VIA MEDICA

SHORT COMMUNICATION

Folia Morphol.
Vol. 62, No. 4, pp. 455–457
Copyright © 2003 Via Medica
ISSN 0015–5659
www.fm.viamedica.pl

The influence of experimental *Bacteroides fragilis* infection on substance P and somatostatin-immunoreactive neural elements in the porcine ascending colon — a preliminary report

Sławomir Gonkowski[1], Barbara Kamińska[2], Agnieszka Bossowska[1], Maria Korzon[2], Piotr Landowski[2], Mariusz Majewski[1]

[1]*Department of Clinical Physiology, University of Warmia and Mazury, Olsztyn, Poland*
[2]*Clinic of Paediatrics, Gastroenterology and Oncology, Medical University, Gdańsk, Poland*

[Received 23 October 2003; Accepted 23 October 2003]

The present study was aimed at disclosing the influence of Bacteroides fragilis *(one of the most important bacterial agents causing colitis in children) experimental infection on the expression of substance P (SP) and somatostatin (SOM) in neurons and nerve fibres within the porcine ascending colon. Distinct differences in the distribution pattern of neural elements immunoreactive to the substances studied were observed between the experimental (Inflam) and control (Contr) pigs. In general, the number of SP-IR neurons and nerve terminals increased, while the expression of SOM decreased after* Bacteroides fragilis*-induced colitis (BFIC). However, distinct differences in the intensity of these alterations were observed between particular compartments of the bowel segment studied. Thus, the present results suggest that SP- and SOM-immunoreactive (SOM-IR) elements of the enteric nervous system play a part in the control of colonic activity during BFIC.*

key words: *Bacteroides fragilis* infection, colitis, substance P, somatostatin, pig

INTRODUCTION

Bacteroides fragilis appears to be one of the most important etiological factors causing acute inflammatory processes in the proximal part of the colon in juvenile patients. This kind of BFIC most often applies to the caecum and ascending colon and is accompanied by recurrent diarrhoea. The morphological basis of neuronal circuits activated by bacterial factors in the human large intestine has not yet been understood in detail. Although profound changes in the expression pattern of SP and SOM were observed in some types of colitis [2, 3], there are no data dealing with such alternations in the colonic wall in BFIC. As the pig, on the basis of its anatomical and physiological similarities to humans, is widely thought to be the animal model most suited to the investigation of such processes, we decided in the present study to elucidate the influence of experimental *Bacteroides fragilis* infection on the expression pattern of SP (one of the most important sensory mediators) and SOM (an important regulator of the mucosal secretion) within the porcine colonic wall.

Address for correspondence: Mariusz Majewski, Department of Clinical Physiology, University of Warmia and Mazury, ul. Oczapowskiego 13, 10–957 Olsztyn, Poland, e-mail: Mariusz.Majewski@uwm.edu.pl

Table 1. Distribution pattern of SP- or SOM-IR neural elements in the porcine ascending colon

Compartment of the colonic wall	SP		SOM	
	Contr	Inflam	Contr	Inflam
Number of fibres in **CML***	28.4 ± 1.4	35.0 ± 2.4	3.1 ± 0.5	2.0 ± 0.45
% of positive neurons in **MP**	3.7 ± 0.5	12.6 ± 1.5	3.0 ± 0.1	2.7 ± 0.3
Density of positive nerve fibres	+++	++	+	++++
% of positive neurons in **OSP**	3.4 ± 0.4	12.0 ± 3.0	5.1 ± 0.2	1.7 ± 0.3
Density of positive nerve fibres	++	++	+	++++
% of positive neurons in **ISP**	6.6 ± 1.5	27.0 ± 4.8	3.25 ± 0.4	1.9 ± 0.3
Density of positive nerve fibres	+	+++	++	++
Number of fibres in the **mucosa**	7.1 ± 1.2	0.5 ± 0.3	11.1 ± 2.1	1.0 ± 0.5
Number of neuroendocrine cells*	0	0	3.1 ± 0.7	0

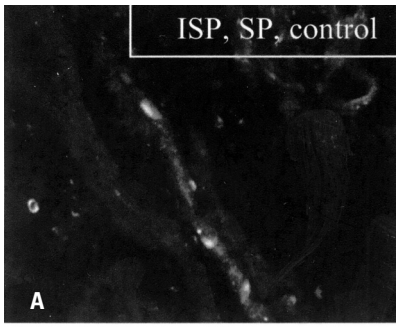
*An average number of nerve profiles/cell bodies *per* area studied (mean ± SEM); Contr — control group, Inflam — experimental group

MATERIAL AND METHODS

Six clinically healthy female piglets were randomly divided into the control (Contr) and experimental (Inflam) groups. In animals of the latter group, experimental colitis was induced by means of injections of bacterial suspension (i.e. *B. fragilis*, isolated from patients hospitalised in the Clinic of Paediatrics) into the ascending colon during laparotomy performed under deep anaesthesia. After 7 days, the animals from both Contr and Inflam groups were re-anaesthetised and transcardially perfused with 4% paraformaldehyde (pH 7.4). The parts of the ascending colon (in the case of experimental pigs, regions with clear signs of inflammation) were collected. 20 μm-thick cryostat sections were subjected to routine single-labelling immunofluorescence, using primary antisera raised in the rat and directed towards SP (Biogenesis, UK, diluted 1:1500) or SOM (Biogenesis, diluted 1:100) and a donkey anti-rat secondary antiserum conjugated to FITC (Jackson Lab, USA, diluted 1:800). Labelled sections were studied with an Olympus BX51 fluorescence microscope equipped with epi-illumination and an appropriate filter set for FITC. Special attention was paid to establishing the detailed distribution pattern of the substances studied in the circular muscle layer (CML), myenteric plexus (MP), outer submucous plexus (OSP), inner submucous plexus (ISP) and within the mucosa. In each group, the relative frequency of neurons containing particular substances was assessed in all the intramural ganglia of a particular enteric plexus on 10 randomly chosen sections, pooled, and presented as a mean ± SEM. Nerve fibres in CML and neuroendocrine cells in the epithelium were counted in 5 different areas of each section studied. The results obtained were then pooled and presented as a mean (± SEM) number of nerve fibres/cells per area. In order to evaluate and compare the density of intraganglionic nerve terminals in the Contr and Inflam groups a subjective rating system was used, which ranged from (–) (no fibres) to (++++) (a very dense mesh of nerve fibres).

RESULTS AND DISCUSSION

The occurrence of SP- and SOM-IR elements was observed in all the compartments studied of the ascending colon in both the control and experimental animals (Table 1). In pigs of the Inflam group, a distinct increase in the number of SP-positive neurons within all 3 enteric plexuses was observed when compared to that found in the control animals (Fig. 1). This was paralleled by an increase in the density of SP-IR nerve terminals in CML (Fig. 2, Table 1) and ISP, while the number of SP-IR fibres within the mucosa drastically decreased. In the case of SOM-IR neural structures a decrease in the number of SOM-positive neurons, with simultaneous increase in the density of SOM-IR intraganglionic nerve terminals, was observed within MP (Fig. 3) and OSP. In contrast, BFIC caused a moderate decrease in the number of SOM-IR neurons in the ISP, although without any changes in the density of SOM-IR intraganglionic nerve fibres. Furthermore, a distinct decrease in the number of SOM-containing neural elements was observed in the mucosal layer, and, to a lesser extent, in the CML. The general increase in SP expression that has been observed in the experimental animals can be attributed either to an increase in its synthesis by colonic neurons or to a diminished release during BFIC. Previous studies have shown that SP is released during different kinds of colitis and interacts with its own receptors, abundantly ex-



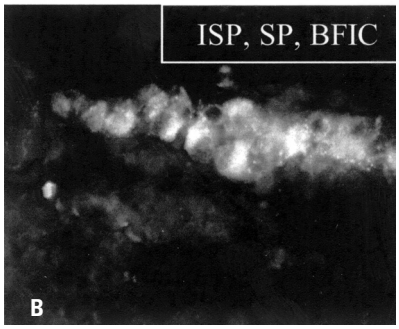
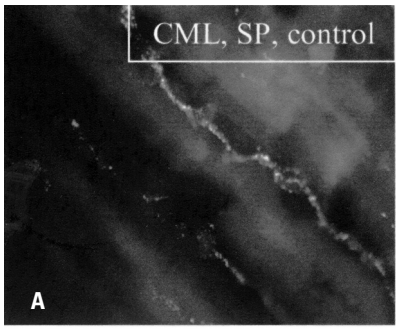


Figure 1. A comparison of the number of SP-IR neurons in ISP of the control (**A**) and BFIC (**B**) animals. × 200.



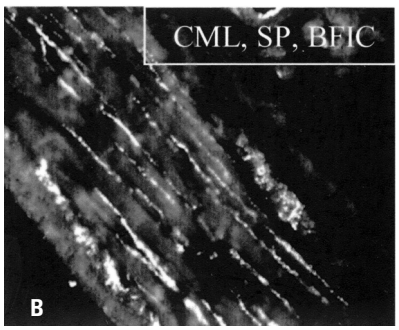


Figure 2. Dramatic BFIC-induced (**B**) increase in the number of SP-IR nerve fibres within the CML of the porcine ascending colon in comparison to the control specimen (**A**). × 200.

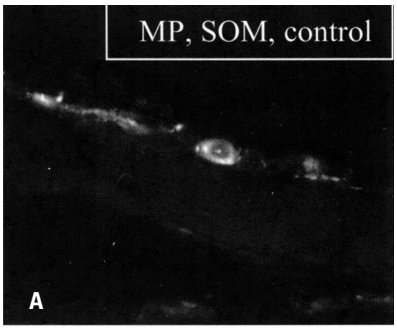


Figure 3. Distribution pattern of SOM-IR nerve terminals within the MP in the control (**A**) and inflamed (**B**) ascending colon of the pig. Note an increase in the number of intraganglionic varicose nerve profiles exhibiting SOM-IR. × 200.

pressed during inflammatory processes in colonic tissues [4]. Furthermore, SP is known to enhance the inflammatory response by stimulating immune cells located in the intestine [5] and by interacting with mast cells [6]. Moreover, sensory nerves (SP is well-known as a sensory neuromediator) may also have a protective function in the gut [1]. There is, however, no clear-cut evidence as yet, as to whether SP may indeed be responsible for this protective mucosal effect. A general decrease in the number of SOM-containing neurons and nerve fibres, as observed in the present study, can most probably be caused by a decrease in the synthesis rate of this peptide during BFIC. SOM is known as an inhibitory factor in the development of inflammatory processes [7], but its functions in the inflamed intestine are still poorly understood. Thus, our results suggest that both substances may play an important role in the pathogenesis of BFIC, but a precise elucidation of their roles during this disease calls for further study.

REFERENCES

1. Li DS, Raybould H, Quintero E, Guth PH (1992) Calcitonin gene-related peptide mediates the gastric hyperaemic response to acid-back-diffusion. Gastroenterology, 102: 1124–1435.
2. Miampamba M, Sharkey KA (1998) Distribution of calcitonin gene-released peptide, somatostatin, substance P and vasoactive intestinal polypeptide in experimental colitis in rats. Neurogastroenterol Motil, 10: 315–329.
3. Neunlist M, Aubert P, Toquet C, Oreshkova T, Barouk J, Lehur PA, Schemann M, Galmiche JP (2003) Changes in chemical coding of myenteric neurones in ulcerative colitis. Gut, 52: 84–90.
4. Reinshagen M, Adler G, Eysselein VE (1995) Substance P gene expression in acute experimental colitis. Regul Pept, 59: 53–58.
5. Scicchitano R, Bienenstock J, Stanisz AM (1988) *In vivo* immunomodulation by the neuropeptide substance P. Immunology, 63: 733–735.
6. Shanahan F, Denburg JA, Fox J, Bienenstock J, Befus D (1985) Mast cell heterogenity: effect of neuroenteric peptides on histamine release. J Immunol, 135: 1331–1337.
7. Watanabe T, Kubota Y, Sawada T, Muto T (1992) Distribution and quantification of somatostatin in inflammatory disease. Dis Colon Rectum, 35: 488–494.