The influence of selected pathological states on the somatostatin-like immunoreactive (SOM-LI) endocrine cells in the mucosal layer of the porcine descending colon

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ABSTRACT. Gonkowski S. & Całka J. 2012. The influence of selected pathological states on the somatostatin-like immunoreactive (SOM-LI) endocrine cells in the mucosal layer of the porcine descending colon. Pesquisa Veterinária Brasileira 32(Supl.1):79-83. Division of Clinical Physiology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowski Str. 13, Olsztyn, 10-957, Poland. E-mail: slawomirgonkowski@uwm.edu.pl

This study reports on changes in the number of somatostatin–like immunoreactive (SOM-LI) endocrine cells in the porcine descending colon, caused by chemically driven inflammation, axotomy and proliferative enteropathy (PE). The distribution pattern of SOM-LI endocrine cells has been studied using the routine single-labelling immunofluorescence technique. Semi-quantitative evaluation of the number of the SOM-immunostained endocrine cells within the mucosal layer of the porcine descending colon has been based on counting of all endocrine cells immunoreactive to SOM per unit area (0,1 mm²). Under physiological conditions the number of SOM-LI endocrine cells has been shown to constitute 3,30±0,22. All applied pathological processes resulted in changes in the SOM–like immunoreactivity, which varied in particular processes studied. The number of SOM-LI endocrine cells increased to 6,28±0,31 and 4,43±0,35 during chemically driven inflammation and proliferative enteropathy, respectively, and decreased to 1,17%±0,16 after axotomy. The obtained results suggest that SOM-LI endocrine cells may participate in various pathological states within porcine descending colon and their functions probably depend on the type of pathological factor.

INDEX TERMS: Immunohistochemistry, inflammation, proliferative enteropathy, axotomy, swine.

INTRODUCTION

The gastrointestinal system has been referred to as the largest endocrine organ of the body and is composed of various types of gastrointestinal endocrine cells dispersed in the gastric glands and epithelia of all parts of the digestive tract (Ahlman & Nilsson 2001). The regional distribution and relative frequencies of these cells vary according to the animal species and feeding habits and till now, they were investigated in gastrointestinal (GI) tract of fishes (Ku et al. 2004), birds (Mendes et al. 2009) as well as in various fragments of the digestive tract in numerous mammals, including human (Ahlman & Nilsson 2001, Ham 2002, Tzaneva 2003, Al Haj Ali et al. 2007). Gastrointestinal endocrine cells contain several biological active substances such as gastrin, somatostatin, histamine, serotonin, ghrelin and neurotropins (Solcia et al. 2000, Ahlman & Nilsson 2001, Lucini et al. 2002, Tzaneva 2003) and play multiple roles, among which the most important are the control of the metabolism of carbohydrates and all the processes associated with digestion and absorption of nutrients, such as secretion of the glands; peristalsis; supply of blood; and reabsorption and kinetics of the epithelium of the gastrointestinal tract as well as the regulation of feeding behavior (for review, Ahlman & Nilsson 2001).

One of the substances, which is distributed in gastrointestinal endocrine cells is somatostatin (SOM). It is a tetradecapeptide (14 amino acids) that was isolated for the first time in 1973 from the ovine hypothalamus and was demonstrated to inhibit growth hormone secretion (Brazau et al. 1973), which is secreted by D type of endocrine cells (Tzaneva 2003). Within the GI tract SOM plays multiple roles mediated by six types of receptors, all of which...
are members of the G protein coupled receptor superfamily and which are involved in various intracellular signaling pathways (for review, see Corleto et al., 2004). First of all SOM is known as the major inhibitory mediator of gastric acid release (Komasaka et al. 2002). SOM also reduces gut secretion and exerts the inhibitory effects on many gut hormones including gastrin, cholecystokinin, vasoactive intestinal polypeptide (for review, Low 2004). Moreover SOM decreases the food intake, reduces the blood flow in the gut and affects the GI tract motility (Li et al. 1996, Scalera & Tarozzi 1998) It is also an important anti-inflammatory and anti-nociceptive agent, which down-regulates lymphocyte proliferation, reduces immunoglobulin production and inhibits the release of proinflammatory cytokines (for review, see Ten Bokum et al. 2000).

It is well known that gastrointestinal endocrine cells take part in adaptive processes of GI tract both under physiological factors such as ageing (Sandström & El-Salhy 1999) and during pathological changes such as ulcerative colitis, Crohn’s disease, gastritis due to Helicobacter pylori infection or irritable colon syndrome (El-Salhy et al. 1997, Kostiukovich et al. 2004; for review, see Lomax et al. 2006). However till now the knowledge about colonic SOM-LI endocrine cells and their functions during various pathological processes is very limited (Watanabe et al. 1992, for review, see Lomax et al. 2006), especially in the pig, which seems to be an optimal species for studies of gastrointestinal tract due to similarities between human an porcine digestive system (Brown & Timmermans 2004).

Therefore the aim of this study has been, for the first time, to investigate and compare the possible alterations of the SOM-LI endocrine cells in the mucosal layer of the porcine descending colon under various physiological conditions, both, experimental such as the chemically-induced inflammation or axotomy and “natural” such as proliferative enteropathy (PE), which is caused by the Lawsonia intracellularis with proliferative changes (Smith & Lawson 2001).

MATERIALS AND METHODS:

Investigations were performed on twelve immature female pigs of the Large White Polish breed (approx. 8 weeks old). The animals were kept in standard laboratory conditions with admission to species-specific chow and water ad libitum. All surgical operations were performed in compliance with the instructions of Local Ethical Committee in Olsztyn (Poland), with a special attention paid to the minimising of any stress reaction during and after the surgery.

Pigs were divided into four experimental groups: control (C group; n=3), animals with chemically induced colitis (inflammation (I) group; n=3), pigs with Lawsonia intracellularis infection (proliferative enteropathy (PE) group; n=3), where the diagnosis of PE was confirmed with a polymerase chain reaction (PCR)-based test performed at a State Veterinary Research institute in Pulawy (Poland) and those subjected to axotomy (axotomy (A) group; n=3).

The animals from I and A groups were pre-treated with Stresnil (Janssen, Belgium, 75μl/kg of body weight given intravenously) 15 min. before application of the main anaesthetic - sodium thiopental (Thiopental, Sandoz, Kundl-Rakúsko, Austria; 20 mg/kg of body weight given intravenously). Then, pigs were subjected to median laparotomy and injected with 80μl of 10% formalin solution (intraperitoneal injection of 5-8μl) into the wall of descending colon (I group) or subjected to the bilateral transection of colonic nerves connected to the inferior mesenteric ganglion (IMG) with the descending colon (A group). After five days, animals (I and A groups) were re-anæsthetised and were euthanized by an overdose of sodium thiopental and then perfused transcardially with 4% buffered paraformaldehyde (pH 7.4) prepared ex tempore. Animals of C and PE groups were euthanized by an overdose of sodium thiopental (Thiopental, Sandoz, Kundl-Rakúsko, Austria; 20mg/kg of body weight given intravenously) and then also perfused transcardially with 4% buffered paraformaldehyde.

Fragments (ca. 3 cm long) of a descending colon were collected from all studied animals. Colitis in the animals of I group has been confirmed by histopathological examination performed at the Laboratory of Histopathology (Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland). Then samples were post-fixed by immersion in the same fixative for several hours and, finally, stored in 18% sucrose and sectioned into 10 μm thick cryostat sections.

These sections were processed for routine single-labelling immunofluorescence. Briefly, after air-drying at room temperature (rt) for 45 min, sections were incubated with a blocking solution containing 10% normal goat serum, 0.1% bovine serum albumin, 0.01% NaN3, Triton X-100 and thimerosal in PBS for 1h (rt). Then, they were incubated (overnight; rt, in humid chamber) with antisera directed towards SOM (rat monoclonal, Biogenesis, UK, 1: 100). The complex of primary antiseraum bound to appropriate antigen was visualized by incubation (1h, rt) with species-specific secondary antiseraum conjugated to FITC (Jackson ImmunoResearch, USA, 1:800). Each step of the immunolabelling was following by rinsing of the sections with PBS (3x10 min, pH 7.4). Negative controls used in the immunofluorescence procedure included pre-absorption of the antibody with an appropriate antigen.

Semi-quantitative evaluation of the number of the SOM-immunostained endocrine cells within the mucosal layer has been based on the counting of all endocrine cells immunoreactive to SOM per observation field (0.11 mm2) under Olympus BX51 microscope equipped with epi-fluorescence and appropriate filter set. SOM-IR cells were counted in 3 observation fields of 10 sections per animal (in the sum: in the 30 observation fields per animal). To prevent double counting the sections were located at least 100 μm apart from each other. The obtained data were pooled and presented as mean. Pictures were captured by a digital camera connected to a PC, analyzed with AnalySIS software (version 3.02, Soft Imaging System, FRG) and printed on a wax printer (Phaser 8200, Xerox, USA).

RESULTS

During present investigation SOM-LI endocrine cells within mucosal layer of the porcine descending colon were observed both in physiological conditions and under all pathological states studied (Table 1, Fig.1). In control group relatively low number of these cells was noted (Table 1). In some observation fields SOM-LI endocrine cells were not observed at all, but in other ones the number of such cells amounted to maximum 5 cells per observation field (Fig.1A).

All pathological states studied produced changes in the number of colonic endocrine cells immunostained to SOM...
and the character of this changes depended on the type of the pathological factor (Table 1, Fig.1).

During chemically-induced inflammation and proliferative enteropathy the increase of the number of SOM-positive endocrine cells was observed (Table 1, Fig.1B,C). In these specimens representing both pathological processes SOM-LI endocrine cells were present in all observation fields and their number fluctuated between 3 to 8 per particular observation field. The most significant changes were noted during chemically-induced inflammation, where the average numbers of studied cells in particular animals were always about twice higher than in the control group and on average in this experimental group amounted to above 6 cells per observation field (Table 1).

Contrary to chemically-induced inflammation and proliferative enteropathy, axotomy caused the significant decrease in the number of SOM-LI colonic endocrine cells (Table 1). In axotomized animals in numerous observation fields they were not observed at all and their number in particular observation fields never exceeded three (Fig.1D).

**DISCUSSION**

The results of this study demonstrate that SOM-like immunoreactivity is present in porcine colonic mucosal endo-

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**Table 1. The number of SOM-LI mucosal endocrine cells in the porcine descending colon in the animals of control group (C group), during chemically induced inflammation (I group), proliferative enteropathy (PE group) and after axotomy (A group)**

<table>
<thead>
<tr>
<th>Animal number</th>
<th>C group</th>
<th>I group</th>
<th>PE group</th>
<th>A group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total number of SOM positive endocrine cells (per 30 fields)</td>
<td>97</td>
<td>111</td>
<td>89</td>
<td>189</td>
</tr>
<tr>
<td>Average number of SOM-LI endocrine cells per observation field in particular animals</td>
<td>2.96</td>
<td>3.23</td>
<td>3.70</td>
<td>6.30</td>
</tr>
<tr>
<td>Average number of SOM-LI endocrine cells within experimental group per observation field ±SEM</td>
<td><strong>3.30±0.22</strong></td>
<td><strong>6.28±0.31</strong></td>
<td><strong>4.43±0.35</strong></td>
<td><strong>1.17±0.16</strong></td>
</tr>
</tbody>
</table>
crine cells. In spite of well known functions of SOM within whole GI tract such as the regulation of gut secretion, motility, blood flow and food intake (Li et al. 1996, Scalera & Tarozzi 1998, for review, Low 2004) and described previous roles of SOM in the large intestine where it stimulates ion transport and inhibits chloride secretion (for review, see Low 2004), the number of SOM – positive endocrine cells in control animals were not large, what is in accordance with previous observations in other species, including human, where such endocrine cells in the colon were not numerous (Watanabe et al. 1992) or they were no observed at all (Ku et al. 2006, Aj Haj Ali et al. 2007).

Moreover, in this study changes in SOM–like immuno-reactivity of the endocrine cells were observed under all pathological factors studied, what strongly suggests the role of SOM in adaptative processes within descending colon. The type of these changes depended on the factor applied. Generally, in this study the inflammation processes both experimental (chemical) and “natural” (PE), caused an increased of SOM-positive colonic endocrine cells, what is contrary to previous investigations in human colon, where inflammation bowel disease caused the decrease of such cells and this decrease was related to the degree of inflammation (Watanabe et al. 1992). These differences as well as not large dissimilarities between two inflammatory processes observed in present investigations probably result from various types of pathological factors. The increase in the number of SOM-LI endocrine cells during inflammatory processes observed in course of this study on one hand may arise from the augmentation of SOM synthesis as an adaptative process in the colon and on the other hand it can indicate an inhibition of SOM release from mucosal endocrine cells. Inhibition of SOM release may escalate symptoms of diarrhea during intestinal inflammations, what can be confirmed by exogenously administered SOM or its analogue in treatment of diarrhea with very positive results (Ruskone et al. 1982, Herder & Lamberts 2003). Our observations on the participation of SOM in the inflammatory processes within descending colon are in accordance with previous studies on other organs, where this substance and its analogues have been described as well known anti-inflammatory factors. SOM down-regulates pro-inflammatory cytokine expression and release (Chowers et al. 2000), limphocyte proliferation and immunoglobulin production (for production, see Ten Bokum et al., 2000) and plays an important role in the reduction of nociception, which is mainly realized by the action on SST4 SOM receptor subtype (Plourde et al.1993; for review, see Pinter et al. 2006).

Although, during these investigations the clear decrease of SOM-positive colonic endocrine cells was observed after axotomy, currently the mechanism of this process is unknown. Till now, the influence of nerve injury on SOM-LI mucosal endocrine cells in the descending colon has not been studied. The obtained results suggest that the nerve fibers supplying the colon may affect the survival of SOM-LI mucosal endocrine cells of this gut fragment and/or play an important role in the regulation of SOM synthesis by these cells. Our observations may initiate a new chapter in therapeutic application of somatostatin and its analogues, which in the future might be applied not only in the cases of endocrine tumors and bowel inflammations (for review, see Low 2004), but also in patients after gut fragments resection in order to restor physiological gut functions. On the other hand the decrease in the number of SOM-LI endocrine cells observed after axotomy may be the consequence of intensification of SOM secretion under noxious stimuli described in previous studies (Antal et al. 2008).

CONCLUSION

To sum up, the results obtained in the this study suggest an important role for SOM-LI mucosal endocrine cells in the function of the porcine descending colon, not only in physiological conditions, but also under pathological factors such as various types of inflammation or nerve injury. Nevertheless, in spite of well known functions of SOM as an anti-inflammatory factor (for review, see Pinter et al. 2006), the physiological role of this substance as well as utilization of SOM and its analogues in various pathological processes is not fully explained and requires further investigation.

REFERENCES


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