CHANGES IN CONTENTS AND LOCALIZATIONS OF CARBONIC ANHYDRASE Ⅱ, PROCHYMOSIN AND PEPSINOGEN IN ABOMASAL MUCOSAE DURING LONG TERM MILK FEEDING GOATS

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Summary

The present paper describes temporal changes of immunohistochemical localization and quantities of carbonic anhydrase isozyme Ⅱ (CA-Ⅱ) prochymosin (PC) and pepsinogen (PN) in goat's abomasal mucosae during long term milk feeding. The CA-Ⅱ was not detected by day 14 after birth and then became positive on day 34 in the parietal cells, suggesting that the excretion of the hydrochloric acid (HCl) begins between days 14 and 34 under a feeding condition without solid materials. The quantity of the PC in the gastric chief cells detected by the ELISA showed rapid increase from the day of birth, making a peak on day 8 and then gradually decreased with age. The decrease in quantity of PC became started during the time period when HCl excretion had not started yet. The quantities of PN in the gastric chief cells were almost stable during the whole period examined. Expressions of these gastric enzymes did not seem to be regulated by the change of feeding condition.
(**Key Words**: Carbonic Anhydrase Ⅱ, Prochymosin, Pepsinogen, Goat Abomasum)

Introduction

Mammalian digestive tract is adapted to its structure and function by the postnatal dietary changes during, before and after weaning periods (in the cattle, Huber, 1968; Tamate et al., 1962; in the pig, Kelly et al., 1991; in the human, Lebenthal 1985; in the rat, Brendsen 1980). Secretions of gastric hormones and enzymes including gastrin in rats (Okahata et al., 1989), prostaglandin F2 alpha in rats (Bedrick and Koldovsk'y, 1987), and gastric aspartic enzymes (chymosin and pepsin) in calves (Amasaki et al., 1990; Andrén et al., 1982; Gozawa et al., 1989), are changed in amount during the weaning periods. When young calves are fed by large amount of milk, chymosin is kept expressed in high level. On the contrary, when adult cattle are fed by large amounts of fiber rich diets, chymosin secretion is reduced, but pepsin is stable (Andrén et al., 1982; Foltmann et al., 1977; Garnot et al., 1974). Andrén et al. (1982) suggested that switching changes in expressions of chymosin and pepsin would be a kind of adapted phenomenon and might be caused by changes of diets at weaning periods. Chymosin has high milk-clotting and low proteoclastic activities with the optimal pH of 3.5, while pepsin (pepsin A; Braudys et al., 1988) has low milk clotting and high proteoclastic activities with the optimal pH of less than 2.0 (Arima et al., 1967). It is apparent that the activities of these two gastric proteoclastic enzymes are influenced by gastric pH conditions *in vivo*. It is difficult to determine abomasal pH directly because the cardiac orifice is so loose that ruminal contents with neutral pH may flow into the abomasum to make abomasal pH very fickle. Since CA-Ⅱ is contributed to the hydrochloric acid (HCl) secretion from parietal cells (Cross, 1970; Sugai and Ito, 1980),

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we used CA-Ⅱ for a marker of HCl secretion in the abomasum. We examined whether a long-term milk feeding have any effects on HCl secretion from parietal cells and on the expression of PC and PN from gastric chief cells in goats at neonatal, infantile and adult stage.

Materials and Methods

Animals

In total 22 male Shiba goats were used in the present studies; 3 newborn animals before suckling milk were sacrificed less than 12 hours after birth, 4 young animals fed by maternal milk (MM) alone were sacrificed on day 8 after birth, 3 young animals fed by MM were sacrificed on day 14 after birth, 4 young animals fed by substituted milk containing with soy'meal and skim milk (SM) were sacrificed on day 34 after birth, 5 young animals fed by SM were sacrificed on day 70 after birth, and 3 adult animals maintained by conventional diets for more than a year were sacrificed.

Anti-bodies and antigens

Anti-sera raised against the equine CA-Ⅱ, Bovine PC and PN were used. Specific cross-reactivities of anti-equine CA-Ⅱ serum was confirmed between the equine CA-Ⅱ and goat's abomasal extracts obtained by gel filteration with TSKgel DEAE-TOYOPEARL 650S TOSOH Co., Japan (Lindskog, 1960). The activities of carbonic anhydrase were determined in each gel fraction by the pH method (Maren, 1967). The levels of immuoreactivities to the anti-sera were analyzed by the ELISA method (Johnson et al., 1978). The cross-reactivities of anti-sera against PN and PC between cattle and goats have been described earlier by Amasaki et al. (1990).

Immunochemical preparations

The crude samples were extracted from the proper gastric glandular mucosae in 0.02 M phosphates buffered saline pH 6.1 (PBS) with proteinase inhibitor (1 mM ethylendiaminetetra-acetic acid disodium salt dehydrate, 1 mM p-chloromercuribenzensulfonic acid, Sigma chemical company, U.S.A., 1 mM dithio-1,4-threitol, Merk, U.S.A.).

Immunohistochemical preparations

Histological samples were excised from proper gastric mucosa in each animal, and immediately fixed in zamboni solutions. Samples were embedded into paraffin, and cut at 5 μm serially according to conventional methods. After removing paraffin and rehydration, tissue samples were immunostained by the anti-equine CA-Ⅱ, anti-bovine PC or anti-bovine PN sera, respectively, according to previous reports (Amasaki et al., 1990, 1991), and observed under the light microscope.

Results

Cross-reactivity between the anti-equine carbonic anhydrase Ⅱ serum and goat's crude extract

Figure 1 shows protein levels in each gel-filtrated fraction. Figure 2 represents immunoreactivities against anti-equine CA-Ⅱ serum measured by the ELISA, and carbonic anhydrase activities determined by the pH method in fractions from 28 to 38. The immunoreactivity and enzymatic activity peaked at fractions 30 and 31, respectively.

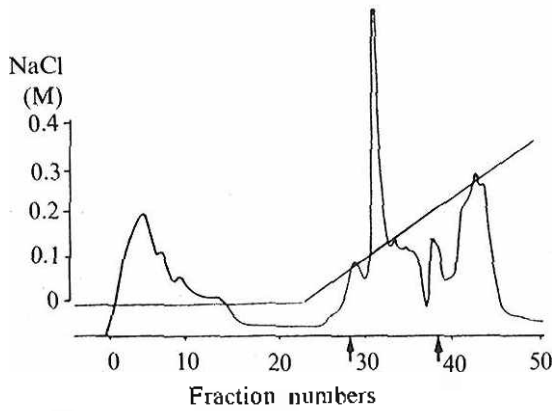


Figure 1. Elution pattern of goat abomasum extractions from a colum (1.5 × 10 cm) of TSKgel DEAE-TOYOPEARL 650S TOSOH Co., JAPAN with 0.05 M phosphate buffer (pH 8.2). A linear NaCl gradient (from 0 to 0.4 M) is used for selective removal of the enzyme. Faction volumes are 1.5 ml on all days examined. Fractions 28 38 (from arrow to arrow) are pooled and analyzed by the ELISA method for immuno-reactivities against anti equine CA-Ⅱ serum, and by the pH-method for carbonic anhydrase activities.

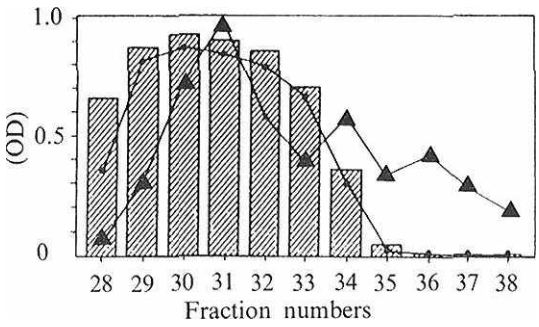


Figure 2. The fractions between arrowed points (figure 1) are analyzed for total carbonic anhydrase activities (•) and immunoreactivities with the ELISA (bar). The levels of total protein in each fraction are expressed in terms of optical density at 280 nm (▲). The immuno-reactivity and enzyme activity shows a peak at fractions 30 and 31 respectively.

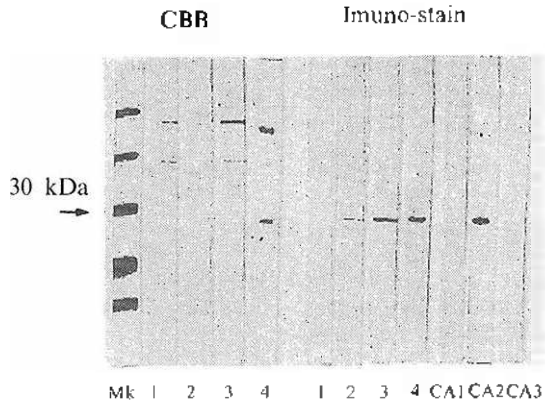


Figure 3. Immuno-blots against the anti-equine carbonic anhydrase isozyme Ⅱ serum after 10% SDS-PAGE. lane MK: maker proteins. lane 1: neonate without feeding. lane 2: 8 day old goat with MM. lane 3: 34 day old goat with SM. lane 4: acult with corvertional feeding. CA 1: purified equine carbonic anhydrase isozyme Ⅰ. CA 2: purified ecuine carbonic anhydrase isozyme Ⅱ. CA 3: purified equine carbonic anhydrase isozyme Ⅲ. CBB: stained with coomassie brilliant blue R250. Immuno-stain: immunochemical staining with anti-equine CA-Ⅱ serum. Immunoreactive bands are detected at about 30 kDa.

Immuno-blotting

The molecular size of goat CA-Ⅱ from crude extracts were detected at approximately 30 kDa on SDS-PAGE. The immunoreactive bands against CA-Ⅱ on the transfer membrane was detected from the samples in the animals on day 34 and adult goats. The immunoreactive levels of CA-Ⅱ were gradually increased with age (figure 3).

Amounts of PC, PN and CA-Ⅱ in the abomasal mucosae

The amounts of PC, PN and CA-Ⅱ detected by ELISA are shown in table 1. The quantity of PC detected by the ELISA showed rapid increase from the day of birth, making a peak on day 8 and then gradually decreased with age. The quantities of PN were almost stable during the whole period examined. CA-Ⅱ was not detected from day of birth to 14 and it was detected in young animals fed by SM on day 34, 70 and adult clarity. The amount of CA-Ⅱ was increased gradually with age.

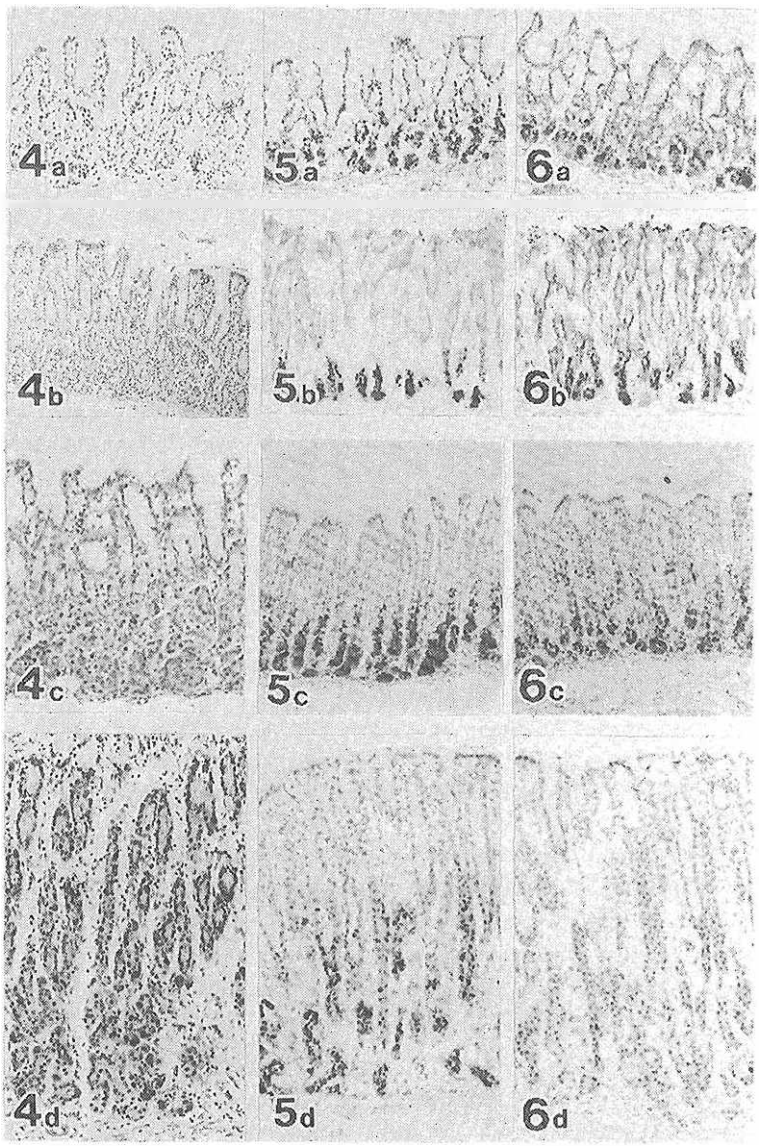
Immuno-histochemistry

The cells immunoreactive to anti CA-Ⅱ was mainly located in the gastric parietal cells in

TABLE 1. OPTICAL DENSITY (O.D.) LEVELS OF IMMUNO REACTIVITIES OF PROCHYMOSIN, PEPSINOGEN AND CARBONIC ANHYDRASE ISOZYME Ⅱ IN THE GOAT'S ABOMASAL MUCOSA BY ELISA (O.D./mg, Mean ± SE)

Stage	Number	Prochymosin	Pepsinogen	CA-Ⅱ
At birth	3	0.788 ± 0.003	1.552 ± 0.058	not detected
8 days	4	2.301 ± 0.108	1.797 ± 0.108	not detected
14 days	3	1.103 ± 0.102	1.614 ± 0.077	not detected
34 days	4	0.635 ± 0.024	1.164 ± 0.017	0.137 ± 0.007
70 days	5	0.502 ± 0.058	1.606 ± 0.132	0.354 ± 0.008
Adult	3	0.034 ± 0.029	1.599 ± 0.045	1.532 ± 0.124

34 days old animals fed by SM (figures 4a, 4b, 4c, 4d). The cells positive to anti-PC and/or anti-PN sera were mainly located in the gastric chief cells. PC was detected in the newborn, 8, 14 and 34 day-old animals. PC was not detected in the abomasal mucosa of 70 day old and adult animals (figure 5a, 5b, 5c, 5d). PN was detected at a stable immunoreactive intensity throughout the whole stages examined (figures 6a, 6b, 6c, 6d).



Figures 4, 5 and 6. Slides of goat's proper gastric mucosa stained by the anti-equine carbonic anhydrase isozyme II (4a, 4b, 4c, 4d), anti-bovine pepsinogen (5a, 5b, 5c, 5d) and anti-bovine chymosin (6a, 6b, 6c, 6d) sera with ABC methods, respectively. a: Neonate without feeding. b: 8 day old goat with MM. c: 34 day old goat with SM. d: Adult with conventional feeding. × 200. Carbonic anhydrase isozyme II are detected at gastric parietal cells. Pepsinogen and prochymosin are detected in gastric chief cells.

Discussion

The present study demonstrates the presence of CA-Ⅱ in the abomasal mucosae of the goat. The anti-equine CA-Ⅱ sera did not cross-react to equine CA-Ⅰ and Ⅲ (figure 3). It reacted to a single band on the immunoblot after SDS-PAGE of the crude extract of the goat abomasum. The molecular weight was estimated about 30 kDa, pretty close to known CA Ⅱ in other species: 29 kDa in sheep (Tanis and Tashian, 1971); 28 kDa in horse (Furth, 1968); 30 kDa in cattle (Nyman and Lindskog, 1964); 29 kDa in *pig* (Tanis et al., 1970). Immunoreactive fraction actually showed carbonic anhydrase activity. Therefore, we believe that anti-equine CA-Ⅱ serum cross-reacted against the goat gastric CA-Ⅱ specifically.

The present study showed that amounts of gastric pepsinogen was kept stable after birth in the abomasal mucosae of the goat, while chymosin showed rapid increase after birth, making a peak on day 8 and decreased with age in spite of long term milk feeding (table 1). These changing patterns of gastric aspartic enzymes were comparable to the data in the conventional weaning calves (Andrén et al., 1982). The decrease in chymosin expression under long term milk feeding suggests that the gene expression of chymosin might be controlled by at least in part inherent processes independent from the diet.

CA-Ⅱ expressions in gastric parietal cells is reflected on amounts of secretions of HCl from the parietal cells (Cross, 1970; Maren, 1967; O'Brien et al., 1977; Sugai and Ito, 1980; Winborn et al., 1974). We used CA-Ⅱ as a marker of gastric HCl secretions. The present study showed that CA-Ⅱ became detectable in 34 day old animals fed by SM. The first expression of CA-Ⅱ would be a day between 15 and 34. The gastric HCl secretions might start somewhere around this period. Since the animals were kept under milk feeding condition in the present study it is apparent that CA-Ⅱ expression was not affected by changes of diets to solid materials. In another words, long term milk feeding with SM could not inhibit HCl secretion in terms of CA-Ⅱ expression. PC has an optimal pH of 3.5 with high milk-clotting and low proteoclastic activities, while PN (pepsin A; Braudys et al., 1988) has the optimal pH of < 2.0 with low milk clotting and high proteoclastic activities (Arima et al., 1967). Therefore, it is apparent that the activities of these two gastric proteoclastic enzymes are influenced by gastric pH conditions *in vivo*. The present study showed that the decrease in quantity of PC initiated during the time period when HCl excretion had not started yet. On the other hand, the quantities of PN in the gastric chief cells were almost stable during the whole period examined. Therefore, expressions of these gastric enzymes did not seem to be regulated by the change in gastric pH condition.

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