16P

MEASUREMENT OF URINARY MEDIUM CHAIN ACYL GLYCINES BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY

S.M.Bonham Carter[1]; D.G.Watson[1]; J.M.Midgley[1]; R.W.Logan[2]

1 Department of Pharmacy, University of Strathclyde, Glasgow G1 1XW
2 Department of Biochemistry, Royal Hospital for Sick Children and Queen Mother's Hospital, Yorkhill, Glasgow G3

Medium chain acyl-CoA dehydrogenase (MCAD) deficiency is an inborn error of fatty acid metabolism which can present as simple hypoglycaemia, intolerance to fasting, lethargy, coma and medium chain dicarboxylic aciduria (Stanley et al 1983). It has been mistaken for Reye's syndrome (Rocchioccioli et al 1984; Taubman et al 1987), and there is good evidence that a significant percentage of cot deaths are due to this deficiency (Howat et al 1985; Roe et al 1986). Recently it has been shown that the urinary excretion of two medium chain acyl glycines, hexanoyl glycine and 3-phenylpropionyl glycine, normally minor metabolites of fatty acid metabolism, were markedly increased in patients with MCAD deficiency not only during acute episodes but also in remission (Rinaldo et al 1988). Urinary suberyl glycine was also increased in some samples.

We report the establishment of a method for measuring urinary acyl glycines using gas chromatography-negative ion chemical ionisation mass spectrometry (GC-NICIMS). We have synthesised a series of acyl glycines and their respective internal standards using $^{13}C_2$-glycine. Hexanoyl, octanoyl and 3-phenylpropionyl glycines were prepared according to Bondi and Eissler (1910) and suberyl glycine according to Gregersen et al (1976). Known amounts of the acyl $^{13}C_2$-glycines were added to the urine samples which were acidified and extracted into ethyl acetate; back extraction into dilute alkali, followed by re-acidification and re-extraction into ethyl acetate was used to reduce interference from the biological matrix. After evaporating the solvent under a stream of nitrogen the extracts were reacted with trifluoroethanol (10 μL) and pentafluoropropionic anhydride (40 μL) at 70° for 1 h. This procedure resulted in conversion of carboxyl groups to trifluorethyl esters and the replacement of the amide hydrogen with a pentafluoropropionyl group, giving highly electron-capturing, volatile derivatives for GC-NICIMS. In the NICI spectra of all the glycines we have made most of the ion current was carried by the loss of hydrogen fluoride from the molecular ion. These mass spectral characteristics give a potential limit of detection at below the picogram level, but in practice the sensitivity is dependent on interfering background from the biological matrix. At present in urine it is possible to detect 10 ng of material above background. The precision of the method is high since the physical properties of the ^{13}C-labelled standards are identical to those of the endogenous compounds which compensates accurately for losses in extraction and derivatisation.

Preliminary results obtained for these acyl glycines in urine samples from normal subjects give the following ranges: (μg/mg of creatinine,(n)); hexanoyl (0.09-1.15, (12)), octanoyl (N.D.- 0.15, (12)), 3-phenylpropionyl (0.01-0.09,(12)), suberyl (0.02-1.53,(10)). N.D. = below the limit of detection.

Bondi, S. and Eissler, F. (1910) Biochem. Z. 23: 499-509
Gregersen, N. et al (1976) Clin. Chim. Acta 70: 417-425
Howat, A.J. et al (1985) Br. Med. J. 290: 1771-1773
Rinaldo, P. et al (1988) New Engl. J. Med. 319: 1308-1313
Rocchioccioli, F. et al (1984) Biomed. Mass Spectrom. 11: 127-131
Roe, C.R. et al (1986) J. Pediat. 108: 13-18
Stanley, C.A. et al (1983) Pediatr. Res. 17: 877-884
Taubman, B. et al (1987) Pediatrics 79: 382-385