Transcriptional regulation of trypsin and chymotrypsin biosynthesis in the calf pancreas during weaning

Isabelle Le Huerou, Paul Guilloteau, R. Toullec, C. Wicker, Antoine Puigserver

TRANSCRIPTIONAL REGULATION OF TRYPSIN AND CHYMOTRYPSIN BIOSYNTHESIS IN THE CALF PANCREAS DURING WEANING

I. Le Huërou, P. Guilloteau and R. Toullec
Laboratoire du Jeune Ruminant, INRA, 65, rue de St-Brieuc,
35042 Rennes Cedex, France

and

C. Wicker and A. Puigserver
Centre de Biochimie et de Biologie Moléculaire, CNRS,
BP 71, 13402 Marseille Cedex 9, France

Introduction

A number of anatomical, biochemical and physiological changes, such as the development of forestomachs and increase in microbial digestion, are known to occur during the weaning period in calves (Ruckebusch *et al.*, 1983). The overall activity of pancreatic proteinases was also found to be modulated by weaning (Guilloteau *et al.*, 1985), but the molecular basis of the adaptative response could not be investigated. On the other hand, Han *et al.* (1986) reported that the development of pancreatic hydrolases in the rat was dependent on that of the corresponding mRNAs. In the present study, we have examined both enzymic activity and specific mRNA levels of two serine proteases, namely trypsin and chymotrypsin, in the calf pancreas as influenced by weaning.

Materials and Methods

Holstein-Friesian male calves were randomly distributed in two groups of five animals each. In the first group, the animals were exclusively milk-fed for 119 days in order to have them maintained in the preruminant state. In the second group, they were offered water, hay as well as a commercial starter concentrate *ad libitum* from day 29 to day 56 of age, while the amount of milk they were given was gradually reduced. All calves were sacrificed on day 119.

Both tryptic and chymotryptic activity levels in pancreatic tissue were measured by spectrophotometric methods according to Bundy (1963) and Erlanger *et al.* (1961), respectively. Levels of the corresponding mRNAs were determined by dot-blot hybridization analysis. Following homogenization of each calf pancreas in guanidinium thiocyanate and repeated extractions with guanidine hydrochloride, total RNA (0.5-3μg) was fixed on nitrocellulose sheets and further hybridized with a specific complementary DNA probe (cDNA) which was previously ^{32}P-labeled by nick-translation. The amount of either trypsin and chymotrypsin probe was 0.3-1.5 x 10^7 cpm/ml hybridization buffer. The cDNA encoding each enzyme was obtained from a bovine pancreatic cDNA library constructed in our laboratory and characterized by partial nucleotide sequence analysis according to the method of Sanger. Trypsin and chymotrypsin cDNA probes were of a 890 and 660 base-pair length, respectively.

Results and Discussion

The effect of weaning on pancreatic trypsin and chymotrypsin activity levels as well as on that of corresponding mRNAs is illustrated in figure 1. Both enzyme specific activities were not significantly different in ruminant calves as compared to preruminant calves, though a 1.4-fold increase in trypsin and chymotrypsin activity was observed. By contrast, the levels of the corresponding mRNAs were actually increased in weaned animals. Representative autoradiography patterns are shown in figure 2, from which it was possible to estimate the content in trypsin and chymotrypsin specific mRNAs by cutting out radioactive spots and counting them. The concentration of trypsin and chymotrypsin mRNAs was increased by 36% and 133%, respectively, as compared to the milk-fed calves.

Thus, the parallel evolution of tryptic activity and relative level of its mRNA could mainly be

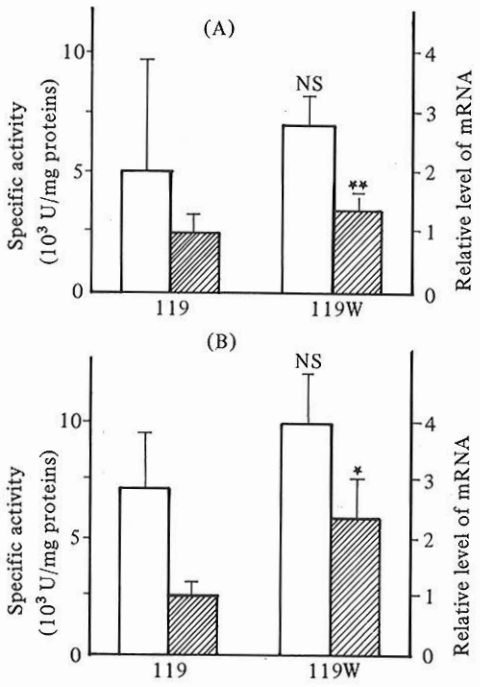

Figure 1. Specific activity (□) and mRNA level (▨) of trypsin (A) and chymotrypsin (B) in the calf pancreas. The levels of specific mRNAs were normalized to the values of milk-fed calves. P values, calculated from Student's t-test comparing weaned animals (W) to non-ruminant calves at 119 days of age were < 0.05 (*) and < 0.001 (**).
NS: non significant.

due to transcriptional modulation of the corresponding gene expression as well as to post-transcriptional events, such as mRNA processing, transport of the messenger from nucleus to cytoplasm or stabilization of mRNA within the cytoplasm compartment. The important increase in chymotrypsin mRNA level, which was not really correlated with the enzyme activity, should be the result of some translational and/or post-translational regulation of the chymotrypsin gene in addition to the transcriptional control mechanism.

This study demonstrates, for the first time, the existence of a regulation on the expression of calf pancreatic protease genes at a pretranslational level and, to a lesser extent, at translational and/or post-translational levels during weaning. Among

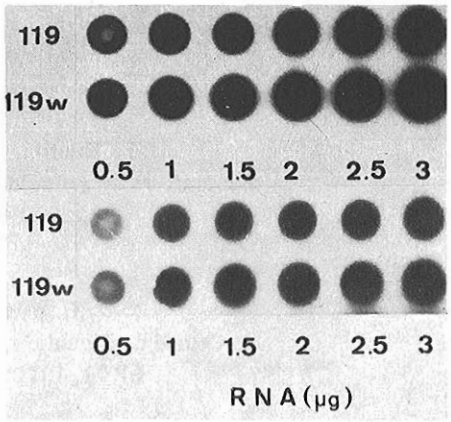

Figure 2. Autoradiography of dot-blot hybridization experiments with trypsin (top) and chymotrypsin (bottom) cDNA probes. W: weaned animals.

possible factors involved in the observed regulation, gastrointestinal hormones such as secretin and/or cholecystokinin may reasonably be considered as good candidates since the latter has previously been shown to modulate pancreatic secretion during post-natal development in animals.

(**Key Words:** Trypsin and Chymotrypsin Biosynthesis, mRNA and cDNA, Weaning)

Literature Cited

Bundy, H.F. 1963. Chymotrypsin-catalysed hydrolysis of N-Acetyl- and N-Benzoyl-L-Tyrosine p. Nitroanilides. Arch. Biochem. Biophys. 102:416-422.

Erlanger, B.F., N. Kolowsky and W. Cohen. 1961. The preparation and properties of two new chromogenic substrates of trypsin. Arch. Biochem. Biophys. 95:271-278.

Guilloteau, P., T. Corring, R. Toullec and R. Guilhermet. 1985. Enzyme potentialities of the abomasum and pancreas of the calf. II. Effects of weaning and feeding a liquid supplement to ruminant animals. Reprod. Nutr. Develop. 25:481-493.

Han, J.H., L. Rall and W.J. Rutter. 1986. Selective expression of rat pancreatic genes during embryonic development. Proc. Natl. Acad. Sci. USA 83:110-114.

Ruckebusch, Y., C. Dardillat and P. Guilloteau. 1983. Development of digestive function in newborn ruminant. Ann. Rech. Vet. 14:360-374.