Does urinary taurine reflect changes in protein metabolism? A study with cycloheximide in rats

Catherine J. Waterfield, Daniel S. Asker and John A. Timbrell

We have previously reported on the changes in urinary taurine levels in rats following treatment with some hepatotoxic agents and compounds reported to affect protein synthesis. This study follows the time course of the elevation of urinary taurine after treatment of rats with cycloheximide which was maximal 8–12 h after dosing and was dose related. [^{3}H]-Leucine incorporation into proteins was used as an indicator of protein synthesis. There was a significant reduction in [^{3}H]-leucine incorporation into acid precipitable proteins 8 h but not 24 h after dosing. The reduction in incorporation was negatively correlated with the raised levels of both serum and urinary taurine 8 h after dosing. Liver glutathione was raised both 8 and 24 h after dosing rats and liver taurine was significantly reduced at 8 h. It is suggested that measuring urinary taurine in collections made continuously might provide a simple, non-invasive biomarker for monitoring the effects of xenobiotics or other external stimuli on the status of protein synthesis.

Keywords: *taurine, protein synthesis, urinary marker, cycloheximide.*

Introduction

Many xenobiotics, particularly those which are hepatotoxic, are known to inhibit protein synthesis. The mechanism of this inhibition varies, depending on the compound. Thus, galactosamine reduces the synthesis of RNA and inhibits the incorporation of amino acids into proteins; this is because of a deficiency of UTP resulting from the formation of galactosamine-1-phosphate. Ethionine replaces methionine and so *S*-adenosyl-ethionine is formed which leads to a trapping of adenine, thus reducing ATP levels and inhibiting amino acid incorporation into microsomal proteins. Carbon tetrachloride (CCl_4) and thioacetamide also inhibit protein synthesis, although the mechanism is different in each case. Cycloheximide is believed to inhibit protein synthesis by blocking the termination of peptide chains. The onset of the inhibition is rapid after treatment with some compounds such as cycloheximide and in this example the inhibition is also reversible (Zimmerman 1978, Higashi *et al.* 1983, Dahlstrom-King and Plaa 1989).

Protein synthesis *in vitro* can be measured by the incorporation of radiolabelled amino acids into acid-precipitable proteins. Similar techniques are utilized *in vivo* although it is more difficult to achieve a true measure of protein synthesis. In order to monitor the rate of protein synthesis it must be decided either to administer a pulse dose given 10 min before necropsy or to use continuous infusion (Garlick *et al.* 1980, Preedy *et al.* 1990). The choice of amino acid, the time at which to measure the incorporation of radiolabelled amino acid into protein, the size of the endogenous unlabelled precursor pool and the rate of protein degradation all lead to difficulties in assessing any effects a compound may have on protein synthesis (Rannels *et al.* 1982, Garlick *et al.* 1994). Another drawback in all of the *in vivo* methods used is that they are invasive techniques often requiring the sacrifice of animals. For pulse labelling the time course for the inhibition of protein synthesis also needs to be determined in advance before measuring the reduced tracer incorporation into proteins. Thus, prospective studies are very difficult and would require the use of large numbers of animals.

Urinary taurine has been reported to increase after hepatic damage and an original hypothesis suggested that this increase resulted from leakage of taurine from damaged hepatocytes (Kocsis *et al.* 1968, Chesney 1985). Several more recent studies with rats established that several hepatotoxic compounds result in an elevation of urinary taurine (Sanins *et al.* 1990, Waterfield *et al.* 1993a, b, Timbrell *et al.* 1994). However, in these studies the total amount of taurine excreted exceeded the amount of taurine calculated to be available by direct leakage. Also, compounds such as ethionine, hydrazine and cycloheximide increased urinary taurine without elevation of serum aminotransferases or any histological evidence of necrosis. The common feature of these three compounds was their reported inhibition of protein synthesis.

Cysteine is preferentially utilized for protein and glutathione synthesis (Higashi *et al.* 1983) and excess cysteine is catabolized to sulphate and taurine (see Figure 1) which may be eliminated in the urine (Worden and Stipanuk 1985, Sturman 1990). Thus, inhibition of protein synthesis will result in an accumulation of amino acids including cysteine both from dietary sources and those resulting from protein degradation. In contrast, we reported reduced urinary levels of taurine with the β-agonists clenbuterol and salbutamol, possibly as a result of an *increase* in protein accretion in muscle tissue and therefore diversion of cysteine away from taurine synthesis (Carvalho *et al.* 1995, Waterfield *et al.* 1995). Thus urinary taurine levels may vary significantly in response to perturbations in protein synthesis.

In the previous studies with rats the time of maximum elevation of urinary taurine varied with the administration of different zenobiotics reported to reduce protein synthesis (Waterfield *et al.* 1993a). Thus, CCl_4 resulted in a rise 16 h after dosing which was sustained for 96 h or longer, thioacetamide elevated urinary taurine 24–48 h after dosing and cycloheximide (2 mg kg^{-1}) increased levels in the first 24 h with taurine excretion falling below control levels 24–48 h after dosing. If these increased levels resulted from an inhibition of protein synthesis, the inhibition of radiolabelled amino acid incorporation would be expected to occur at a similar time as the maximum elevation of taurine in the urine.

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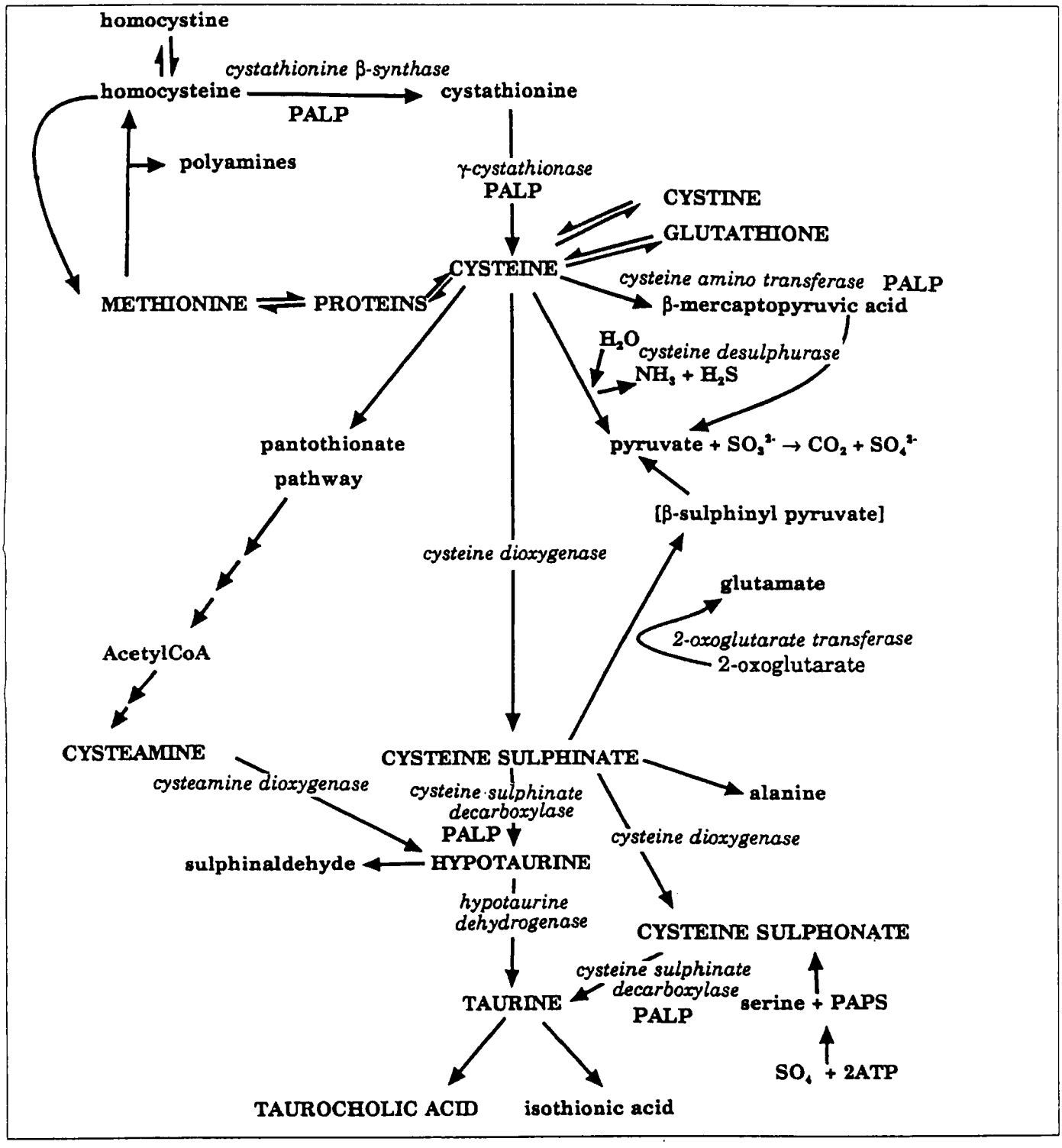

Figure 1. Metabolic pathways involved in the synthesis of taurine. PALP, pyridoxal phosphate; PAPS, 3'-phosphoadenosine-5'-phosphosulphate (Waterfield *et al.* 1993b)

The following studies were carried out using cycloheximide to inhibit protein synthesis (Rothblum *et al.* 1976) and to correlate the inhibition with increased levels of urinary taurine in order to assess urinary taurine as a potential marker for the status of protein synthesis.

Two studies were carried out. The first investigation determined the most likely time for the maximum inhibition of protein synthesis by following the urinary excretion of taurine. The incorporation of [^{3}H]-leucine into protein was also measured at the end of the study. The second study examined the incorporation of [^{3}H]-leucine at the time of maximum taurine excretion which was determined from the first study.

METHODS

Chemicals

The following compounds were supplied by the Sigma Chemical Company (Poole, Dorset, UK): cycloheximide, *o*-phthalaldehyde (OPA; HPLC grade), taurine (cell culture tested), Dowex resins, 5,5'-dithiobis-2-nitrobenzoic acid (DTMB) for measurement of total non-protein sulphydryls (TNPSH), glutathione, L-leucine and bovine serum albumin. Mercaptoethanol, sodium hydroxide (Aristar), sulphosalicylic acid, boric acid and Folin's reagent were obtained from Merck Ltd (Lutterworth, Leicestershire, UK); methanol (HPLC grade) from Rathburn (Wakeburn, UK). Coomassie Plus Protein Assay Reagent, for measurement of urinary protein, was purchased from Pierce and Warriner, Chester, UK. Water was of ultra high quality (UHQ), prepared using an Elgastat water purifier. Other chemicals were Eco-lite from ICN Biochemicals Ltd (Thame, Oxfordshire, UK) and [3,4,5-^{3}H]-L-leucine (168 Ci mmol^{-1}) from Amersham International plc (Aylesbury, Buckinghamshire, UK).

Animals and husbandry

Both studies used male rats (Sprague–Dawley stock Glaxo Research and Development, Ware), weighing 185–250 g (study 1) and 250–300 g (study 2). They were acclimatized for 8 days in communal cages after delivery then housed in individual metabolism cages during the studies (Waterfield *et al.* 1995). The rats were allocated to treatment groups (four in each group), with the same mean body weights. Powdered diet 41B (Special Diet Services, Witham, Essex, UK) and drinking water were allowed *ad libitum*. Lighting was controlled to give a regular 12 h light–dark cycle (8 am on–8 pm off) and room temperature was maintained at 20±1.5 °C.

Animals were weighed and food and water intake were measured daily for 3 days. Three pre-dose urine collections were made (24 h) over ice, made up to 25 ml with UHQ water and stored in 5 ml aliquots at –80 °C for later analysis.

Study 1

On day 4 of the study approximately 1 μmol of [^{3}H]-leucine (0.25 μCi g^{-1} body weight) was administered in the drinking water (35 ml) for 24 h before necropsy; food was not withdrawn. As the time course for the inhibition of protein synthesis was not known a continuous intake of labelled leucine may enable any differences in protein synthesis to be measured. At 10 am, animals were dosed with 0, 0.5, 1.0, 1.5 or 2.0 mg kg^{-1} cycloheximide in phosphate buffered saline (PBS; 1 ml kg^{-1} ip). Urine collections were made 0–4, 4–8, 8–12 and 12–24 h after dosing as previous studies had shown maximum taurine excretion 24 h after dosing rats with cycloheximide (Waterfield *et al.* 1993b).

Study 2

After analysis of the data from study 1, a second study was carried out. Two groups of rats (four per group) were housed in individual metabolism cages and 24 h pre-dose urine collections were made as before. At 10 am, one group was given PBS (1 ml kg^{-1}) and the second was given cycloheximide (1.5 mg kg^{-1} in PBS, 1 ml kg^{-1}, ip). Food and water were withdrawn. A single dose of [^{3}H]-leucine (0.125 μCi, 0.5 μmol g^{-1} body weight) was given ip 4 h after the dose of cycloheximide to each rat. Animals were killed 8 h after dosing with cycloheximide (4 h after [^{3}H]-leucine was given) and tissues taken *post mortem* as in study 1.

Blood and tissue sampling

At the end of the study, the animals were anaesthetized (Hypnorm:Hypnoval: water; 1:1:2, 3.33 ml kg^{-1} ip.) and exsanguinated from the abdominal aorta. Blood was collected into Microtainer serum separators (Beckton Dickinson and Co., Rutherford, NJ, USA) and centrifuged (13000 *g*, 1 min) to separate the serum which was frozen until analysed (–80 °C). The liver was removed, weighed and the right lobe frozen in liquid nitrogen for subsequent analysis of taurine, total non-protein sulphydryl groups (TNPSH), protein and incorporation of [^{3}H]-leucine into acid-precipitable protein. Tissues were stored at –80 °C until analysed.

Serum chemistry

Any tissue damage resulting from the treatment was assessed by measuring serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase, albumin, total protein, glucose, total bilirubin, cholesterol, bile acids, urea, triglycerides and creatinine and other parameters at 37 °C, using the appropriate kits (Boehringer Mannheim GmbH Diagnostica) with a centrifugal analyser (IL Monarch 2000, Instrumentation Laboratory, UK, Ltd). Previous studies (Waterfield *et al.* 1993b) had shown that the doses of cycloheximide used (in these studies) were unlikely to result in overt tissue damage. Histology was not, therefore, carried out in these studies.

Taurine and total non-protein sulphydryls (TNPSH)

Taurine was extracted from diluted urine (25 μl) using Dowex resins similar to the method previously described (Waterfield 1994) modified from Larsen *et al.* (1980). Taurine and TNPSH were measured in the supernatants from liver homogenates (300 mg in sulphosalicylic acid 0.2 M, 4 ml, 4 °C) (Ellman 1959, Waterfield 1994). As reduced glutathione (GSH) constitutes > 95% of liver TNPSH, this was used as a measure of GSH (DeMaster and Refern 1987, Potter and Tran 1993). Serum taurine was measured after deproteinating serum samples (200 μl) with sulphosalicylic acid (200 μl, 0.4 M, 11000 *g*, 4 °C). Taurine was measured using fluorometric detection in the eluates from the ion exchange columns using HPLC after derivatization with OPA and mercaptoethanol.

[^{3}H]L-leucine incorporation into protein

[^{3}H]L-leucine incorporation into liver and serum acid-precipitable proteins was measured in the sulphosalicylic acid-precipitated protein pellets prepared when making acid soluble extracts to measure taurine and TNPSH. The pellets were resuspended and washed with sulphosalicylic acid (4 °C, 4 times), then the pellets were dissolved in NaOH (1–10 ml, 1 M, 60 °C) and sonicated to aid dissolution. Aliquots (200 μl, in duplicate) were mixed with Eco-lite scintillation fluid (3.5 ml) and counted for 15 min in a scintillation counter (Beckman LS 6000 Series Liquid Scintillation System, Beckman Instruments Inc., Fullerton, CA, USA). Protein was measured in diluted samples (50–500×) of the same NaOH digests by the method of Lowry *et al.* (1951) using bovine serum albumin standards in the range 0–100 μg ml^{-1}.

Urinary protein

Urinary protein was measured in diluted urine (1+49 or 1+99) and assayed for protein using Coomassie Brilliant Blue G-250 (Pierce Protein Assay Reagent) and a microassay procedure. The absorbance was determined at 595 nm and compared with bovine serum albumin standards (0–25 μg ml^{-1}).

Statistical analysis

Urinary levels of taurine in samples taken from the same animal on different days were compared with pre-dose values for each animal using a paired Student's 't' test. For all other data, Dunnett's test for multiple comparisons with a single control was used to determine significance between treatment groups and the control group for study 1 and Student's 't' test was used to compare the treatment group with the control group in study 2.

Results

Body weights, food and water intake

Study 1

Animals treated with the highest dose of cycloheximide (2 mg kg^{-1}) developed diarrhoea 4 h after dosing and their weight gain was reduced but not significantly. Food intake was significantly reduced in all animals given cycloheximide, except the lowest dose group (0.5 mg kg^{-1}) but water intake was not significantly affected (Figure 2).

Study 2

There was no significant effect on body weight 8 h after dosing with 1.5 mg kg^{-1} cycloheximide (data not shown). Water and food were withdrawn after dosing.

Organ weights

There were no significant effects on liver or kidney weights in either study (data not shown).

Serum chemistry

None of the serum parameters measured 24 h after dosing (study 1) were different from control values (data not shown).

In study 2, 8 h after treatment with cycloheximide (1.5 mg kg^{-1}) significant changes were seen in the serum biochemistry of these animals (Table 1). Thus total bilirubin, urea and triglyceride levels were significantly increased and cholesterol and glucose were reduced.

Taurine levels

Urinary taurine

Study 1. Urinary taurine was elevated above control levels by all doses of cycloheximide during the first 4 h after dosing (Figure 3A), an effect which was significant with 1.5 mg kg^{-1} cycloheximide. Urinary taurine continued to increase between 4 and 8 h after dosing and then declined from 8 to 12 h (0.5 and 1 mg kg^{-1}) and after 12 h at the higher doses. The urine collections 12–24 h after dosing had taurine levels which were similar to control levels. The total increase in urinary taurine excretion was dose related and maximal after 1.5 mg kg^{-1} cycloheximide (Figure 3B).

Study 2. Urinary taurine was raised significantly 4–8 h after dosing with cycloheximide (1.5 mg. kg^{-1}) to a level higher than study 1 (Table 2).

Hepatic taurine

Liver taurine concentrations were not significantly different from the control group of animals 24 h after dosing animals with all doses of cycloheximide (study 1, data not shown). However, the concentration of taurine in the liver taken 8 h after dosing animals with cycloheximide (1.5 mg kg^{-1}) was significantly reduced (study 2, Table 2).

Serum taurine

In study 1, serum levels of taurine were not significantly different between the treatment groups 24 h after dosing (data

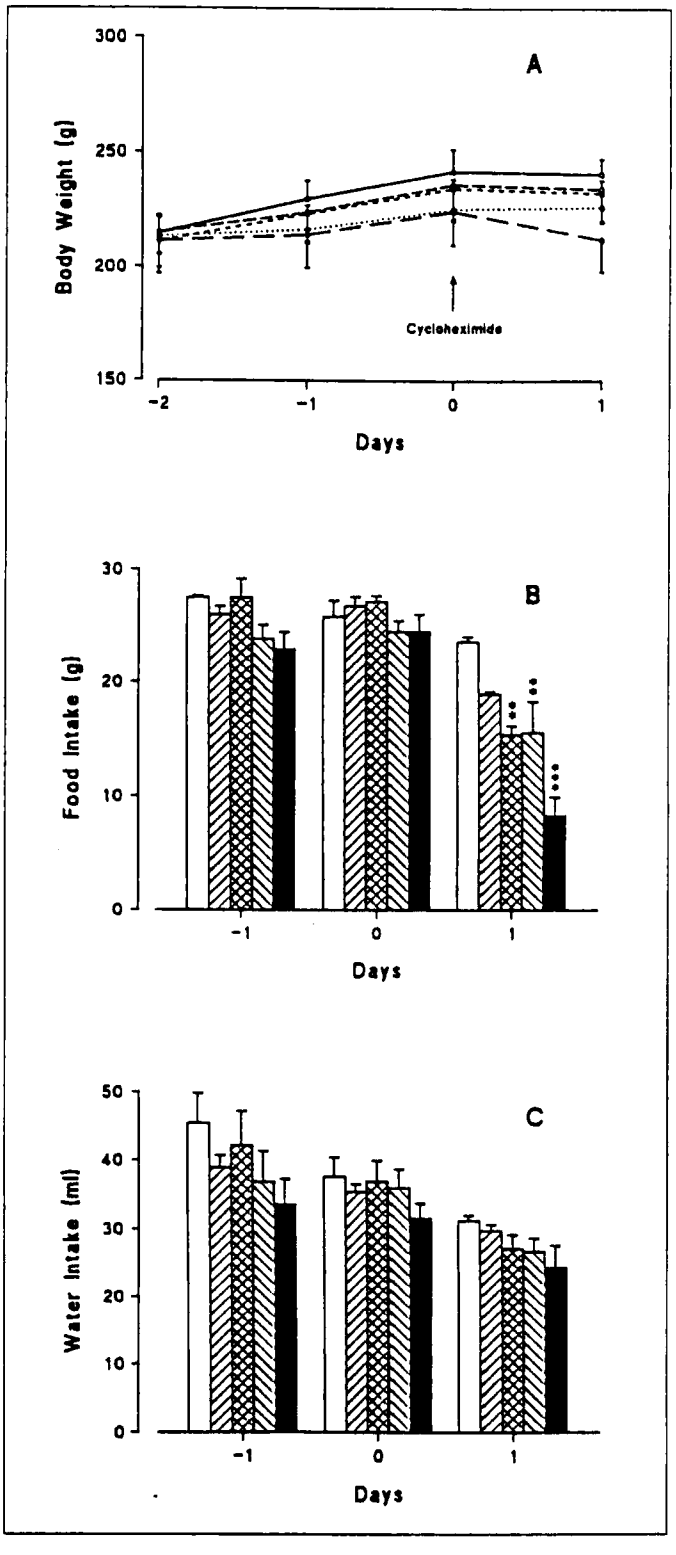

Figure 2. (A) Study 1: Body weights of rats before and after dosing with cycloheximide. Control ——; cycloheximide 0.5 mg kg^{-1} – – – –; 1.0 mg kg^{-1} ·····; 1.5 mg kg^{-1} . . . . . .; 2.0 mg kg^{-1} — —. Values are means ± SEM; $N = 4$. (B) and (C) Study 1: Food (B) and water (C) consumption in rats before and after dosing with cycloheximide. Control □; cycloheximide 0.5 mg kg^{-1} ▨; 1.0 mg kg^{-1} ▩ 1.5 mg kg^{-1} ▧; 2.0 mg kg^{-1} ■. Values are means ± SEM; $N = 4$; $^{**}p < 0.01$, $^{***}p < 0.001$ using Dunnett's test.

Treatment	Glucose (mmol l^{-1})	Total bilirubin (μmol l^{-1})	Cholesterol (mmol l^{-1})	Urea (mmol l^{-1})	Triglycerides (mmol l^{-1})
Control	9.78 ± 0.03	2.50 ± 0.23	1.56 ± 0.03	3.80 ± 0.08	0.57 ± 0.10
Cycloheximide	5.77 ± 0.28***	15.53 ± 1.92**	1.14 ± 0.10*	7.03 ± 0.49**	1.76 ± 0.26*

Table 1. Study 2: Mean serum chemistry values at 8 h after treatment with cycloheximide (1.5 mg kg^{-1}). Only parameters showing significant differences between control values have been tabulated. Serum enzyme levels, albumin and total protein levels were unaffected by the treatment. $N = 4$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ using Student's 't' test.

Treatment	Urinary taurine (μmol kg^{-1} h^{-1})		Samples taken 8 h post-dose				
	0–4 h post-dose	4–8 h post-dose	Liver TNPSH (μmol g^{-1})	Liver taurine (μmol g^{-1})	Serum taurine (mmol l^{-1})	Serum [^{3}H]-leucine (dpm mg^{-1} protein)	Liver [^{3}H]-leucine (dpm mg^{-1} protein)
Control	7.89 ± 1.66	7.84 ± 1.66	4.9 ± 0.26	5.88 ± 0.58	0.22 ± 0.01	153.6 ± 8.4	30.3 ± 2.01
Cycloheximide	16.17 ± 4.64	65 ± 15.83*	6.88 ± 0.09***	3.43 ± 0.26**	0.44 ± 0.03***	64.8 ± 7.1***	12.42 ± 0.77***

Table 2. Study 2: Taurine and total non-protein sulphydryl (TNPSH) levels in tissues and [^{3}H]-leucine incorporation into acid-precipitable protein from tissues taken 8 h after dosing with cycloheximide (1.5 mg kg^{-1}). $N = 4$; *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, Student's 't' test.

not shown). However, after 8 h, serum taurine values in animals treated with 1.5 mg kg^{-1} cycloheximide were double the control levels (Table 2).

Hepatic total non-protein sulphydryls (TNPSH)

Total non-protein sulphydryl levels were elevated in liver tissue taken 24 h after dosing animals with 0.5, 1.0 and 1.5 mg kg^{-1} but not with 2 mg kg^{-1} cycloheximide (Figure 4). This elevation was also seen 8 h after treatment with 1.5 mg kg^{-1} cycloheximide (Table 2).

[^{3}H]-Leucine incorporation into protein

Study 1

There was no correlation between the incorporation of [^{3}H]-leucine (as a percentage of the total intake of [^{3}H]-leucine) into liver or serum protein 24 h after dosing with cycloheximide and the levels of urinary taurine excreted by individual animals. There was also no significant difference in the incorporation of [^{3}H]-leucine into acid-precipitable serum and liver proteins after 24 h with any dose of cycloheximide (data not shown).

Study 2

Eight hours after treating animals with cycloheximide (1.5 mg kg^{-1}) there was a significant reduction in the incorporation of [^{3}H]-leucine into acid-precipitable proteins for both serum and liver (Table 2). This reduction was negatively correlated in individual animals with elevated levels of urinary taurine 4–8 h after dosing (Figure 5 A and B). Serum levels of taurine were also negatively correlated with [^{3}H]-leucine incorporation into serum proteins (Figure 5C).

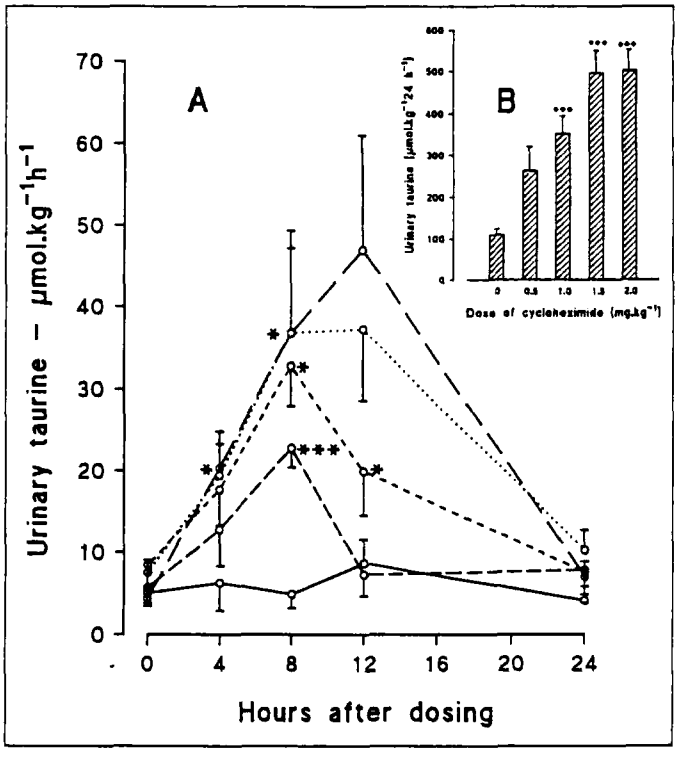

Figure 3. (A) Study 1: Urinary taurine measured in 4 h urine samples following dosing with cycloheximide. Control ——; cycloheximide 0.5 mg kg^{-1} – – – –; 1.0 mg kg^{-1} ------; 1.5 mg kg^{-1} . . . . . ; 2.0 mg kg^{-1} — —. Values are means ± SEM; $N = 4$, *$p < 0.05$, ***$p < 0.001$ using paired 't' test, pre-dose values compared with post-dose values. (B) Total excretion of urinary taurine in rats for 24 h following treatment with cycloheximide. Values are means ± SEM; $N = 4$; ***$p < 0.001$, using Dunnett's test.

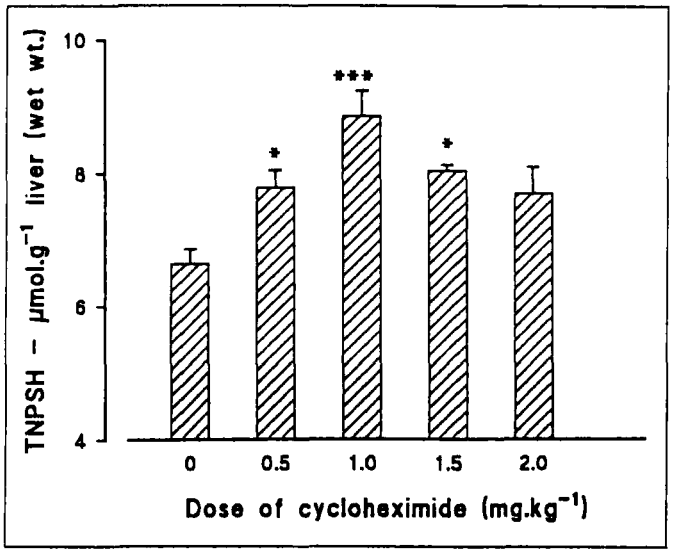

Figure 4. Concentration of total non-protein sulphydryls (TNPSH) in liver 24 h after dosing with cycloheximide. Values are means ± SEM, $^{*}p < 0.05$, $^{***}p < 0.001$ using Dunnett's test; $N = 4$.

Urinary protein

Some protein is normally lost into the urine of rats. These levels appear to be reduced when there is an inhibition of protein synthesis and is particularly apparent when serum total protein and albumin are also reduced (Waterfield *et al.* 1993b). There was a high degree of variability between the urinary levels of protein excreted in study 1. However, all doses of cycloheximide resulted in a significant reduction in urinary protein excreted for the 24 h following dosing, the effect being seen initially 4–8 h after dosing and then sustained for the remainder of the study (Figure 6).

Discussion

The aim of these investigations was to assess the value of taurine as a biomarker of protein metabolism by correlating the cycloheximide-induced inhibition of leucine incorporation into protein with elevations of urinary taurine in the absence of tissue damage. Serum enzyme levels supported the lack of overt cellular damage. Absolute protein values were measured in serum and urine and [^{3}H]-leucine incorporation into proteins was measured.

Although we have previously reported a marked reduction in serum proteins, albumin and enzymes 48 h following cycloheximide treatment of rats (2 mg kg^{-1}) (Waterfield *et al.* 1993b) there were no significant changes seen in the absolute values of serum proteins 8 or 24 h after treatment in these studies (data not shown). However, levels of urinary protein were significantly reduced in study 1 despite the lack of effect on serum protein levels. This highlights the advantages of measuring parameters in urine which can be constantly monitored rather than in blood samples which may change transiently. The lack of effect on serum protein levels 8 and 24 h after dosing may indicate that protein degradation was

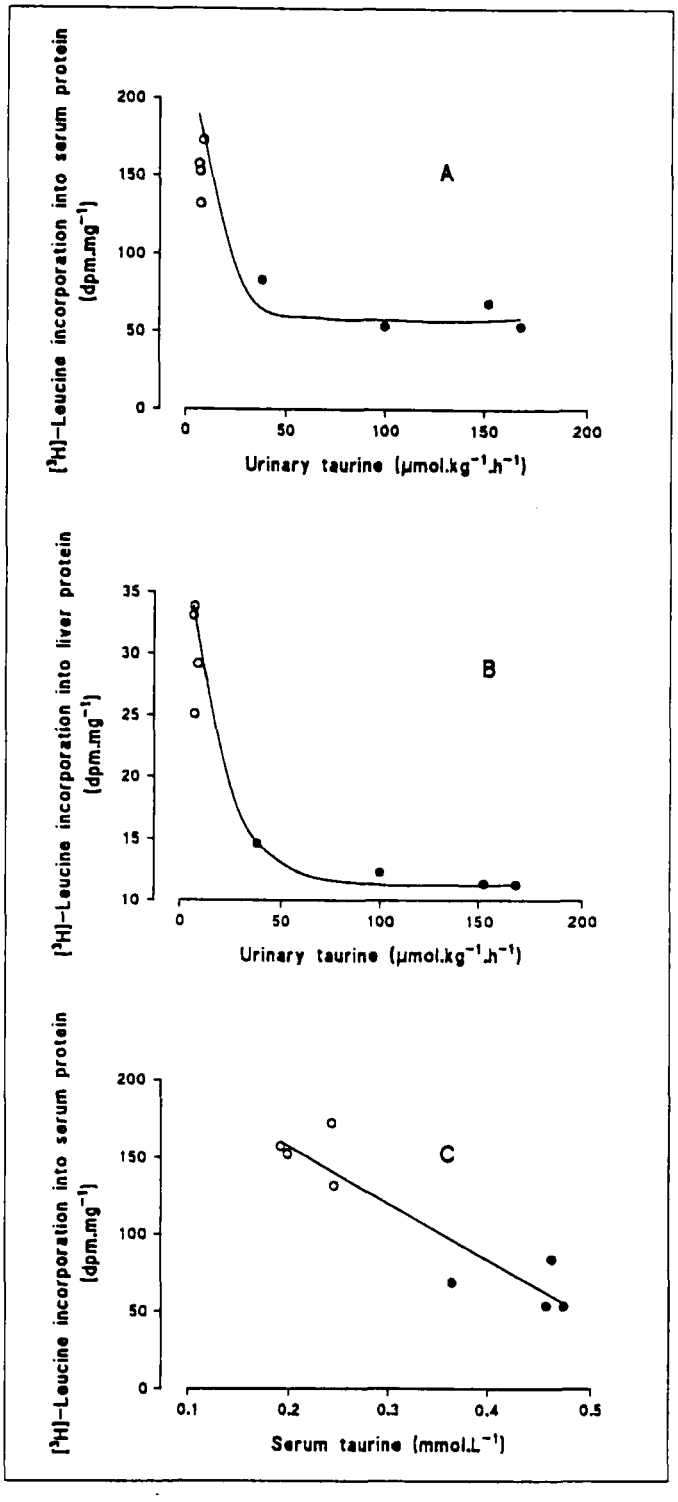

Figure 5. Study 2: Correlations between [^{3}H]-leucine incorporation into acid-precipitable proteins 8 h after dosing with cycloheximide (1.5 mg kg^{-1}) and taurine levels. Each point represents data from an individual animal. Control ○ ; treated ● . (A) Correlation between [^{3}H]-leucine incorporation into serum proteins and urinary taurine (4–8 h collection after dosing). Linear correlation on $\log_{10}/\log_{10}$ conversio, $r = -0.96$, $p < 0.001$. (B) Correlation between [^{3}H]-leucine incorporation into liver proteins and urinary taurine (4–8 h collection after dosing). Linear correlation on $\log_{10}/\log_{10}$ conversion, $r = -0.97$, $p < 0.001$. (C) Correlation between [^{3}H]-leucine incorporation into serum proteins and serum taurine 8 h after dosing; $r = -0.92$, $p < 0.001$.

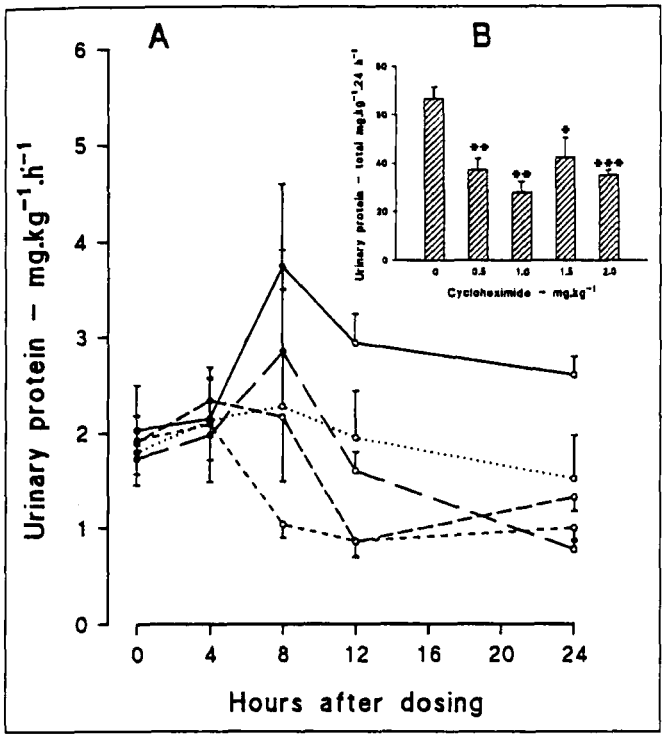

Figure 6. (A) Study 1: Urinary protein in 4 h urine collections after dosing with cycloheximide. Control ——; cycloheximide 0.5 mg kg^{-1} — —; 1.0 mg kg^{-1} – – – –; 1.5 mg kg^{-1} . . . . .; 2.0mg kg^{-1} — — —. Values are means ± SEM; $N = 4$. (B) Study 1: Total excretion of urinary protein in rats for 24 h following treatment with cycloheximide. Values are means ± SEM; $N = 4$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ using Dunnett's test.

not taking place rapidly enough to be affected by an inhibition of protein synthesis. There was, however, a significant reduction in the incorporation of [^{3}H]-leucine into serum and liver acid-precipitable proteins 8 h after dosing (1.5 mg kg^{-1}). Although neither the precursor pools of leucine nor the time course of labelling were measured this observation is compatible with the reported inhibition of protein synthesis by cycloheximide 2–4 h after administration (Indacochea-Redmond *et al.* 1973) when protein synthesis remains low for 18 h after dosing. This effect, however, would also be observed if the rate of protein degradation was increased by the administration of cycloheximide.

Urinary taurine was significantly elevated 4–12 h after dosing with cycloheximide despite the fact that the treated animals had a significant reduction in their food intake at the highest doses (study 1) and had food withdrawn in study 2. Reduced food intake would normally reduce urinary output of taurine (Waterfield *et al.* 1993a).

The increased levels of total bilirubin found in serum 8 h after dosing rats with 1.5 mg kg^{-1} cycloheximide was not a result of haemolysis but may suggest that cycloheximide had affected bile flow, possibly reducing it. The rise in bilirubin may also have been a result of a reduction in albumin available to bind bilirubin, although serum albumin levels did not appear to be reduced. The elevated level of serum triglycerides may have resulted from a mobilization of fats from adipose tissue or reduced uptake into hepatocytes. There was also a rise in serum urea which would also be consistent with a change in protein status resulting in the deamination of excess amino acids not incorporated into proteins as a result of changes in amino acid utilization in protein metabolism. As serum creatinine levels were normal and urinary protein was reduced, the raised level of serum urea (8 h after dosing) was unlikely to be caused by reduced kidney function. However, serum transaminase enzymes were not increased indicating that there had not been overt hepatocellular damage which could have resulted in overflow of cellular taurine into the bloodstream and then into the urine.

It seems likely, therefore, that the elevation of urinary taurine was caused by a change in protein synthesis, possibly an inhibition of synthesis. The decrease in the rate of utilization of both methionine and cysteine as a result of inhibition of protein synthesis would lead directly to an increase in taurine synthesis. Indeed, a similar effect on GSH synthesis has been reported by Higashi *et al.* (1983) after administration of cycloheximide to rats. They reported an increase in GSH synthesis 4 h after dosing and a maximum two-fold increase in liver GSH levels 6 h after dosing with raised levels of GSH still apparent 24 h later. This increase in GSH synthesis paralleled the increase in cysteine levels as a result of protein synthesis inhibition and tracer studies with ^{35}[S]-cysteine showed that incorporation of cysteine into GSH was stimulated by cycloheximide treatment (Higashi *et al.* 1983). The data presented here for increased hepatic TNPSH (which includes GSH) increased both 8 and 24 h after dosing with cycloheximide could reflect increased GSH synthesis due to raised cysteine levels.

The lower levels of liver taurine 8 h after dosing are difficult to account for. Similar studies following the administration of ethionine to rats (Waterfield *et al.* 1993a) resulted in an increase in liver taurine levels 72 h (but not 48 h) after administration, with increased hepatic GSH levels at both time points. Liver levels of taurine do vary more than those in any other tissue (Hirai *et al.* 1987) probably as a result of the effects of bile acid conjugation and biliary excretion as well as the rate of transportation into the blood stream to other tissues. Thus, liver taurine has a short half-life and urinary taurine maybe better at reflecting the body pool for taurine. A similar increase in urinary taurine accompanied by a reduction in liver taurine levels (despite higher cysteine dioxygenase activity) has also been reported by Hosokawa *et al.* (1988). It is possible that the synthesis of hepatic taurine is regulated by serum levels which were significantly raised 8 h after dosing with cycloheximide.

These data reinforce the hypothesis that the hypertaurinuria which develops in rats treated with some hepatotoxic compounds in previous studies is either enhanced by the inhibition of protein synthesis (e.g. CCl_4 and galactosamine) or is a direct result of the inhibition of protein synthesis (e.g. hydrazine and ethionine) (Waterfield *et al.* 1993a). As there appears to be a good correlation between the incorporation of leucine into protein and the urinary excretion of taurine, the measurement of urinary taurine may provide a useful non-invasive method for monitoring the effects of xenobiotics or other stress-induced alterations in protein synthesis such as surgical trauma.

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