Red kidney bean (*Phaseolus vulgaris*) lectin stimulation increases the number of enterochromaffin cells in the small intestine of suckling piglets

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Abstract

The quantities and distribution patterns of serotonin-immunoreactive (serotonin-IR) enterochromaffin cells (EC) were studied immunohistochemically in the small intestine of suckling piglets stimulated with red kidney bean lectin, and in non-stimulated, control animals. The co-expression patterns of serotonin with somatostatin (SOM) or corticotropin releasing-factor (CRF) were also studied. After the lectin treatment, the increased numbers of EC were noted in the duodenum of experimental animals. Lectin stimulation did not change the proportions of EC in the jejunum and ileum. In the duodenal epithelium of the lectin-stimulated piglets, the vast majority of serotonin-IR EC were distributed at the basis of crypts. After the lectin administration, the proportions of serotonin-IR/SOM-IR EC were statistically similar in all sections of the small intestine. No upregulation of CRF was found in duodenal, jejunal, and ileal EC of lectin-treated animals. The findings demonstrated that red kidney bean lectin increased the serotonin reservoir in the duodenum, and thus may be an effective stimulant of the gut maturation in suckling mammals.

Key words: pig, small intestine, serotonin, enterochromaffin cells, red kidney bean lectin.

Introduction

More than 90% of the total body content of 5-hydroxytryptamine (serotonin) is located in mucosa of the gastrointestinal tract, and a subset of enteroendocrine cells (EEC), called enterochromaffin cells (EC), are its main source (2). The vast majority of EC are bottle-shaped, with basal location of serotonin granule stores, and very long luminal extensions with short microvilli; however, other types are also noted (11). Morphological studies revealed region-specific EC distribution pattern, with the highest abundance in the proximal part of the small intestine (10). EC-like cells are also present in the mammalian stomach; although, these cells predominantly secrete histamine and calbindin (6). In the healthy gut, serotonin released from EC controls local blood flow through different receptors, and participates in the regulation of motility and fluid secretion (10). Under pathological conditions, EC may undergo carcinogenic transformation leading to overproduction of serotonin and in result to carcinoid syndrome (8). Recent studies have clearly demonstrated that different sub-populations of EEC (including EC) may be also plastic, and various conditions may evoke functional changes. It has been found that in the bovine rectum the number of L-type EEC expressing peptide YY and glucagon-like peptide 1 depends on the developmental stage (23). In lines of human EEC, the exposition to fatty acids, flagellin or bacterial lipopolysaccharide resulted in up-regulation of pro-differentiation genes as well as cholecystokinin synthesis (26). The distribution and number of rat EEC were locally changed dependently on the diet type and the presence of the gut microflora (27). Recently, it has been also found that the number of calbindin-containing EEC increases in suckling pigs treated with lectin (30).

In animal feeding, dietary lectins have been recognised as promising nutritional stimulants able to evoke beneficial effects including stimulation of the
small intestine growth, modulation of innate immune system, and changes in secretion of gut hormones (22). The aim of the study was to determine whether the numbers of EC are changed in the small intestine of suckling piglets. The changes in the numbers of EC in the duodenum, jejunum, and ileum of suckling piglets treated with lectin were examined immunohistochemically to understand better the mode of action of lectin in the small intestine. The second aim of the study was to compare immunohistochemically the co-expression patterns of somatostatin (SOM) and corticotropin releasing-factor (CRF) in serotonin-containing EC of the small intestine in normal and lectin-stimulated piglets.

Material and Methods

The experimental design and animal care were approved by the Local Animal Ethics Committee and the animals were euthanised in agreement with Principles of Laboratory Animal Care. Studies were performed on ten three-day-old crossbreed Polish Landrace × Pietrain piglets (weighing approx. 4 kg) of both sex, born at term and housed in standard farming conditions. Each piglet from the experimental group (n = 5) was given orally 160 HU/kg b.w. of a red kidney bean lectin prepared according to the Pusztaí and Watt method (21). Control animals (n = 5) received 1 ml of saline in order to mimic the volume of the lectin administration. Ten days later, all animals were deeply sedated with azaperone (Stresnil, Janssen-Cilag GmbH, Germany; 0.5 mg/kg b.w.) and euthanised with an overdose of sodium pentobarbital (Morbital, Biowet Pulawy, Poland; 50 mg/kg b.w.). Approx. 10 cm long samples of the small intestine (duodenum, jejunum, and ileum) were dissected out, rinsed with 0.01 M phosphate-buffered saline (PBS; pH = 7.3), and immediately fixed for 1 d in Stefanini’s solution and cryoprotected for next 4 d with 16% sucrose. The material was cut into smaller pieces of 10 mm length in both transversal and longitudinal direction. Cryostat sections (10 µm thick) were made. Every fifth section was placed on a glass slide (SuperFrost Plus, Menzel GmbH & CoKG, Germany) and stored at -20°C for immunohistochemical studies. Mouse antibodies against serotonin (1:100, Abcam UK, code number 16007) were used as a specific marker for EC. Immunohistochemical staining procedure used in the study was already described (1). In brief, the slides were washed three times (10 min each) in PBS, and blocked for 1 h in PBS, supplemented with 10% normal goat serum, 0.25% bovine serum albumin (BSA, Sigma-Aldrich Germany), and 0.25% Triton X-100 (Sigma-Aldrich), at room temperature (RT). Afterwards, the slides were placed in humid chamber and incubated overnight (RT) with mouse anti-serotonin sera. For co-localisation, mouse anti-serotonin sera were mixed with either rabbit antibodies against CRF (1:80 Sigma-Aldrich, Germany, code C5348) or rat antibodies against SOM (1:300, Biogenesis UK, code number 8330-0009). On the next day, the sections were rinsed in the same buffer, and incubated for 1 h at RT with FITC-conjugated anti-mouse goat IgG (dilution 1:400; MP Biomedicals, USA) combined either with Texas Red-conjugated anti-rabbit goat IgG (1:400; MP Biomedicals) or Texas Red-conjugated anti-rat goat IgG (1:400; MP Biomedicals). Following a final washing step with PBS, coverslips were mounted on microscope slides using a glycerol-based medium, and sections were examined under a spinning disk confocal microscope (BX-DSU Olympus, Japan) equipped with appropriate interference filters for detection of Texas Red (545-580 nm; MWIY2) and FITC (470-490 nm; MNIBA2). The specificity of primary antibodies was tested by preabsorption experiment as well as substituting the primary antibody by a non-immune serum. Control sections showed no detectable labelling. Previously described counting protocol was applied in the image analysis (30). Briefly, in the sections with no visible damage of mucosa, all EC were identified and counted. In each segment of the small intestine, the mean from at least five slides from each animal (n = 5 in control and n = 5 in experimental group) was calculated. Finally, the numbers of EC found in 1 cm of mucosa were expressed as a mean ± S.E.M. The proportions of EC co-storing somatostatin or CRF were calculated by cell counting, and were presented as a percentage of the relative to the total number of EC. Paired t-test followed by one way ANOVA was used to evaluate statistically significant differences between control and lectin-treated groups. P values <0.05 were considered statistically significant.

Results

Fluorescent microscopic observations showed that serotonin-immunoreactive (IR) EC were found at the basis of crypts and throughout the epithelial villi of the small intestine of control and lectin-treated piglets. Serotonin-containing EC were very bright and easy to distinguish from the dark background. In animals from both groups, the vast majority of serotonin-positive EC (more than 95%) were pyramid shaped with apical process reaching the gut lumen (Fig. 1). A small population of EC (approx. 5%) with apical and more or less visible basolateral extensions was also visualised in the small intestine from control and lectin-treated piglets. In the duodenum of control piglets, the mean number of EC distributed throughout 1 mm of the mucosa was 126 ± 19. In control piglets, statistically similar proportions of EC were also found in the mucosa of the jejunum (109 ± 22) and ileum (92 ± 17). In general, in all portions of the small intestine of control animals EC were evenly distributed at the basis of crypts and gastrointestinal villi. In the lectin treated piglets, the number
of EC in the duodenum significantly increased to 180 ± 25 (P < 0.05; Fig. 2). Substantially higher proportions of EC were observed at the basis of duodenal crypts in the lectin-treated animals when compared to the controls (Fig. 2). The average numbers of EC in the jejunum and ileum of the lectin-treated piglets (116 ± 24 and 105 ± 23 respectively) were statistically similar when compared to controls. In the lectin-treated animals, the number of EC in the duodenum was statistically different when compared to those in the jejunum and ileum (P < 0.05).

In the duodenum and jejunum from the control piglets, less than 1% of EC simultaneously showing immunoreactivity to SOM (Fig. 3) were found. After the lectin treatment, the number of EC additionally co-expressing SOM in the duodenum and jejunum (approx. 1% in each segment) were statistically comparable to the control values. It was impossible to visualise the co-existence of SOM in EC in the ileum of normal and lectin-treated piglets. In control and lectin-stimulated animals, the EC located throughout intestinal villi and were frequently placed next to SOM-expressing EEC. None of EC from the small intestine of control animals (all portions) co-expressed CRF. Lectin stimulation did not induce the expression of CRF in duodenal, jejunal, and ileal EC (Fig. 4).

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**Fig. 1.** Fluorescent micrographs showing serotonin-containing “open” type EC in the jejunum of control (left) and lectin-stimulated (right) piglets

**Fig. 2.** Immunohistochemical labelling of serotonin-IR EC in the duodenum of control (up) and experimental (down) piglets. Lectin administration markedly increased the number of EC in the duodenum
Fig. 3. (Up) In transverse section of the duodenum of control piglets, EC additionally co-expressing SOM (arrowhead) are seen in the apical portion of the vilus. (Down) In the duodenum of lectin-treated piglets, serotonin-IR/SOM-IR (arrowheads) EC are still present. Note the presence of SOM-IR EEC lacking serotonin (arrow)

Fig. 4. Transverse section through the duodenum of lectin-treated piglet showing the distribution pattern of EC and CRF-containing EEC (arrows). CRF-IR nerve fibres are present in close vicinity to EC

Discussion

A higher number of EC in the duodenum of suckling piglets stimulated with red kidney lectin were demonstrated in the study. Several previous reports clearly indicated that the number of EC in the mammalian gut can be changed. A dramatic change in EC amount (correlated with alterations in serotonin content) has been noted in numerous diseases including diabetes induced by dexamethasone or streptozotocin treatment (9, 29), irritable bowel syndrome (18), colitis induced by TNBS or dextran sodium sulfate treatment (14, 20), as well as parasite infection (19). Additionally, nearly two-fold increase in the number of EC was noted in the small intestine of fasted rats 1 d after food deprivation (28). In the small intestine of neonatal guinea pigs, the number of EC is relatively low and starts to increase at 9th week of postnatal life, indicating that the mammalian gut serotonin-signalling system is not entirely developed at birth (31). From this functional point of view, every factor, which is potentially able to expedite the gut maturation process, is of importance in production of farm animals, particularly pigs. In previous studies in rats and suckling piglets, the effects of lectins action on the small intestine mucosa morphology and function(s)
have been described in details (15, 24). Interestingly, it has been found that lectin administration increased the number of EC in the duodenum (but not in jejunum and ileum) in suckling piglets. It seems that this phenomenon could have an additional beneficial influence on porcine gut maturation. It can be assumed that in lectin-stimulated duodenum, the higher number of EC leads to increased luminal serotonin content. In the healthy gut, peristalsis is initiated by release of serotonin from EC, and this mechanism involves direct serotonin action on smooth muscle cells, and activation of 5-HT₂-bearing receptors that mediate the activation of intrinsic primary afferent neurons (participating in the gut reflexes) (7). Moreover, serotonin also regulates pacemaker activity of interstitial cells of Cajal via 5-HT₁ receptors (16). Serotonin-induced secretory response, manifested by elevated Cl⁻ and prostaglandin secretion, and inhibited Na⁺ absorption, has been noted in porcine small intestine (12). Further in vitro studies in rat ileum demonstrated that electrogenic Cl⁻ secretion involves non-neuronal pathway, most likely via direct activation of enterocytes (25). It becomes apparent that increased serotonin content may accelerate intestinal transit, and serotonin per se may be used as a potential drug in the treatment of gastrointestinal disorders. It has been well known that intestinal transit in neonates is slower and more chaotic mainly due to immaturity of small intestinal motor activity (3). Therefore, it is possible that red kidney bean lectin given to suckling piglets may improve food passage time in immature duodenum by increasing the amount of EC. Recent findings demonstrated that serotonin signalling system additionally influences function of the gut immune system. Serotonin released from EC directly regulates the activity of immune cells including enhanced phagocytosis in macrophages, increased chemotaxis in dendritic cells, and proliferative effect on CD+4 T cells (17). It must be kept in mind that this may lead to negative consequences since lectin preparations given to suckling piglets may stimulate (by serotonin-dependent pathway) immune cells, and increase inflammatory processes frequently occurring during weaning.

In has been previously found that the binding effect of lectins to intestinal villi is accompanied with an increased plasma corticosterone level (15). It is worth considering whether overexpression of biologically active CRF or SOM can be found in EC of the lectin-stimulated piglets. Unfortunately, in the present study, no up-regulation of CRF and SOM in serotonin-IR EC was noted after the lectin-treatment, which may suggests that these substances play a minor role (if any) in the gut regulating serotonin-dependent mechanisms. However, in previous experiments serotonin release was found to be dose-dependently inhibited by SOM (4) and SOM analogs (13). Moreover, it seems that CRF and serotonin may predominantly co-operate at the central level, since CRF and CRF-related peptides modulate secretory activity of serotonin-IR neurons of dorsal raphe nucleus, and thus influence stress-related feeding behaviour (5).

To conclude, lectin administration increased the number of serotonin-producing EC in the duodenum (but not jejunum and ileum) of suckling piglets. These results suggest that red kidney bean lectins may be efficient factors influencing serotonin-dependent gut maturation processes.

References