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The biosynthesis of taurine from *N*-acetyl-L-cysteine and other precursors *in vivo* and in rat hepatocytes

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Summary. The synthesis of taurine from *N*-acetylcysteine has been examined in rats *in vivo* and in rat hepatocyte suspensions *in vitro*. In rats *in vivo*, administration of *N*-acetylcysteine significantly increased urinary taurine (3 fold) 24 h after dosing and liver glutathione levels. Liver taurine was not increased significantly. In hepatocytes incubated in the presence of *N*-acetylcysteine, glutathione concentration increased to a maximum after 1 hour but the increase was not dependent on the concentration of *N*-acetylcysteine. In contrast, after an initial lag phase, taurine synthesis increased in relation to the concentration of *N*-acetylcysteine and continued for 3 hours. Glutathione synthesis seems to be preferential to taurine synthesis. Taurine synthesis from cysteine sulphinate was greater and from hypotaurine was greatest and maximal after 1 hour. Implications for the mechanism of protection by *N*-acetylcysteine are discussed.

Keywords: Amino acids – Taurine synthesis – Glutathione – Hypotaurine – Hepatocytes – Cysteine sulphinate

Introduction

The β-amino acid taurine, occurs in high concentrations in most animal tissues (mM). It is often the most abundant free amino acid in cells and its' functions appear to vary depending on the tissue (Huxtable, 1992). Thus it conjugates bile acids in the liver, has an important role in the heart as a modulator of calcium levels and acts as an osmolyte and neuroinhibitor in the brain (Gaull, 1989). It has also been shown to act as a protective agent both *in vivo* and *in vitro* (Timbrell et al., 1995). For example, the cardiotoxicity of isoprenaline and adriamycin can be ameliorated in rats by taurine administration (Azuma et al., 1987); a deficiency of taurine has been shown to result in retinal degeneration and poor growth in cats (Sturman, 1993) and bleomycin-induced pulmonary fibrosis in hamsters can be ameliorated by a combination of

taurine and niacin (Wang et al., 1991). We have shown that there is a correlation between urinary and liver taurine and susceptibility to various hepatotoxicants (Waterfield et al., 1993a) and that depletion of liver taurine increases the susceptibility of rats to carbon tetrachloride hepatotoxicity (Waterfield et al., 1993b). Taurine can protect lymphoblastoid cells against retinoid damage (Pasantes-Morales et al., 1984) and isolated hepatocytes are partially protected from carbon tetrachloride, hydrazine and 1,4-naphthoquinone toxicity (Waterfield et al., 1993c). Therefore, taurine appears to have protective properties both as an endogenous compound and when administered therapeutically. Although taurine is obtained in the diet from animal products it is also synthesised by the oxidation of cysteine. Low intracellular levels of L-cysteine are maintained (30–250μM) as cysteine is one of the most toxic protein amino acids. Excess cysteine that is not required for protein or glutathione synthesis will be catabolised via pyruvate to CO_2 and sulphate or to taurine (Weinstein et al., 1988).

The principal properties of *N*-acetyl-L-cysteine are related to its ability to act as a free radical scavenger and effective precursor for intracellular cysteine, the rate limiting amino acid in glutathione synthesis. Hence it is used routinely to treat paracetamol over-dose in patients (Prescott et al., 1977; Peterson and Rumack, 1977). There are also reports of *N*-acetylcysteine being effective in limiting liver injury after the acute ingestion of CCl_4 (Mathieson et al., 1985; Ruprah et al., 1985), as well as limiting the toxic effects of compounds as diverse as halothane, nitrogen oxides and zinc and in *Amanita phalloides* poisoining.

Not all of the protective properties of *N*-acetylcysteine are understood. The increased levels of glutathione, maintenance of protein thiols, direct binding to the toxic compound and the prevention of lipid peroxidation may all be involved (Miners et al., 1993). However, it is possible that some protection could be afforded by the increased synthesis of taurine from raised intracellular cysteine levels following *N*-acetylcysteine administration. Alternatively the excess cysteine may give rise to increased levels of sulphate. As excessive doses of paracetamol deplete sulphate, the production of sulphate from *N*-acetylcysteine may facilitate conjugation of paracetamol.

Rat hepatocytes, incubated with cysteine will synthesise taurine, although the percentage catabolised through the taurine pathway varies according to the diets on which rats are fed as well as other factors (Stipanuk et al., 1992a,b). *N*-acetylcysteine is also able to increase intracellular cysteine and taurine plus hypotaurine levels in hepatocytes but glutathione synthesis is preferential to taurine synthesis during 60 minute incubations (Banks and Stipanuk, 1994).

Thus *de novo* synthesis of taurine from *N*-acetylcysteine and from the metabolic intermediates cysteine sulphinate and hypotaurine has been investigated *in vitro* in relation to glutathione synthesis over 3 hours to assess the possibility of protection by NAC in isolated suspensions via synthesis of taurine. The effect of *N*-acetylcysteine on taurine and glutathione levels *in*

vivo has also been investigated. The doses and concentrations used were those relevant to the use of *N*-acetylcysteine as an antidote (1.84mmol·kg^{-1} over 20h) (Ellenhorn and Barceloux, 1988).

Materials and methods

Reagents

The following compounds were supplied by the Sigma Chemical Company (Poole, Dorset, U.K.): collagenase type IV, HEPES, *N*-acetyl-L-cysteine, hypotaurine, taurine, cysteine sulphinate, *o*-phthalaldehyde (OPA; HPLC grade), taurine (cell culture tested), Dowex resins, fire fly lantern extract, NADH, pyruvic acid, DTNB (5,5′-dithiobis-2-nitrobenzoic acid) for measurement of total non-protein sulphydryls (TNPSH) and glutathione. Mercaptoethanol, sodium hydroxide (Aristar), sulphosalicylic acid and boric acid were obtained from Merek Ltd., (Lutterworth, Leicestershire, U.K.); methanol (HPLC grade) from Rathburn (Wakeburn, Scotland, U.K.). Water was of ultra high quality, prepared using an Elgastat water purifier.

In vivo study

Animals and treatment

Eight male rats (Sprague-Dawley stock, Glaxo Research and Development), weighing 230–290g, were acclimatised for 10 days after delivery. During the experiment animals were housed in individual metabolism cages designed to separate and collect faeces and urine (Techmate Ltd, Milton Keynes, U.K.) and given powdered diet (691 diet, Quest Nutrition Ltd, Wingham, Kent, U.K.) and water *ad libitum*. Lighting was controlled to give a regular 12h light-dark cycle (7 am on – 7 pm off); room temperature was maintained at 21 ± 2°C. Three pre-dose 24h urine collections were made. Four animals were given N-acetylcysteine (4mmol·kg^{-1} i.p.) in phosphate buffered saline (PBS, 8ml·kg^{-1}, pH adjusted to 7.4), controls were given PBS alone. A 24h urine collection was made after dosing and analysed for taurine (see below). All urine samples were collected over ice and then diluted to 25ml, centrifuged and stored (−80°C) in aliquots for future analysis. The body weight and general condition of animals were monitored daily; food and water intake were measured.

Animals were post-mortemed 24h after dosing by exsanguination from the abdominal aorta under anaesthesia. Blood samples were put into Microtainers (Beckton Dickinson and Co., Rutherford, N.J., U.S.A.) for the separation of serum and subsequent taurine measurement. The liver was removed, weighed and the right lobe frozen in liquid nitrogen and stored at −80°C for analysis of taurine and total non-protein sulphydryls (TNPSH).

Biochemical measurements

Taurine and total nonprotein sulphydryls (TNPSH)

Taurine was extracted from diluted urine (25μl) using Dowex resins using a method similar to that previously described (Waterfield, 1994). Taurine and TNPSH were measured in the supernatant prepared from liver samples (300mg) homogenised in sulphosalicylic acid (4ml, 0.2M, 4°C). Samples of supernatant were applied to Dowex columns for taurine extraction (25μl) or assayed for TNPSH (Ellman, 1959). As reduced glutathione (GSH) constitutes >95% of TNPSH in the liver, this was used as a measure of GSH (De Master et al., 1987; Potter and Tran, 1993). Taurine was measured in the eluates from the Dowex ion exchange columns using HPLC with fluorometric detection (Waterfield, 1994 modified from Larson et al., 1980).

Statistical analysis

Urinary levels of taurine in samples taken from the same animal on different days were compared with predose values for each animal using a paired "t" test. A Student's "t" test was used to compare treated animals with control animals for other parameters.

In vitro investigations

Taurine synthesis

Rat hepatocytes were isolated by two step collagenase perfusion (Moldeus et al., 1978) and used if the viability was 87% or more as measured by the exclusion of Trypan blue (0.2%). Cells were pre-incubated for 1 h (in order to allow recovery from the isolation procedures) in Krebs Henseleit buffer under humidified O_2:CO_2, (95:5%) in 50 ml round bottomed flasks (1.5×10^6 cells·ml^{-1}, 15 ml). This system rotates the flasks which are open to the atmosphere and are gassed continuously (Moldeus et al., 1978). Samples were taken to measure LDH leakage into the supernatant (1 ml aliquots, centrifuged 13,000 g, 10 sec) by a modification of the method of Bergmeyer (1965). The pellet was washed twice with Krebs Henseleit buffer (4°C) and intracellular reduced glutathione (GSH) was determined in the pellet and after the addition of 6.5% TCA (Hissen and Hilf, 1976). A second aliquot of cell suspension (0.5 ml) was added directly to TCA (0.5 ml, 4°C) for the measurement of ATP. This method utilises a fire fly lantern extract with a computer controlled Thorn EMI photon detection system with an air cooled photomultiplyer tube (Stanley and Williams 1969). Cellular and extra-cellular taurine levels were measured in acid precipitated cells and medium as previously described (Waterfield, 1994). Cells were then incubated with different concentrations of *N*-acetylcysteine (added in 1 ml of incubation buffer) to give final concentrations of 0, 0.5, 1.0 or 5.0 mM. Incubations with *N*-acetylcysteine (2 mM), cysteine sulphinate (2 mM) or hypotaurine (2 mM) were carried out for 3 h and samples for taurine, ATP and GSH measurements and LDH leakage were taken after 1, 2 and 3 h. Trypan blue uptake was assessed after 0 and 3 h.

Wet weight of hepatocytes per 10^6 cells was calculated as 6.5 mg·10^6 cells^{-1} (wet weight) by the method of Berry et al. (1991). This figure was also used for conversion of published values for comparison.

Statistical analysis

Levels of *N*-acetylcysteine metabolites in incubations at the start of experiments were compared with samples taken from the same flasks at later time points using a paired "t" test. This was done as there was variation between taurine levels for each isolation making statistical analysis between individual experiments difficult. Dunnett's test was used for comparison of multiple treatments with a single control for all other comparisons.

Results

In vivo

There were no effects detected on body weight, food or water intake in animals dosed with *N*-acetylcysteine (data not shown).

Animals dosed with *N*-acetylcysteine had significantly higher levels of TNPSH in the liver than control animals (8.8 v 8.0 μmol·g^{-1} wet weight tissue) but the difference in taurine content in the liver was not significant (9.3 v 7.6 μmol·g^{-1} wet weight) (Fig. 1). Urinary levels of taurine (0–24 h) were significantly raised (3×) in those animals dosed with *N*-acetylcysteine (Fig. 2).

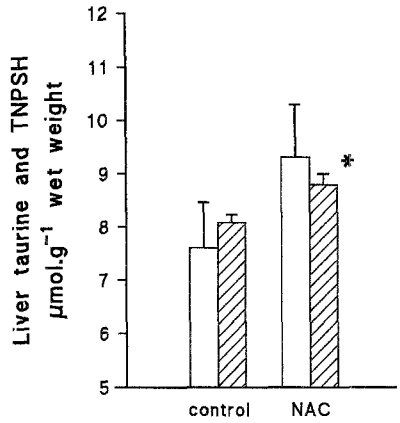

Fig. 1. Liver taurine and TNPSH levels in rats 24 after the administration of *N*-acetylcysteine (NAC; 4mmol·kg^{-1}); □ taurine; □ glutathione. Results are means ± SEM, n =4. *p < 0.05 (Student's "t" test)

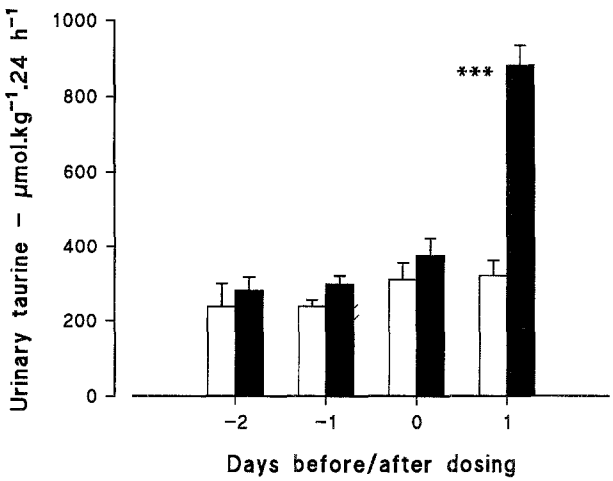

Fig. 2. Mean 24hr urinary taurine excretion in rats before and after treatment with a single dose of *N*-acetylcysteine (4mmol·kg^{-1}) on Day 0, ■. □ control group dosed only with vehicle. Results are means ± SEM, n = 4. ***p < 0.001 (paired "t" test)

This increase in urinary taurine represented about 15% of the *N*-acetylcysteine dose on a molar basis.

In vitro

Lactate dehydrogenase leakage and ATP levels

There was no significant increase in LDH leakage in any of the flasks (Fig. 3). ATP levels were maintained throughout the incubation period at a level above 25nmol·10^6 cells^{-1} or 3.5µmol·g^{-1} cells (Fig. 4).

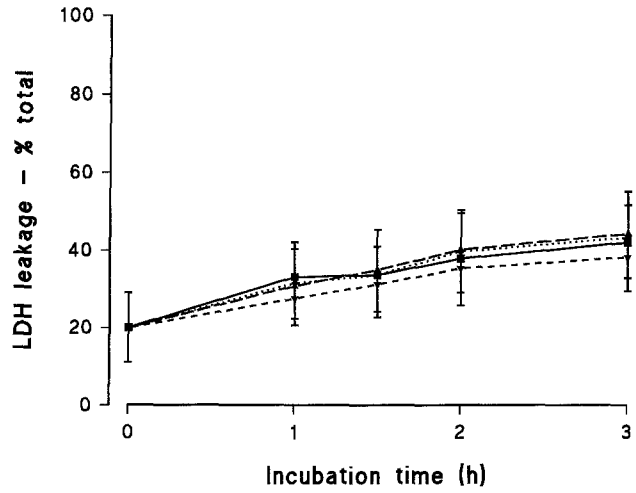

Fig. 3. Lactate dehydrogenase leakage (% of total) in hepatocytes incubated with no additions ——; *N*-acetylcysteine (2mM) ——; cysteine sulphinate (2mM) ---; and hypotaurine.....; values are means ± SEM from 4 separate incubations

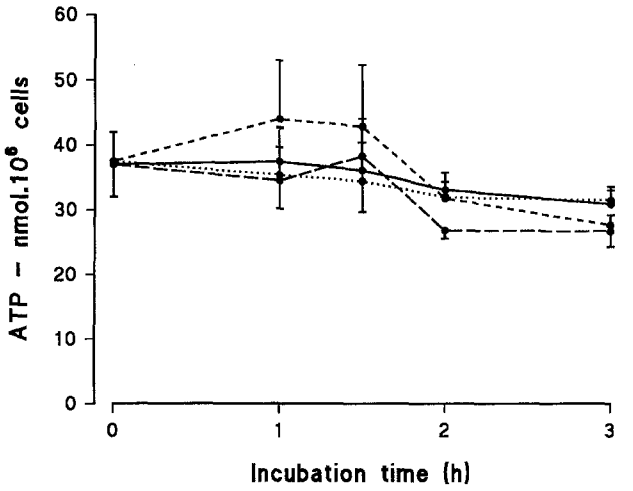

Fig. 4. ATP levels in hepatocytes incubated with no additions ——; *N*-acetylcysteine (2mM) ——; cysteine sulphinate (2mM) ----; and hypotaurine.....; values are means ± SEM from 4 separate incubations

Glutathione synthesis

Celluar GSH was raised by all concentrations of *N*-acetylcysteine to levels which correspond to maximal levels found in livers of control rats, i.e. $40\,nmol \cdot 10^6$ cells^{-1} or approximately $6.2\,\mu mol \cdot g^{-1}$ wet weight of tissue. The levels reached were independent of the concentration of *N*-acetylcysteine in the incubation media (Fig. 5). This observation is in agreement with Thor et al. (1979). Also, the maximum level was reached in the first hour, with no significant increase after this time. The average rate of synthesis for all three concentrations used was $15.6\,nmol \cdot h^{-1} \cdot 10^6$ cells^{-1} (accounting for 3% of 0.5mM *N*-acetylcysteine). Control levels remained constant throughout the 3h incubation.

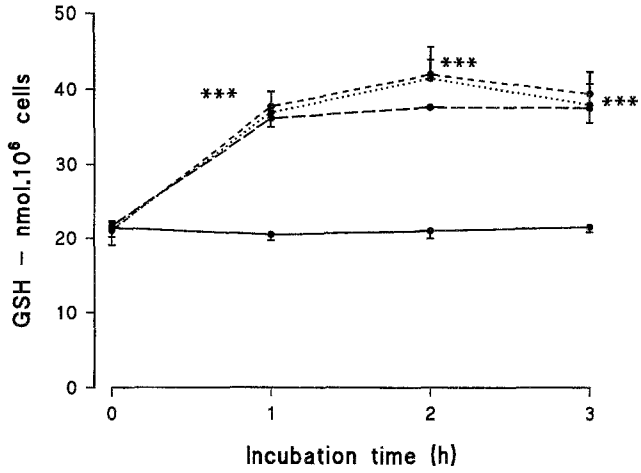

Fig. 5. Glutathione levels in isolated hepatocytes incubated with various concentrations of *N*-acetylcysteine (mM); ——0; — —0.5; – – –1.0;.....5.0. Results are means ± SEM of 3 separate incubations; ***$p < 0.001$ (paired "t" test)

Taurine synthesis

Taurine synthesis increased in relation both to *N*-acetylcysteine concentration and incubation time over the three hour incubation period. There appeared to be an initial lag phase in the synthesis of taurine, then the rate of synthesis increased (Table 1, Figs. 6 and 7). Thus the intra-cellular concentrations of taurine increased over the 3 h incubation period (Fig. 6) and during this time taurine also appeared in the medium, although the increase apparent 3 h after the addition of *N*-acetylcysteine (Fig. 7) was not significant. The ratio of taurine in the cells to that in the buffer after 3 hours was between 2.1 and 2.3 to 1 for all three *N*-acetylcysteine concentrations. After a 3 h incubation with *N*-acetylcysteine (5 mM), a total of 53 nmol·ml^{-1} of taurine could be measured in both cells (10^6 cells) and media, representing a conversion of 1.6% of the *N*-

Table 1. Taurine synthesis in hepatocyte suspensions from *N*-acetylcysteine

	Total taurine synthesised per hour measured in cells and medium (nmol·10^6 cells^{-1}·h^{-1})		
Concentration *N*-acetylcysteine (mM)	0–1 h	1–2 h	2–3 h
0	−0.48 ± 0.39	0.44 ± 0.22	1.05 ± 0.49
0.5	1.61 ± 0.83	4.35 ± 1.40	4.68 ± 1.60*
1.0	3.02 ± 1.58	7.19 ± 2.09	9.19 ± 2.43#
5.0	8.19 ± 2.68#	13.25 ± 4.82*#	20.94 ± 2.79**###

Total taurine was measured in 1 ml aliquots of cell suspension after precipitation of proteins with SSA. Values have been calculated from a mean starting value in the incubations of 10.0 nmol·10^6 cells^{-1}. Values are means ± SEM of the taurine synthesised in a 1 hour period; 4 separate hepatocyte preparations. *$p < 0.05$, **$p < 0.01$ increase over initial values; #$p < 0.05$, ###$p < 0.001$, Dunnett's test.

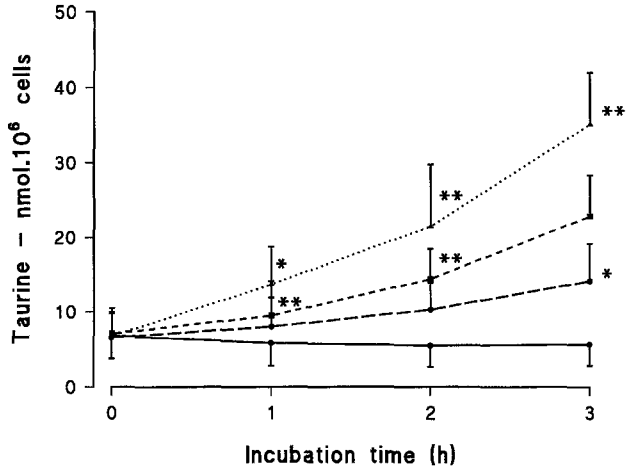

Fig. 6. Intracellular taurine levels in hepatocytes incubated with various concentrations of *N*-acetylcysteine (mM); —— 0; —— 0.5; --- 1.0;.....5.0. Results are means ± SEM of 4 separate incubations. *p < 0.05; **p < 0.001 (Paired "t" test)

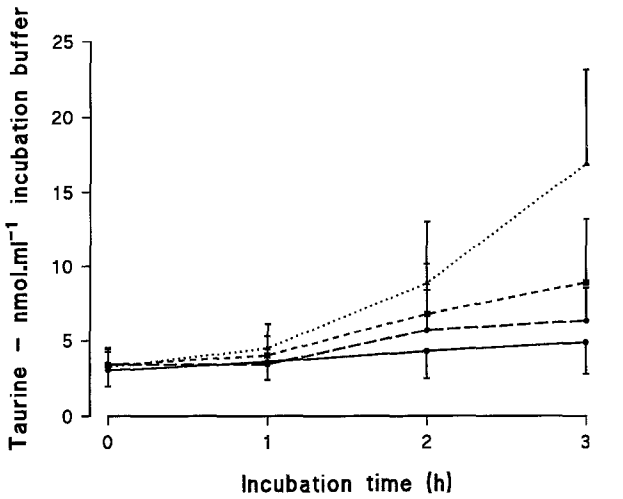

Fig. 7. Taurine concentrations in the incubation buffer from hepatocytes incubated with various concentrations of *N*-acetylcysteine (mM) —— 0; ——0.5; ---- 1.0;..... 5.0. Results are means ± SEM of 4 separate incubations

acetylcysteine present whereas 4.5% of the *N*-acetylcysteine present in the media was converted to taurine when the concentration of *N*-acetylcysteine was 1 mM. The synthesis of taurine was concentration dependent (Fig. 8).

After 1 h incubation with hypotaurine, levels of taurine were maximal in hepatocytes (≈250 nmol·ml^{-1} incubation containing 1.5×10^6 cells or approximately 12% of the hypotaurine). This represented an intracellular concentration of taurine of ≈23 mM. Metabolism of cysteine sulphinate to taurine continued to increase over the three hour incubation period, reaching 108 nmol·1.5×10^6 cells^{-1} (approximately 5% of the cysteine sulphinate). Although the rate of accumulation of taurine intracellularly was decreased by 3 h incubation, an intracellular concentration of 11 mM was achieved after 3 h. When the metabolism of *N*-acetylcysteine to taurine is compared to that of

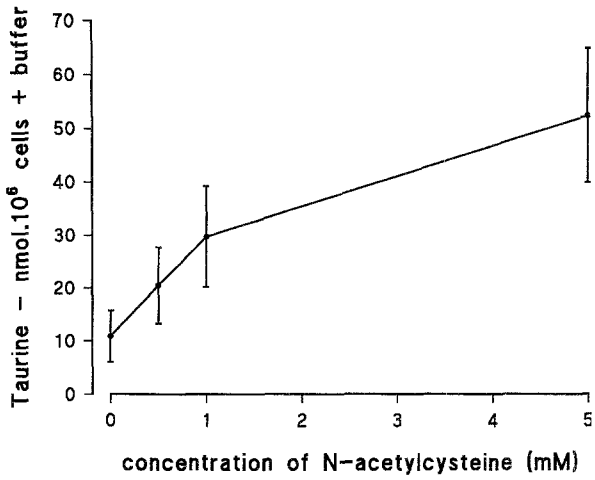

Fig. 8. Synthesis of taurine over 3 hours by hepatocytes exposed to various concentrations of *N*-acetylcysteine. Values are total taurine levels at 3 h, and are means ± SEM of 4 incubations

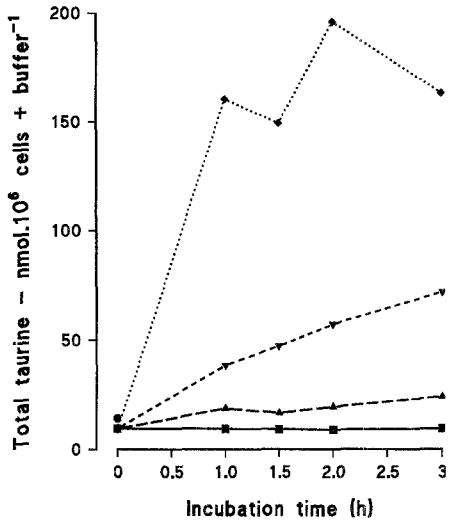

Fig. 9. Comparison of taurine synthesis measured in cells and medium over 3 h from taurine precursors; ——■—— control; ——▲—— *N*-acetylcysteine (2 mM); ---▼--- cysteine sulphinate (2 mM) and...◆...hypotaurine (2 mM). Values are means from 4 separate incubations

the taurine precursors the difference in the rate of taurine synthesis is clear (Fig. 9). Thus the synthetic rate adjusted for 10^6 cells·ml^{-1} over 3 hours from hypotaurine was 51 nmol·h^{-1}·10^6 cells^{-1} from cysteine sulphinate 24 nmol·h^{-1}·10^6 cells^{-1} and 4.7 nmol·h^{-1}·10^6 cells^{-1} from *N*-acetylcysteine.

Discussion

It is likely that the raised levels of urinary taurine found in animals treated with *N*-acetylcysteine were the result of an increased intracellular cysteine

concentration (generated by *N*-acetylcysteine) being catabolised to taurine. In the situation presented here, there were no extra demands made on GSH in the rats as no toxic or GSH depleting agents were administered to the animals and the animals were fed *ad libitum*. *N*-acetylcysteine would have been metabolised primarily to cysteine which would then have been incorporated into glutathione or protein or catabolised. However, as liver levels of GSH should have been normal at the time of *N*-acetylcysteine administration any excess cysteine would have been catabolised to pyruvate, sulphate and taurine (De La Rosa et al., 1989), excess taurine and sulphate being excreted into the urine.

Levels of GSH in livers used for hepatocyte isolation would also have been uncompromised (approximately 6–7 mM) although there was some loss of GSH at the time of isolation as 23 nmol·10^6 cells represents 3.5 μmol·g^{-1} wet weight of liver. Levels were raised within the first hour of incubation with *N*-acetylcysteine (approximately 40 nmol·10^6 $cells^{-1}$) and then maintained. These results are similar to the observations reported by Banks and Stipanuk (1994). After this time excess cysteine would have been metabolised to pyruvate, sulphate and taurine.

The values for the synthetic rates of taurine are very similar to those reported by Stipanuk et al. (1992a,b) and Banks and Stipanuk (1994) for the synthesis of taurine directly from cysteine and *N*-acetylcysteine. They found that the % of casein in the diet had a profound effect on the partitioning of cysteine to taurine and sulphate, high casein diets favouring taurine synthesis. After 1 h of incubation with *N*-acetylcysteine (1 mM) Banks and Stipanuk (1994) measured a total of 18 pmol·min^{-1}·mg cells (wet wt)$^{-1}$ taurine and hypotaurine synthesised by hepatocytes. In the studies discussed here, 2.8 nmol·h^{-1}·10^6 $cells^{-1}$ (7.2 pmol·min^{-1}·mg cells) taurine were synthesised in the first hour of incubation increasing to 7.2 nmol·h^{-1}·10^6 $cells^{-1}$ (18.5 pmol·min^{-1}·mg cells) during the second hour of the incubation (Table 1). The increased rate of taurine synthesis may have been due to the increased cysteine available as glutathione levels were maintained at 40 nmol·10^6 $cells^{-1}$ after the first hour of incubation. Alternatively this may have been due to accumulation of precursors. Whether the raised levels of taurine would have been sufficient to have any protective effect against a toxic insult can only be speculated at the present time. It is also likely that compounds such as paracetamol which deplete GSH would result in the diversion of cysteine synthesis away from taurine in favour of GSH, resulting in lower percentage of *N*-acetylcysteine being metabolised to taurine.

There is clearly a major difference between the synthesis of glutathione and taurine in hepatocytes *in vitro*. The intracellular GSH concentration reached a plateau within one hour which was independent of the *N*-acetylcysteine concentration. Taurine synthesis, however, continued, for at least 3 hours and was dependent on the *N*-acetylcysteine concentration. The increasing rate of taurine synthesis over the 3 h incubation period was probably the result of an initial preferential metabolism of *N*-acetylcysteine to GSH via cysteine (Banks and Stipanuk, 1994) which continued until levels of GSH were maximal. Taurine synthesis did not appear to reach maximal levels

within the 3h incubation period, although proportionally more taurine appeared in the incubation buffer as the incubation time continued. The taurine in the medium was unlikely to be the result of cells dying during the incubation period as cell death was minimal as indicated by LDH leakage. However, the rate of synthesis from *N*-acetylcysteine was lower than that reported by Stipanuk et al. (1992) from cysteine, 48–52 pmol·min^{-1}·mg cells^{-1}, at 1 mM, (with bathocuproine disulphonate included in the incubation).

Also of importance in the rate of taurine synthesis is the possible variation in synthetic rate due to initial glutathione levels and the predominant lobular origin of the hepatocytes at the time of isolation. Thus Pentilla (1990) found significant differences in taurine synthesis between periportal and perivenous hepatocytes; 16 and 117 pmol·min^{-1}·mg cells^{-1}, in periportal and perivenous hepatocytes respectively.

Synthesis of taurine from both cysteine sulphinate and hypotaurine was greater than from *N*-acetylcysteine. This could be because alternative pathways are available for *N*-acetylcysteine catabolism or simply reflect the position of the intermediates in a linear sequential series of reactions. Similarly, synthesis from hypotaurine was greater than that from cysteine sulphinate and again there is an alternative pathway for the latter although it precedes hypotaurine in the sequence. The rate of uptake of the precursors may also be a factor but was not monitored in this study.

In conclusion, the data shows that taurine synthesis is increased by *N*-acetylcysteine both *in vivo* and *in vitro* in hepatocytes. Other pathways for metabolism of *N*-acetylcysteine such as synthesis of glutathione are also operative as GSH levels were raised in both systems in the presence of *N*-acetylcysteine. Catabolism of *N*-acetylcysteine to sulphate will also occur. Thus only a small proportion of the *N*-acetylcysteine is metabolised to taurine. However, it is not known whether this is sufficient to account for some of the protective properties of *N*-acetylcysteine which are reported *in vitro* and *in vivo* and warrants further investigation.

Acknowledgements

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References

Azuma J, Hamaguchi T, Ohta H, Takihara K, Awata N, Sawamura A, Harada H, Tanaka Y, Kishimoto S (1987) Calcium overload-induced myocardial damage caused by isoproterenol and by adriamycin: possible role of taurine in its prevention. Adv Exp Med Biol 217: 167–179

Banks MF, Stipanuk MH (1994) The utilization of *N*-acetylcysteine and 2-oxothiazolidine-4-carboxylate by rat hepatocytes is limited by their rate of uptake and conversion to cysteine. J Nutr 124: 378–387

Berry MN, Edwards AM, Barritt GJ (1991) Isolated hepatocytes, preparation, properties and applications, 1st edn, ch. 6. Elsevier, Amsterdam

Bergmeyer HU, Bernt E, Hess B (1965) Lactate dehydrogenase. In: Bergmeyer HU (ed) Methods in enzymatic analysis. Academic Press, New York, pp 736–743

DeMaster EG, Redfern B (1987) High-performance liquid chromatography of hepatic thiols with electrochemical detection. In: Jakoby WB, Griffith OW (eds) Methods in enzymology. Academic Press, New York, pp 110–114

De la Rosa J, Drake MR, Stipanuk MH (1987) Metabolism of cysteine and cysteine-sulfinate in rat and cat hepatocytes. J Nutr 117: 549–558

Ellerhorn MJ, Barceloux DG (1988) Medical toxicology. Elsevier, Amsterdam, p 64

Ellman GL (1959) Tissue sulfydryl groups. Arch Biochem Biophys 82: 70–77

Hissen PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissue. Anal Biochem 74: 214–226

Huxtable RJ (1992) Physiological actions of taurine. Physiol Rev 72: 101–163

Gaull GE (1989) Taurine in pediatric nutrition: review and update. Pediatrics 83: 433–442

Larsen BR, Grasso DS, Chang SY (1980) A rapid method for taurine equantitation using high performance liquid chromatography. J Chromatogr Sci 18: 233–236

Mathieson PW, Williams G, Macsweeney JE (1985) Survival after massive ingestion of carbon tetrachloride treated by intravenous infusion of acetylcysteine. Hum Toxicol 4: 627–631

Miners JO, Drew R, Birkett DJ (1984) Mechanism of action of paracetamol protective agents in mice *in vivo*. Biochem Pharmacol 33: 2995–3000

Moldeus P, Hogberg J, Orrenius S (1978) Chapter 4. Isolation and use of liver cells. Methods Enzymol 52: 60–71

Pasantes-Morales H, Wright CE, Gaull GE (1984) Protective effect of taurine, zinc and tocopherol on retinol-induced damage in human lymphoblastoid cells. J Nutr 114: 2256–2261

Peterson R, Rumack BH (1977) Treatment of acetaminophen poisoning with *N*-acetylcysteine. Veter Human Toxicol 19: 270–280

Pentilla KE (1990) Role of cysteine and taurine in regulating glutathione synthesis by periportal and perivenous hepatocytes. Biochem J 269: 659–664

Potter DW, Tran T-B (1993) Apparent rates of glutathione turnover in rat tissues. Toxicol Appl Pharmacol 120: 186–192

Prescott LF, Ballantyne A, Park J, Adriaenssens P (1977) Treatment of paracetamol (acetaminophen) poisoning with *N*-acetylcysteine. Lancet ii: 432–433

Ruprah M, Mant TGK, Flanagan RJ (1985) Acute carbon tetrachloride poisoning in 19 patients: implications for diagnosis and treatment. Lancet i: 1027–1029

Stanley PE, Williams SG (1969) Use of the liquid scintillation spectrometer for determining adenoisine triphosphate by the luciferase enzyme. Anal Biochem 29: 381–392

Stipanuk MH, Bagley PJ, Coloso RM, Banks MF (1992a) Metabolism of cysteine to taurine by rat hepatocytes. In: Lombardini JB, Schaffer SW, Azuma J (eds) Taurine. Nutritional value and mechanisms of action. Plenum Press, New York, pp 413–421

Stipanuk MH, Coloso RM, Garcia RAG, Banks MF (1992b) Cysteine concentration regulates cysteine metabolism to glutathione, sulfate and taurine in rat hepatocytes. J Nutr 122: 420–427

Sturman JA (1993) Taurine in development. Physiol Rev 73: 119–147

Thor H, Moldeus P, Orrenius S (1979) Metabolic activation and hepatotoxicity – effect of cysteine, *N*-acetylcysteine and methionine on glutathione biosynthesis and bromobenzene toxicity in isolated hepatocytes. Arch Biochem Biophys. 192: 405–413

Timbrell JA, Seabra V, Waterfield CJ (1995) The *in vivo* and *in vitro* protective properties of taurine. Gen Pharmac 26: 453–462

Wang Q, Giri SN, Hyde DM, Li C (1991) Amelioration of bleomycin-induced pulmonary fibrosis in hamsters by combined treatment with taurine and niacin. Biochem Pharmacol 42: 1115–1122

Waterfield CJ (1994) Determination of taurine in biological samples and isolated hepatocytes by high performance liquid chromatography with fluorimetric detection. J Chromatog B 657: 37–45

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1993a) The correlation between urinary and liver taurine levels and between pre-dose urinary taurine and liver damage. Toxicology 77: 1–5

Waterfield CJ, Turton JA, Scales MDC, Timbrell JA (1993b) Reduction of liver taurine in rats by β-alanine treatment increases carbon tetrachloride toxicity. Toxicology 77: 7–20

Waterfield CJ, Mesquita M, Parnham P, Timbrell JA (1993c) Taurine protects against the cytotoxicity of hydrazine, 1,4-naphthoquinone and carbon tetrachloride in isolated rat hepatocytes. Biochem Pharmacol 46: 589–596

Weinstein CL, Haschemeyer RH, Griffith OW (1988) In vivo studies of cysteine metabolism: use of D-cysteine sulfinate, a novel cysteine sulfinate decarboxylase inhibitor to probe taurine and pyruvate synthesis. J Biol Chem 263: 16568–16579

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