Immunohistochemical Localization of Carbonic Anhydrase Isoenzymes in Salivary Gland and Intestine in Adult and Suckling Pigs

Tomoko AMASAKI¹⁾, Hajime AMASAKI^{2)*}, Jun NAGASAO³⁾, Nobutsune ICHIHARA¹⁾, Masao ASARI¹⁾, Toshiho NISHITA¹⁾, Kazumi TANIGUCHI³⁾ and Ken-ichiro MUTOH³⁾

¹⁾Department of Anatomy and Physiology, School of Veterinary Medicine, Azabu University, Sagami-hara-shi 229-8501, ²⁾Department of Veterinary Anatomy, Nippon Veterinary and Animal Science University, Musashino-shi 180-8602 and ³⁾Department of Veterinary Anatomy, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada-shi 034-8628, Japan

(Received 30 December 2000/Accepted 17 May 2001)

ABSTRACT. Localizations of carbonic anhydrase isoenzymes (CA I, CA II and CA III) were investigated immunohistochemically in the salivary glands and intestine of mature and suckling pigs. Carbonic anhydrase isoenzymes were not detected in the salivary glands of sucklings, but were present in the adult. Bicarbonate ion in saliva might be important for the digestion of solid foods in mature pigs, but unnecessary for the digestion of milk in sucklings. Expressions of CAI and CAII were detected strongly in the large intestine of the adult and sucklings, and faintly only at duodenum in the small intestine. CA I and CA II isoenzymes in the large intestine may be involved, at least in part, in ion absorption and water metabolism during digestion and absorption of milk in suckling pigs. In addition, CA I and CA II expression in the duodenal villus enterocyte may support the process of bicarbonate absorption in the duodenum.

KEY WORDS: adult and suckling pig, carbonic anhydrase, intestine, salivary gland.

J. Vet. Med. Sci. 63(9): 967–970, 2001

Carbonic anhydrase (CA) isoenzymes involved in the hydration of carbon dioxide are ubiquitous in the living world [8, 13–17]. In animals, there are at least 5 carbonic anhydrase isozymes: CA-I, CA-II, CA-III, CA-IV, and CA-VI, each with its own molecular structure. The luminal surface of the digestive tract is covered with an epithelial layer from the oral cavity to the anus, and this layer contains cells that synthesize and secrete mucus substances and digestive enzymes. Mucus substances covering the luminal surface provide protection for the epithelial surface and aid in the absorption of substances such as sugar, amino acids, fatty acid and ions. The properties of epithelial cells in digestive tract (including expression of CA isoenzymes) vary according to animal species and region of the tract. CA isoenzymes are widely involved in the functions of the digestive tract, and investigators have reported different patterns of expressions of these enzymes in the digestive organs of cattle [6, 14], horses [3], rats [4, 7, 9] and dogs [5], which represent significant variation in diet. However, no reports to date have investigated such expression in pigs. To this end, we examined immunohistochemical expressions of CA I, CA II and CA III in salivary glands and intestine in mature and suckling pigs. We subsequently compared such expression between these two groups of animals in the differences of the digestive function between suckling and mature pigs.

MATERIALS AND METHODS

Samples: The animals used in this study were five 2-week-old male suckling pigs with body weights of 7.3–9.2

kg, and one 1-year-old mature female pig. All animals were deeply anesthetized by intraperitoneal injection of nembutal sodium solution (70 mg/kg body weight; Dainippon Pharmacy Co.). We excised tissue from parotid, mandibular and mono-sublingual glands, duodenum, jejunum, ileum, cecum, colon and rectum of all 6 animals.

Immunohistochemistry: Excised tissue samples were immediately fixed in Bouin solution and embedded in paraffin wax. They were then sliced to a thickness of 6 μ m. Immunohistochemical staining was carried out using the anti-horse CA polyclonal antibodies which cross reacted to porcine isoenzymes CAI, CAII and CAIII, respectively, as supported by Western blot analysis (data not shown). The working dilution of each antibody was 1:3,000. Detection of the first antibody was carried out using the avidin-biotin peroxidase complex (ABC) method and 3,3'-diaminobenzidine (DAB) coloration. Sections were observed and photographed using an optical microscope after application of the nuclear contrast stain, methyl green.

RESULTS

We did not detect any signals of the immunoreactions of CA I, CA II or CA III in the salivary glands of the sucklings (Figs. 1 and 2, Table 1). In the mature pig, there was an absence of these signals in the acinar cells of the parotid gland. However, we detected faintly stained CA I and CA III in the liner epithelial cells of the striated duct of the sublingual glands, faintly stained CA I also in the striated duct of the mandibular gland, moderately stained CA II in the striated duct of the sublingual gland, and faintly stained CA II only in the serous demilunes cells of the sublingual gland (Fig. 3, Table 1). Therefore, CA isoenzymes were detected at the submandibular gland and the sublingual gland in the

* CORRESPONDENCE TO: DR. AMASAKI, H., Department of Veterinary Anatomy, Nippon Veterinary and Animal Science University, 1–7–1 Kyonan-cho, Musashino-shi, Tokyo 180–8602, Japan

Table 1. The localization of isoenzymes of carbonic anhydrase in suckling and mature pigs

Sit/CA isozymes	CAI		CAII		CAIII	
	suckling pig	mature pig	suckling pig	mature pig	suckling pig	mature pig
Parotid gland						
Serous demilune	—	—	—	—	—	—
Serous acinar cell	—	—	—	—	—	—
Ductal epithelial cell	—	—	—	—	—	—
Mandibular gland						
Serous demilune	—	—	—	—	—	—
Mucous acinar cell	—	—	—	—	—	—
Ductal epithelial cell	—	±	—	—	—	—
Sublingual gland						
Serous demilune	—	—	—	±	—	—
Mucous acinar cell	—	—	—	—	—	—
Ductal epithelial cell	—	±	—	+	—	±
Small intestine						
Duodenum	± ^{a)}	± ^{a)}	± ^{a)}	± ^{a)}	—	—
Jejunum	—	—	—	—	—	—
Ileum	—	—	—	—	—	—
Large intestine						
Cecum	++ ^{b)}	++ ^{b)}	++ ^{b)}	++ ^{b)}	+ ^{c)}	+ ^{c)}
Colon	++ ^{b)}	++ ^{b)}	++ ^{b)}	++ ^{b)}	+ ^{c)}	+ ^{c)}
Rectum	++ ^{b)}	++ ^{b)}	++ ^{b)}	++ ^{b)}	+ ^{c)}	+ ^{c)}

a) Villous surface epithelial cell. ++ : intense stain.

b) Upper half of intestinal gland. + : moderate stain.

c) Reticular fiber of fat tissue. ±: faint stain.
— negative stain.

mature pig, but not in the sucklings (Table 1).

In both the sucklings and the mature, CA I and CA II were faintly stained at the luminal cytoplasm of enterocytes of the duodenal villi (Fig. 4, Table 1). In the suckling (Figs. 5, 6, Table 1) and mature animals (Fig. 7, Table 1), CA I or CA II was strongly expressed in the epithelial cells of the upper half of the intestinal glands in the large intestine. CA III was detected in both the mature and suckling animals only at the reticular fiber of adipose tissue of the large intestine (Fig. 8, Table 1).

DISCUSSION

The various regions of the digestive tract exhibit distinctive features; each designed to facilitate different functions in digestion, absorption and secretion. CA isoenzymes are involved in ion transportation and water metabolism, and have been detected in many organisms [15–17]. These enzymes are known to be expressed in association with transportation and absorption of calcium and other ions in erythrocytes [13], the pancreas [11], muscles [12] and kidneys [8]. Expression of CA isoenzymes in epithelial cells of the digestive tract seems to reflect their role in maintaining the alkaline environment in this tract, and varies not only among animal species but also among regions of the digestive tract. In the salivary glands [3–5], stomach and intestine [6, 7, 9, 14], patterns of expression have been reported to correlate with diet. It has also been reported that temporal and spatial expressions of these enzymes are related to cell differentiation during developmental stages [1, 2]. In this

study, we investigated the distributional patterns of CA isoenzymes in the digestive tract and salivary glands of suckling and mature pigs. These organs are very active in ion transportation. Distributional patterns of CA isoenzymes would be important to reveal the activity of CA isoenzymes as a function of dietary differences and topological function within the digestive tract. We detected no expression of CA isoenzymes in the salivary glands of the suckling pigs, but faintly or moderately expression in mature pigs. This finding indicates that the presence of bicarbonate ion in saliva is important for digestion in the mature pig, but is unnecessary for the digestion of milk in the suckling pig. Expression of the CA isoenzymes in the large intestine was detected in both stages of pigs. CA I and CA II isoenzymes in the large intestine may be involved in ion transport, ion absorption and water metabolism; functions which are important for digestion and absorption of milk in newborns. In addition, we observed the positive immuno-reactions for anti-CA I and II in absorptive epithelial cells of the duodenal villi. Recent report on the absorption of bicarbonate, sodium and hydroxyl ions through the small intestinal cells in the eels [10]. The same mechanism may be at work in the proximal small intestinal epithelia of both eels and mammalian species. Immunoreaction to CAIII against the reticular fiber of adipose tissue is also observed in the other organs [unpublished data], but no reason for those CAIII expressions has been revealed.

The findings of the present study suggested a correlation between differences in function of salivary glands in suckling and mature pigs and expression of CA isoenzymes.

Figs. 1, 2. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the salivary gland of suckling pig. Any CA isoenzymes are not detected. 1; Non-localization of CA I in the parotid gland. bar, 100 μ m. 2; Non-localization of CA II in the mandibular gland. bar, 100 μ m.

Fig. 3. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the salivary gland of a mature pig. The localization of CA II in the sublingual gland. A slightly positive reaction is detected in the serous demilunes (arrow heads), and a moderate positive reaction is detected in the striated duct (arrows). bar, 100 μ m.

Fig. 4. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the small intestine of a mature pig. CA I is slightly detected in the surface epithelial cells (arrow) in the duodenum villi. Cross section of upper part of intestinal villi. bar, 100 μ m.

Figs. 5, 6. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the large intestine of suckling pig. 5; The localization of CA I in the cecum. CA I is detected in the epithelial cells in the upper half zone of intestinal glands. bar, 100 μ m. 6; The localization of CA II in the colon. CA II was detected in the epithelial cells in the upper half zone of intestinal glands. bar, 100 μ m.

Fig. 7. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the large intestine of a mature pig. CA I is detected in the epithelial cells of the upper half zone of intestinal glands in the colon. bar, 100 μ m.

Fig. 8. The immunohistochemical localization of carbonic anhydrase (CA) isoenzymes in the large intestine of suckling pig. The localization of CA III in the colon. A positive reaction is detected only in the reticular fibers of the adipose tissue (arrow) but not in the epithelium. bar, 100 μ m.

Furthermore, the secretion of bicarbonate ion in salivary glands and absorption and secretion of water and minerals and bicarbonate ions in duodenum and large intestine appear to be related to the activity of CA.

REFERENCES

1. Amasaki, H., Matsumoto, S. and Daigo, M. 1991. The distributions of the fibronectin, laminin, type I and IV collagens and the carbonic anhydrase isozyme III during bovine ruminal epithelial development. *Acta Anat.* **140**: 169–174.
2. Amasaki, H., Matsumoto, S., Takanosu, M. and Daigo, M. 1991. Distribution of matrix and carbonic anhydrase isozymes III during bovine palatine ridge. *J. Vet. Med. Sci.* **53**: 1031–1036.
3. Asari, M., Sasaki, M., Kano, Y. and Nishita, Y. 1991. Immunohistolocalization of the carbonic anhydrase isozymes I, II and III in equine salivary glands. *Okajima Folia Anat. Jpn.* **67**: 467–472.
4. Asari, M., Igarashi, S., Sasaki, K., Amasaki, A., Nishita, T. and Amasaki, H. 1991. Localization of carbonic anhydrase III in the rat parotid gland. *J. Anat.* **179**: 9–14.
5. Asari, M., Kimura, H., Ichihara, N., Kasuya, T. and Nishita, T. 2000. Immunohistochemistry of carbonic anhydrase isoenzymes (CA-I, II and III) on canine salivary glands: A distributional and comparative assessment. *Anat. Histol. Embryol.* **29**: 9–12.
6. Carter, M.J. 1971. The carbonic anhydrase in the rumen epithelial tissue of the ox. *Biochem. Biophys. Acta* **235**: 222–236.
7. Igarashi, S., Kano, Y., Nishita, T., Amasaki, H. and Asari, M. 1992. Comparison of the distribution of carbonic anhydrase isozymes (CA-I, CA-II, CA-III) in the rat gastrointestinal tract. *J. Vet. Med. Sci.* **54**: 535–539.
8. Kaphukorpi, E-K. 1991. Carbonic anhydrase II in rat acid secreting cells: comparison of osteoclasts with gastric parietal cells and kidney intercalated cells. *Acta Histochem.* **90**: 11–20.
9. Lacy, E.R. and Colony, P.C. 1985. Localization of carbonic anhydrase activity in the developing rat colon. *Gastroenterology* **89**: 138–150.
10. Maffia, M., Trischitta, F., Lionetto, M.G., Storelli, C. and Schettino, T. 1996. Bicarbonate absorption in eel intestine: evidence for the presence of membrane-bound anhydrase on the brush border membranes of the enterocyte. *J. Exp. Zool.* **275**: 365–373.
11. Marino, L.R. and Cotton, C.U. 1996. Immunolocalization of bovine pancreatic duct epithelial cells. *Am. J. Physiol.* **270**: 676–683.
12. Nishita, T., Matsushita, H. and Kai, M. 1987. Immunocytochemical localization of carbonic anhydrase III in equine skeletal muscle. *Equine Vet. J.* **19**: 509–513.
13. Nishita, T., Kondo, S., Ishida, S., Ochiai, H. and Asari, M. 2000. Isolation and measurement of carbonic isoenzymes (CA-I and CA-II) in the erythrocyte of the dog. *Am. J. Vet. Res.* (in press.)
14. Sasaki, K., Igarashi, S., Amasaki, T., Amasaki, H., Nishita, T., Kano, Y. and Asari, M. 1993. Comparative immunohistolocalization of carbonic anhydrase isozymes I, II and III in the equine and bovine digestive tract. *Histochem. J.* **25**: 304–311.
15. Tashian, R.E. 1989. The carbonic anhydrases: widening perspectives on their evolution, expression and function. *Bioessays* **10**: 186–192.
16. Veitch, F.P. and Blankenship, L.C. 1963. Carbonic anhydrase in bacteria. *Nature (Lond.)* **197**: 76–77.
17. Wayggod, E.R. 1955. Carbonic anhydrase (plant and animal). *Methods. Enzymol.* **2**: 836–846.