Journal of Chromatography, 593 (1992) 99–101
Elsevier Science Publishers B.V., Amsterdam

CHROM. 2405

Short Communication

Rapid determination of amino acids by high-performance liquid chromatography: release of amino acids by perfused rat liver

F. Zezza*, J. Kerner, M. R. Pascale, R. Giannini and E. Arrigoni Martelli

Department of Biochemistry, Research and Development, Sigma-tau SpA, Rome (Italy)

ABSTRACT

Perfused rat liver can be considered as one of the most suitable *ex vivo* models for studies of liver metabolism. To assess the possible effect of L-carnitine and some of its acyl esters on proteolysis in the rat liver, the amino acid derivatization and high-performance liquid chromatographic separation of Tapuhi *et al.* [*Anal. Biochem.*, 115 (1981) 123] was modified.

INTRODUCTION

The method for amino acid derivatization and separation by high-performance liquid chromatography (HPLC) of Tapuhi *et al.* [1] has been modified and applied to determine amino acids released by perfused rat liver *in situ*. The amount of L-valine in the perfusate was taken as an indicator of liver proteolysis because its metabolism in the liver is very slow and quantitatively insignificant.

The aim of this work was to evaluate the possible inhibitory effect of different acyl-L-carnitine derivatives on liver proteolysis in normal rats.

EXPERIMENTAL

Male Wistar rats (130–150 g body weight) were used and were maintained on standard laboratory chow and water *ad libitum*. The animals were anaesthetized with ketamine (Inoketam), 3 μl/g body weight, and heparinized (200 μl of a 5000 U/ml solution).

The perfusion was performed according to the method of Mortimore *et al.* [2]. In the first step (non-recirculating), Krebs Ringer hydrogencarbonate buffer containing 4% bovine serum albumin (fraction V, Sigma) was used for 40 min. This was followed (recirculating step) by a second Krebs Ringer buffer (50 ml) without glucose, but containing 18 μ*M* cycloheximide, to stop hepatic protein synthesis.

The acyl-L-carnitine derivatives to be tested were added to the recirculating buffer at 0.22 or 0.88 m*M* concentrations. Control livers were perfused as described, but without the carnitine derivatives.

The buffer pH was adjusted to 7.40–7.45 by gassing for 1 h before the perfusion with a mixture of oxygen and carbon dioxide (95:5) and the solutions were filtered through a 0.45-μm Millipore filter. After 15 min of recirculating perfusion, samples were taken and 750 μ*M* L-norvaline was added as an internal standard. A 1-ml volume of perfusate was deproteinized with 75 μl of ice-cold perchloric acid (60% solution). The supernatant was then neutral-

0021-9673/92/$05.00

ized with 200 m*M* potassium carbonate. A 0.5 *M* solution of potassium carbonate–potassium hydrogencarbonate (30:70, v/v) was added and the pH adjusted to 9.40–9.50 with 5 *M* potassium hydroxide. Dansyl chloride was prepared (1.25 mg/ml final concentration) in acetonitrile and 100 μl of this solution were added to 200 μl of each sample. Derivatization was carried out at room temperature for 1 h in the dark. The reaction was stopped by adding methylamine (6 μl of a 0.2% solution in water) to neutralize any excess dansyl chloride.

Acetic acid was then added to the samples (3% final concentration) to avoid the possible formation of bubbles following sample injection and mixing with the mobile phase.

A Varian high-performance liquid chromatograph was used for the analysis. This consisted of a STAR 9095 autosampler, a STAR 9010 solvent delivery system, a Merck-Hitachi F-1050 fluorescence spectrophotometer, a Biosil C_{18} reversed-phase ODS-5S, 250 × 4 mm column (Biorad) with a Biosil ODS-5S guard column with a microguard refill cartridge (30 × 4.6 mm). The whole system was controlled by a Compaq personal computer.

Dansylated amino acids were separated using mobile phases of: (a) water–methanol (85:15, v/v), containing 1% (v/v) of glacial acetic acid and 0.030% (v/v) of triethylamine; and (b) methanol–acetonitrile (70:30, v/v) containing 3% (v/v) of glacial acetic acid and 0.030% (v/v) triethylamine.

For elution the following gradient was used: 0–52 min, linear increase from 30 to 50% solvent B; 52–73 min, linear increase from 50 to 75% solvent B. Solvent B (75%) was maintained for 5 min, followed by a linear decrease of solvent B to 30% in 7 min (return to initial conditions). The flow-rate was held at 1.0 ml/min throughout the analysis. The separation was performed at room temperature and the effluent monitored and recorded at 340 and 520 nm (excitation and emission wavelengths).

RESULTS

Using this method it was possible to evaluate the release of amino acids (proteolysis) in rat liver. L-Valine, considered to be the best indicator of liver proteolysis, was calculated as the total amount released in the total recirculating buffer plus liver wa-

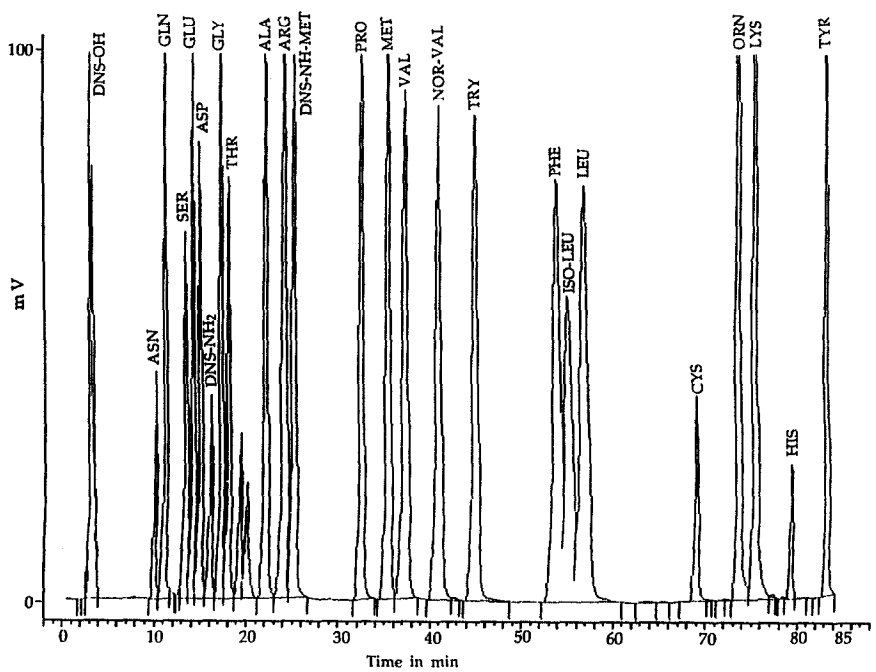

Fig. 1. Representative chromatogram for the standard analysis of amino acids with norvaline as the internal standard. The injection volume was 20 μl and the amino acid concentration 37.5 μ*M*. Dansylated amino acids were separated as described under Experimental.

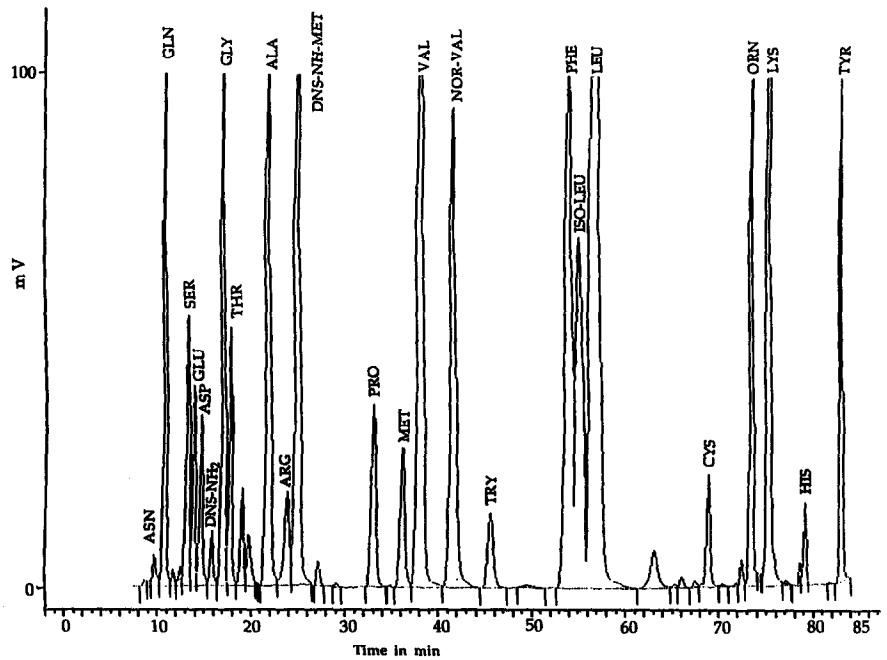

Fig. 2. Representative HPLC analysis of liver perfusate. Experimental conditions as in Fig. 1.

ter [3]. Representative chromatograms are shown in Figs. 1 and 2.

The rate of L-valine release into the perfusate buffer was expressed as nmol/ml of perfusate per g liver wet weight.

For each group of rats (control and those treated with L-carnitine, L-leucyl-L-carnitine or isovaleryl-L-carnitine-γ-hydroxybutyrate) at least four livers were perfused. Each compound was tested at two different concentrations (0.22 and 0.88 m*M*).

In these experiments L-carnitine, L-leucyl-L-carnitine and isovaleryl-L-carnitine-γ-hydroxybutyrate inhibited the release of L-valine acids from rat liver, lowering the proteolysis by over 20% at the higher concentration. The results obtained with L-leucyl-L-carnitine are similar to those found with L-leucine by other workers [4].

DISCUSSION

The aim of this study was to evaluate the possible effect of L-carnitine and some of its acyl derivatives on rat liver proteolysis. It has been shown that leucine is a strong inhibitor of rat liver proteolysis, suppressing proteolysis by about 50% at a concentration four times its normal plasma level [5]. It has also been shown that isovaleryl-L-carnitine, and to a lesser extent L-carnitine, inhibits proteolysis in perfused rat liver induced by amino acid deprivation [4].

In these experiments L-carnitine and two of its acyl esters (L-leucyl-L-carnitine and isovaleryl-L-carnitine-γ-hydroxybutyrate) were studied and it was found that both esters have a pronounced inhibitory effect on liver proteolysis. The inhibitory action of isovaleryl-L-carnitine seems to be very specific, as neither isovalerate alone nor isovalerate plus L-carnitine have a comparable effect [4].

It is intended to use this method to test other carnitine derivatives to find a compound (or compounds) which are superior in their antiproteolytic effects to those compounds studied here.

REFERENCES

1 Y. Tapuhi, D. E. Schmidt, W. Lindner and B. L. Karger, *Anal. Biochem.*, 115 (1981) 123.
2 G. E. Mortimore, A. R. Pösö, M. Kadowaki and J. J. Wert, *J. Biol. Chem.*, 262 (1987) 16322.
3 G. E. Mortimore, K. H. Woodside and J. E. Henry, *J. Biol. Chem.*, 247 (1972) 2776.
4 G. Miotto, R. Venerando and N. Siliprandi, *Biochem. Biophys. Res. Commun.*, 158 (1989) 797.
5 A. R. Pösö, J. J. Wert and G. E. Mortimore, *J. Biol. Chem.* 257 (1982) 12 114.