Development of some intestinal endocrine cell populations in water buffalo

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ABSTRACT: The frequency and distribution of 5-hydroxytryptamin (5HT), somatostatin, cholecystokinin (CCK), motilin, gastric inhibitory polypeptide (GIP) and glucagon/glicentin (glu/gli) endocrine cells were immunohistochemically studied in the intestinal tract of 3, 6, 8 month old buffalo foetuses (m.f.) and of 2 day old (d) animals.

Key word: Water buffalo, Gut development, Immunohistochemistry, Endocrine cells.

INTRODUCTION - The occurrence and distribution of different endocrine cell types in the gastrointestinal tract of large and small domestic mammals have been extensively studied (Ceccarelli et al. 1995; Agungpriyono et al. 2000). Some studies have been also carried out on the ontogeny of gut endocrine cells in mammals (Ono et al. 1994), and only few in ruminant. (Kitamura et al. 1985; Guilloteau et al. 1997). In order to complete a previous study regarding postnatal development of intestinal endocrine cells (Lucini et al. 1999), in this study we report the appearance and distribution of some endocrine cell types in the gut of water buffalo during foetal development.

MATERIAL AND METHODS - Specimens of the duodenum, jejunum, ileum, caecum, colon and rectum were taken from 3, 6 and 8 month old foetuses (m.f.) and from 2 day old (d.) buffalos. The intestinal samples, obtained immediately after butchering at the local slaughterhouse, were fixed in Bouin’s fluid, dehydrated and embedded in paraffin wax. Serial sections (5-7 µm) were cut and tested using the peroxidase-antiperoxidase (PAP) method (Sternberger, 1986). The dewaxed sections were reacted with 3% H₂O₂ at room temperature (RT) for 20 min, rinsed in phosphate buffered saline (PBS) 0.5 M pH 7.3 and incubated with normal goat serum (NGS S-100, Vector, Burlingame, CA, USA) 1/5 for 30 min at RT. After drawing off the excess serum, consecutive sections were reacted with the primary antisera shown in Table. All antisera were raised in rabbit. Incubation was performed in a humid chamber over night at 4°C for each primary antiserum. After 3 baths in PBS, the sections were further incubated with goat anti-rabbit IgG (GAR- IgG AI-1000 Vector) 1/50 for 30 min at RT. Succesively the sections were rinsed in PBS, incubated with rabbit PAP complex (PAP A200/V, UCB, Braine-l’Alleud, Belgium) 1/100 for 30 min at RT and rinsed in PBS. The sections were rinsed again, and the immunoreactive sites were visualized using a freshly prepared solution of 10 µg of 3,3’-diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemicals Co.)

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RESULTS AND CONCLUSIONS - Immunohistochemical techniques revealed the presence and distribution of neuroendocrine cells containing 5HT, somatostatin, CCK, glu/gli, GIP, and motilin irregularly distributed throughout the intestinal tract. All the intestinal endocrine cell types were more frequently distributed in the lower half of the crypts as single elements, at times in 2 or 3 element groups. They were round, ovoid, pyramidal or spindle shaped and sometimes with cytoplasmic processes running along the basement membrane or between neighbouring cells. Some cells reached the intestinal lumen. The immunoreactivity was distributed throughout the cytoplasm (in round or ovoid cells) or in the supra- and infranuclear regions (in pyramidal or elongated cells) as well as in the cytoplasmic processes.

5-Hydroxytryptamine (5HT) immunoreactive cells (i.c.) were detected in all intestinal segments of various aged animals and the duodenum (figure 1) was the intestinal region with the largest number of 5HT i.c. Specifically, regarding the small intestine, 5HT i.c. were numerous in the duodenum and scarce in the jejunum and ileum of 3 m.f. and progressively increased during development. In the large intestine the rectum was the region with the largest number of 5HT i.c. They increased in number from 3 to 6 m.f. and then decreased until the birth in all regions.

Somatostatin (Som) i.c. were numerous in the duodenum and few in the jejunum of 3 m.f. In 6 m.f. Som i.c. appeared in the rectum. At 2d the jejunum represented the tract with the largest number of Som i.c., the duodenum and ileum the regions with a moderate cell number and the rectum the poorest (figure 2).
Glucagon/Glicentin (Glu/gli) i.c. were few in number in all intestinal tracts of 3 m.f. and increased during development, mainly in the large intestine. In 2 d glu/gli i.c. were rare in the duodenum, numerous in the jejunum and ileum, moderately represented in the caecum and colon, numerous in the rectum (figure 3).

Cholecystokinin (CCK) i.c. were detected only in the small intestine of all aged animals. Specifically, they were rare in the duodenum of 3 m.f. and progressively increased during development until 2 d. Moreover, CCK i.c. were frequently detected in the jejunum (figure 4) and ileum in 8 m.f., and were scarcely represented in 2 d.

Gastric inhibitor peptide (GIP) i.c. were detected only in the small intestine of 6 m.f. to newborn animals. In 6 m.f. few GIP i.c. were observed in the duodenum, rare in the jejunum and ileum. They little increased during development (figure 5), so after the birth GIP i.c. were frequent in the duodenum, and progressively less numerous from the jejunum to the ileum.

Motilin (Mot) i.c. were detected only in the small intestine of all aged animals. Few motilin i.c. were detected in the duodenum of 3 m.f. They increased during development and became numerous in 8 m.f. (figure 6). A moderate number of Mot. i.c. was observed in the duodenum of 2 d. In the jejunum and ileum rare Mot. i.c. were firstly detected in 6 m.f. and little increased during development.

All the results allowed to stress some aspects of the endocrine cell ontogenesis. Most of the endocrine cells detected throughout all the digestive tract (5HT and Som), or peculiar of the small intestine (CCK, GIP and Mot) appeared firstly in the proximal tracts (duodenum, jejunum). These results are similar to the results reported in other mammals (Sundler and Hakanson, 1984; Baltazar et al., 1998; Agungpriyono S et al., 2000).

Some endocrine cells as 5-HT, Som, Mot, CCK, appeared early during gut development, other as glu/gli appeared more numerous in foetuses than at the birth. These results may suggest a trophic role in the intestinal mucosal growth and maintenance (Zachary et al. 1987).

Figure 1-6 Endocrine cells in 8 m.f. gut (500X): 5-HT i.c. in the duodenum (1); Som i.c. in the rectum (2); Glu/gli i.c. in the rectum (3); CCK i.c. in the jejunum (4); GIP i.c. in the duodenum (5); Mot i.c. in the duodenum (6).
The absence of glu/gli i.c. in the duodenum of 3 m.f. could be ascribed to transient occurrence of this cell type before this stage, as described in other species during development (Sundler and Hakanson, 1984). The results in animal at birth confirm that also in buffalo the presence of different endocrine cell type is greatest in the duodenum and gradually reduces distally in the small intestine (Baltazar et al. 1998; Agungpriyono et al. 2000).

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REFERENCES - Agungpriyono S., Macdonald A.A., Leus K.Y.G., Kitamura N., Adnyane I.K.M., Gooda ll G.P., Hondo E. and Yamada J. 2000. Immunohistochemical study on the distribution of endocrine cells in the gastrointestinal tract of the Babirusa, Babyrousa babyrussa (Suidae). Anat.Histol:Embryol.29: 173-178. Baltazar ET., Kitamura N., Hondo E., Yamada J., Maala CP., Simborio LT. 1998. Immunohistochemical study of endocrine cells in the gastrointestinal tract of the Philippine carabao (Bubalus bubalis). Anat.Histol:Em- bryol.27(6): 407-411. Ceccarelli P., Pedini V., Gargiulo AM. 1995 The endocrine cells in the gastro-enteric tract of adult fallow deer (Dama dama L) Anat, Hist, Emb 24, 171-174. Guil-loteau P., Huerou-Luron IL, Chyvialle JA, Toullec R, Zabielsk Blum JW 1997 Gut regulatory peptides in young cattle and sheep Lab Jeune Rum, Rennes 44(1) 1-23. Kitamura N, Yamada J, Calingsan NY, Yamashita T 1985 Histologic and immunocytochemical study of endocrine cells in the gastrointestinal tract of the cow and calf Am J of Vet Res 46, 1381-1386. Lucini C., de Girolamo P., Coppola L., Paino G. and Castaldo L. 1999. Postnatal development of intestinal endocrine cell populations in the water buffalo. J.Anat. 195: 439-446. Ono E., Doi Y, Furukawa H, Fujimoto S 1994 The differentiation of entero-endocrine cells of pre- and postnatal rats: lighth and electron microscopy and immunocytochemistry Acta Anat 149, 81-88. Sternberger LA, 1986 Immunohistochemistry 2nd edn New Work: John Wiley & Sons. Sundler F. and Hakanson R., 1984. Gastro-entero-pancreatic endocrine cells in higher mammals, with special reference to their ontogeny in the pig. In: Evolution and tumour pathology of the neuroendocrine system. Falkmer S, Hakanson R. and Sundler V. ed.- Elsevier Science Publishers B.V. 111-135. Zachary I, Penella JW, Rozengurt E 1987 A role for neuropeptides in the control of cell proliferation. Develop Biol 124, 295-308.