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SUBCELLULAR LOCALISATION OF DI- AND TRIPEPTIDASES IN GUINEA PIG AND RAT ENTEROCYTES

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Summary

Enterocytes were isolated from rat and guinea pig jejunum and subcellular fractions were prepared by density gradient centrifugation. Gradient fractions were assayed for principal organelle marker enzymes and for di- and tripeptidases. The hydrolases showed a dual localisation with both brush border and cytosol components. In the rat, approximately equal portions of dipeptidase activities were found in the two fractions but, in the guinea pig, three times more activity was found in the soluble than in the brush border fractions. Cytosol components in the rat were markedly inhibited by *p*-hydroxymercuribenzoate. In both species tripeptidase, leucyl-2-naphthylamidases and γ-glutamyltransferase activities were found predominantly in the brush border fractions.

Introduction

The subcellular localisation of peptidases in the intestine has been the subject of some controversy and conflicting results have been obtained by different workers [1—5]. These studies used differential centrifugation on whole intestinal mucosal scrapings as starting material. In the presence studies, isolated intestinal epithelial cells (enterocytes) were used and a one-step density gradient fractionation was used to separate the organelles.

Materials and Methods

Animals. Adult male Wistar rats (250—300 g) and adult male Duncan-Hartley guinea pigs (300—400 g) were used. The animals were killed by a blow on the head, and a 30 cm segment of jejunum removed for enterocyte isolation.

The enterocytes from rat and guinea pig were isolated by procedures previously described [6,7]. Enterocytes from both species were than treated in the same way.

The enterocytes were washed in 20 ml ice-cold 0.3 M sucrose, containing 3 mM imidazole-HCl, pH 7.2 (SI medium) by repeated resuspension and centrifugation. The final pellet was suspended in 10 ml SI medium and disrupted in a medium-sized Dounce homogeniser with ten strokes of a loose-fitting (type A) pestle. This preparation was centrifuged at 800 × *g* for 10 min and the supernatant fraction removed, stored on ice and subjected to analytical subcellular fractionation. In some experiments, 0.82 mM digitonin, a selective membrane perturbant [8], was included in the homogenisation medium.

Analytical subcellular fractionation [6]. Approx. 5 ml supernatant fraction was layered onto 28 ml sucrose density gradient extending, linearly with respect to volume, from a density of 1.05 to 1.28, and resting on a 6 ml cushion of density 1.32 in the Beaufay [9] automatic zonal rotor. All solutions contained 3 mM imidazole-HCl buffer (pH 7.2). The rotor was accelerated to 35 000 rev./min and run for 35 min. It was then slowed to 8000 rev./min for automatic unloading. Some 15 fractions were collected into pre-weighed tubes, thoroughly mixed, re-weighed and the density determined with an Abbé refractometer with reference to conversion table [10]. All fractions were stored at −20° C.

All di- and tripeptide substrates and L-amino acid oxidase reagents were obtained from Sigma (London) Chemicals Co. All other chemicals were of Analar grade.

Fluorescent method for estimating peptidase activity. We used a two-step assay with L-amino acid oxidase to estimate the concentration of L-amino acids released after peptidase hydrolysis [11]. Substrates used included: Phe-Gly, Gly-Phe, Leu-Leu, Phe-Gly-Gly, Leu-Leu-Leu. Assays for peptidase activity against Phe-Gly and Gly-Phe were carried out in the presence and absence of 0.5 mM *p*-hydroxymercuribenzoate. All substrates were used at 5 mM in 0.1 M Tris-HCl buffer (pH 8.0).

Marker enzymes. The gradient fractions were assayed for the principal subcellular organelles using marker enzymes. The enzymes assayed were neutral α-glucosidases (brush border), *N*-acetyl-β-glucosaminidase (lysosomes), leucyl-2-naphthylamidase (brush border), γ-glutamyltransferase (brush border), malate dehydrogenase (mitochondria), catalase (peroxisomes) and lactate dehydrogenase (cytosol). Conditions for these assays are described previously for rat [6] and for guinea pig [12]. Protein was estimated by the method of Schacterle and Pollack [13].

Results

Table I shows the activities of the various marker enzymes and peptidases in the enterocyte homogenates.

Marker enzymes for the brush border (Zn^{2+}-resistant and Tris-sensitive α-glucosidase), lysosomes (*N*-acetyl-β-glucosaminidase) and endoplasmic reticulum (Tris-resistant α-glucosidase) showed no species differences. Catalase (peroxisomes) was significantly higher in the guinea pig, whereas malate

TABLE I

ENZYME ACTIVITIES OF ISOLATED RAT AND GUINEA PIG ENTEROCYTES

Mean ± S.E. specific activities (munits/mg protein) for duplicate estimations on five preparations. 1 munit corresponds to the hydrolysis of 1 nmol substrate/min. Statistical analysis was performed by unpaired Student's *t*-test.

Enzyme (subcellular location)	Rat (mean ± S.E.)	Guinea pig (mean ± S.E.)	*P*
Zn^{2+}-resistant α-glucosidase (brush border)	16.7 ± 2.0	18.2 ± 2.1	> 0.05
Tris-sensitive α-glucosidase (brush border)	14.4 ± 1.63	16.9 ± 1.73	> 0.05
Tris-resistant α-glucosidase (endoplasmic reticulum)	1.35 ± 0.22	1.62 ± 0.19	> 0.05
N-Acetyl-β-glucosaminidase (lysosomes)	3.26 ± 0.18	4.54 ± 0.67	> 0.05
Catalase (peroxisomes)	8.92 ± 0.57	56.1 ± 8.8	> 0.001
Malate dehydrogenase (mitochondria)	4 120 ± 1 020	2 010 ± 363	> 0.05
Lactate dehydrogenase (cytosol)	1 420 ± 190	501 ± 79	> 0.002
Phe-Gly dipeptidase	37 500 ± 9 000	41 900 ± 9 900	> 0.05
Gly-Phe dipeptidase	7 250 ± 880	56 200 ± 4 700	< 0.001
Leu-Leu dipeptidase	3 550 ± 416	19 750 ± 2 600	< 0.001
Leu-Leu-Leu tripeptidase	1 420 ± 164	2 600 ± 430	> 0.05
Phe-Gly-Gly tripeptidase	3 430 ± 440	4 130 ± 490	> 0.05
γ-Glutamyltransferase	19.1 ± 1.2	6.21 ± 1.90	< 0.001
Leucyl-2-naphthylamidase	44.5 ± 3.0	113 ± 14	< 0.001

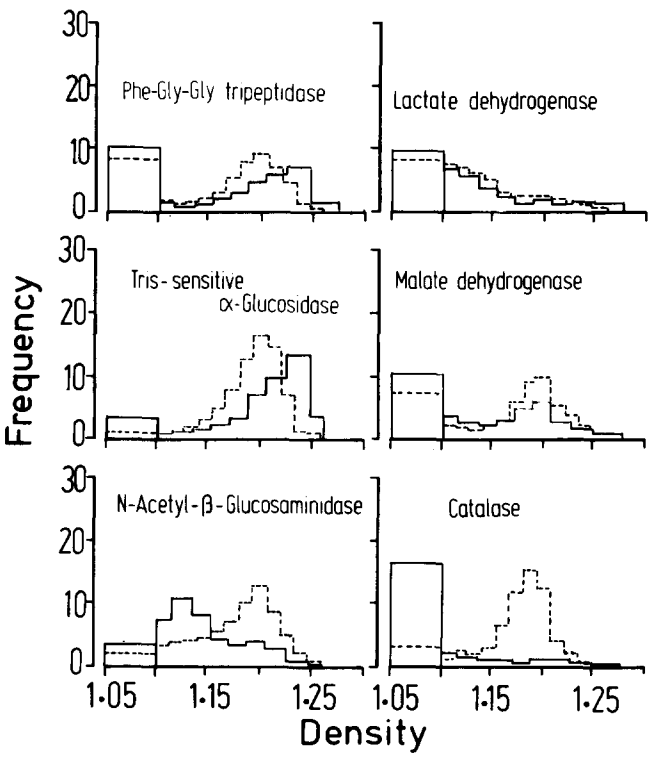

Fig. 1. Isopycnic centrifugation of 8000 × *g*-min supernatant from rat enterocytes: ———, digitonin-treated preparations; - - - - - -, control preparations. The graphs show frequency-density histograms for marker enzymes. Frequency is defined as the fraction of total recovered activity present in the subcellular fraction divided by the density span covered. The activity in the density span 1.05—1.10 is regarded as the enzyme remaining in the sample layer and presumed to reflect the cytosol. Histograms show mean distribution of three experiments in each group. Recovered enzyme activity 78—96%.

dehydrogenase (mitochondria) and lactate dehydrogenase (cytosol) were higher in the rat.

Phe-Gly dipeptidase had similar activity in both species, but Gly-Phe dipeptidase was higher in the guinea pig and Leu-Leu dipeptidase higher in the rat.

Leu-Leu-Leu tripeptidase and Phe-Gly-Gly tripeptidase had similar activities in the two species. Leucyl-2-naphthylamidase was higher in the guinea pig and γ-glutamyltransferase was higher in the rat.

Fig. 1 shows the distribution of the principal marker enzymes for rat enterocytes in the sucrose density gradients after homogenisation in the absence or presence of 0.82 mM digitonin. This membrane perturbant had a selective effect on each of the organelles and was most useful in assigning the particular peptidase to the brush border. The brush border marker showed a

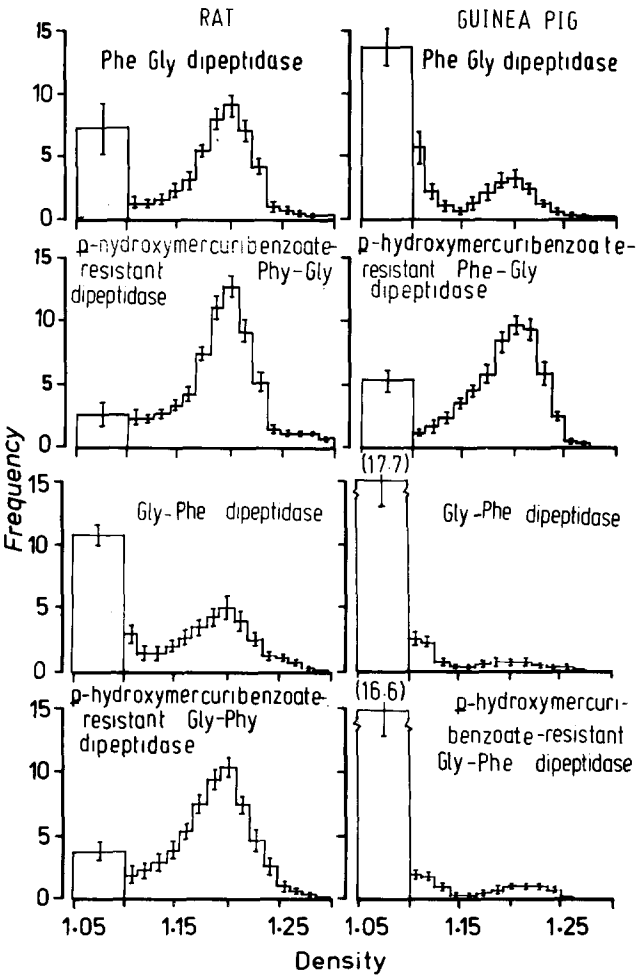

Fig. 2. A comparison of the results of isopycnic centrifugation of 8000 × *g*-min supernatants from rat and guinea pig enterocyte preparations. Graphs show frequency-density histograms for Phe-Gly and Gly-Phe dipeptidases in the presence and absence of 0.5 mM *p*-hydroxymercuribenzoate. Further details as given for Fig. 1. Distributions are the mean of three experiments. Recovered enzyme activity 80—107%.

increase in median density following digitonin treatment, and the particulate component of the Phe-Gly-Gly tripeptidase and other peptidases showed a similar density shift. Lactate dehydrogenase and malate dehydrogenase were unaffected, *N*-acetyl-β-glucosaminidase showed a decrease in density and particulate catalase was completely solubilised by the digitonin. Similar results were obtained with guinea pig enterocytes, except that the lysosomes and peroxisomes were less affected by the digitonin treatment.

Fig. 2 shows the distribution of two dipeptidases in rat and guinea pig enterocytes. In rat, for both dipeptidases, there was a larger particulate (brush border) component. Guinea pig Gly-Phe dipeptidase was almost exclusively localised in the cytosol. *p*-Hydroxymercuribenzoate treatment inhibited the cytosolic dipeptidase activity in the rat and, consequently, the distribution of dipeptidase activity was altered when assays were performed in the presence of

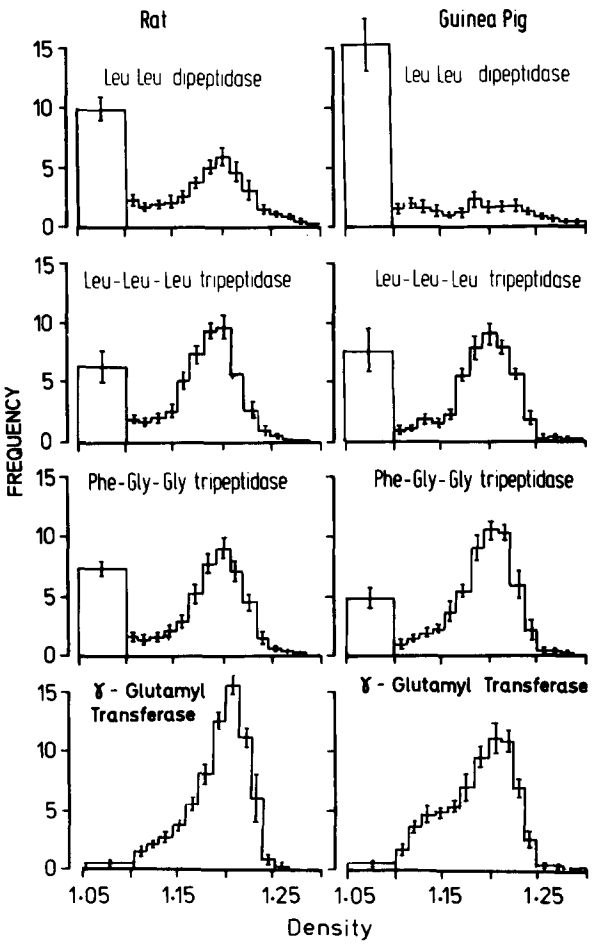

Fig. 3. A comparison of the results of isopycnic centrifugation of 8000 × *g*-min supernatants from rat and guinea pig enterocyte preparations. Graphs show frequency-density histograms for di, tripeptidase and γ-glutamyltransferase. Details as Fig. 1. Distributions are the mean of three experiments. Recovered enzyme activity 87—108%.

this reagent. In contrast, guinea pig cytosol dipeptidase activity was less affected by this reagent and the distribution of activity when Gly-Phe was used as substrate was not altered.

The distributions of peptidases in the two species are shown in Fig. 3. Leu-Leu dipeptidase had a larger brush border component in the rat than the guinea pig. The tripeptidases, γ-glutamyltransferase and leucyl-2-naphthylamidase had a major brush border localisation in both species.

Discussion

The present study showed that, in isolated enterocytes from rat and guinea pig, di- and tripeptidase activities both have a cytosol and particulate distribution. Similar results were obtained by Peters [5] and Kim et al. [4], who both used mucosal scrapings as starting material and used differential centrifugation to separate the various organelles, rather than the density gradient procedure used in this study.

Both types of fractionation technique indicated that the particulate component is localised in the brush border and this is confirmed in this study by the digitonin experiments. This perturbant binds selectively to cholesterol-rich membranes increasing their equilibrium density [8]. Thus, plasma membranes show an increase in density but other organelles are unaffected or show a reduced density, presumably due to the detergent action of the digitonin [14,15].

There are species differences in the distribution of dipeptidases. For all substrates studied, there was, in the rat, a significant brush border component up to 50% of the total activity. Studies on whole rat mucosa using differential centrifugation procedures [4] suggested that less than 20% of the activity is found in this organelle. Differences in starting material and in subcellular fractionation techniques probably account for the different results. In the guinea pig, less dipeptidase activity was found in the brush border and, for certain substrates (Leu-Leu, Gly-Phe), only trace amounts were localised in this organelle. This is in agreement with the results of Peters [5], who showed, for a series of dipeptides, that less than 10% of the activity was found in the brush border fraction. Our data agree with the results of Heizer et al. [17] in the rat and Fottrell et al. [18] in the guinea pig, which showed that *p*-hydroxymercuribenzoate is a more potent inhibitor of the dipeptidase activity from rat than from guinea pig cytosol.

The tripeptidase, and particularly leucyl-2-naphthylamidase and γ-glutamyltransferase, were mainly located in the brush border in both species. This is in agreement, certainly for the guinea pig, with previous studies, where 40—80% of the tripeptidase activity was found in the brush border [5]. For longer peptide substrates, it was found in this species that there was a progressively greater brush border concentration of hydrolase activity with increasing number of residues in the peptide substrate [16].

The present study, thus, clarified the apparent controversy which existed concerning the dual localisation of peptidases in rat guinea pig intestine. There are differences in the localisation of dipeptidase in the two species and

physiological studies of peptide absorption are also likely to yield differing conclusions.

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