Conversion of taurine into *N*-chlorotaurine (taurine chloramine) and sulphoacetaldehyde in response to oxidative stress

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N-Chlorotaurine (taurine chloramine), formed by treating taurine with hypochlorous acid, was shown to decompose to sulphoacetaldehyde with a first-order rate constant of $9.9 \pm 0.5 \times 10^{-4} \text{ h}^{-1}$ at 37 °C in 0.1 M phosphate buffer, pH 7.4. Rat liver homogenates accelerated this decay in a process that was proportional to tissue-protein concentration and saturable, with maximum velocity (V_{max}) and K_m values of $0.28 \pm 0.01 \text{ nmol/min per mg}$ of protein and $37 \pm 9 \mu\text{M}$ respectively. This activity was found to be lost on heat denaturation, but retained after dialysis. There was no detectable formation of sulphoacetaldehyde when taurine itself was incubated with the tissue homogenates under the same conditions. Activation of human neutrophils (1.67×10^6 cells/ml) with latex beads resulted

in a respiratory burst of oxygen-radical production, the products of which were partially sequestered by 12.5 mM taurine. Under these conditions sulphoacetaldehyde was generated at a constant rate of $637 \pm 18 \text{ pmol/h per ml}$ for over 7 h. A non-activated neutrophil suspension contained constant levels of $1.42 \pm 0.02 \text{ nmol/ml}$ sulphoacetaldehyde, as did activated cells incubated in the absence of taurine, a basal level which may indicate a steady turnover of taurine in these cells. Such formation of chlorotaurine and its decay to the aldehyde may be the first steps in the metabolism of taurine to isethionate (2-hydroxyethanesulphonate) that has been demonstrated by various authors to occur *in vivo*.

INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is present in millimolar concentrations in most mammalian tissues. Although it has been variously described as a neurotransmitter [1], a regulator of calcium fluxes [2,3], an osmoregulator [4–6], an antioxidant and cytoprotectant [7–9], a thermoregulator [10] and an anti-convulsant [11], no consensus on its fundamental role(s) in mammalian systems has been reached. The evidence for the various proposed functions of taurine has been extensively reviewed by Huxtable [12].

Taurine was, for many years, considered to be an innocuous end product of the metabolism of sulphur-containing amino acids, and some researchers went so far as to state that mammalian systems could not metabolize taurine [13]. Taurine metabolism was shown, however, by the finding of the alcohol isethionic acid (2-hydroxyethanesulphonic acid) in dog heart [14] and in rat brain [15]. The latter study demonstrated the flow of radioactively labelled sulphur from methionine through to isethionic acid, via cysteine, hypotaurine and taurine, at a rate of about 2 nmol of isethionic acid formed/h per g of tissue. That study also provided some evidence that the conversion is enzymic, demonstrating the ability of the supernatant of a 5000 *g* centrifugation to catalyse the conversion of taurine into isethionate and its inability to do so after heat inactivation.

The most likely intermediate in the conversion of taurine into isethionate would be sulphoacetaldehyde. Although the presence of this aldehyde has never been reported in any mammalian system, it has been shown to be produced from taurine by enzyme-catalysed processes in a number of bacteria. The conversion is catalysed by transaminases in *Achromobacter super-*

ficialis [16] and *Pseudomonas aeruginosa* [13] and by a taurine dehydrogenase in an unidentified bacterium [17].

Mammalian tissues contain a number of amine oxidases, including monoamine oxidase (EC 1.4.3.4; [18]) and the semicarbazide-sensitive amine oxidase family of enzymes (EC 1.4.3.6; see [19]) that might catalyse the oxidative deamination of taurine to form the corresponding aldehyde. There are also numerous transaminases that may exhibit varying degrees of substrate specificity and thus may accept taurine as a substrate. One obvious candidate would be γ -aminobutyric acid (GABA) transaminase (EC 2.6.1.19). GABA is structurally similar to taurine and taurine has been shown to bind to a class of GABA_A receptors in some studies [20]; however, GABA transaminase has already been shown not to accept taurine as a substrate [21]. Furthermore, we were unable to detect any metabolism of taurine added to rat liver homogenates.

An alternative possibility for the production of sulphoacetaldehyde from taurine exists, since chloramines are known to decay to form the corresponding aldehydes [22,23]. Chlorotaurine is now well known to be produced during the respiratory burst in activated neutrophils and in macrophages [24,25] via the scavenging of myeloperoxidase-produced hypochlorous acid (HOCl). This scavenging accounts for the observed decrease in the chemiluminescence response in the presence of exogenous taurine [26]. Although the rate of decay of chloramines of β -amino acids is known to be considerably slower than that for α -amino acids [22,23], chlorotaurine might decay to sulphoacetaldehyde under physiological conditions, particularly under the highly oxidative and acidic conditions prevalent during the respiratory burst [27].

The aim of the present study was to investigate the chlorination

Abbreviations used: DIH, 2-(diphenylacetyl)indane-1,3-dione 1-hydrazone; Nbs₂, 5,5'-dithiobis(2-nitrobenzoic acid); Nbs[−], 5-mercapto-2-nitrobenzoic acid (5-thio-2-nitrobenzoic acid); GABA, γ -aminobutyric acid; EB, ethidium bromide; AO, Acridine Orange; TNF, tumour necrosis factor.

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of taurine and the subsequent decay of the chloramine to sulphoacetaldehyde as a possible route for mammalian taurine metabolism. The highly sensitive assay for sulphoacetaldehyde, using HPLC with fluorescence detection, which can detect as little as 500 pmol/ml of this compound [28], was used for this purpose.

MATERIALS AND METHODS

Chemicals and reagents

2-(Diphenylacetyl)indane-1,3-dione 1-hydrazone (DIH) was obtained from Aldrich Chemical Company, Gillingham, Dorset, U.K. Romil HPLC-grade acetonitrile was obtained from Lennox Ltd., Dublin. Taurine, Histopaque 1055, 5,5'-dithiobis-(2-nitrobenzoic acid) (Nbs₂), latex beads (0.8 µm in diameter, 10 % suspension, blue-dyed), ethidium bromide (EB), Acridine Orange (AO) and luminol were all obtained from Sigma Chemical Co., Poole, Dorset, U.K. Sulphoacetaldehyde and chlorotaurine were prepared by procedures outlined below. All other chemicals were purchased from either Sigma or BDH Chemicals (Poole, Dorset, U.K.).

Assay procedure

The assay procedure used was a modification of that of Rideout et al. [29] for the quantification of blood acetaldehyde levels; it uses the fluorescent reagent DIH [30], which has also been used to determine a wide variety of aldehydes in car exhaust fumes [31]. The assay is based on the reaction of the hydrazone group of DIH with aldehydes to form fluorescent azines, which may be separated from the excess reagents and any other derivatives by HPLC. A 0.3 mg/ml solution of DIH was prepared by dissolving 30 mg of DIH in approx. 30 ml of acetonitrile at 37 °C for 10 min, and then adjusting the solution up to 100 ml with acetonitrile. Samples for assay (0.25 ml) were added to ice-cold DIH solution (1 ml) and left for 5 min on ice. HCl (2 M, 50 µl) was then added and the mixture was incubated at 50 °C for 90 min to ensure complete reaction of the aldehyde. Samples were then centrifuged at 12000 *g* for 10 min to remove precipitated protein, and the supernatants were decanted and subjected to quantitative analysis by HPLC.

HPLC conditions

The HPLC equipment comprised a Beckman 110B solvent-delivery system, a Rheodyne manual sample injector with 50 µl sample loop, a Uvikon SFM25 fluorimetric detector and a Spectra-Physics SP4290 integrator. A Waters C₁₈ µBondapak reverse-phase column (3.9 mm × 300 mm) was used. The column was eluted isocratically with 60 % (v/v) acetonitrile, 30 % (v/v) H₂O and 10 % (v/v) 0.1 M ammonium acetate, final pH 5.0. A flow rate of 1 ml/min was used until the sulphoacetaldehyde peak had been eluted, at approx. 3.7 min. Subsequently the flow rate was increased to 3 ml/min for approx. 8 min to elute the excess reagent and any other derivatives while simultaneously injecting acetonitrile to clean the injection loop. The fluorescence of the azine peak was measured at an excitation wavelength of 415 nm and an emission wavelength of 525 nm.

Preparation of sulphoacetaldehyde

An aqueous solution (100 ml) containing 23.6 mmol of cysteic acid and 59.1 mmol (2.2 molar equivalents) of ninhydrin was heated at 80 °C for 5 h, cooled to room temperature and the precipitate was filtered off. The product was extracted four times

with ethyl acetate, and the volume of this solution was decreased by rotary evaporation at 40 °C and passed through a strongly acidic ion-exchange resin (Dowex 50; 50 cm × 3 cm) in the free acid form. Following elution with water the resulting solution was adjusted to pH 4 with NaOH and 23.6 mmol of NaHSO₃ was added (as 11.8 mmol of Na₂S₂O₅, which hydrolyses to NaHSO₃ in water). Ethanol was then added to this and the resulting crystals were collected and recrystallized from 50 % ethanol. The yield was 3.1 g (52 %). This product is the bisulphite addition compound of sulphoacetaldehyde and must be treated before use as an aldehyde standard. This was done by the method of White [32]. To a solution of chosen concentration were added 2 equiv. of HCl, and then 2 equiv. of NaHCO₃. N₂ was then passed through this solution for approx. 30 min to ensure that all sulphite had been removed. This sample could then be adjusted to the desired concentration.

Synthesis of chlorotaurine

Chlorotaurine was synthesized freshly each day by a modification of a previously reported method [22] as follows. Taurine and sodium hypochlorite were separately prepared in 0.1 M potassium phosphate buffer, pH 7.4, and allowed to react in a 1:1 molar ratio. Chlorotaurine formation was monitored by UV absorbance. This compound has an absorbance maximum at 252 nm [22], whereas hypochlorite absorbs maximally at 291 nm. The concentrations of these two compounds in the reaction mixture could be calculated from their absorption coefficients: 429 M⁻¹·cm⁻¹ at 252 nm for chlorotaurine, and 142 M⁻¹·cm⁻¹ at 291 nm for hypochlorite. The absorbance of HOCl/OCl⁻ varies greatly with pH, so solutions must be accurately adjusted to pH 7 to allow quantification. The reaction was found to be essentially instantaneous and complete at room temperature. Determination of the absorbance spectra of reaction mixtures (see [22]) showed that there was no detectable presence of either residual hypochlorite or *N,N*-dichlorotaurine (taurine dichloramine) after the reaction was complete.

Preparation and use of 5-mercapto-2-nitrobenzoic acid (Nbs⁻)

In addition to sulphoacetaldehyde, chlorotaurine was also found to react with DIH, giving a derivative which ran close to the aldehyde-azine derivative. For this reason, under conditions where chlorotaurine was present in high (millimolar) concentrations, it was necessary to destroy this excess, both to prevent the complete exhaustion of DIH during the derivatization reaction and to simplify sulphoacetaldehyde quantification.

This could be achieved by exploiting the oxidation of Nbs⁻ to the corresponding disulphide Nbs₂ by chlorotaurine according to the reaction:

This reaction forms the basis of a commonly used spectrophotometric assay for chlorotaurine [34]; this depends on the low reactivity of the reagent as a thiol, due to its *p*-nitro group, so that it does not reduce typical disulphide bonds, as well as on the ease of measuring its concentration.

A solution of Nbs⁻ was prepared by dissolving 25 mM Nbs₂ in 50 mM potassium phosphate buffer, pH 7.4, and titrating this solution to pH 12 with NaOH to facilitate alkaline hydrolysis. After 5 min the solution was re-adjusted to pH 7.4 with HCl. This procedure would give an Nbs⁻ concentration of about 37 mM if the sulphenic acid produced together with the thiol by alkaline hydrolysis dismutated to thiol and sulphinic acid [35].

The Nbs⁻ solution was prepared freshly each week and stored in the dark, under nitrogen, in the presence of 1 mM EDTA.

The Nbs⁻ solution was added to the sample for assay immediately before the addition of DIH, in a volume sufficient to destroy all chlorotaurine. The volume added was maintained constant throughout any batch of assays and was also included in all standards, which were run in parallel. Typically a volume of 30 μ l was added.

Chlorotaurine decay

Various concentrations of freshly synthesized chlorotaurine were incubated in 0.1 M potassium phosphate buffer, pH 7.4, at 37 °C for periods of up to 12 h. Samples (0.25 ml) were taken at various times, mixed with Nbs⁻ (30 μ l) and immediately subjected to derivatization and subsequent quantitative analysis.

Isolation of human neutrophils

Approx. 20 ml of human blood was collected in a plastic syringe and immediately added to a Sterilin tube containing sodium heparin. The heparinized blood was then added to 5 ml of grade B dextran (M_r 150 000–200 000; 6%, w/v, in 0.9% NaCl) and left for 45 min at room temperature to facilitate erythrocyte sedimentation. The upper leucocyte-rich fraction was then layered on to 5 ml of Ficoll gradient (Histopaque 1055) and centrifuged at 400 *g* for 30 min at room temperature in a Sorvall RT 6000D centrifuge. The peripheral-blood-mononuclear-cell fraction, which formed a layer at the interface between the plasma and the Ficoll gradient, was discarded. The neutrophil fraction was in the pellet, but this also contained residual erythrocytes. These were lysed by the addition of 3 ml of water, rapidly mixing and leaving the mixture for 1 min at room temperature. NaCl (3.6%, 1 ml) and 0.1 M PBS, pH 7.3, containing 1 mM glucose (1 ml), were then added. The cells were then centrifuged at 200 *g* for 5 min and the supernatant discarded. The pellet was resuspended in 0.9% NaCl, containing 1 mM glucose, and could be kept at room temperature for several hours.

Microscopic cell counting

The neutrophil concentration and viability were assessed using an EB/AO stain in which viable neutrophils stain green and non-viable cells appeared orange under fluorescent light using the plan 16/0.35 objective of a Zeiss M35 fluorescence microscope. Stock solutions of EB (4 mg/ml) and AO (1 mg/ml) were prepared in 0.1 M PBS, pH 7.3, and stored in the dark at room temperature. The working EB/AO mixture was prepared by adding 20 μ l of each solution to 4.96 ml of 0.1 M PBS, pH 7.3. Cells were diluted 1:20 in the EB/AO mixture and their numbers counted on an improved Neubauer haemocytometer. The cell concentration was calculated by counting the mean number of viable cells in an area of 16 squares and multiplying by the dilution factor (20) and the inverse of the volume contained in 16 squares (10 000/mm³). The stock cell suspension was then adjusted to the required cell concentration using 0.9% NaCl containing 1 mM glucose.

Chemiluminescence assays

Reactive-oxidant production by the isolated neutrophils was measured by monitoring chemiluminescence responses at 37 °C using a Lumac M2010 biocounter. A latex bead suspension was used as stimulus to induce phagosome formation [36]. A 10%

latex-bead suspension was diluted 1:200 in 0.1 M PBS, pH 7.3, and the cell stock was diluted to 5×10^6 cells/ml in 0.9% NaCl. A stock solution of luminol (3 mM in 0.1 M NaOH) was diluted 1:10 in 0.1 M PBS immediately before use. Luminol (100 μ l) was added to cells (100 μ l), with gentle mixing, before the addition of the latex-bead suspension (100 μ l) and recording of the chemiluminescence response at 15 s intervals. The latex-bead suspension was replaced with 5 mM EGTA in non-activated controls as activation of the respiratory burst is Ca²⁺-dependent, and partial activation of the burst was found to be unavoidable in the absence of latex beads. To assess the effect of taurine on reactive oxidant production, 20 μ l of 200 mM taurine in 0.1 M PBS, pH 8, was included before activation.

Neutrophil incubations

When conditions for activation of the respiratory burst had been optimized, the cells (1 ml of a solution containing 5×10^6 cells/ml) were incubated at 37 °C and stimulated to effect activation, by the addition of 1 ml of the 0.5% (w/v) latex-bead suspension in the presence of either 1 ml of 0.1 mM PBS or 1 ml of 30 mM taurine in 0.1 mM PBS. Non-activated controls were prepared by the addition of PBS (1 ml) and 5 mM EGTA (1 ml) to the cell suspension. All incubations were done at 37 °C. Samples (0.25 ml) were taken at intervals and subjected to immediate derivatization with DIH and subsequent quantitative analysis by HPLC. The viability of the neutrophils was tested, as described above, at each time point.

Homogenate-enhanced degradation of chlorotaurine

Rats were killed and their livers immediately excised and placed in ice-cold 0.1 M potassium phosphate buffer, pH 7.4, containing 0.2 M sucrose. The tissue was washed, and then homogenized in this buffer (20 ml) using a Dounce homogenizer. The protein concentration was estimated using the absorbance at 280 nm, in a concentration range where the absorbance was linear with respect to concentration, assuming a 1 mg/ml solution to give an absorbance of 1.0. Homogenates (1 ml) of various concentrations were preincubated at 37 °C for 10 min and the reaction was initiated by the addition of 1 ml of freshly synthesized chlorotaurine (3.7 mM). Samples (0.25 ml) were taken at intervals, mixed with 50 mM Nbs⁻ (30 μ l) and then subjected to derivatization, protein precipitation and subsequent HPLC analysis. A similar procedure was used to assess the effect of increasing concentrations of chlorotaurine on the initial rate of sulphoacetaldehyde production at a constant protein concentration of 1 mg/ml. Denaturation and removal of protein was achieved by incubation of the homogenate at 100 °C for 45 min and subsequent centrifugation at 12 000 *g* for 10 min. Dialysis was carried out for two 8 h periods, each time against 2 litres of 10 mM phosphate buffer, pH 7.4.

RESULTS

Production of sulphoacetaldehyde from chlorotaurine

Freshly synthesized chlorotaurine was found to decay spontaneously to form sulphoacetaldehyde at 37 °C, whereas taurine incubated under the same conditions did not yield any detectable aldehyde. The aldehyde peak was clearly visible after about 4 min under the conditions used in the present study. Its identity as sulphoacetaldehyde was confirmed by its coincidence with the aldehyde standard under a variety of HPLC elution conditions. This production of aldehyde was found to be constant for at least 8 h, at a rate proportional to the initial concentration of

Figure 1 Production of sulphoacetaldehyde from freshly synthesized chlorotaurine (top panel) and replot of the rate of sulphoacetaldehyde generation (from top panel) versus initial chlorotaurine concentration (bottom panel)

Top panel: chlorotaurine, at concentrations of 3.65 mM ($\square$), 2.7 mM ($\blacklozenge$), 1.82 mM ($\blacktriangle$), 0.96 mM ($\bullet$) and 0.56 mM ($\blacksquare$), was incubated at 37 °C and pH 7.4, under the conditions described in the text, and the concentration of sulphoacetaldehyde was determined after fixed times. Each point represents the mean $\pm$ S.E.M. from at least four separate determinations from two or more independent experiments. Bottom panel: replot.

chlorotaurine (Figure 1). The amount of sulphoacetaldehyde formed over this incubation time was a small fraction ($< 2\%$) of the total concentration of chlorotaurine, and the decrease of chlorotaurine was therefore not monitored in parallel. However, determination of the absorbance spectra of the chlorotaurine after a reaction time of 12 h showed that the decrease in its concentration was comparable with the amount of sulphoacetaldehyde formed. A first-order rate constant (k) of $9.9 \pm 0.5 \times 10^{-4} \text{ h}^{-1}$ was calculated from the data represented in Figure 1, according to the equation:

$$kt = \ln(A_0/A)$$

Here A_0 represents the starting concentration of chlorotaurine and A is its concentration at time t . This corresponds to a half-life of approx. 29 days. The decay proceeded about five times as fast when the assay sample was adjusted to pH 5 with HCl.

Catalysis of chlorotaurine decay

The rate of decay of chlorotaurine to sulphoacetaldehyde was enhanced approx. 40-fold in the presence of rat liver homogenates

Figure 2 Dependence of the rate of sulphoacetaldehyde formation from chlorotaurine on the concentration of rat liver homogenate

Incubations were at 37 °C and pH 7.4, under the conditions described in the text, and the concentration of sulphoacetaldehyde was determined after fixed times. The initial chlorotaurine concentration was 1.85 mM. Each point represents the mean $\pm$ range for two separate determinations.

Figure 3 Dependence of the rate of sulphoacetaldehyde formation on chlorotaurine concentration in the presence of rat liver homogenate

Different concentrations of freshly synthesized chlorotaurine were incubated at 37 °C and pH 7.4, as described in the text, with 1 mg/ml rate liver homogenate. Each rate is the mean $\pm$ range from two independent experiments.

(1 mg/ml). The initial rate of aldehyde formation, after subtraction of the uncatalysed rate, was directly proportional to the concentration of homogenate present, as shown in Figure 2. No production of sulphoacetaldehyde could be detected when taurine itself was incubated with the homogenates under identical conditions. The dependence of the rate of homogenate-catalysed sulphoacetaldehyde formation upon the chlorotaurine concentration obeyed saturation kinetics (Figure 3). The limiting velocity (V_{max}) and K_m values were calculated by non-linear regression to be 0.28 ± 0.01 nmol/min per mg of protein and $37.3 \pm 9.2 \mu\text{M}$ respectively. This rate enhancement was abolished by heat denaturation, but was retained after dialysis.

Activation of respiratory burst in neutrophils

The suitability of latex beads as a stimulus for the initiation of the respiratory burst was tested using the chemiluminescence response at 37 °C to monitor the release of reactive oxidants from the cell during the phagocytic process. Latex beads were chosen since they stimulate the formation of phagolysosomes [36] and hence provide the ideal conditions for the formation of sulphoacetaldehyde from chlorotaurine. A latex-bead concentration of 0.0167% (w/v) gave consistent activation of the

Figure 4 Chemiluminescence response of neutrophil suspensions to latex beads in the presence and absence of taurine

Final concentrations of 1.67×10^6 neutrophils/ml were used in the presence of 0.0167% (w/v) latex beads (■), latex beads and 12.5 mM taurine (●) or 1.67 mM EGTA (◆). Each point represents the mean $\pm$ S.E.M. from at least three separate determinations.

Figure 5 HPLC traces illustrating the sulphoacetaldehyde peak (*) produced by (a) activated neutrophils in the presence of 10 mM taurine, (b) a 5 μ M standard sulphoacetaldehyde solution, (c) non-activated neutrophils in the presence of 1.67 mM EGTA and (d) activated neutrophils in the absence of taurine

All incubations were carried out at a concentration of 1.67×10^6 cells/ml. The peak corresponding to 2-(diphenylacetyl)indane-1,3-dione 1-hydrazone (DIH) is also indicated.

neutrophil suspension, peaking at approx. 38000 luminescence counts after 4.5 min (Figure 4) and declining rapidly thereafter, presumably because of the completion of phagosome formation and the internalization of the beads. As shown in Figure 4, the

Figure 6 Production of sulphoacetaldehyde from chlorotaurine by activated neutrophils

Neutrophils at a final concentration of 1.67×10^6 cells/ml were stimulated with 0.0167% (w/v) latex beads in the presence (■) or absence (□) of 10 mM taurine. Latex was replaced with 1.67 mM EGTA to act as a negative control (◆). Each point represents the mean $\pm$ range from two separate determinations, with similar results being obtained in three independent experiments.

inclusion of 12.5 mM taurine in the incubation resulted in a significant decrease in the chemiluminescence response; this is consistent with removal of HOCl by reaction with the taurine to form chlorotaurine. There was no significant activation of neutrophils when the latex beads were replaced with EGTA. Neutrophils were activated and incubated for extended time in an attempt to measure the production of sulphoacetaldehyde from chlorotaurine generated in the respiratory burst. Neutrophils activated in the presence of exogenous taurine generated sulphoacetaldehyde at a rate of 637 ± 18 pmol/h per ml over a period of at least 7 h (Figures 5 and 6). At least 65% of the neutrophils were viable after that time. Neutrophils incubated in the absence of exogenous taurine, or in the presence of EGTA to prevent activation, showed no significant rate of increase of sulphoacetaldehyde, although there was a constant basal level of 1.42 ± 0.02 nmol/ml. A similar sulphoacetaldehyde concentration was determined as the zero-time value (i.e. before initiation of the respiratory burst) in cells incubated in the presence of taurine. This concentration thus appears to represent a basal level of the aldehyde in these cells under the conditions of the present experiments, suggesting constant turnover of taurine.

DISCUSSION

Peck and Awapara [15] demonstrated the flow of [35 S]methionine through to isethionic acid via taurine, at a rate of 2 nmol/h per g of tissue in rat brain. These results clearly suggest the existence of a complete route for taurine catabolism. They also demonstrated this conversion to be enzyme-catalysed and suggested that the putative intermediate, sulphoacetaldehyde, might be formed by transamination. However, both that study and experiments in our laboratory, employing the HPLC assay described here, have failed to provide any evidence for such a conversion. We were unable to detect conversion of taurine into sulphoacetaldehyde by tissue homogenates in the presence of added pyridoxal phosphate and the 2-oxo acids pyruvate, glyoxylate, 2-oxoglutarate and oxaloacetate, and also failed to show any oxidative deamination by the isolated amine oxidases (EC 1.4.3.4 and EC 1.4.3.6) (C. Cunningham and K. F. Tipton, unpublished work).

It is known that most chloramines decompose rapidly to form their corresponding aldehydes [23]. Although the chloramines of

Scheme 1 Pathways for chloramine breakdown

Pathway 1 shows the loss of a hydrogen as H^+ from the C-2 carbon of a chloramine formed from a β -amino acid, whereas pathway 2 shows the more facile breakdown of that formed from an α -amino acid, requiring only the loss of CO_2 .

β -amino acids decompose more slowly, the formation of chlorotaurine does provide one possible route of aldehyde formation. Chlorotaurine is formed by the direct reaction of taurine with HOCl, which is generated by the myeloperoxidase-catalysed oxidation of H_2O_2 during the respiratory burst [37]. The generation of chlorotaurine by neutrophils was demonstrated by Weiss et al. [38]. The extracellular concentration of chloramines 1 h after neutrophil activation is approx. 0.1 mM, most of which is thought to be chlorotaurine [24]. Chlorotaurine has been described as a long-lived oxidant, undergoing less than 5% decomposition/h at pH 7 and 37 °C [22], although this process has been reported to be accelerated at lower pH [39], a factor that would be relevant during phagocytosis [27].

As expected, the first-order rate constant for the decay of chlorotaurine reported here is considerably lower than that for the chloramines of α -amino acids, which decompose over a period of minutes to yield aldehydes [23]. A possible explanation for this is that the CO_2 lost in the formation of aldehydes from chloramines of α -amino acids is a better leaving group than the H^+ of β -amino acid chloramines. This is shown in Scheme 1. The observation that the decay proceeds more quickly at a lower pH suggests that the H-2 may be lost more easily when the $-NH-Cl$ group is also protonated ($-NH_2^+-Cl$). This would accord with the observation by Thomas and Learn [23] that chlorination of an amine drastically lowers the pK; acidification of a pH 7.4 incubation buffer would increase the very small fraction of chloramine protonated and hence increase the rate of decay.

Taurine is thought to have anti-inflammatory effects, by scavenging of HOCl to form chlorotaurine, and, in doing so, preventing feedback inhibition of myeloperoxidase by HOCl [40]. HOCl also has severe cytotoxic effects, and taurine protects against these by sequestration of the oxidant [41].

In the present study we have demonstrated the spontaneous formation of sulphoacetaldehyde from chlorotaurine at 37 °C and pH 7.4, with a first-order rate constant of $9.9 \pm 0.5 \times 10^{-4} \cdot h^{-1}$. This rate would generate less than 400 nM aldehyde/h from a typical post-respiratory-burst chlorotaurine concentration of

0.1 mM (see [24]), but this rate is greatly enhanced in the presence of liver homogenates (approx. 40-fold in the presence of a 1 mg/ml homogenate). Preliminary results suggest that this rate-enhancing factor is also present in rat brain homogenates. With liver homogenates the rate enhancement is directly proportional to the protein concentration, and is saturable, with an estimated K_m of $37.3 \pm 9.2 \mu M$. Furthermore, we show the activity to be heat-denaturable, but not to be lost on dialysis. These findings not only suggest enzyme catalysis of the conversion, but also a novel route for the generation of sulphoacetaldehyde *in vitro*, in a process where taurine is released and turned over in response to the oxidative stress caused by the invasion of tissues by microbes or other foreign bodies or by cell debris. We have now shown that human neutrophils produce sulphoacetaldehyde when activated in the presence of taurine. We have also shown an apparently constant level of sulphoacetaldehyde in both non-activated neutrophils and in cells activated in the absence of taurine, suggesting the possibility of a basal rate of taurine metabolism in these cells. These findings constitute the first report of sulphoacetaldehyde in any mammalian system and therefore provide a possible pathway for taurine turnover.

The discovery of sulphoacetaldehyde in an inflammatory cell may be significant in the light of accumulating evidence for taurine as a neuroprotectant [42,43]. Taurine exerts a protective effect in neutrophils by removing HOCl; it now seems that in addition chlorotaurine may itself have protective roles. NO and its oxygen-containing radical metabolites, as well as tumour necrosis factor (TNF) are major mediators of oxidant-induced cell damage [44,45]. Recently, chlorotaurine has been reported to decrease both nitric oxide (NO) and TNF secretion by activated macrophages [46] in a manner that involves changes at the transcriptional and translational levels of inducible NO synthase and TNF expression respectively, as well as by inhibiting NO synthase itself. This suggests that chlorotaurine may be sufficiently long-lived to affect such cellular events as gene expression; this adds importance to further knowledge of the factors that govern its breakdown to sulphoacetaldehyde.

Although neutrophils have been reported not to be normally resident in the brain, their accumulation in brain regions with low blood flow has been observed in the early post-ischaemic period [47]. Activated microglia could also be a source of chlorotaurine in the brain. Microglia are the ontogenic and functional equivalents of mononuclear phagocytes in somatic tissues [48] and have been shown to migrate and differentiate at central-nervous-system sites of inflammation and to participate in phagocytosis [49].

Secretion of reactive oxygen species by activated microglia could have both protective and destructive roles, and the reaction of these species with taurine to form chlorotaurine could be important for their removal. The generation of superoxide anions by microglia has been demonstrated [50] and shown to be similar to that of neutrophils and macrophages. Although one study has reported myeloperoxidase, which catalyses the conversion of H_2O_2 into HOCl, not to be present in microglia [51], the gene for this enzyme has been reported to be co-localized with the glial marker glial fibrillary acidic protein [52]. In view of the many states of differentiation of microglia, it is certainly possible that these cells may express myeloperoxidase in certain situations. A recent report that astrocytes protect neurons from H_2O_2 toxicity via increases in catalase and glutathione peroxidase activities pointed out that, even during complete inhibition of these enzyme activities, H_2O_2 clearance was still observable [53], perhaps implicating myeloperoxidase.

Further studies on the possible generation of chlorotaurine in the brain and other tissues, as well as on the factors controlling its conversion into sulphaacetaldehyde and the further conversion of that compound to isethionic acid, are clearly now required.

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