crichton RR, Srail K, Pirreaux C, Hue
31 L, Piette J, Kleon D, Summer K. Iron supplementation to pregnant rats: effects on
32 pregnancy outcome, iron homeostasis and immune function 2009 *Biomaterials* 22; 211-
33 223.
34
35
- 36 [21]. Corradin SB., Mauel J., Donini SD., Quattrocchi E., Ricciardi-Castagnoli P.
37 Inducible nitric oxide synthase activity of cloned murine microglial cells. *Glia* 1993,
38 7: 255-262.
39
40
- 41 [22]. Kingham PJ., Pocock JM. Microglial apoptosis induced by chromogranin A is
42 mediated by mitochondrial depolarisation and the permeability transition but not by
43 cytochrome c release. *J Neurochem* 2000, 74: 1452-1462
44
45
- 46 [23]. Taylor DL., Jones F., Kubota ES., Pocock JM. Stimulation of microglial
47 metabotropic glutamate receptor mGlu2 triggers tumor necrosis factor alpha-induced
48 neurotoxicity in concert with microglial-derived Fas ligand. *J Neurosci* 2005,
49 16:2952-2964.
50
51
- 52 [24] Ward RJ, Colivicchi MA, Allen R, Schol F, Lallemand P, De Witte P, Ballini C,
53 Della Corte L, Dexter D. Neuro-inflammation induced in the hippocampus of 'binge
54 drinking' rats may be mediated by elevated extracellular glutamate content 2009 *J*
55 *Neurochem* 111; 1119-1128.
56
57
- 58 [25]. Zhao Z, Satsu H, Fujisawa M, Hori M, Ishimoto Y, Nambu A *et al.* Attenuation
59 by dietary taurine of dextran sulfate sodium-induced colitis in mice and of THP-1-
60
61
62
63
64
65

1 induced damage to intestinal Caco-2 cell monolayers. *Amino Acids* 2007; 35: 217-
2 224

3 [26]. Marcinkiewicz J, Grabowska A, Berata J, Bryniarski K, Nowak, B. Taurine
4 chloramine down-regulates the generation of murine neutrophil inflammatory
5 mediators. *Immunopharmacol* 1998; 40: 27-38.

6
7
8 [27]. Kim C, Park E, Quinn MR, Schuller-Levis G. The production of superoxide
9 anion and nitric oxide by cultured murine leukocytes and the accumulation of TNF-
10 alpha in th conditioned media is inhibited by taurine chloramine. *Immunopharmacol*
11 2003; 34: 89-95.

12
13
14 [28]. Chorazy M, Kontny E, Marcinkiewicz J, Maslinski W. Taurine chloramine
15 modulates cytokine production by human blood mononuclear cells. *Amino Acids*
16 2002; 23: 407-413.

17
18
19 [29]. Park E, Jia J, Quinn MR, Schuller-Lewis G. Taurine chloramine inhibits
20 lymphocyte proliferation and decreases cytokine production in activated human
21 leukocytes. *Clin Immunol* 2002; 102: 179-184.

22
23
24 [30]. Ganapathy V, Leibach FH. Expression and regulation of the taurine transporter
25 in cultured cell lines of human origin. *Adv Exp Med Biol*.1994; 359:51-57.

26
27
28 [31]. Ito T, Fujio Y, Hirata M, Takatani T, Matsuda, T., Muraoka S *et al.* J.
29 Expression of taurine transporter is regulated through the TonE (tonicity-responsive
30 element)/TonEBP(TonE-binding protein) pathway and contributes to cytoprotction
31 in HepG2 cells. *Biochem J* 2004; 15: 177-182.

32
33
34 [32]. Park KK, Jung E, Chon SK, Seo M, Kim HW, Park T. Finding of TRE (TPA
35 responsive element) in the sequence of human taurine transporter. *Adv Exp Med Biol*
36 2003; 526: 159-166.

37
38
39 [33]. Hartwell LH, Kastan MB. Cell cycle control and cancer. *Science* 1994; 266:
40 1821-1828.

41
42
43 [34]. Mochizuki T, Satsu H, Shimizu M. Tumor necrosis factor α stimulates taurine
44 uptake and transporter gene expression in human intestinal Caco2-cells. *FEBS Lett*
45 2002; 517: 92-96.

46
47
48 [35]. Kim H, Jeon H, Kong H, Yang Y, Choi B, Kim YM, Neckers L, Jung Y. A
49 molecular mechanism for the anti-inflammatorrt effect of taurine-conjugated 5-
50 aminosalicylic acid in inflamed colon. *Mol Pharmacol* 2006; 69: 1405-1412.

51
52
53 [36]. Dahchour A, Lallemand F, Ward R.J, De Witte P. Production of reactive oxygen
54 species following acute ethanol or acetaldehyde and its reduction by acamprosate in
55 chronically alcoholized rats. *Eur J Pharmacol* 2005; 520: 51-58.

56
57
58 [37]. Barger SW, Goodwin ME, Porter MM, Beggs ML. Glutamate release from
59 activated microglia requires the oxidative burst and lipid peroxidation. *J Neurochem.*
60 2007; 101:1205-13.

1 [38]. Brown GC, Neher JJ. Inflammatory neurodegeneration and mechanisms of
2 microglial killing of neurons Mol Neurobiol 2010; 41 :242-7.
3

4 [39]. Liang J, Takeuchi H, Jin S, Noda M, Li H, Doi Y, Kawanokuchi J, Sonobe Y,
5 Mizuno T, Suzumura A. Glutamate induces neurotrophic factor production from
6 microglia via protein kinase C pathway. Brain Res 2010; 1322:8-23.
7
8

9 [40]. Page MI. Beta-sultams mechanism of reactions and use as inhibitors of serine
10 proteases. Acc Chem Res 2004; 37: 297-303.
11
12

13 [41]. Gupta RC, Win T, Bittner S. Taurine analogues; a new class of therapeutics:
14 Retrospect and prospects. Current Med Chemistry 2005; 12: 2021-2039.
15
16

17 [42] Li Q, Verma IM. NF-kappaB regulation in the immune system Nat Rev Immunol.
18 2002; 2:725-34.
19
20

21 [43].Kim JK, Kim C .Inhibition of LPS-induced NO production by taurine
22 chloramines in macrophages is mediated through Ras-ERK-NF-kappaB. Biochem
23 Pharmacol 2005; 70:1352-1360.
24
25

26 [44]. Fan C, Li Q, Ross D, Engelhardt JF. Tyrosine phosphorylation of IkBa
27 activated NFkB through a redox-regulated and c-Src-dependent mechanism following
28 hypoxis reoxygenation. J Biol Chem 2003; 278: 2070-2080.
29
30

31 [45].Ward RJ, Crichton RR, Taylor DL, Corte LD, Srai SK, Dexter DT. Iron and the
32 innate immune system J Neural Transm 2010; PMID 20878427
33
34

35 [46]. Jeevanandam K, Young DH, Ramias L, Schiller WR. Effect of major trauma on
36 plasma free amino acid concentration in geriatric patients. Am J Clin Nutr 1990; 51:
37 1040-1045.
38
39

40 [47]. Eppler B, Dawson R. Dietary taurine manipulations in aged male Fischer 344
41 rat tissue: taurine concentrations, taurine biosynthesis and oxidative markers.
42 Biochem Pharmacol 2001; 62: 29-39.
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure Legends

1
2
3
4
5 **Figure 1.** The hydrolysis of ethane- β -sultam to taurine .
6
7

8
9
10 **Figure 2.** Rate of hydrolysis of ethane- β -sultam to taurine from pH2- pH10. . The
11
12 pK_a of ethane β -sultam was determined by titration using the reversible
13
14 chromophoric change at 230nm, while hydrolysis of ethane β -sultam to the β -amino
15
16 acid taurine as a function of pH was followed at 300nm.
17
18
19
20
21

22 **Figure 3a.** Release of nitrite and glutamate from alveolar macrophages after
23
24 stimulation with LPS, 1 μ g/ml[dark grey] or LPS + IFN γ 100 units/ml [light grey].
25
26 The data shown were obtained in one representative experiment of at least three
27
28 independent experiments. Significance is represented by *, P <0.05, **, P<0.01.
29
30
31

32
33
34 **Figure 3b.** Release of nitrite from alveolar macrophages after ex vivo incubation
35
36 with taurine, 10 mM and 50 mM, or ethane β -sultam, 10 mM and 50 mM for 24 h
37
38 followed by stimulation with 1 μ g/ml LPS for 24 h. The data shown were obtained in
39
40 one representative experiment of at least three independent experiments. Significant
41
42 reductions were calculated for each dose, **, P <0.01.
43
44
45
46
47
48

49
50 **Figure 4a.** Release of nitrite and glutamate from N9 microglial cells after stimulation
51
52 with LPS, 1 μ g/ml or LPS + IFN γ 100 units/ml. The data shown were obtained in one
53
54 representative experiment of at least three independent experiments. Significance is
55
56 represented by *, P <0.05, **, P<0.01.
57
58
59
60
61
62
63
64
65

1
2 **Figure 4b.** Nitrite release into the culture media from N9 microglial cells after
3
4 incubation with taurine or ethane- β -sultam, 1000 nM, 100 nM and 50 nM for 24h hr
5
6 [open blocks] followed by LPS stimulation, 1 μ g/ml, for a further 24 h [dark grey].
7
8 The results are representative of at least 3 separate incubation studies. Significance is
9
10 represented by *, P <0.05, **, P<0.01.
11
12
13
14
15
16

17 **Figure 4c.** Glutamate release into the culture media from N9 microglial cells after
18
19 incubation with taurine or ethane- β -sultam, 1000 nM, 100 nM and 50 nM for 24h hr
20
21 [open blocks] followed by LPS stimulation, 1 μ g/ml, for a further 24 h [dark grey]..
22
23 The results are representative of 3 separate incubation studies. Significance is
24
25 represented by **, P <0.01 by comparison to the non-stimulated controls; \$ P <0.01
26
27 by comparison to stimulated controls.
28
29
30
31
32

33 **Figure 5.** Release of nitrite from alveolar macrophages isolated from rats, n=6 in each
34
35 group, which had received a daily dose of taurine or ethane- β -sultam 0.15mmol/kg/l
36
37 /day by gavage for 7 days. The alveolar macrophages were left for 24h (open blocks
38
39 non-stimulated] before being stimulated ex vivo and with LPS, 1 μ g/ml, (black blocks]
40
41 for 24h. Significance is represented by **, P <0.01; * P<0.05 by comparison to the
42
43 stimulated controls; \$ P <0.05 by comparison to un-stimulated controls.
44
45
46
47
48
49

50 **Figure 6.** Changes in the release of NO, **Figure 6a** and IL-6, **Figure 6b**, from
51
52 alveolar macrophages isolated from rats, n=6 in each group, which had received
53
54 intermittent ethanol administration 3x/day , +/- one daily dose of 0.15 mmol/kg/l of
55
56 ethane- β -sultam by gavage. The alveolar macrophages were left for 24h [open blocks]
57
58
59
60
61
62
63
64
65

1 before being stimulated ex vivo with LPS, 1 µg/ml, [black blocks] for 24h.

2
3 Significance is represented by **, P <0.01; * P<0.05 by comparison to the
4
5 corresponding stimulated and non-stimulated cells.
6
7

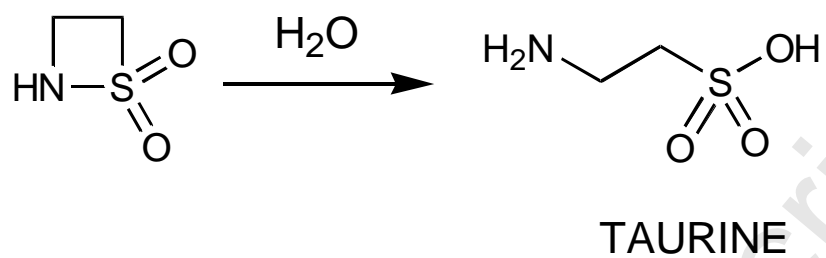
8
9
10 **Figure 7a.** Electromobility shift showing NFkappaB activation in alveolar
11
12 macrophages isolated from control rats and rats which had received taurine
13
14 supplementation in their drinking water, (12.5 g/l), after their stimulation with LPS, 1
15
16 µg/ml, and TNFα, 4U/ml, at different time points. Nuclear pellets were prepared and
17
18 incubated with P32 labelled primers.
19
20

21
22 **Figure 7b.** Density intensities for each NFkappaB band
23
24

25
26
27 **Figure 8a.** Western blot showing stabilisation of IkappaBα in alveolar macrophages
28
29 isolated from rats which had received oral administration of taurine, 6.25, 12.5 or 25
30
31 g/l, for 7 days and then stimulated with LPS, 1 µg/ml, and TNFα, 4U/ml.
32
33

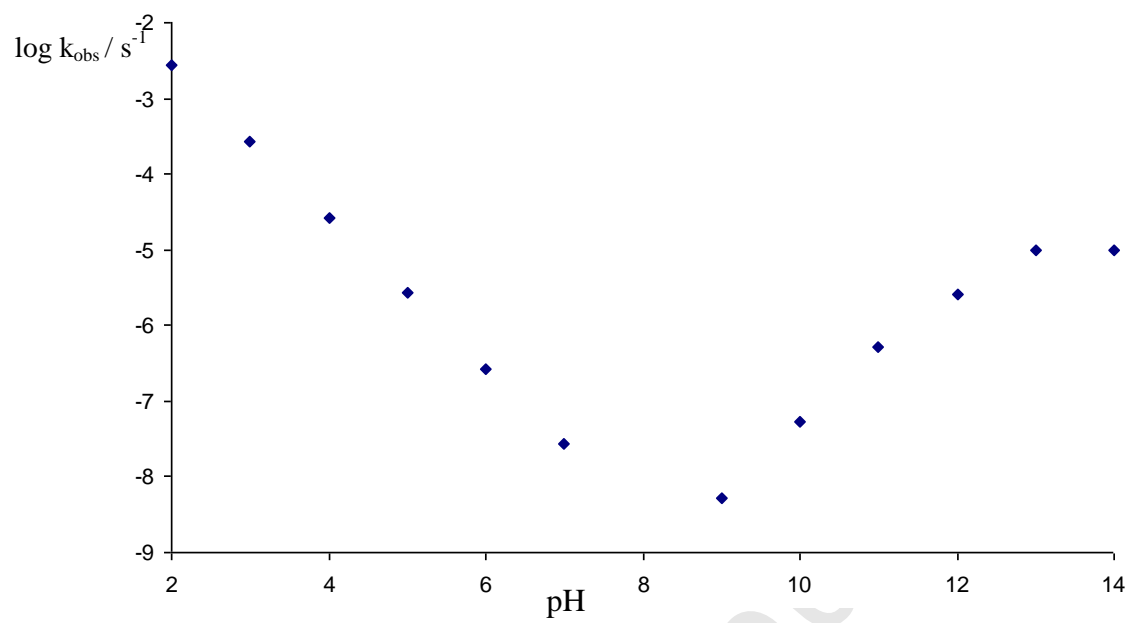
34
35 **Figure 8b.** Density intensities for IkappaBα, three individual bands from each
36
37 concentration were scanned. The mean and standard deviation for each concentration
38
39 is represented in the Figure.
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Figure 1.



Accepted Manuscript

Figure 2



Accepted Manuscript

Figure 3a

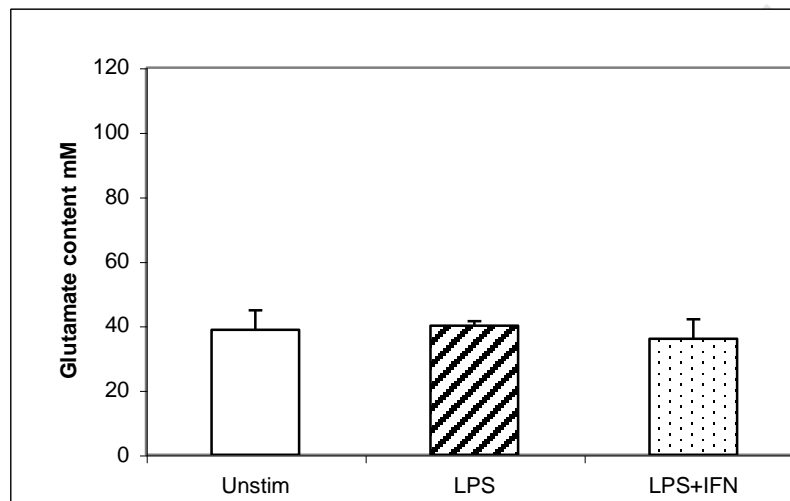
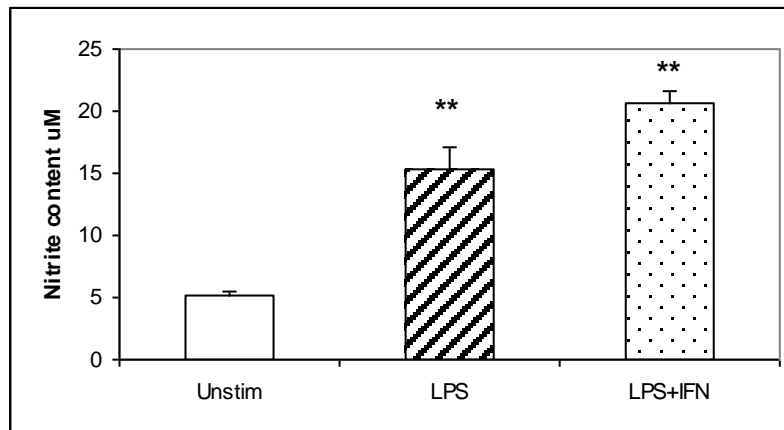


Figure 3b

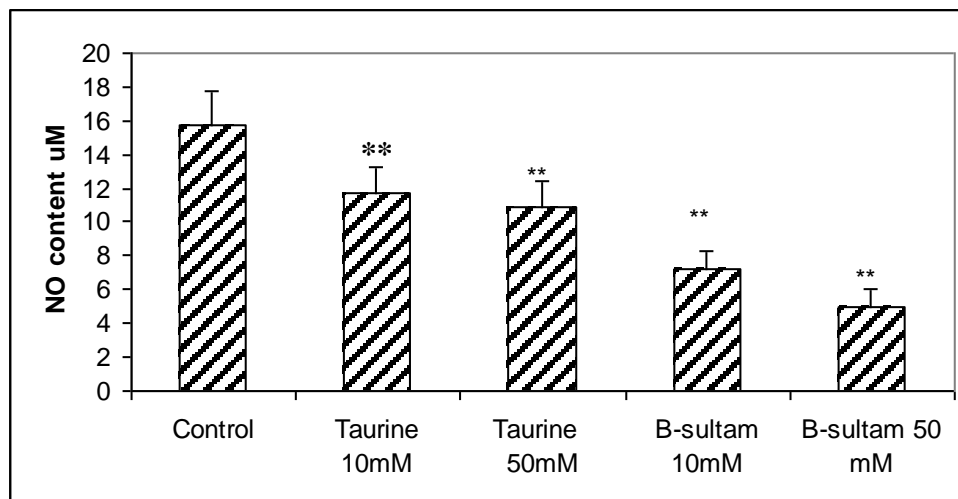


Figure 4a

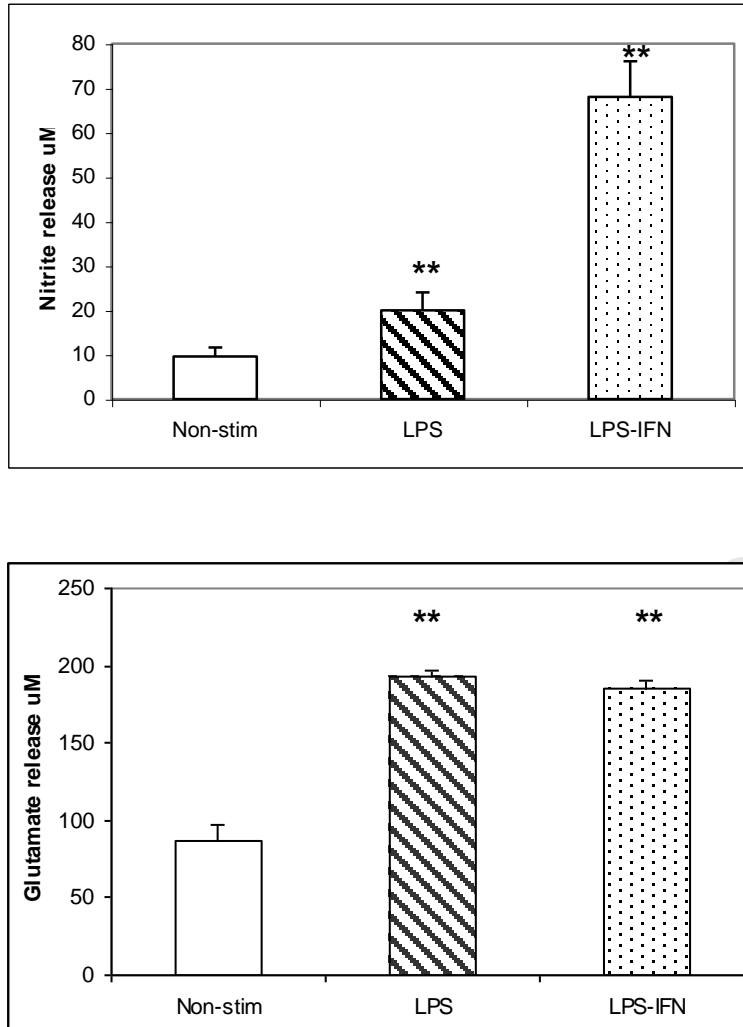


Figure 4b.

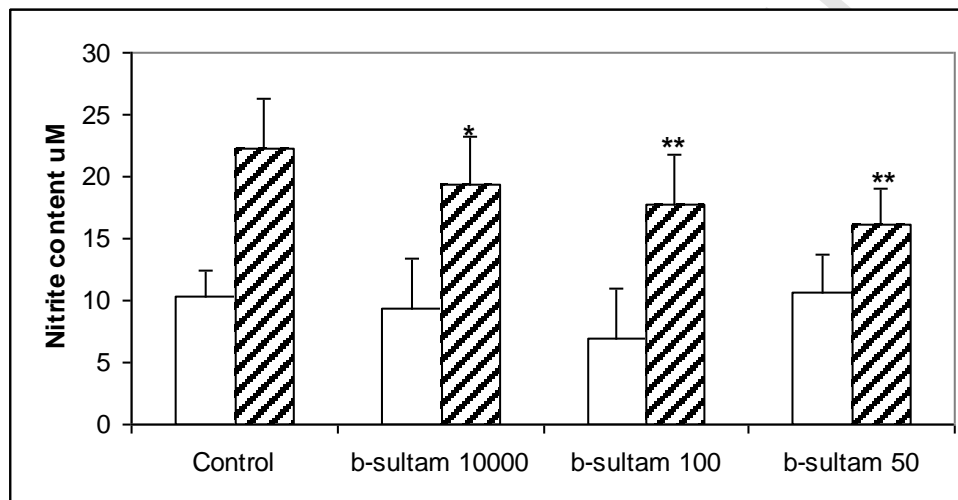
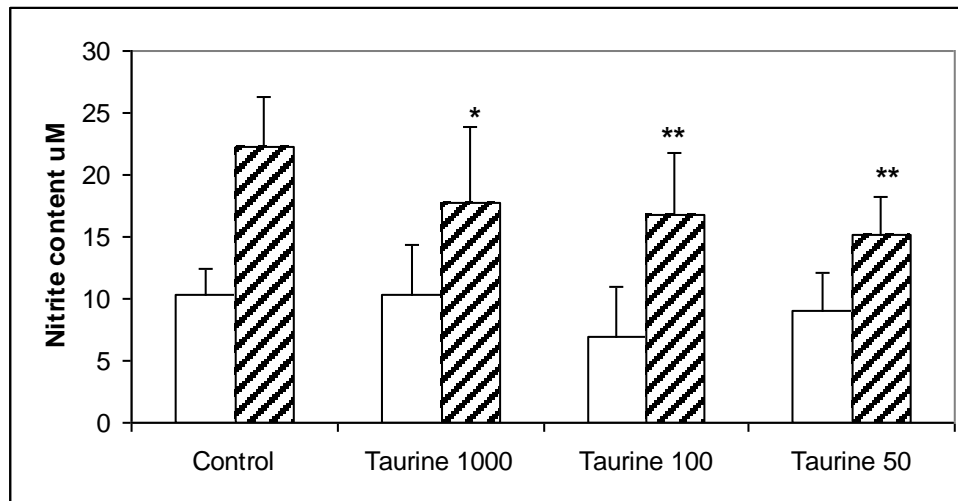


Figure 4c

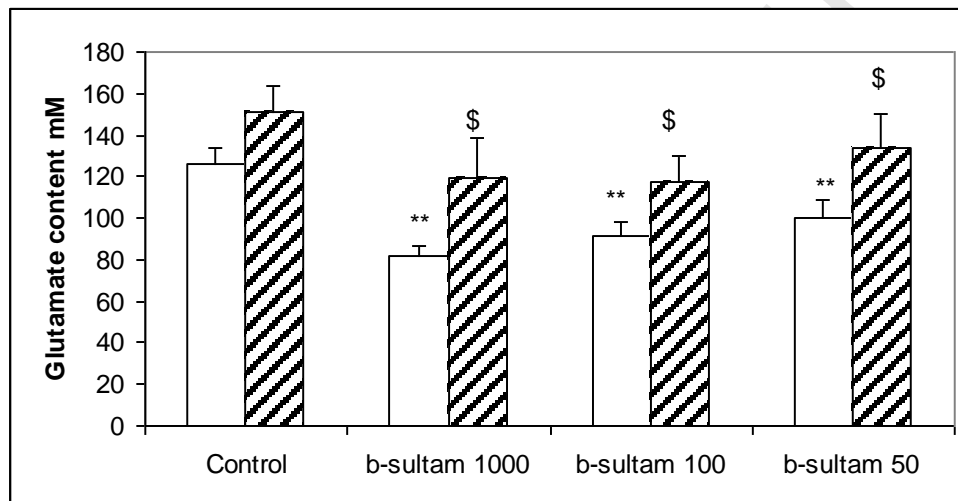
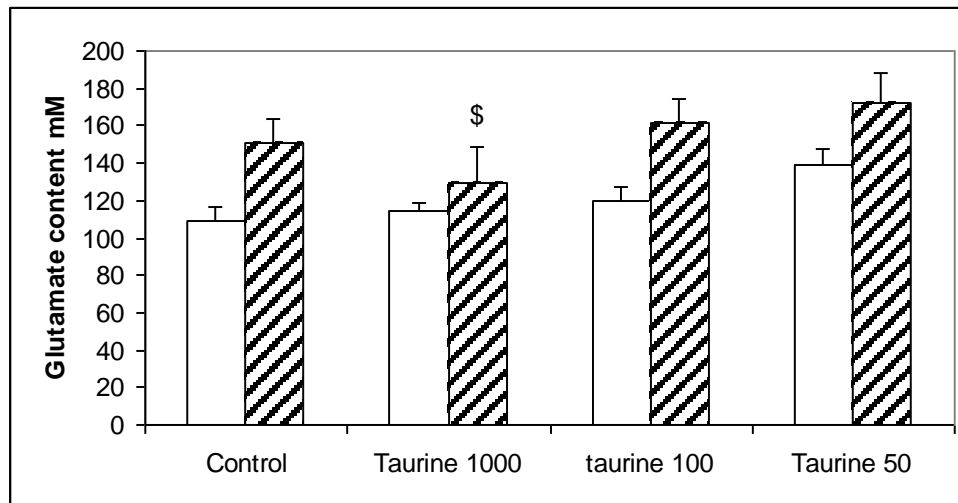
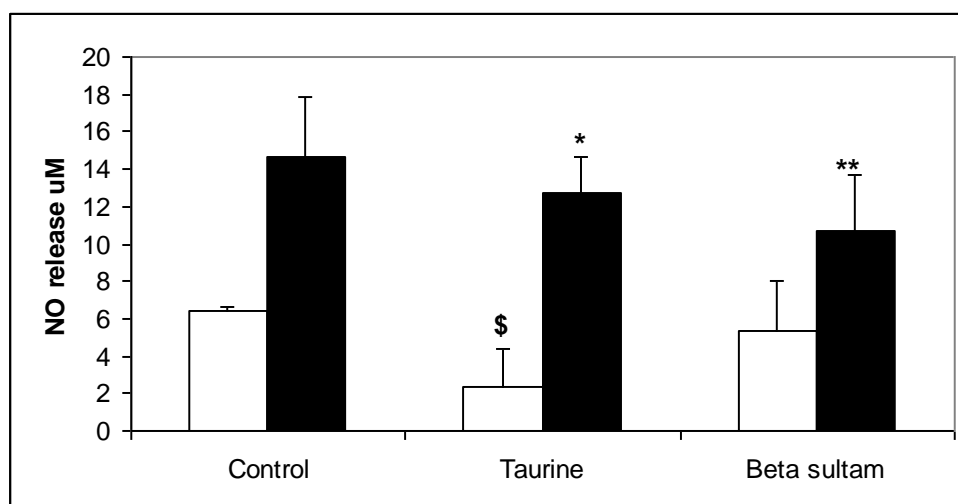


Figure 5.



Accepted Manuscript

Figure 6a

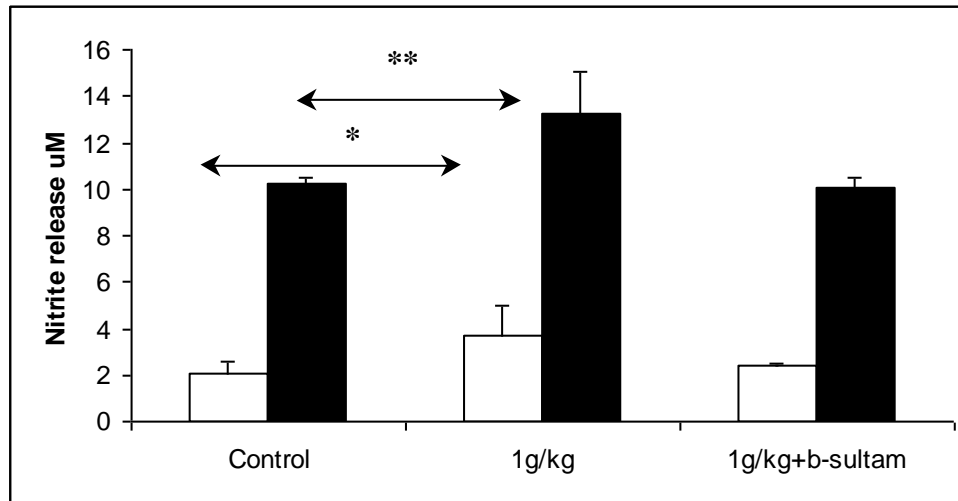


Figure 6b

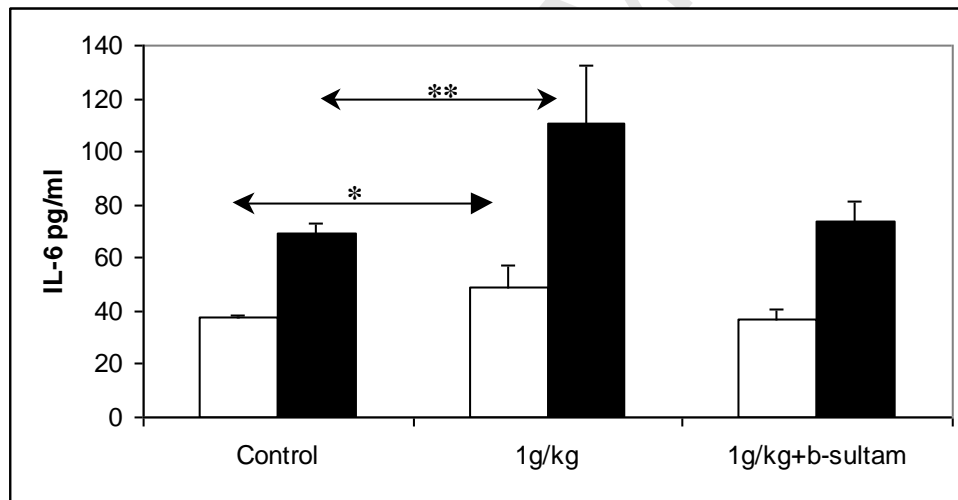
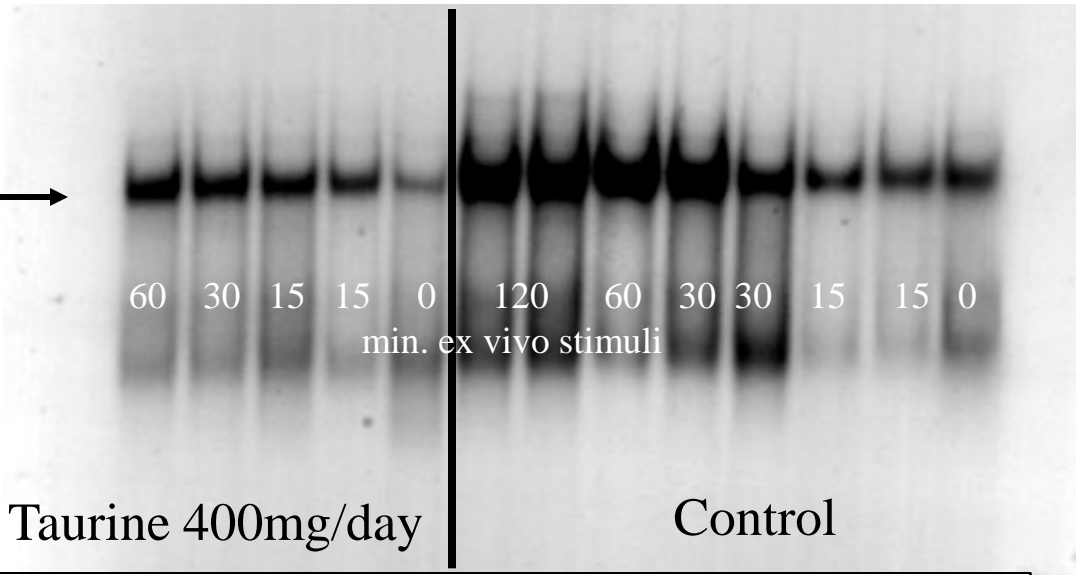


Figure 7

A

NF κ B complex



B

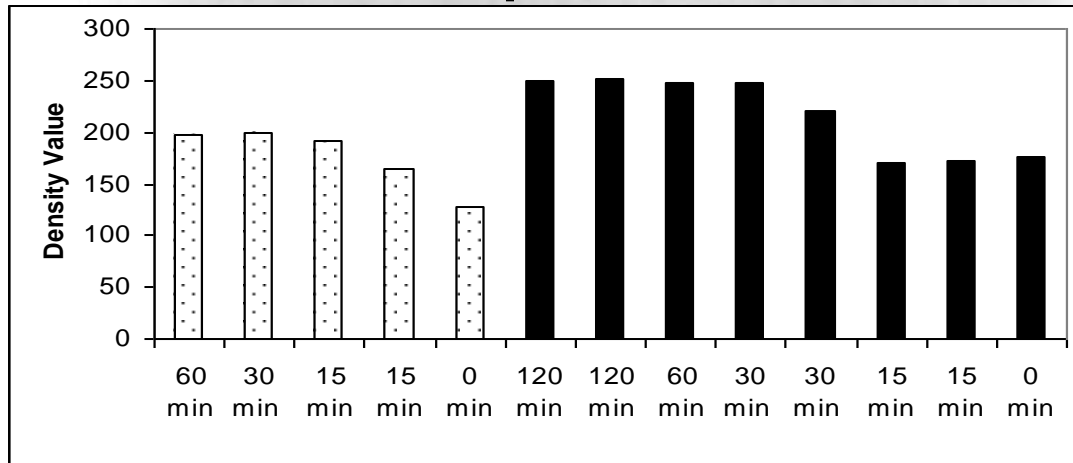


Figure 8a

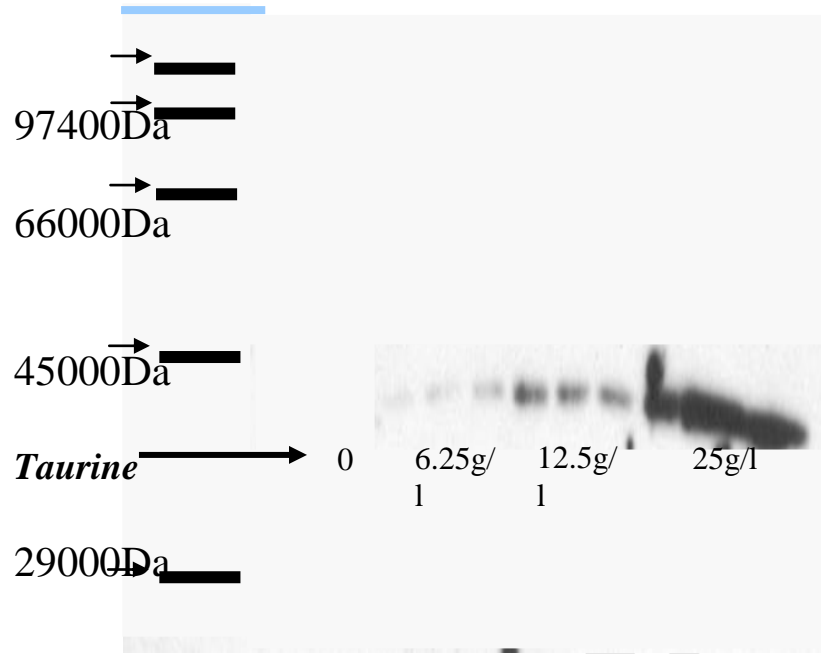
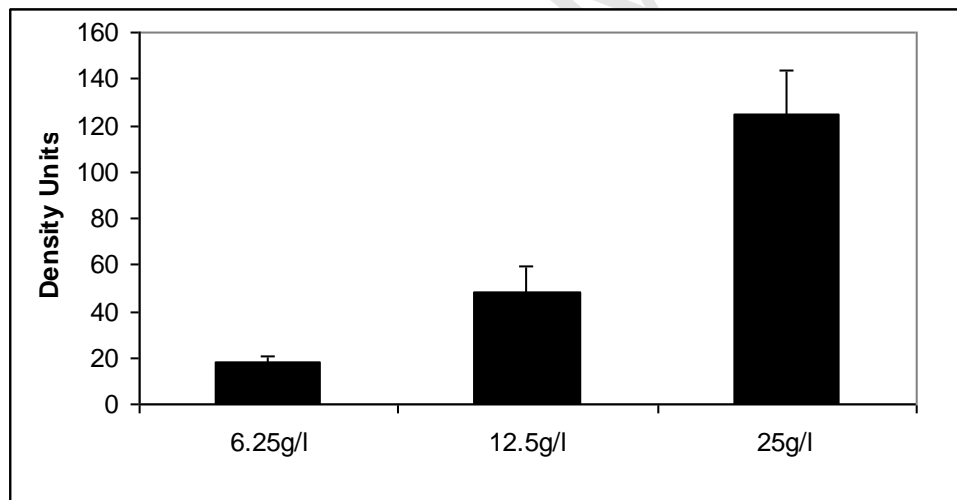


Figure 8b



Phagocytic cell

Inflammatory stimulus

NADPH oxidase



HClO₄



Oxidative stress



Reduction in cystine



Glutamate release

Ethane-β-sultam



Taurine Modulation

IκappaBα



NFκappaB activation



NO, IL-6 release

