WORLD RABBIT SCIENCE 2000, VOL.8 (4),59-162.

HISTOCHEMICAL DISTRIBUTION OF LEUCINE AMINOPEPTIDASE (LAP-ase) IN THE YOUNG RABBIT INTESTINE

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ABSTRACT : The distribution of leucine aminopeptidase in normal duodenal, jejunal, ileal, cæcal and colonic mucosa of the rabbit has been studied using a histochemical method. Thirty New Zealand White rabbits were used ranging from 26-day old foetuses to 43-day old young (3 rabbits per age). Small intestine sites performed a leucine aminopeptidase reaction starting from the 30th day of foetal life. After birth the reaction was variable in intensity up to 19 days of age, while in older animals it became very strong. Similarly, the large intestine followed the same pattern with the exception that from the 24th day of age onwards it performed no reaction.

RÉSUMÉ : Étude histochimique de la répartition de la leucine aminopeptidase dans l'intestin du lapereau.
La méthode histochimique a été utilisée pour étudier la répartition de la leucine aminopeptidase dans les muqueuses du duodénum, du jéjunum, de l'iléon, du caecum et du colon de jeunes lapins. Trente lapins néo-zélandais blancs allant de fœtus âgés de 26 jours jusqu'à de jeunes lapereaux âgés de 43 jours ont été utilisés (3 par stade d'observation). La leucine aminopeptidase a été détectée dans l'intestin grêle à partir du 30ème jour de vie fœtale. Après la naissance l'intensité de la réponse est variable jusqu'à 19 jours d'âge, tandis qu'elle devient très forte chez les animaux plus âgés. Le gros intestin suit le même modèle avec une exception notable : à partir de l'âge de 24 jours il n'y a plus d'activité détectable.

INTRODUCTION

Aminopeptidases are widely distributed in nature and have medical and biological importance due to their function in the modification and degradation of proteins. Aminopeptidases are classified in two groups, exopeptidases and endopeptidases. Exopeptidases hydrolyse peptide bonds adjacent to terminal α-amino or terminal α-carboxyl groups, and include enzymes like aminopeptidase and carboxypeptidase. Endopeptidases hydrolyse centrally located as well as terminal peptide bonds and include pepsin, trypsin, chymotrypsin and cathepsin.

The histochemical aminopeptidase reaction depends upon the hydrolysis of a peptide bond adjacent to a terminal α-amino group, which results in the liberation of a chromogenic moiety (BURSTONE, 1962).

The enzyme which hydrolyses the leucyl compound is leucine-aminopeptidase (Leucyl naphthylamidase thereafter named LAP-ase), a metalloenzyme, catalyzing the following reaction :
L-leucyl-β-naphtylamide + H_2O → leucine + β-naphtylamine.
This enzyme is present in a number of tissues and intestinal secretions. As far as the intestine is concerned it had been studied in rat, dog, guinea pig, man and hamster (NACHLAS *et al.*, 1957, SHNITKA, 1960, DAWSON and DAVIS, 1963, ANDREWS, 1973).

With regards to the rabbit, few reports have been published on this subject. Using a biochemical assay method on foetal intestine, MORIN and POTIER (1981) reported little proteolytic activity before 25 days of gestation, and little proteolytic activity in rabbit serum concentration using chromatography (CACCIONI *et al.*, 1964).

The role of LAP-ase is very important in the metabolism of various amino acids (tryptophan, phenylalanine, histidine, tyrosine) at the intestinal level.

We aimed to establish whether the dynamic enzymatic changes of LAP-ase, described for the intestinal epithelium of rodents and chicks, also occurred in the rabbits.

MATERIALS AND METHODS

For this study, 30 New Zealand White rabbits were used ranging from 26-day old foetuses to 43-day old young, and more specifically from 26, 28 and 30 days old foetuses and 1, 11, 15, 19, 24, 27 and 43 day-old kits. Three rabbits were observed at each age. In addition, the average litter size was 7 kits. All animals were sacrificed by the use of chloroform in a glass container. The abdominal cavity was immediately opened and the alimentary tract from the cardia of the stomach to the rectum was removed. The intestine was then unraveled and freed from the mesentery. The contents of the small and large intestine were washed out with formol-calcium solution. Segments (each from two sections per rabbit) of duodenum, jejunum, ileum, caecum and proximal colon were placed and fixed in formol-calcium at 4°C. Appropriate samples were trimmed, orientated and mounted in a support

medium on metal chucks and were immediately immersed in liquid nitrogen until frozen. Samples were then sectioned on a cryostat microtome- Bright cryostat model OTF/AS/M- at 6 µm of thickness and at -20°C temperature. The sections were fixed on slides without adhesive and were dried at room temperature.

Cryostat sections of the small and large intestine were incubated for two hours at room temperature in the following incubating medium :

Stock substrate solution (L-leucyl-4 methoxy-b-naphthylamide in distilled water, 1 mg/1ml)..	1 ml
Acetate buffer 0,1 M pH 6.5	10 ml
Sodium chloride (0,85%)	8 ml
Potassium cyanide	1ml
Fast blue B salt	10 mg

After incubation, sections were rinsed in saline for 2 minutes, immersed in 0,1 M-cupric sulfate for 2 minutes, and rinsed again in saline (2) and mounted, without dehydration, in glycerin jelly. A bright red color indicated the site of the leucine aminopeptidase reaction product.

RESULTS

1. Duodenum:

On the 26th and 28th day of fetal life there was no leucine- aminopeptidase reaction. By the 30th day, however, there was a strong LAP-ase positive reaction along the brush border of the cells of the villi. The cells of the crypts were either negative or only weakly positive to this treatment.

When the reaction for leucine- aminopeptidase was carried out after birth, the brush border was seen to be positive and has a granular appearance. The reaction was of variable intensity up to 19 days-old and at older ages became stronger, except at the apices of the villi. In later stages, the cells of the apices of the villi were negative for leucine- aminopeptidase. The cells lining the crypts exhibited a reaction. Furthermore, the cytoplasm of the enterocytes in the newborn, 1 and 27 day-old rabbits contained LAP-ase positive granules. Brunner' s glands were negative (table 1).

2. Jejunum:

The jejunum of the newborn (Fig.1) showed a very strong leucine-aminopeptidase positive granular reaction in the cells of the villi. From this age, except in the case of the 11 day-old animal where no reaction was observed, up to 19 days a rather weak reaction was demonstrated, and in older animals there was a strong reaction. The cytoplasm contained LAP-ase positive granules in the newborn, 1, 24, 27 and 43 day-old rabbits. The cells lining the crypts showed a mild reaction in the newborn, a negative one in the 1 and 11 day-old, and a mild positive reaction in the 15 and 19 day-old rabbits. In older animals, the reaction was strongly positive with the exception of the 24 day-old which exhibited only a positive reaction (table 1).

Figure 1 : Jejunum-new born (x 140)
Strong leucine amino-peptidase positive reaction along the brush border. Method for LAP-ase

3. Ileum:

A strongly positive granular leucine - aminopeptidase reaction was seen along the brush border only at the 30th day of foetal life. The crypts

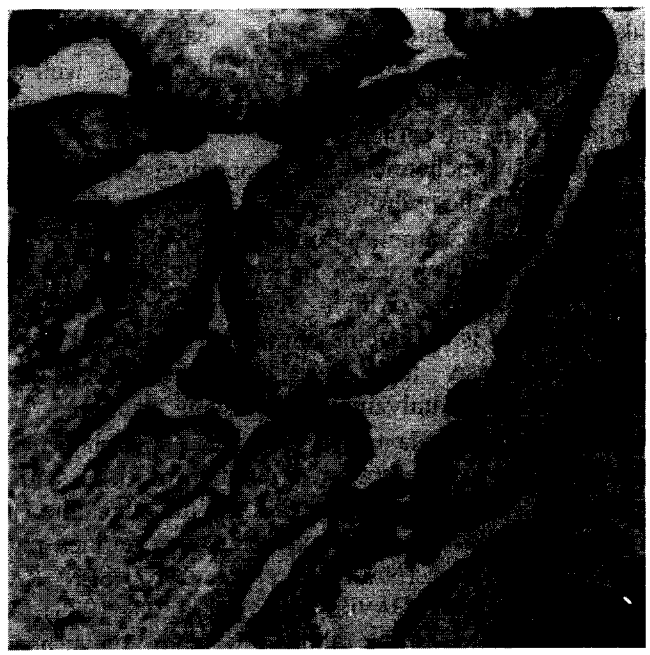

Figure 2 : Ileum – 1 day old
Leucine amino-peptidase positive reaction along the brush border. Method for LAP-ase

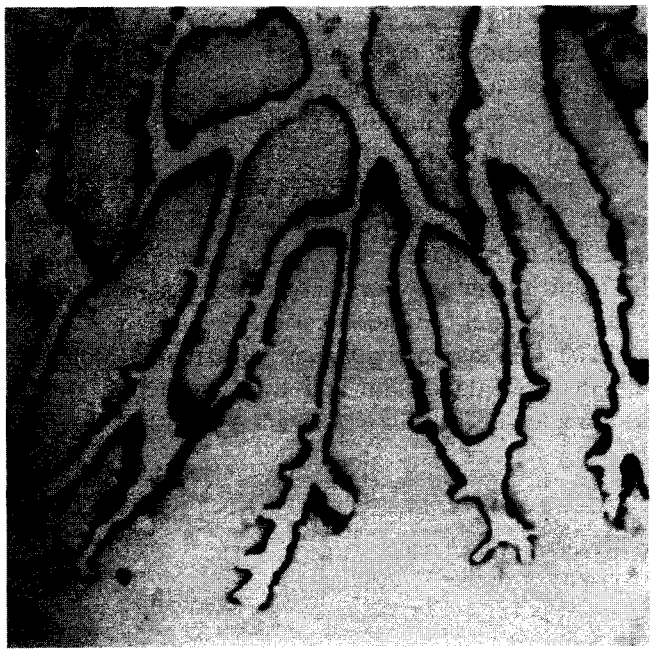

Figure 3 : Ileum – 43 day old
Strong leucine amino-peptidase positive reaction along the brush border. Method for LAP-ase

Figure 4 : Caecum – newborn
Strong leucine amino-peptidase positive reaction along the brush border. Method for LAP-ase

were either negative or exhibited a mild reaction.

The ileum showed a strong leucine-aminopeptidase positive granular reaction along the brush border in the newborn (Fig. 2). This became weaker up to 19 days of age and in the older animals the reaction was very strong (Fig. 3). In addition to the brush border, most of the cytoplasm of the newborn, 1 and 15 day-old animals contained LAP-ase positive granules. The cells lining the crypts showeda positive reaction in the newborn and 1 day-old and then are negative up to 19 days, with the exception of the 15 day-old, which showed a mild reaction. In older animals up to the 43 day-old, the brush border showed a very strong reaction (table1).

4. Caecum:

At the 26th and 28th day of foetal life, there was no reaction to treatment for leucine-aminopeptidase. At the 30th day, however, there is a LAP-ase positive granular reaction along the brush border of the epithelial cells. This reaction was absent from the cells of the deep parts of the glands.

The caecum showed a strong leucine-

Table 1 : Average intensity of the leucine aminopeptidase reaction

			Days of Gestation			Days after Birth						
			26	28	30	1	11	15	19	24	27	43
DUODENUM	Villi	Apex of villi	-	-	++	+	+	++	++	-	-	-
		Sides of villi	-	-	++	+	+	++	++	+++	+++	+++
		Bases of villi	-	-	++	+	+	++	++	+++	+++	+++
	Crypts		-	-	+	+	+	++	++	++	+++	+++
JEJUNUM	Villi	Apex of villi	-	-	+++	++	-	+	+	+++	++++	++++
		Sides of villi	-	-	+++	++	-	+	++	+++	++++	++++
		Bases of villi	-	-	+++	-	-	+	++	+++	++++	++++
	Crypts		-	-	+	-	-	+	+	++	++++	++++
ILEUM	Villi	Apex of villi	-	-	+++	+++	+	++	-	++++	++++	++++
		Sides of villi	-	-	+++	+++	+	++	++	++++	++++	++++
		Bases of villi	-	-	+++	+++	+	++	-	++++	++++	++++
	Crytps		-	-	+	++	-	+	-	+	++	++++
CAECUM	Surface	epithelial cells	-	-	+++	+++	++	+	-	-	-	-
	Glands		-	-	+++	+++	++	+	-	-	-	-
COLON	Surface	epithelial cells	-	-	+++	+++	+	-	+	-	-	-
	Glands		-	-	+++	+++	++	-	+	-	-	-

Reaction : +mild, ++positive, +++strong, ++++very strong, - no reaction

aminopeptidase positive reaction along the brush border of the epithelial cells of those animals ranging from the newborn (Fig. 4) to the 15 day-old. The reaction is either granular (newborn, 1 day-old) or granular in parts (11 day-old) or granular in parts along the outer edge only of the brush border (15 day-old). After the 19th day-old,there was no reaction (table 1).

5. Colon:

As far as the leucine-aminopeptidase reaction is concerned, there was no reaction on the 26th and 28th day of gestation. At the 30th day, however, there was a strong LAP-ase positive reaction, granular along the brush border of the epithelial cells except towards the bases of the glands, a reaction similar in it's distribution to the caecum. After birth, the observed reaction was strong (1 day old), mild (11 day old), negative (15 days old), mild (19 days old) and after the 24th day of age negative (table 1).

DISCUSSION

The strong LAP-ase reaction confined to the latest stage of gestation was at variance with the biochemical findings of MORIN and POTIER (1981) who identified LAP-ase in the younger foetus on the 25th day of gestation. They considered that the protein present was in the meconium, which originates from swallowed amniotic fluid and desquamated intestinal cells. In the present findings, LAP-ase reaction was not identified before the 30th day of foetal life. Subsequently, up until the 19th day of life, there was a low level of proteolytic activity in the small intestine and a relatively higher level in the large intestine, a situation that became reversed after that age. However, it must be pointed out that the apices of the villi were negative for LAP-ase in the duodenum after the 24th day of age onwards.

As far as the LAP-ase distribution in different animal species , it has to be mentioned that the superficial epithelium of the villi of the jejunum in the dog was strongly LAP-ase positive (NACHLAS *et al.*, 1957). The striated border of the columnar epithelial cells of the small intestine in the rat and man performed intense LAP-ase activity, while the colonic mucosa in the rat was negative (SHNITKA, 1960). The small intestine of man showed only an intermittent moderate to a weakly positive reaction on the surface epithelium and in the crypts (SHNITKA, 1960, DAWSON and DAVIES, 1963).

Duodenum and jejunum exhibited intense activity in the cytoplasm and the brush border of surface epithelial cells and of cells covering the villi, while in the ileum the activity of these cells was moderate or slight in hamster (ANDREWS, 1973).

The LAP-ase activity was distributed along the villi in chick duodenum and was less active in the distal region of the villi (GREY and LE COUNT, 1970).

Received : August 4th, 2000
Accepted : November 3rd, 2000

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