Title: The roles of pepsinogen and agmatine in normal human gastric acid production and tissue protection

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Abstract

The production of concentrated acid by the human stomach with the absence of any harmful effects of this concentrated acid on the normal stomach has lacked satisfactory explanation. Evidence is presented for the decarboxylation of the basic amino acid arginine being involved in this process. The arginine is derived from the activation segment of pepsinogens. The decarboxylation of arginine is extracellular and occurs in the parietal cell canaliculi. The production of gastric acid by the hydration of this carbon dioxide is extracellular and occurs in the parietal cell canaliculi. Agmatine, which is formed by the decarboxylation of arginine, contributes to the defence of the tissues against the concentrated acid. This mechanism of acid production provides a satisfactory explanation as to why normal gastric acid production does not have an adverse effect on gastric tissues.

Keywords: Pepsinogen, arginine, agmatine, gastric acid.

Introduction

Human gastric acid is produced by the chemical hydration of carbon dioxide, a reaction which is catalysed by the enzyme carbonic anhydrase. When considering the ingredients for the hydration of carbon dioxide, there is good evidence relating to the source of water and the presence of the enabling enzyme carbonic anhydrase but evidence relating to the source of carbon dioxide is lacking. Physiology text books state that gastric acid is produced in the cytoplasm of parietal cells and is secreted by these parietal cells. The pH of this concentrated acid achieves a level of 1·2 which would be capable of tissue destruction but this destruction does not normally occur.

It is accepted that gastric acid secretion is associated with the transfer of a large quantity of water through the gastric mucosa (Durbin & Moody 1965). The localization of carbonic anhydrase in gastric epithelial cells is well-established

(Cross 1970; Winborn et al. 1974). This enzyme is found on the outer surface of mitochondria in chief cells and in parietal cells. In addition, this enzyme has been identified in significant quantity on the luminal surface of the cell membrane of the parietal cell canalicular microvilli (Cross 1970). The enzyme has not been located on the cytoplasmic surface of the cell membrane of parietal cell canalicular microvilli.

The hydration of carbon dioxide resulting in the production of gastric acid requires a significant supply of carbon dioxide. Interstitial fluid is thought to be the source of this carbon dioxide but the arterial blood supply to the stomach would be unable to provide sufficient carbon dioxide. It was therefore decided to investigate other sources of carbon dioxide. In exploring other sources of carbon dioxide the molecule with the greatest potential to act as a source for the production of carbon dioxide is an amino acid. Any acid/base equilibrium difficulty created by producing carbon dioxide from an amino acid can be overcome by involving a basic amino acid in this process (Steer 2005). A plentiful supply of basic amino acids is required. The pepsinogens which are secreted by the chief cells of the human stomach would fulfill this requirement.

Why can the pepsinogens act as a source of basic amino acids? Pepsinogens are inactive proenzymes which are activated by the separation of a molecular fragment from the pepsinogen molecule. This molecular fragment is termed activation segment. The biochemical structure of the activation segment of human pepsinogen 1 has been determined (Kageyama & Takahashi 1980). This molecule consists of forty seven amino acids of which fifteen are basic amino acids. Six of the fifteen basic amino acids are arginine. Arginine is the most basic amino acid with an isoelectric point (pI) of 11·15 making the arginine molecule significantly basic at physiological pH (7·4). Thus, pepsinogens could act as a source of basic amino acids.

It was decided to examine the distribution of pepsinogen, which could act as the source of arginine, and the distribution of agmatine which is an end product of the decarboxylation of arginine. The cytochemical studies carried out involve using the technique of resin embedding of mucosal biopsies (Britten Howarth & Roche 1993). This research was originally published in 2005 (Steer 2005).

Material and methods

This investigation of gastric acid secretion has involved studying biopsies obtained at upper gastrointestinal endoscopy from thirty eight patients with a normal stomach when assessed by endoscopy and by histology. The absence of *Helicobacter pylori* infection of the stomach has been determined by a negative CLO test (Kimberly-Clark, Ballard Medical Products, Utah, USA) and on immunohistology of the gastric biopsies using a rabbit polyclonal antibody to *Helicobacter pylori* (Steer 2005).

All patients have had biopsies taken from high on the greater curve of the stomach, fifteen patients have had additional biopsies from the distal oesophagus and the first part of the duodenum. For the glycol methacrylate (GMA) processing the following procedure has been adopted (Britten Howarth & Roche 1993). Immediately following their retrieval endoscopic biopsies have been placed in ice acetone containing 2mM phenyl methyl sulphonyl fluoride and 20mM iodoacetamide. Following overnight fixing at -20°C, the fixative is replaced with acetone at room temperature for 15 minutes, then methyl benzoate at room temperature for 15 minutes. The tissues have been infiltrated

with processing solution (5% methyl benzoate in glycol methacrylate solution – GMA solution A) at 4°C. There have been three changes of processing solution with two hours in each change before embedding in freshly prepared embedding solution (GMA solution A / benzoyl peroxide). The capsules containing resin embedded biopsies have been polymerized at 4°C for 48 hours and stored in airtight boxes at -20°C. Sections, 2µm thick, have been cut from the resin embedded specimens and have been stained with polyclonal antibodies (Steer 2005). This study has involved using a sheep polyclonal antibody to human pepsinogen 11 (Abcam, Cambridge, U.K.), a rabbit polyclonal antibody to agmatine (1-amino-4-guanidobutane) (Chemicon International, Chemicon Europe, Chandlers Ford, Hampshire, U.K.) and a rabbit polyclonal antibody to *Helicobacter pylori* (DakoCytomation, Ely, Cambridge, U.K.).

The following staining procedure has been used for staining sections of the GMA embedded tissues with polyclonal antibodies. Endogenous peroxidase has been inhibited using a solution of 0·1% sodium azide and 0·3% hydrogen peroxide in reverse osmosis water for 30 minutes. The sections have been washed three times with tris buffered saline (TBS) at five minute intervals prior to inhibiting the non-specific binding sites by incubating the sections with 20% foetal calf serum in Dulbecco's modified Eagle's medium containing 1% bovine serum albumen for 30 minutes. The slides have been drained and then incubated with the primary antibody (either antibody to human pepsinogen 11 or antibody to agmatine) at the appropriate dilution in TBS for 18 hours at 4°C. Evaporation of the antibody solution has been prevented by placing a coverslip over the section with care being taken to exclude any air bubbles. Subsequently, the sections have been rinsed three times with TBS at five minute intervals. The slides have been drained and the appropriate biotinylated second stage antibody applied at the appropriate dilution for two hours at room temperature. The slides have been rinsed three times with TBS at five minutes intervals prior to incubation with streptavidin-biotin horseradish peroxidase complexes at appropriate dilution in Tris/HCl for two hours at room temperature. The slides have been rinsed three times with TBS at five minute intervals before applying AEC solution for 20 minutes at room temperature. The slides have been rinsed with TBS and washed in running tap water for two minutes. The sections have been counterstained with Mayer's Haematoxylin for two minutes and washed in running tap water for five minutes to blue. Crystalmount has been applied and the sections baked for approximately 10 minutes at 80°C until the crystalmount was dry. The slides have been allowed to cool to room temperature prior to mounting with a coverslip in DPX.

Ethical approval for the study has been obtained. Permission to obtain the endoscopic biopsies as well as perform the cytochemical analyses have been obtained from the patients. The patients have been undergoing endoscopic examinations as part of the investigation of their presenting symptoms.

Results

This study has been confined to observations relating to gastric parietal cells, gastric mucus secreting epithelial cells, the oesophageal epithelium and the goblet cell area of the duodenal epithelium. The control slides for pepsinogen II, agmatine and *Helicobacter pylori* are all negative with no positive chromogen staining.

Pepsinogen 11 is found in the canaliculi of the parietal cells (figures 1A and 1B). The canaliculi can be either nondilated (figure 1A) or dilated (figure 1B). A dilated parietal cell canaliculus is seen opening into the lumen of a gastric gland (figure 1B). The presence of pepsinogen 11 in the parietal cell canaliculi is not universal.

The activation segment of pepsinogens has a high concentration of arginine (Kageyama & Takahashi 1980). The decarboxylation of arginine results in the formation of agmatine. This process is shown in figure 2.

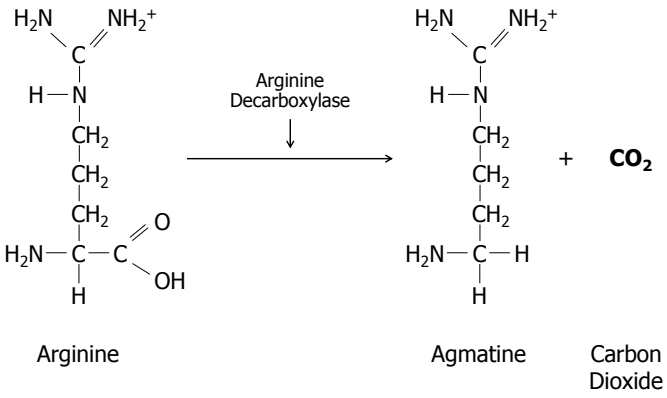

Figure 2. The decarboxylation of arginine.

Agmatine is present in the parietal cell canaliculi (figure 1C) but, as noted for pepsinogen 11, this localization is not universal and is similar to the findings for indicator dyes (Bradford & Davies 1950). There is intense staining for agmatine in the mucus of the gastric mucus secreting epithelial cells located at the pit area of the gastric glands and on the luminal surface of the gastric mucosa (figure 1D).

Agmatine is absent from the normal oesophageal epithelium (figure 3A) but is found in goblet cells of the duodenal mucosa (figure 3B). Agmatine is absent from the brush border columnar epithelial cells of the duodenal epithelium except at the brush border of these cells.

Discussion

The presence of pepsinogen 11 in parietal cell canaliculi and the fact that not all parietal cell canaliculi contain pepsinogen is consistent with the patchy localization of indicator dyes found by Bradford and Davies (1950) in their classical studies leading to the establishment of the role of parietal cells in acid secretion. Previously, a coagulable material has been noted in parietal cell canaliculi (Stohr 1882; Zimmerman 1925; Hoerr & Bentley 1936). This coagulable material would be consistent with the presence of a protein in the canaliculi. The association of pepsinogens with acid secretion is interesting when one considers that pepsinogens are produced and secreted by the same cells that "secrete" acid in birds, fish, amphibians and reptiles (Hirschowitz 1984).

Agmatine has been recognized since the work of Professor Albrecht Kossel (Kossel 1910), in the year that he was awarded the Nobel Prize. Kossel isolated agmatine from herring roe and for many years agmatine had been considered to be absent from mammalian tissues. This misapprehension was corrected in 1994 with the identification of agmatine in mammalian tissue (Li et al. 1994). An examination of various mammalian organs has revealed that the greatest concentration of agmatine is found in the stomach (Raasch et al. 1995).

The presence of pepsinogen 11 in the parietal cell canaliculi can act as a source of arginine at this site. The process of decarboxylation of this arginine in the stomach has been confirmed in the present study by finding the end product of this reaction, namely agmatine, in the parietal cell canaliculi. The decarboxylation of arginine provides a source of carbon dioxide. The hydration of this carbon dioxide enables hydrogen ions to be released. The decarboxylation of another basic amino acid, lysine, may also provide carbon dioxide for this process but lysine has not been studied. In human pepsinogen 1, the activation segment has fifteen basic amino acids out of a total of forty seven amino acids. As stated six of these basic amino acids are arginine but of the remaining nine basic amino acids eight are lysine (Kageyama & Takahashi 1980) which has an isoelectric point of 9·59.

The diagrammatic representation of the extracellular production of gastric acid by the hydration of carbon dioxide which has been derived from the decarboxylation of arginine is shown in figure 4.

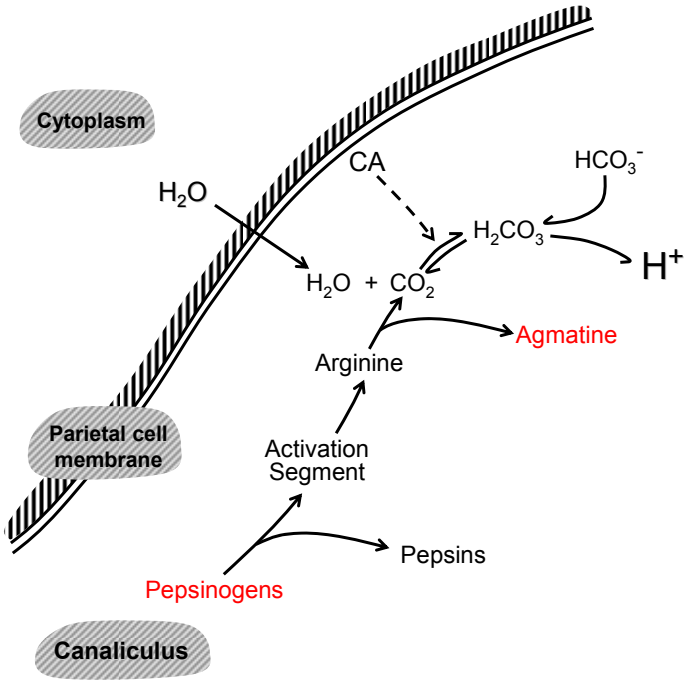

Figure 4. Diagrammatic representation of the extracellular production of gastric acid. Carbonic anhydrase (CA) is located on the luminal aspect of the parietal cell membrane (Cross 1970). The molecules investigated in this study are indicated in red.

This extracellular, rather than intracellular, hydration of carbon dioxide and the formation of agmatine would explain why the parietal cells are not destroyed by the production of the strong acid.

The current study has demonstrated significant agmatine in the mucus of gastric mucus secreting epithelial cells (figure 1D). It has been shown that radio-labeled agmatine is absorbed from the lumen of the intact and isolated rat stomach (Molderings et al. 2002). The agmatine formed by the decarboxylation of arginine at the gastric parietal cells could be absorbed by the gastric mucus secreting epithelial cells at the luminal aspect of the gastric mucosa and in the pit region of the gastric glands. This absorbed agmatine can biochemically assist in the protection of the stomach from the concentrated acid. There is less agmatine in the gastric mucus secreting epithelial cells of patients infected with *Helicobacter pylori* (Steer 2005). This decrease in the amount of agmatine would lessen gastric protection in patients infected with *Helicobacter pylori*.

The decrease in the amount of gastric epithelial agmatine with *Helicobacter pylori* infection coincides with the decrease in the amount of mucus found in these gastric epithelial cells (Steer and Colin Jones 1975; Steer 1985; Steer 2005). The agmatine is located at the mucus containing area of these cells. The decrease in the amount of epithelial agmatine with *Helicobacter pylori* colonization of the stomach is contrary to the known effect of *Helicobacter pylori* on the local agmatine production as assessed in gastric juice (Molderings et al 1999). The present findings of decreased epithelial agmatine in *Helicobacter pylori* infection would indicate that the increased agmatine in gastric juice in *Helicobacter pylori* infection (Molderings et al. 1999) is not being absorbed to a sufficient extent by the gastric epithelial cell mucus. Agmatine produced by the *Helicobacter pylori* would therefore not be beneficial to the host but may be beneficial to the bacterium.

The absence of agmatine from the stratified squamous epithelium of the normal oesophagus makes this epithelium liable to damage when gastric acid refluxes into the lumen of the oesophagus. This liability of the oesophagus to damage is the subject of a further publication (Steer 2007).

Conclusion

The presence of pepsinogen and agmatine in the parietal cell canaliculi supports the conclusion that decarboxylation of arginine occurs in these canaliculi. The carbon dioxide produced by the decarboxylation of arginine can be hydrated with the production of acid in these canaliculi. This hydration of carbon dioxide is catalysed by the enzyme carbonic anhydrase which has been located on the luminal surface of the cell membrane lining these parietal cell canaliculi. The agmatine produced by the decarboxylation of arginine would help biochemically to protect tissues against the harmful effects of the strong acid.

The activation of pepsinogens, the decarboxylation of arginine and the production of agmatine in the normal stomach are all part of the same biochemical process which is inextricably linked to the production of concentrated gastric acid and the protection of tissues against this strong acid.

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Figure 1.

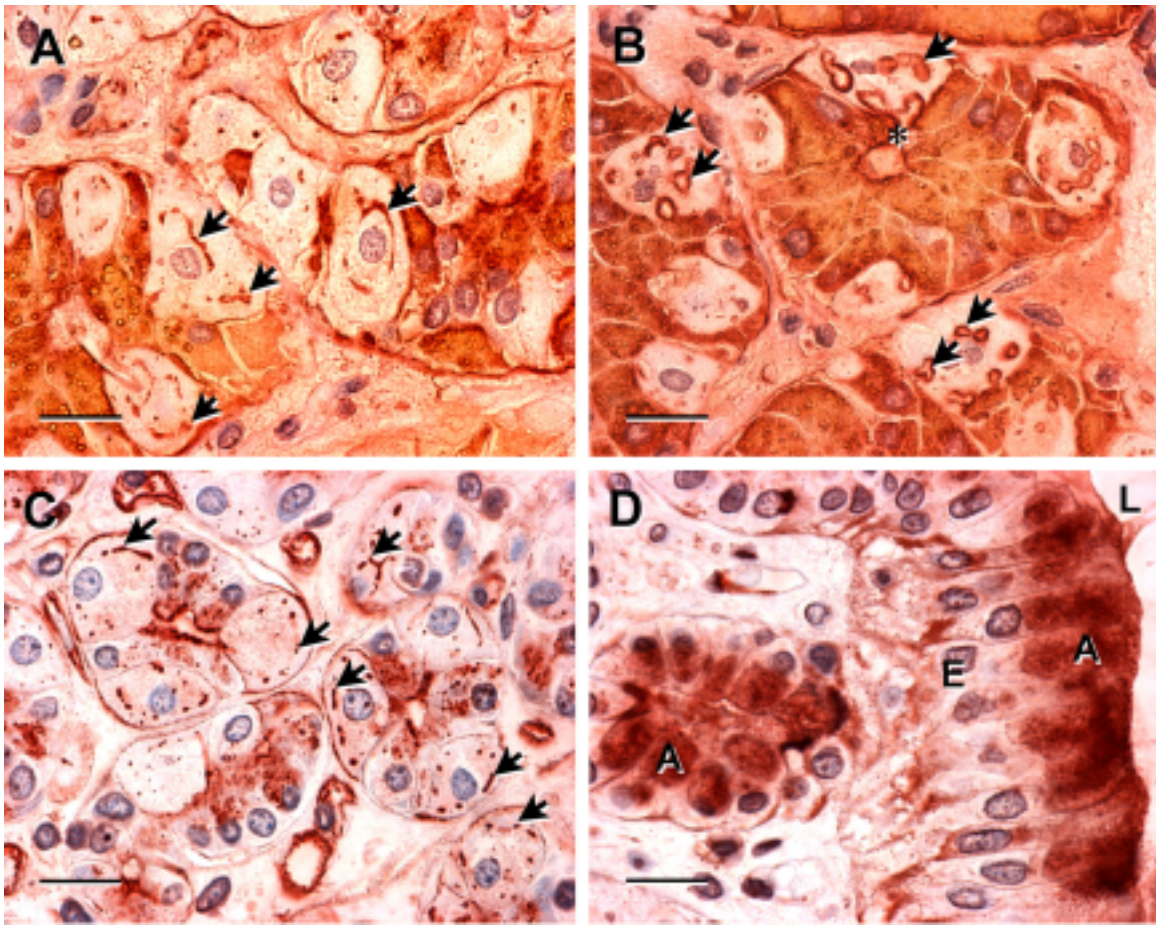

Figure 1.

Biopsies from high on the greater curve of the stomach. Sections A and B stained for pepsinogen 11. Sections C and D stained for agmatine. Sections A, B and C are from the parietal cell area of the gastric mucosa. Section D is from the luminal area of the gastric gland.

A – agmatine. E – epithelial cells. L – lumen.
Arrows in sections A B and C – parietal cell canaliculi containing pepsinogen 11(sections A and B) or agmatine(section C).
Asterisk in section B – marks the site of opening of the parietal cell canaliculus into the gastric gland lumen.

Scale bar is 20µm.

Figure 3.

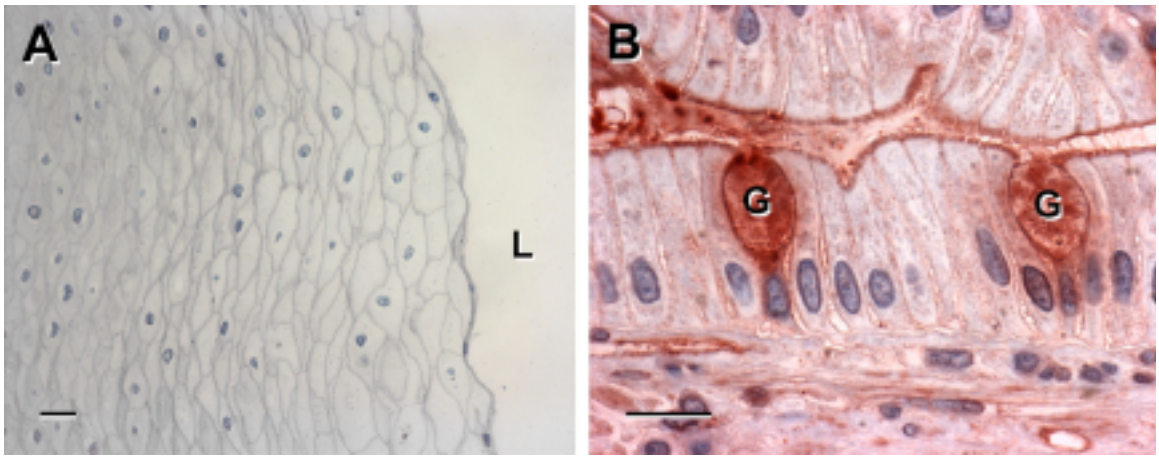

Figure 3.

Section A – biopsy from the distal oesophagus.
Section B – biopsy from the first part of the duodenum.
Section A and B stained for agmatine.
L – lumen. G – goblet cell.

Scale bar is 20µm.