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Immunohistochemical study on the expression of serotonin and 5HTR3 in gastrointestinal tract of rat embryos and newborns

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ABSTRACT

Serotonin takes part in the regulation of the gastrointestinal motility, secretion and sensitivity. It produces its effects by binding specific receptors thus being one of the important neurotransmitters and signal molecules in the gut. There are different types of specific serotonin receptors, recognized and cloned into seven groups (from 5HT1 to 5HT7) with various subtype numbers. The aim of this work is to determine the presence and localization of immunohistochemical expression of serotonin and serotonin receptor 3 (5-HTR3) in the developing gastrointestinal tract of rats. 20 rat embryos (16th - 20th day of gestation) and fragments of the stomach and small intestine of 10 one-day-old rats were used for the study. On paraffin sections immunohistochemical reactions for serotonin (primary antibody rabbit polyclonal antibody, Chemicon, USA) and serotonin receptor 5-HTR3 (primary antibody goat polyclonal antibody, Santa Cruz Biotechnology, USA) were carried out using the ABC. Immunohistochemical expression for serotonin and serotonin receptor 3 (5-HTR3) were not detected in the gastrointestinal tract of rat embryos. In the gastrointestinal tract of the one-day-old rats there was positive reaction for serotonin. Serotonin-producing cells were present in the covering epithelium of the villi in the small intestine. They were small in numbers and separately located. The immunohistochemical reaction for 5-HT3 receptors was positivated in the cytoplasm of a great number of smooth muscle cells in both transverse and longitudinal muscle layer of the stomach wall of the newborn rats. Serotonin production and secretion starts after birth and through its 5-HT3 receptors takes part in the regulation of the morphological and functional maturation of the gastrointestinal tract which starts in the embryonal period but continues postnatally.

Key words: serotonin, 5-HT3 receptors, rats

Introduction

Fetal and embryological development of the gastrointestinal tract is a complex process. The morphological and functional maturity of the secretion, sensitivity and the motor function is achieved through the regulating function of a big number of biologically active substances one of which is serotonin. Serotonin produces its effects by binding specific receptors. Nowadays, serotonin receptors 5HTR are recognized and cloned into seven groups of from 5-HTR1 to 5HTR7 with various subtype numbers. Most of these receptors are coupled with G-protein, which

activates membrane-gated adenocyclase or phospholipase. 5-HTR3 receptors are ion channels. Some of 5HTR receptors are presynaptic and others postsynaptic.

In the smooth muscle cells of the gastrointestinal tract 5-HTR3 and 5-HTR4 receptors have been established (Lee *et al.*, 1991). In the smooth muscle cells of stratum circulare of the colon are found 5-HTR4 receptors and in stratum longitudinale 5-HTR7 receptors (De Ponti & Tonini, 2001). 5-HTR1p receptors are verified in afferent somatic nerve fibers from plexus submucosus (Gershon, 2003). 5-HTR3 receptors are found in afferent somatic fibers of plexus myentericus (Wood *et al.*, 1999).

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The aim of the study is to determine the presence of the immunohistochemical expression of serotonin and serotonin receptor 3 (5-HTR3) in the developing gastrointestinal tract of rats.

Materials and Methods

The study is carried out on from 20 rat embryos in the 16th - 20th gestation day and fragments of the stomach and small intestine of 10 one-day-old newborn rats. The study was made of 24 female white rats, fertilized by a time-confined contact with male rats.

Paraffin slides are investigated by the ABC method with primary antibody for MAB352 serotonin (rabbit polyclonal antibody – Chemicon USA) and primary antibody for serotonin receptor 5-HTR3 (goat polyclonal antibody Serotonin Receptor sc – 19150 Santa Cruz Biotechnology USA). Serotonin and serotonin receptor 5-HTR3 was expressed by brown granular substance in the secretory granules of the cells. Negative controls, with buffer (PBS) or normal non-immune serum used instead of the specific antibodies, were used to verify the specificity of the immunohistochemical reactions to any examined antigen. In those samples no product from the corresponding reaction was present.

Results

Expression of serotonin and its receptor 5HTR3 is negative in rat fetal material. The reactions became positive in the gastrointestinal tract of one-day-old newborn rats. The immunohistochemical examination revealed presence of differentiated enteroendocrine EC cells by expression of serotonin in them. The reaction was positive in small number of cells in the intestinal wall. These were scatter singularly between the resorptive cells in the epithelial lining of the small intestine villi (Figure 1). They can be found along the villi and in their apical area. The enteroendocrine EC cells were of cone shape. The serotonin expression was localized in the basal part of the cells (Figure 2).

The serotonin receptor 5-HTR3 is expressed in the stomach wall of one-day-old newborn rats. The reaction is visualized as brown granulation filling the cytoplasm of a big number of smooth muscle cells in both longitudinal and transversal layers (Figure 3).

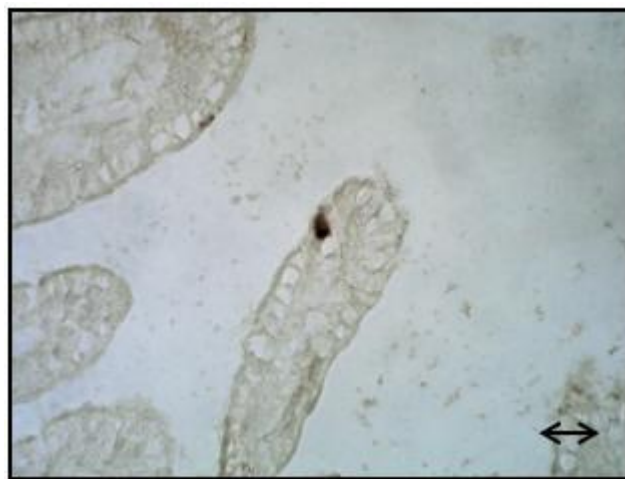


Figure 1. Longitudinal section of a villus from small intestine of one-day-old newborn rat. Serotonin expression in enteroendocrine EC cell from the lining epithelium. Paraffin preparation. x40.



Figure 2. Cross section of a villus from small intestine of one-day-old newborn rat. Serotonin expression in the widened basal part of enteroendocrine EC cell. Paraffin preparation. x40.

We found extensive extracellular expression of 5-HT3 on the border between the muscle layers. The 5-HT3 receptors are probably localized along pl. myentericus (Figure 4). 5-HTR3 receptors were not found in other gastrointestinal organs.

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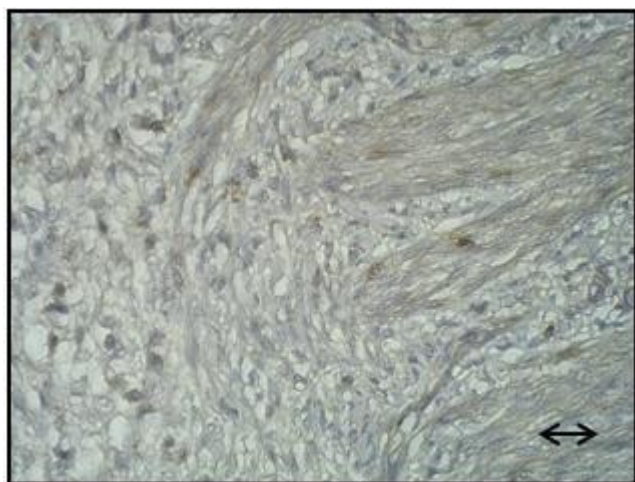


Figure 3. Tunica muscularis from stomach wall of one-day-old newborn rat - brown granulation filling the cytoplasm of a big number of smooth muscle cells in both longitudinal and transversal layers. Immunohistochemical expression of 5-HTR3. Paraffin preparation. x40.



Figure 4. Tunica muscularis from stomach wall of one-day-old newborn rat - extensive extracellular expression of 5-HT3 on the border between the muscle layers. Paraffin preparation. x40.

Discussion

During examination of serotonin expression we found differentiated enteroendocrine EC cells in the intestine of one-day-old newborn rats. The cells are scanty, located singularly in the covering epithelium of the villi. Their low number is probably due to the unfinished maturation in the intestinal wall. In adult individuals the intestinal epithelium is

organized in villi projecting into the intestinal lumen. Between the bases of the villi open the Lieberkühn crypts, which contain a compartment of stem cells. According to some authors this small group of proliferating nondifferentiated stem cells gives origin to several cell phenotypes – resorptive, goblet, endocrine cells - that migrate to the adjacent villi (Quinlan et al., 2006). Migration of the cells along this proximal-distal crpto-villous axis is achieved by interaction of superficial receptors of the epithelial cells with proteins of the extracellular matrix, which undergoes constant changes in its content (Pacha, 2000). During examination of the reparative processes in superficial intestinal lesions Rao et al. (1999) have established that the regeneration of the damaged enterocytes of the small intestine villi happens by migration of differentiated intestinal cells from the crypts towards the villi. The factors destroying the intestinal epithelial differentiation cause a decrease in the cell migration after superficial mucosal lesions. In comparison with our results in newborn rats Glatzle et al. (2002) established 5-HT3 immunoreactivity in both – stomach and duodenal wall in adult rats. 5-HTR3 receptors are expressed in circular and longitudinal smooth muscle fibers, functionally different classes of neurons in pl. myentericus and pl. submucosus as well as in Kahal and endocrine cells. Frieling et al. (1991) made farmacological analysis of serotonin antagonists using intracellular microelectrodes in pl. myentericus and pl. submucosus in the distal colon of a guinea pig. The application of serotonin leads to different effects one of witch – the suppression of acetylcholin release is carried out through 5-HT3 receptors. The inhibition of 5-HTR3 receptors also effects the motor function of the gastrointestinal tract. Takhari et al. (2001) study the effect of the 5-HT3R-antagonist *azasetron* on the duodenum of rats with streptozotocin-induced diabetes. The effects of serotonin on the motor function are carried out through 5-HT3R localized in cholinergic neurons that stimulate smooth muscle cells.

Conclusion

In the mature small intestine wall of adult individuals the EC cells are located mainly in the the crypts. In the early postnatal period, despite the presence of crypts, although scanty and shallow, EC cells are found mainly along the villi. The observed location of the EC cells is probably associated with intensive processes of enterocyte migration from the crypts to the villi, in that period of accelerated growth.

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Through studying the expression of 5-HTR3 receptors in the gastrointestinal tract during the fetal development and postnatal period, we established immunohistochemical activity only in the stomach wall in one day old rats. The expression of 5-HTR3 receptors is localized in the cytoplasm of a big number of smooth muscle cells in both – transversal and longitudinal muscle layers of the stomach. The intensive extracellular expression of 5-HTR3 receptors in the border of the muscle layers is probably along the neurons of pl. myentericus.

The morphological and functional maturation of the gastrointestinal tract starts in the fetal period and continues postnatally. Serotonin takes part in the regulation of this process. It's effects are carried out through the 5-HTR3 receptors.

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