γ-GLUTAMYL TRANSFERASE ACTIVITY IN THE PIG PROXIMAL COLON DURING EARLY POSTNATAL DEVELOPMENT

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1. Introduction

The function of the γ-glutamyl cycle in kidney and other tissues has attracted attention. Part of the cellular uptake of amino acids may be mediated by γ-glutamyl transferase (GGT), a membrane-bound enzyme that catalyses one of the reactions of the cycle [1]. GGT has been found at highest activities in plasma membranes of epithelia specialised in absorptive functions, such as the proximal nephron and the small intestine, where the enzyme seems to be localised in the brush border membrane [2–5]. In contrast GGT is virtually absent from colonic epithelium [6].

Here we report high activities of GGT in the proximal colon of the newborn pig, a tissue which has the unusual ability to actively transport amino acids during the first few days of postnatal life [7,8]. The activities of other brush border membrane markers, as well as GGT have also been studied during neonatal development.

2. Methods

2.1. *Animals*

Pigs were taken from a herd of large Whites bred at Babraham. They were either used at birth, before they could suckle the sow, or at 2 or 7 days postpartum. Parturition was induced as in [9].

2.2. *Membrane preparation*

Brush border membranes were obtained by the Ca^{2+}-precipitation technique [10], with the modifications in [11]. The frozen (−20°C) proximal colons from 5–10 piglets were used for each preparation. This represents 10–20 g fresh tissue from which 80–160 mg mucosal protein were obtained. After thawing, in 300 mM mannitol, 12 mM Tris–HCl (pH 7.1) the mucosa was isolated by vibration with a vibro-mixer and homogenised in a Waring blender. A brush border fraction was then obtained by differential centrifugation as in [11]. Homogenates and brush border fractions could be stored at −20°C for up to 3 weeks without any loss of GGT of L-leucyl-β-naphthylamidase (LAP) activity. Alkaline phosphatase (AP) activity was assayed in the freshly obtained fractions.

2.3. *Enzyme assays*

Assays were carried out in the presence of excess substrate using incubation times short enough to ensure measurements of linear kinetics. GGT activity was determined at 37°C using 3 mM γ-L-glutamyl-*p*-nitroanilide as donor substrate and 40 mM diglycine as acceptor [12] in a buffer containing 5.5 mM $MgCl_2$, 75 mM NaCl and 50 mM Tris–HCl (pH 8.0).

Alkaline phosphatase was estimated using 1 mM *p*-nitrophenylphosphate [13] in a buffer containing 5 mM $MgCl_2$ and 50 mM Tris–HCl (pH 10.1). Leucyl-β-naphthylamidase was measured with L-leucyl-β-naphthylamide as the substrate using a modification of the Bratton-Marshall reaction (Sigma Technical Bulletin no. 251) to determine the release of β-naphthylamine. Lactase activity was measured as in [14], using 10 mM lactose as substrate and measuring the release of D-glucose by the glucose oxidase method. Protein concentration was measured as in [15].

3. Results

Preliminary experiment revealed the presence of GGT activity in homogenates of proximal colon of the newborn pig. Alkaline phosphatase (AP) and leucine β-naphthylamidase (LAP) were also present in

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Table 1
Activities of brush border enzymes of the proximal colon of the pig during post-natal development

Day after birth	Enzyme	Homogenate	Brush border fraction	Enrichment
0	GGT	12 ± 2	113 ± 15	7.3 ± 0.8
	AP	140 ± 20	1340 ± 190	11.3 ± 2.8
	LAP	41 ± 7	287 ± 51	7.3 ± 0.7
2	GGT	9 ± 2.8	51 ± 19	6.9 ± 3.7
	AP	41 ± 8	510 ± 110	9.0 ± 1.4
	LAP	23 ± 2	74 ± 25	3.2 ± 1.0
7	GGT	1.9 ± 0.1	15.2 ± 3.8	8.2 ± 1.9
	AP	11.1 ± 0.8	53.0 ± 8.0	8.3 ± 1.7
	LAP	24.2 ± 1.8	34.9 ± 3.4	1.5 ± 0.2

Abbreviations: GGT, γ-Glutamyl transferase; AP, alkaline phosphatase; LAP, leucyl-β-naphthylamidase

Specific activities of the various enzymes are expressed as nmol . min^{-1} . mg $protein^{-1}$. Values represent means ± SEM of determinations carried out in 3–6 separate preps

proximal colon homogenates. When the Ca^{2+}-precipitation technique for brush border isolation [10] was applied to this tissue a fraction was obtained in which the 3 enzyme activities were enriched by a factor of 7–11 (table 1). Activities of all the enzymes decreased by day 2 and 7 after birth. Enrichment remained constant with development for GGT and AP, but decreased sharply for LAP. The decrease of GGT and LAP activity in the brush border fraction occurred in a parallel fashion.

The characteristics of GGT activity in the brush border fraction of the newborn tissue was examined in further detail. As reported for other tissues activity was inhibited by the natural donor glutathione (reduced) and had a pH optimum at pH ~ 8.0. A series of amino acids can serve as acceptors (table 2); as for the enzyme of other sources, highest activity was observed with diglycine as donor (table 2).

GGT activity is known to increase in the small intestine of rats during the foetal period and to be present at birth at high levels [16]. In table 3 we have compared the activities of brush border enzymes in the small intestine and proximal colon of the new born pig. GGT, AP and LAP activities were 2-fold higher in the small intestine. Enrichment of these enzyme activities on purification of brush border membranes was similar in the 2 tissues. One striking difference was the complete absence of lactase activity in the proximal colon. Activity of GGT in the proximal colon is also comparable with that observed in the small intestine of adult rats [5,16].

Table 2
Effect of various L-amino acids and diglycine on GGT activity of brush border fraction from newborn pig proximal colon

Acceptor	Activity
Diglycine	100.0
Glycine	34.5
Methionine	56.9
Alanine	58.6
Isoleucine	72.4
Leucine	74.0
Phenylalanine	51.7
Serine	37.9
Valine	34.5
Lysine	46.6

Activity is expressed as a % of that observed in the presence of 40 mM diglycine; acceptors were all used at 40 mM

Table 3
Comparison of enzyme activity in small intestine and proximal colon of newborn unsuckled pigs

Activity	Homogenate		Brush border fraction	
	SI	PC	SI	PC
GGT	28.1	12.4	218.9	112.8
AP	530	140	4200	1340
LAP	79.0	41.0	560	287
Lactase	71	n.d.	375	n.d.

Abbreviations: SI, small intestinal; PC, proximal colon; n.d., non-detectable

Details as in table 1. Lactase activity expressed as nmol . min^{-1} . mg $protein^{-1}$

4. Discussion

We show here that the proximal colon of the newborn pig possesses high activities of GGT, AP and LAP. These enzymes are known to be located in the brush border membrane of epithelial cells of the small intestine [5,9,10]. When a method for small intestinal brush border purification is applied to the proximal colon a preparation is obtained in which the brush border enzymes are enriched. The fact that a similar enrichment is observed for the small intestine and the proximal colon suggests that cells in both organs at this stage of maturation are organized in a similar fashion.

The activity of the brush border enzymes declines in the first few days following birth. It is interesting to compare the way in which GGT activity declines with the postnatal decrease in amino acid transport. If the data on methionine transport in [7] are considered, a fall to 30% and 10% of newborn values is observed by days 2 and 7, respectively. The corresponding figures for GGT are 45% and 11%. This correspondence cannot be taken as evidence for the involvement of GGT in amino acid transport, however, as a similar relation is observed with AP and LAP activities. The decrease in enzyme activities observed here is also paralleled by a reduction in the number and size of microvilli of the brush border membrane as observed by electron microscopy [17].

One difference between the composition of proximal colon brush borders and that from small intestine is the absence of lactase activity in the former. This activity is high in the small intestine at birth and disappears shortly after birth [18]. The absence of lactase from the proximal colon in the newborn pig correlates with the low capacity of this tissue to actively transport sugars [19]. This dichotomy suggests that separate signals may mediate postnatal development in the small and the large intestine.

Our results suggest that immediately after birth the proximal colon of the pig might perform some of the functions of the small intestine. This could compensate for the general inhibition of neonatal small intestinal function that has been attributed to its endocytotic uptake of large amounts of colostral proteins [20]. The proximal colon may perform a useful physiological function at this stage, participating in the digestion and absorption of oligopeptides and amino acids not taken up by the small intestine.

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