Distribution of the carbonic anhydrase isoenzymes I, II, and VI in the human alimentary tract

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Abstract

The distribution of carbonic anhydrase isoenzymes I, II, and VI was studied in the human alimentary tract using specific antibodies to human isoenzymes in conjunction with the immunoperoxidase technique to elucidate the physiological role and possible functional interplay of carbonic anhydrases (CAs) in alimentary canal functions. From the isoenzymes studied, CA II was found to be the most widely distributed in the various epithelia throughout the alimentary canal. In addition to the acinar cells of the parotid and submandibular glands and the duodenal Brunner's glands, it was present in the mucosal epithelium of the oesophagus, stomach, duodenum, and colon. The epithelial cells of the hepatic bile ducts, gall bladder, and pancreatic ducts also contained CA II in abundance. In contrast, CA VI was present only in the serous acinar and ductal cells of the parotid and submandibular glands, and CA I in the mucosal epithelium of the colon and A cells of the pancreatic Langerhans' islets. These results suggest that CA II as a widely distributed isoenzyme in the epithelium of the alimentary canal and CA VI as secreted into saliva, may form a mutually complementary system protecting oesophageal, gastric, and intestinal mucosa from acidity.

Carbonic anhydrase (EC 4.2.1.1; CA) is a widespread catalyst of the reversible hydration of carbon dioxide and participates in the control of the ion, fluid, and acid base balance in various epithelia.1 The enzyme occurs in at least six isoenzymes with different activities and immunological specificities. The best characterised of the isoenzymes are the cytoplasmic forms, CA I, II, and III.1 The membrane bound, mitochondrial and secreted isoenzymes are termed CA IV, V, and VI, respectively.1,2

CA has been detected in several glandular and mucosal epithelia in the mammalian alimentary tract by biochemical and histochemical methods,3-8 suggesting that it plays an important part in various gastrointestinal functions. The distribution of the isoenzymes, however, has not been comprehensively elucidated in the human alimentary tract. At present the best known is the location of the cytoplasmic high activity isoenzyme, CA II. By immunohistochemical techniques it has been shown to be present in the parietal cells of the human gastric glands where its function is probably linked to proton secretion.9,10 CA II has also been located in the gastric, duodenal, and colonic surface epithelia, in the serous acinar and ductal structures of the human salivary glands, and in the pancreatic and bile ducts where it is suggested to supply the secretions with bicarbonate.11,12 CA VI has been shown to be located in the serous acinar cells of the human parotid and submandibular glands and to be secreted at high concentrations into saliva,13 but its presence elsewhere in the human alimentary tract has not been studied. Membrane bound CA IV is a component of the apical plasma membrane of several epithelia and participates in the regulation of the bicarbonate concentration in the secretions.14 Its location and role, however, in the alimentary canal has not been established.

To achieve a comprehensive view of the physiological role and possible interplay of the different CA isoenzymes in the alimentary canal functions, additional information is needed, in the first place, about their expression in the alimentary tract. This study was undertaken to elucidate the differential location of CA I, II, and VI in the human alimentary tract by isoenzyme specific immunohistochemical examination.

Methods

ANTI-SERUM SAMPLES

Polyclonal rabbit antiserum samples to human CA I and II have been produced and characterised by Parkkila et al15 and that to human CA VI by Parkkila et al.16 The antiserum samples showed no cross reactivity in immunoblotting.

PREPARATION OF TISSUES

Samples of the human parotid and submandibular glands, oesophagus, stomach, duodenum, colon, liver, gall bladder, and pancreas were obtained together with routine histopathological specimens taken during surgical operations carried out at Oulu University Hospital. Each tissue sample was divided into several small pieces, about 5 mm thick. The specimens were fixed in Carnoy's fluid (absolute ethanol + chloroform + glacial acetic acid 6:3:1) for six hours, in Bouin's fluid (saturated aqueous picric acid + formaldehyde solution (36-40%)+glacial acetic acid 15:5:1) for 18 hours, or in 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS) for 18 hours. All fixations were carried out at 4°C. The samples were then dehydrated, embedded in paraffin wax in a vacuum oven at 58°C, and sections of 5 μm were placed on gelatin coated microscope slides. Carnoy's fluid preserved best the antigenicity of the enzymes and also gave a satisfactory tissue morphology. The secretary
granules of the parotid and submandibular glands, however, were not preserved leading to a diffuse staining pattern when compared with the fixations with Bouin's fluid or paraformaldehyde. Bouin's fluid preserved well the tissue morphology and the enzyme antigenicity while 4% paraformaldehyde destroyed most of the CA I and II antigenicity.

IMMUNOHISTOCHEMISTRY
The sections were stained using the immunoperoxidase technique. The steps in the staining were (1) Pretreatment of the sections with undiluted swine serum for 40 minutes and rinsing in PBS; (2) Incubation for one hour with the primary rabbit antiserum diluted 1:100 in 1% bovine serum albumin (BSA) in PBS (BSA-PBS); (3) Treatment with swine serum for 40 minutes and rinsing in PBS; (4) Incubation for one hour in biotinylated affinity purified swine immunoglobulins to rabbit immunoglobulins (Dakopatts, Copenhagen, Denmark) diluted 1:300 in BSA-PBS; (5) Treatment with swine serum for five minutes and rinsing in PBS; (6) Incubation for 30 minutes in a 1:600 dilution of peroxidase conjugated streptavidin (Dakopatts) in PBS; (7) Incubation for three hours in 3,3'-diaminobenzidine tetrahydrochloride (DAB; Fluka, Buchs, Switzerland) (9 mg DAB in 15 ml PBS plus 10 μl 30% H2O2).

The sections were washed in PBS three times for 10 minutes after incubation steps 2, 4, and 6. All incubations and washings were carried out at room temperature and the sections were finally mounted in Permount (Fisher Scientific, Fair Lawn, NJ). The sections were viewed with a Leitz Aristoplan microscope (Leitz, Wetzlar, Germany) and photographed on black and white negative film (Agfapan 25 ISO).

Results

LOCATION OF CA I
The erythrocytes stained intensely for CA I in all tissues studied. CA I positive staining was also distinct in the non-goblet epithelial cells of the colon (Fig 1A) and the A cells of the pancreatic Langerhans's islets (Fig 1B). The location of CA I in the A cells was confirmed by subjecting serial sections of the pancreas to staining for CA I and glucagon, which showed colocalisation of the proteins (Figs 1B and C).

LOCATION OF CA II
CA II was, in contrast with CA I, widely distributed in the epithelia throughout the alimentary tract. It was present in the serous acinar cells of the submandibular gland (data not shown) where it was granularly located in the apical portion of the cells. A faint but clear positive staining was also detected in the stratified squamous epithelium of the oesophagus (Fig 2B) while the oesophageal mucous glands failed to stain (Fig 2C). In the stomach, an intense positive staining for CA II was found in the surface epithelial cells (Fig 2D). The parietal cells of the gastric glands also showed a positive staining while the zymogenic cells remained unstained (data not shown). The epithelial cell staining was most intense in the glandular pit areas. CA II was also detected in the epithelium of the duodenum (Fig 2E) and mucus Brunner's glands (data not shown) where the enzyme was located mainly in the basal cytoplasm and the cell nuclei. In the colon, anti-CA II serum showed a distinct cytoplasmatic staining in the non-goblet epithelial cells (Fig 2F). CA I and II clearly colocalised to these cells (Figs 1A and 2F), and their staining intensities decreased from the surface towards the base of the crypt of Lieberkühn. In the liver, the most intense staining for CA II was seen in the hepatic bile ducts, and a faint but distinct staining was also found in the hepatocytes (Fig 2G). The epithelial cells of the gall bladder (Fig 2H) and the pancreatic ducts (Fig 2I) also contained the enzyme.

LOCATION OF CA VI
CA VI was detected in the serous acinar cells and parotid (Figs 3A and B) and parotid (Fig 3C) glands where the enzyme was located in granules probably representing the secretory granules. Some epithelial cells of the striated ducts also showed a faint staining (Fig 3C). Interestingly, the other tissues studied showed no specific staining for CA VI (Figs 3D-I).

Discussion
Carbonic anhydrases are present in various epithelia where they catalyse the reversible hydration of metabolic carbon dioxide (CO2 + H2O→H+ + HCO3-), and participate in the control of the ion, fluid, and acid base balance.12

Figure 1: Immunohistochemical staining of CA I (A,B) and glucagon (C) in the human colon (A) and pancreas (B,C). In the colon, CA I is found in the epithelial cells located in the outer part of the mucosa (A). In the pancreas, CA I positive staining is present in the A cells of the Langerhans's islets as CA I and glucagon colocalise in the serial sections (B,C). Carnoy's fluid fixation. Original magnifications (A×250; B,C×500).
Figure 2: Immunohistochemical staining of CA II in the human submandibular gland (A), oesophagus (B,C), stomach (D), duodenum (E), colon (F), liver (G), gall bladder (H), and pancreas (I). In the submandibular gland, the serous acinar cells show granular staining (A). The epithelial cells of the oesophagus are slightly stained (B) while the mucous glands failed to stain (C). In the stomach, the enzyme is present in the surface epithelial cells of gastric pits (D). The surface epithelial cells show positive staining both in the duodenum (E) and colon (F). The sections in Figs 1A and 2F are serial sections. In the liver, the small (G) and large (insert) hepatic bile ducts show an intense positive immunoreaction and a faint positive staining is also found in the hepatocytes. CA II is also present in the epithelial cells of the gall bladder (H) and ductal epithelium of the pancreas (I). Carnoy's fluid fixation. Original magnifications (A,D,F-H × 250; B,C × 500; E × 200; I × 400).

To understand the physiological role and possible interplay of different CA isoenzymes in alimentary canal functions, we studied the differential location of the low activity isoenzyme (CA I), the high activity isoenzyme (CA II), and the secretory isoenzyme (CA VI) in the human alimentary tract. Our results showed that from these isoenzymes, CA II was the most widely distributed in the epithelia of the human alimentary canal (Table). This suggests that it is mainly responsible for supplying the alimentary canal secretions with bicarbonate. The surface epithelia throughout the alimentary tract displayed an intense staining for this isoenzyme. It was also present in the serous acinar cells of the parotid and submandibular glands, the parietal cells of the gastric glands, the Brunner's glands, the hepatocytes and the epithelia of the bile and pancreatic ducts and gall bladder. On the contrary, CA VI was present only in the serous acinar and ductal cells of the parotid and submandibular glands, and CA I in the colonic surface epithelium and the A cells of the Langerhans's islets (Table). CA IV is present in the apical plasma membrane of the epithelial cells of several tissues. Although its location and role has not yet been established in the human alimentary tract, it is probable that CA IV also participates in the regulation of the bicarbonate concentration of the secretions in the alimentary tract.

Although CA II showed a granular staining pattern like CA VI in the serous acinar cells of the submandibular and parotid glands, it is not secreted into saliva. Thus, its main role in the salivary glands, regardless of its cellular location, is to supply saliva with bicarbonate. Interestingly, CA VI was found to be expressed only in the salivary glands. Although, its exact physiological role in saliva is not yet known, its
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Figure 3: Immunohistochemical staining of CA VI in the human submandibular (A,B) and parotid glands (C), and in the esophagus (D), stomach (E), duodenum (F), colon (G), gall bladder (H), and pancreas (I). A positive granular staining is present only in the submandibular and parotid glands (A,B,C). The demilunes (D) of the submandibular gland also contain CA VI. A faint staining is also found in some occasional epithelial cells of the striated ducts (arrow). All other tissues are negative (D-I). Paraformaldehyde (A), Bouin’s (B,C), and Carnoy’s fluid (D-I) fixations. Original magnifications (A,B×500; C-I×250).

Presence there is probably linked to removal of acid as carbon dioxide in the mouth and elsewhere in the alimentary canal by catalysing the reversible reaction $\text{H}^+ + \text{HCO}_3^- \rightarrow \text{H}_2\text{O} + \text{CO}_2$. In gastro-oesophageal reflux, the oesophageal mucosa is exposed to acid gastric juice, which may lead to a severe mucosal damage and oesophagitis. It has been suggested that bicarbonate, both locally produced in the oesophagus and found in swallowed saliva, is an important protective factor in the oesophagus. Meyers et al. recently reported that the oesophageal mucus contains bicarbonate that is possibly secreted by the oesophageal mucus glands. Our finding here suggests, however, that bicarbonate is delivered into the oesophageal mucus by the stratified squamous epithelium, which was found to contain CA II.

The gastric mucosa secretes high concentrations of bicarbonate and protons. Bicarbonate ions mainly originate from the surface epithelial cells and protons from parietal cells of the gastric glands that both contain CA II.$^{13,14}$ The resistance of the gastric and duodenal mucosa to acid has been proposed to result from a parallel mucus and bicarbonate secretion by the surface epithelial cells.$^{22}$ The role of CA VI delivered in saliva into stomach and duodenum may also be important in protection of the gastric and duodenal mucosa from acidity. The mean (SD) enzyme content in human saliva is 6.8 (4.3) μg/ml,$^{24}$ and the daily saliva volume is 1.5-2.0 l. Accordingly, the total amount of CA VI delivered daily in saliva into the stomach is about

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