

PYY, Serotonin Expression Immunoreactive Cells in Adult Goose's Small Intestine (ANSER ANSER): An immunohistochemistry study on frequency and distribution

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Abstract

Enteroendocrine cells serve as the endocrine components by hormone secretion in certain parts of the small intestinal tract. We play important roles in regulating the secretions of only a few large bodies. For how effective those hormones are in maintaining the Organism's internal climate, we have had Investigating the production and distribution of certain Adult goose (*ANSER ANSER*) cells in such small intestines. Regional localization of immunoreactive endocrine cells (IRC) was inspected using immunohistochemistry techniques in some parts of adult goose's small intestine tract. Specimens of ten adult goose of both sexes, aged 1-2 years, have been studied. In an immunohistochemistry process, Chromogranin A (ChA), the popular EEC indicator and serotonin, PYY were extensively used. The results exposed the expression of serotonin along the crypt villus axis on subset-epithelial-cell basis and PYY-immunoreactive cells were found in the lower and middle portions of the villi between the epithelial cells of duodenum and ileum and were never seen in the crypts and jejunum. In addition, These birds ' intestines formed flask or triangular hormonal cells, supporting similar expression in the EECs. The entire small intestine in the immunoactive serotonin and PYY-cells showed declines. It is concluded that the pattern of distribution of the entero-endocrine cells in goose is similar to that of most species of mammals and other poultry. ChA was found to be expressed in the EECs with serotonin and peptide PYY confirming the specific expression.

Key words: serotonin, PYY, gut, goose, Chromogranin A

Introduction

Small intestine (SI) was the place where the nutrients are digested and ingested. Early bird development reveals some physiological and morphological improvements in the SI (Konarzewski, et al. 1990). Many active amines and peptides are synthesized and secreted throughout the gastrointestinal tract (GIT) by certain immunoreactive-endocrine cells (IRECs) (Rodrigues, et al. 2012). A very complex system is formed by the digestive endocrine cells and their chemical products. These system's physiological function is to control all processes relating to the digestion and resorption of food (Grube,1986). Peptide YY is a hormone with 36 amino-acids, Synthesized mainly in Enteroendocrine cells of type L (Kadhim, 2017). The EECs are distributed unequally in the bird GIT than those in Mammals due to GIT ripening differences, depending on the duration of the gestational period (Zghair, 2018). CCK, 5-hydroxytryptamine, PYY, and somatostatine are Certain of the big GIT hormones (El-Salhy, et al. 1985; SALVI, E, et al. 1995). Most intestinal commonly, endocrine cells are elongated with basal granules and called Open cells due to the presence on the apical surface of a microvillus tuft dedicated to the recognition of intestinal chemical data. Closed endocrine cells of round type without any luminous relationship are also present in the gastrointestinal tract (Mohammad et al., 2014) .

Materials and methods

A wax-embedded-tissue method was used to prepare the SI samples for slide. Dewaxing processes were produced for 3x10min each in 100 per cent xylene. Then thereafter, steps based on ethanol were performed for 10min each two-time-100 percent (Luna, 1968). Preceded by an ImmEdge-Hydrophobic-Pen-dependent circling step was an air-dry step for 10min. Earlier, tissue soaking steps of two-fold-7 percent-ethanol were rendered for 5min each. Next, steps of two times the rehydration is induced for 5min each in distilled water (DW). Afterwards Use (10 mMTris / HCl pH 10.0) followed by antigenic recovery buffering autoclaving at 121 and 15psi for 2 times/15min each. Afterwards, In the latest buffer, a cooling-down process was generated at room temperature for 30-60min, Then wash steps 3 times/5min in PBS. By dipping Incubated at room temperature for 1hr with certain humidity, tissues in 10% donkey serum, Blocking of

certain non-specific binding antibody sites has been caused. Key polyclonal antibodies (Biotechnology at Santa Cruse, USA) were incubated at 4 C overnight at 1:100 dilutions against serotonin and PYY. The primary or secondary antibody samples This included 2.5% (v / v) donkey serum, 0.25% (w / v) of Na azide, and 0.2% (v / v) of X-100 in PBS(Bancroft, and Stevens, 2010). Each "Stratech, Scientific Limited, Suffolk, UK" was used per slide for 5 times/5min. Lastly, Those slides were washed in PBS for 5 times/5min each and then assembled with DAPI (Vector Laboratories Ltd, Peterborough, UK) in Vectashield Hard Set Mounting Press. Such sections were visualized and imaged using a Hamamatsu digital camera using an epifluorescence microscope. The primary antibody omission was control. Readings were classified as follows for the IRC frequency: not observed(-), (uncommon (+;Mean estimates were less than 2/filed), some (\pm ;Mean estimates were less than 5 / filed); mild (+ +; Mean standards inferior to 10/filed) and several (+ + +).

Results

Serotonin, PYY and chromogranin A Immunoreactive Cells Expression In the SI

Using a method of immune-histochemistry, the sections of SI tissue were used as an indicator for sections of the adult goose from the sections of duodenum, jejunum, and ileum in the small intestine is used to demonstrate the-expression. Serotonin's expression (Fig.1,2,3). And then, PYY. The results revealed the Serotonin's expression in every part of the small intestine. And PYY's expression only in ileum and few in duodenum (Fig.4). No staining was reported When the primary antibodies were deleted in the control group (Fig. 1,2,3). Typical picture indicates an expression of the gut hormone. It has been demonstrated the role of bowel hormones in goose small intestine. Flask or triangular hormonal cells have been found in these birds ' intestines, however (Fig. 5). Those cells ' form indicated they could have been EECs. Hence, ChA has been used to investigate the type of cell that expresses gut hormones using immunohistochemistry(Fig.5). In this study, in the intestinal tract of the geese, entero-endocrine immunoreactive cells for serotonin, PYY and Chromogranin A were identified. Immunocytochemical studies of many avian species on the intestinal tract showed a number of forms of endocrine cells close to those of mammals. Though various types of endocrine cells are

found in the small intestine (Ceccarelli et al.,1995; Salvi et al. 1996; Baltazar et al., 1998). In our research we found the open class endocrine cells with luminal contact were observed throughout the intestinal tract of the goose via their apical cytoplasmic phase.

Table 1: Enteroendocrine cell distribution, in which adult goose is found in the small intestine as an immunoreactive to regulatory peptides.

| Hormone | Duodenum | Jejunum | Ileum |
|-----------|----------|---------|-------|
| Serotonin | +++ | ++ | ++ |
| PYY | + | - | +++ |

High frequencies:+++ , moderate frequencies:++ , few frequencies:+ , rare:± , and not detected:- :
Relative frequencies

Co-location of intestinal hormone chromogranin A

chromogranin A and Dual immunotisation methods had been used to distinguish PYY presence and serotonin was used to further label with two secondary antibodies to different animal specimens, Anti-chicken IgG and IgG with anti-rabbit each labelled with a fluorochrome. They had been painted singularly Like the green and red dots. In those tissues the co-expression was found when merging as areas of orange / yellow colour. Parts of IS of goose incubated with antibodies to serotonin were read, PYY and ChA. The serotonin and PYY-containing EECs showed co-expression of serotonin and PYY in goose along with ChA in the SI EECs (Fig.6,7) as follow. Serotonin-immunoreactive cells have been found throughout the goose small intestine. Similar distribution in mammals has been reported (Rawdon, 1984; Ceccarelli, 1995; Baltazar et al., 1998; Dall'Aglio et al., 1998). The PYY-immunoreactive cells were found in the lower and middle portions of the villi between the epithelial cells of duodenum and ileum and were never seen in the crypts and jejunum, find as EL-Salhy, et al., (1982) in chicken.

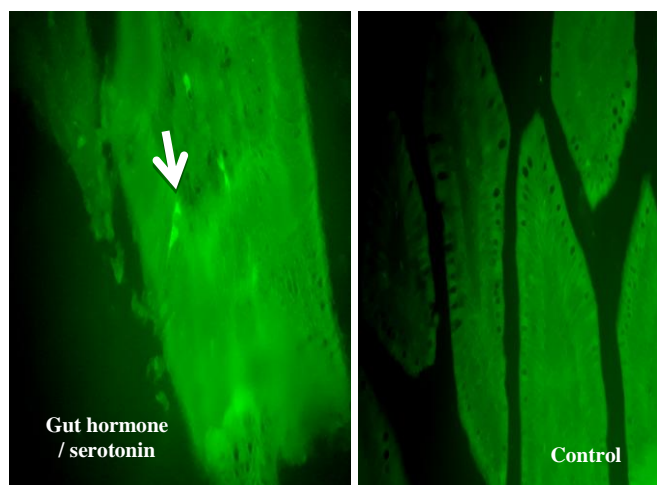


Figure 1: Sections of wax embedded duodenal tissue were examined for gut hormone (serotonin) with the antibody. Typical picture that indicates that the gut hormone (green cells) is expressed on a subset-epithelial basis. In the control section no staining as reported for the gut hormones when the primary antibodies were omitted. 400X.

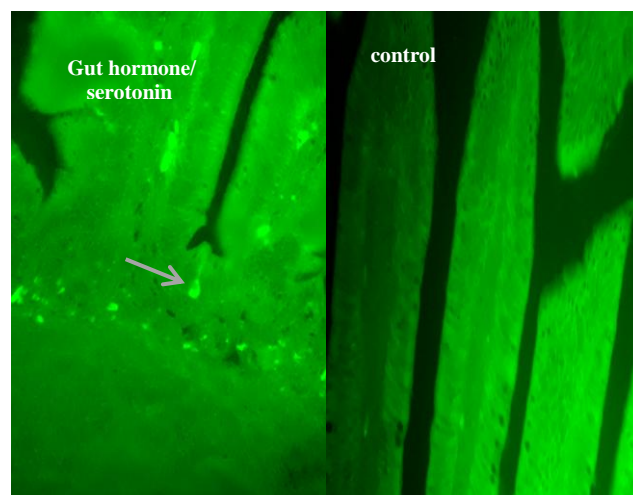


Figure 2: Sections of wax embedded jejunum tissue were examined for gut hormone (PYY) with the antibody. Typical picture that indicates that the gut hormone (green cells) is expressed on a subset-epithelial basis. In the control section no staining was reported for the gut hormones when the primary antibodies were omitted. 200X.

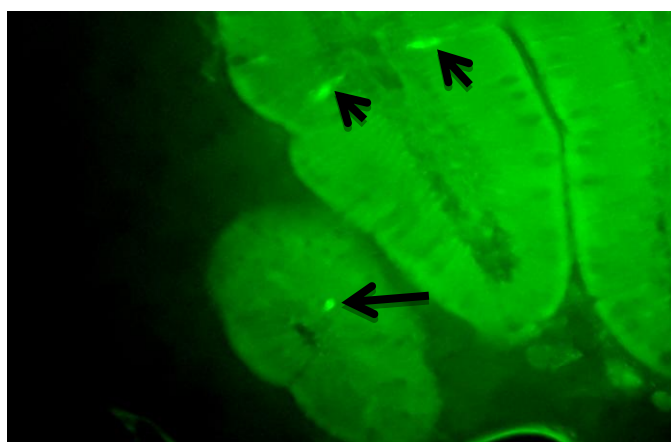


Figure 3: Sections of wax embedded ileum tissue were examined for serotonin. Typical picture that indicates that the gut hormone (green cells/black arrows) is expressed on a subset-epithelial basis. 400X.

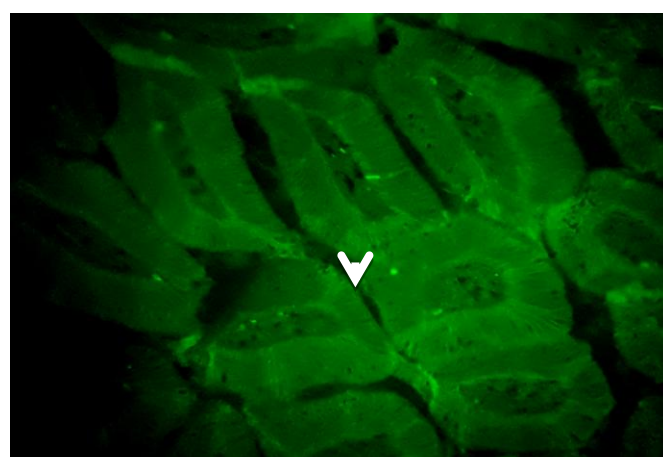


Figure 4: Sections of wax embedded ileum tissue were examined for PYY. Typical picture that indicates that the gut hormone (green cells/white arrow) is expressed on a subset-epithelial basis. 400X.

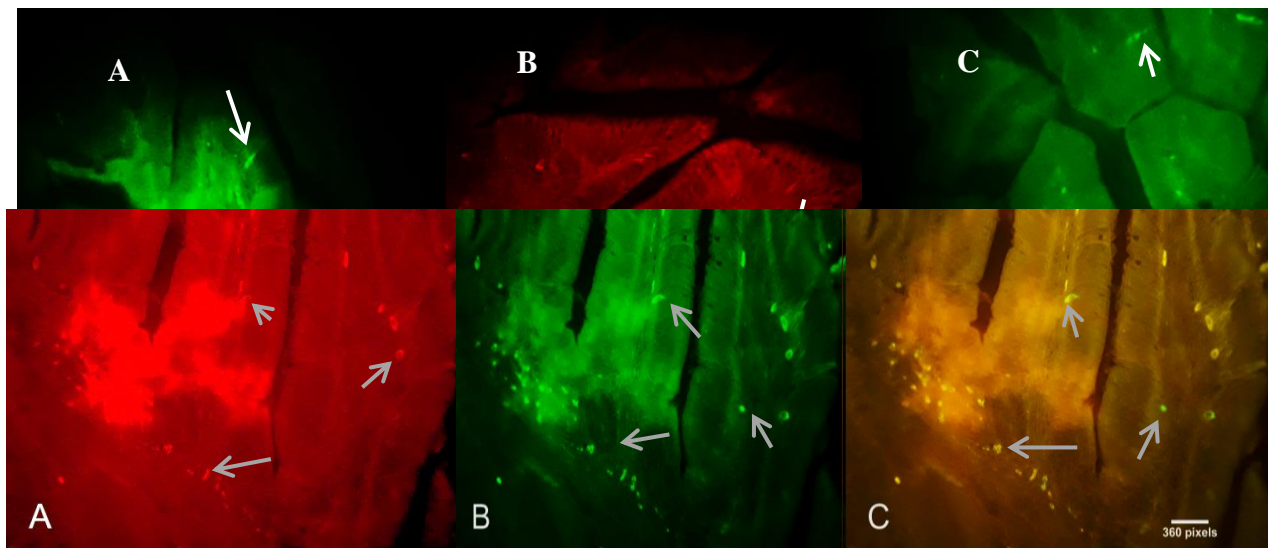


Figure 6: The intestinal parts Have been branded with main (serotonin) To (red), and to (green) ChA. antibody. When does the parts Overlaid, gut hormones and ChA in the same (yellow) cell were shown to be co-expressed. 200 X

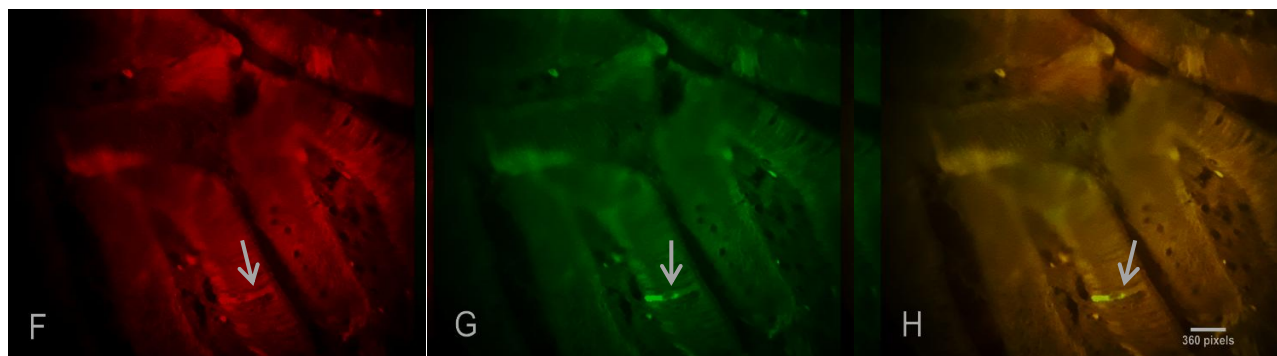


Figure7: The intestinal parts were identified as having between (red) and (green) the primary antibody (PYY). A typical picture of immunofluorescence Shows ChA (green) co-location with intestinal (red) hormone. When does the pieces Was overlaid, intestinal hormones and ChA were shown to co-express in the Same (orang) cell. 400 X,

Reference

- 1- Baltazar, E. T., Kitamura, N., Hondo, E., Yamada, J., Maala, C. P. and Simborio, T. (1998): Immunohistochemical study of endocrine cells in the gastrointestinal tract of the Philippine carabao (*Bubalisbubalis*). *Anat. Histol.Embryol.*27, 407–411.
- 2- Bancroft, J. D. and Stevens, A. (2010). In *Theory and Practice of Histological Techniques*. 2nd(Ed), Churchill Livingstone. New York.
- 3- Ceccarelli, P., Gargiulo, A. M. and Pedini, V. (1995): The endocrine cells in the gastroenteric tract of adult fallow deer (*Damadama L.*). *Anat. Histol. Embryol.* 24, 171–174.
- 4- Dall’Aglia, C., Scocco, P., Ceccarelli, P. and Pedini, V. (1998): Neuroendocrine cells in the gastrointestinal tract of the wild boar. *Anat. Histol. Embryol.* 27,381–385.
- 5- El-Salhy, M., Wilander, E., Lundquist, M.(1995). "Comparative studies of serotonin-like immunoreactive cell in the digestive tract of vertebrates". *Biomed. Res.*, 6:371-375.

- 6- Mohammad M. M., Kohzy H, Kei N., Chihiro T. and Takafumi W.(2014): Distribution of Glucagon-Like Peptide (GLP)-2-Immunoreactive Cells in the Chicken Small Intestine: Antigen Retrieval Immunohistochemistry. doi: 10.1292/jvms.13-0513; J. Vet. Med. Sci. 76(4): 565–568.
- 7- Grube, D. (1986): The endocrine cells of the digestive system: amines, peptides, and modes of action. Anat. Embryol. 175, 151–162.
- 8- Konarzewski, M., C. Lilja, J. Kozłowski, and B. Lewonczuk. "(1990). On the optimal growth of the alimentary tract in avian postembryonic development". J. Zool. 222, 89–101.
- 9- Rawdon, B. B. (1984): Gastrointestinal hormones in birds: Morphological, chemical, and developmental aspects. J.Exp. Zool. 232,659–670.
- 10- Salvi E., Buffa R. and Tindaro G. R. (1996). Ontogeny, distribution and amine/peptide content of chromogranin A- and B-immunoreactive endocrine cells in the small and large intestine of the chicken, AnatEmbryol, 194:89-98.
- 11- Rodrigues, M. N., Abreu, J. A. P., Tivane C., Wagner, P. G., Campos, D. B., Guerra, R. R., Rici, R. E. G. and Miglino, M. A.(2012). Microscopical study of the digestive tract of Blue and Yellow macaws. Current Microscopy Contributions to Advances in Science and Technology (A. Méndez-Vilas, Ed.).
- 12- Kadhim A. B.)2017). An Immunohistochemical Localization of Endocrinal Cells In The Epithelium of the Duodenum Mucosa Of Turkey (*Meleagrisgallpava*)"Bas.J.Vet.Res. Vol.16, No.1.
- 13- Zghair, F. S.(2018). Expression of CCK, GIP and GLP-2 Immunoreactive Cells in the Small Intestine of the Adult Guinea Fowl (*Numidameleagris*): Frequency and Distribution an Immunohistochemistry Study. Indian Journal of Natural Sciences. Vol.9 /Issue 51 /.
- 14- Luna, L. G. (1968). Manual of histologic staining methods of 5th armed forces institute of pathology. 3rd McGraw-Hill book Company-New York.