107P

CYSTEINE CONJUGATE SUBSTRATES FOR RAT RENAL β-LYASE: AN EXAMINATION OF THE STRUCTURE-ACTIVITY RELATIONSHIPS

Ian. S. Blagbrough, Barrie W. Bycroft, David C. Evans, *Trevor Green, P. Nicholas Shaw, Department of Pharmacy, University of Nottingham, Nottingham NG7 2RD, *ICI plc, Central Toxicology Laboratory, Alderley Park, Macclesfield SK10 4TJ

Rat renal β-lyase is implicated in the generation of potentially nephrotoxic and mutagenic metabolites. Compounds which have been previously studied as substrates for this enzyme have included fluorinated and chlorinated S-alkyl L-cysteine conjugates (Green and Odum, 1985). The chlorinated substrates have been shown to be both mutagenic and cytotoxic whereas cytotoxicity alone was displayed by the fluorinated analogues. It has been postulated that the toxicity of these cysteine conjugates is mediated via the formation of a reactive thioacylium species. Cleavage of the substrate by the enzyme also yields pyruvate and ammonia. Measurement of either of the latter compounds allows ready assay of enzyme activity. Previous workers have concentrated upon hepatic β-lyase, the renal enzyme has however been the subject of only one SAR study (Green and Odum, op.cit.) which demonstrated the requirement of a halogen containing cysteine conjugate.

The present work is directed towards the systematic replacement of the halogen by a variety of electron withdrawing groups whilst still maintaining enzyme activity. These variations, together with modifications in the carbon skeleton will enable a definition of the structural requirements for renal β-lyase activity. Several S-mono- and dinitrobenzene substituted L-cysteines were prepared in good yield from an appropriately substituted chloronitrobenzene and the L-cysteine thiolate generated with sodium methoxide in methanol.

Rat renal β-lyase was isolated using a modificaton of the method of Tateishi et al (1978). This procedure involved renal sub-cellular fractionation, protein denaturation (58^{o}C) and four chromatographic purifications over DEAE-cellulose, DEAE-Sephacel, PBE 94 chromatofocussing gel (pH 9-4) and Sepharose 6B-CL. Throughout this procedure enzyme activity was monitored using S-(Z-1,2-dichloroethenyl) L-cysteine as the substrate.

S-2,4-Dinitrophenyl L-cysteine has been established as a substrate for hepatic β-lyase (Stevens and Jakoby, 1983) as has the 2-benzothiazolyl substituted analogue (Dohn and Anders, 1982). These compounds together with the 2- and 4-mononitro and the 3,4-dinitro substituted benzenoids were prepared and fully characterised. On incubation of these aromatic thioethers with renal β-lyase no activity was detected. It is clear from these preliminary studies that haloalkenes cannot be effectively replaced by nitrobenzenoids and this work demonstrates a marked difference in substrate specificities between the renal and hepatic enzymes.

We thank the Health and Safety Executive and ICI for financial and technical support.

Dohn, D.R., Anders, M.W. (1982) Anal. Biochem. 120: 379-386.
Green, T., Odum, J. (1985) Chem.-Biol. Interactions 54: 15-31.
Stevens, J., Jakoby, W.B. (1983) Mol. Pharmacol. 23: 761-765.
Tateishi, M. et al (1978) J. Biol. Chem. 253: 8854-8859.