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SEROTONIN IMMUNOREACTIVE CELLS AND NERVE FIBERS IN THE MUCOSA OF THE RAT STOMACH

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ABSTRACT

The current study has showed the morphological features of serotonin (5-hydroxytryptamine) immunoreactivity in the pyloric mucosa of the rat stomach. The immunoreactive elements included the endocrine cells, mast cells and mucosal nerve fibers in the lamina propria. The immunopositive endocrine cells were oval in shape and located mostly in the basal part of the gastric glands. The cell contained a central non-staining area and the peripheral staining zone. The mast cells stained homogenously and appeared to be oval-round in shape. They were located in the upper, middle and basal parts of the mucosa. Serotonin immunoreactive nerve fibers with varicosities were located in the space between the gastric glands in the lamina propria. They appeared not to be in contact with the immunopositive endocrine and mast cells. The current study shows that serotonin may be released by the immunoreactive elements in the stomach and that future work is needed to characterize the ultrastructural features of serotonin positive nerve fibers in the pyloric mucosa.

Keywords: Stomach, Pylorus, Serotonin, Immunohistochemistry, Wistar rats

INTRODUCTION

The gut contains the enteroendocrine cells that stain positive for a number of substances such as gastrin, serotonin (5-hydroxytryptamine), α-Aminobutyric acid (GABA), secretin, substance P (motilin) and cholecystokinin (CCK) that regulate the various activities related to digestion, absorption of food and differentiation and maintenance of the gut epithelium (Johson 1976; Davanger 1989, Koh *et al.*1997, Oomori *et al*. 1997, Taniyama *et al*. 2000, Takeda *et al*. 2000, Vincze *et al*. 2001). Gastrin, serotonin and GABA containing cells have been localised in the small and large intestines of various animals and are more numerous in the pyloric antrum of the stomach (Davanger *et al*. 1989; Gesase 2001). GABA- and serotonin-immunopostive have been described to have a similar morphology (Oomori *et al*. 1992). The cells appear oval or round in shape and many of them have cell processes that extend to reach the lumen of the gastric glands (Oomori *et al*. 1986, Davanger *et al*. 1994, Toole *et al*. 1998). There is limited data on the morphological characterization of serotonin immunopositive cells. One study indicated the presence of nerve fibers and deeply staining and faintly staining cells against serotonin (Yu *et al*. 2001). Such findings need to be clarified and will add knowledge to the morphology of serotonin immunoreactive elements in the stomach.

The current study used the antibody against serotonin to investigate the morphology of serotonin immunoreactivity in the pyloric mucosa of the rat stomach.

MATERIALS AND METHODS

The study was carried out with seven male Wistar rats (about 200g body weight), which

had lived under normal laboratory conditions. The animals received commercial food and water and were kept under constant conditions in the animal house at Muhimbili University College of Health Sciences.

The animals were anaesthetized with ether, thoracotomized, and then perfused via the left cardiac ventricle with 250 ml of physiological saline followed by 150 ml of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) containing 4% paraformaldehyde at 4ϒ C for 10 minutes. The pyloric part of the stomach was removed and cut into small pieces and stored in the same fixative at a temperature of 4ϒC for 12-24 hours. After rinsing with PBS, the specimens were left overnight in PBS containing 30% sucrose at 4ϒC for 1 day. The tissues were frozen and cut about 12µm thick using a cryostat, and then mounted on gelatine coated glass slides.

The sections were incubated with a rabbit anti-serotonin (UCB-bioproducts, Brussels, Belgium) diluted 1:10,000 for 24 hours at room temperature. After incubation with primary antibodies the slides were treated with goat biotinylated anti-rabbit IgG and ABC complex (Vector Laboratories, Burlingame, Calif., USA) for 1 hour at room temperature. The antigen-antibody reaction sites were made visible by incubating the sections with diaminobenzidine tetrahydrochloride (DAB) and 0.01% hydrogen peroxide in 2.5mM Tris-HCl buffer (pH 7.6) for 10 minutes. The specificity of the immunohistochemical staining was confirmed by omission of the primary or secondary antibody. No immunostaining was observed under these conditions through out the experiment.

RESULTS

Observation of the sections revealed serotonin immunoreactive cells and nerve fibers in the mucosa layer of the pylorus (Figs. 1, 2 and 3). The immunoreacted cells were located in the apical, middle and basal parts of the mucosa and close observations revealed the presence of two cell population (Figs. 1 and 2). The first group of cells appeared to have a non-staining central area and the deeply staining peripheral part (Figs. 2 and 3). These cells appeared to be the endocrine cells of the stomach and were located mainly at the basal portion of the mucosa. The endocrine cells were oval in shape, with elongated ends that pointed at the lumina of the gastric glands. The second group of cells that also stained positive against serotonin antibody were randomly distributed in the mucosa of the pylorus, but many of them appeared to be in the middle and upper mucosa (Figs. 2 and 3). These cells stained deeply and uniformly for serotonin antibody and they appeared to be the mast cells in the lamina propria of the stomach wall. They appeared oval-round in shape and did not show cytoplasmic processes. Close observation of the sections stained with serotonin antibody also revealed the presence of immunoreactive nerve fibers in the pyloric mucosa (Fig. 3). Serotonin immunopositive fibers with varicosities were seen to run in the space between the gastric glands and did not appear to establish contact with the immunopositive endocrine cells.

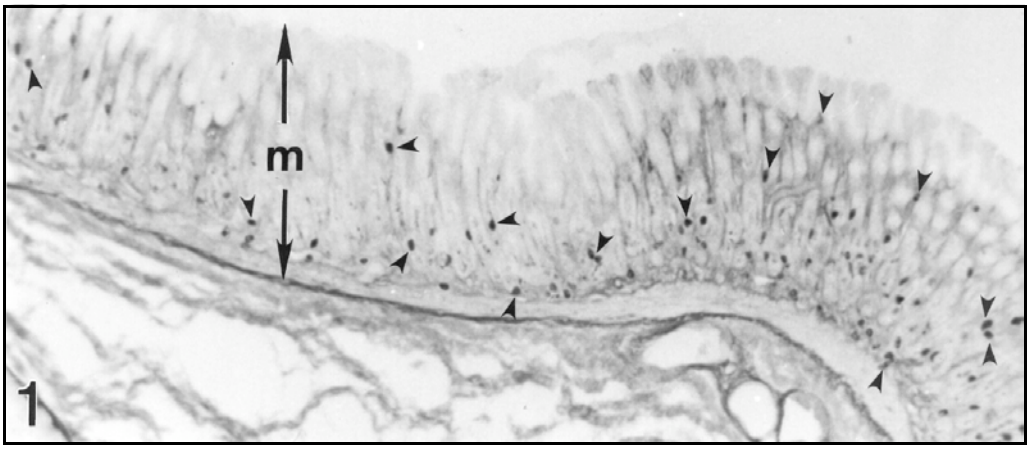

Figure 1: Light micrograph of serotonin (5-hydroxytryptamine) immunoreactivity in the rat stomach showing the mucosa wall (*m*) and immunoreactive cells (*small arrowheads*) that are distributed in the upper, middle and basal parts of the mucosa (m). x120

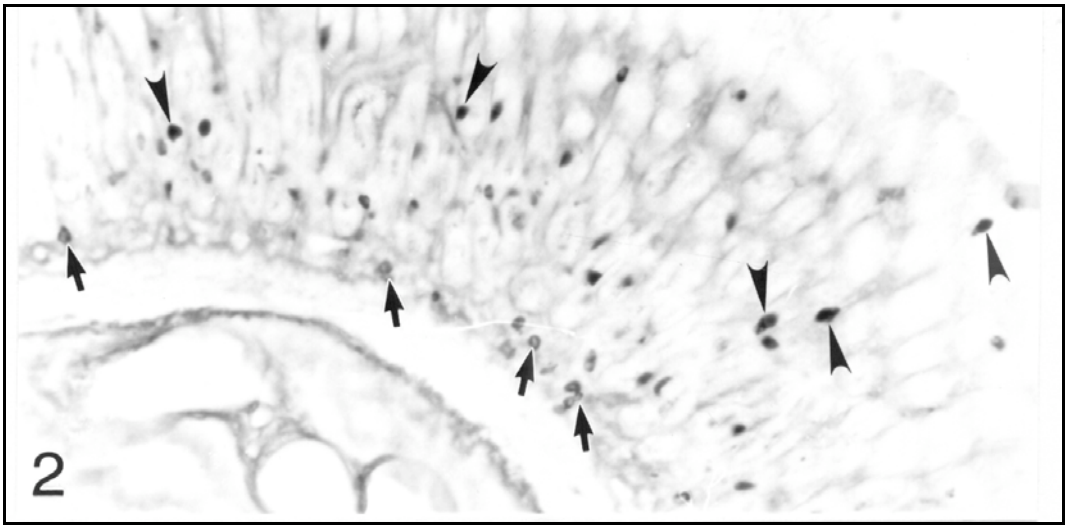

Figure 2: The light micrograph of the rat pyloric mucosa showing the homogenous deeply staining mast cells (*arrowheads*) and endocrine cells (*short thick arrows*) with non-staining central area and the peripheral staining zone. x180

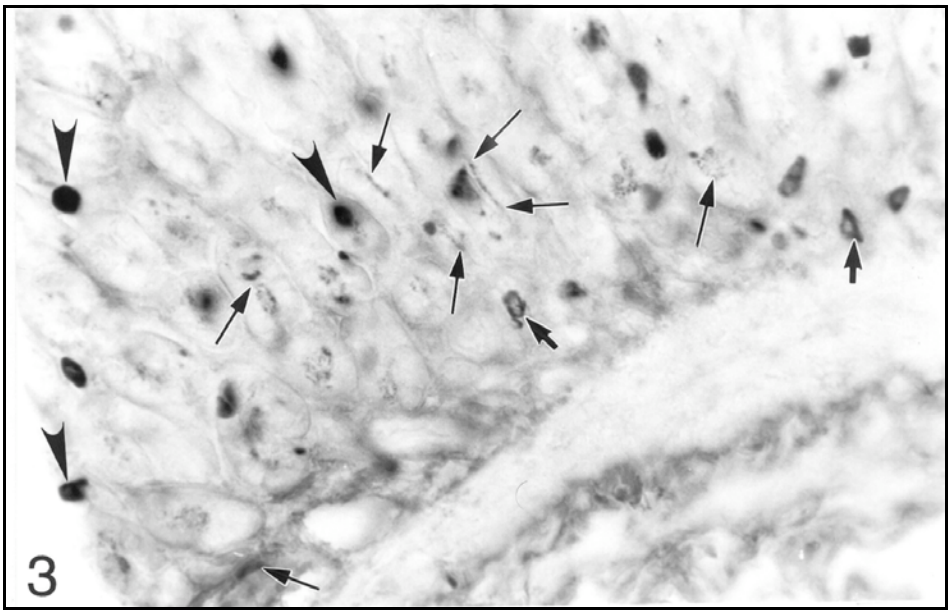

Figure 3: The light micrograph showing the immunopositive nerve fibers (*thin long arrows*) in the rat pyloric mucosa; it also shows the mast cells (*arrowheads*) and immunoreactive endocrine cells (*short thick arrows*). x260

DISCUSSION

The current study has showed the presence of two cell population that stained positive for serotonin antibody. One cell population stained homogenously and appeared to be the mast cells; the endocrine cells showed the central non-staining area and the peripheral staining zone. The results have also showed the presence of serotonin-immunopositive nerve fibers in the pyloric mucosa. The serotonin immunopositive elements may be the source of serotonin that works to regulate various activities in the gut such as smooth muscle contraction and secretion of gastric acids. The mucosal serotonin positive nerve fibers may also play the role of regulating the functions of the epithelia cells in the stomach.

The current results revealed the serotonin immunopostive cells with a central non-staining area that appeared to be the endocrine cells and the deeply homogenously staining mast cells in the pyloric mucosa. These findings are in agreement with the previous observation in the rat stomach (Yu *et al*. 2001). Other studies also observed the serotonin immunopositive cells but did not report on the presence of deeply staining mast cells (Yamada *et al*. 1999, Bordi *et al*. 2000). These observations indicate that serotonin that is secreted in the gastric wall may come from the endocrine cells and the mast cells; the other source is the immunopositive nerve terminals which have been observed in this study. Serotonin is an important hormone that has been shown to control smooth muscle contraction in the gut and in the stomach it also regulates secretion of gastric acid (Canfield and Spencer 1983, Koop and Arnold 1984, Stephens *et al*. 1989, Wilmer *et al*. 1993, Briejer *et al*. 1997, Takeda *et al*. 2000, Taniyama *et al*. 2000). It has also been shown to play a paracrine role in the stimulation of pancreatic secretion via a vagal cholinergic pathway and may also enhance the growth of

neuritis by stimulating 5-HT_{2B} receptors (Fiorica-Howells *et al*. 2000, Li *et al*. 2001; 2000). Previous work have suggested that the largest source of serotonin to be the endocrine cells of the gut and that mast cells and immunopostive fibers may not have greater contribution to the total amount of serotonin released in the stomach (Schworer *et al*. 1987, Li *et al*. 2001). The current results indicated that both cells showed strong signals for serotonin an indication that they may have an equal role during serotonin secretion. Mast cells are thought to release serotonin during immune response (Askenase *et al*. 1980). It is open for future work if the released serotonin can have other extra immune role.

The results have showed the presence of serotonin immunopositive nerve fibers in the pyloric mucosa. This finding is in agreement with past observations that were made in the gastric mucosa of the rat, guinea pig and golden hamster (Mawe *et al*. 1989, Fujita *et al*. 1995, Fujimiya *et al*. 1997, Toole *et al*. 1998, Chen *et al*. 2001, Yu *et al*. 2001). It has been demonstrated that the cell bodies for the serotonin immunopositive nerve fibers are located in the myenteric plexus (Toole *et al*. 1998, Mawe *et al*. 1989). The role played by the serotonin immunopositive fibers in the stomach is not clearly known. Some authors have suggested that the nerve fibers may exert an important role in the epithelial function of the gastrointestinal tract (Fujimiya *et al*. 1997). Such a suggestion needs to be clarified and is open for future work. Past studies that have been done in other tissues have showed the presence of intraepithelial nerve fibers in the vallate papillae, ejaculatory duct, urethra, tracheobronchial and nasal mucosae that stained positively for calcitonin gene-related peptide (CGRP) immunoreactive and substance P (Lundberg *et al*. 1984, Uddman *et al*. 1985, Yokoyama 1989, Fujita *et al*. 1995, Lee *et al*. 1995, Huang and Lu 1996). It has been suggested that intraepithelial nerve fibers may have a protective role against noxious stimuli that come from the external environment (Fujita *et al*. 1995). Observations have showed that the gut does not have the intraepithelial nerve fibers that establish contact with the luminal materials (Fujita *et al*. 1995, Chen *et al*. 2001); instead, it contains a large population of the endocrine cells (Yamada *et al*. 1999, Agungpriyono *et al*. 2000, Bordi *et al*. 2000, Vincze *et al*. 2001) that may act as sensor paraneurones in the epithelium that detect noxious materials in the lumen and send the information to the underlying nerve fibers. The serotonin immunopositive nerve fibers observed in the lamina propria may have a role that is yet to be identified. Future work is needed to characterize the ultrastructural features of the serotonin immunopositive nerve fibers in the pyloric mucosa.

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