Changes in the Expression of Some Neuropeptides in the Intestines and Nerve Ganglia During the Porcine Dysentery

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Abstract

The study was designed to investigate the changes in the expression of GAL, VIP, SOM, NPY, SP and CGRP in the porcine intestines (ileum and colon), as well as nerve ganglia (IMG, CSMG, DRG), in the course of swine dysentery in comparison with control animals. Experimental animals were infected per os with Brachyspira hyodysenteriae. After the clinical symptoms of the disease developed, the animals were sacrificed and the above mentioned tissues, as well as relevant tissues from control animals, were dissected out. In the tissue samples the concentrations of the neuropeptides were assayed with ELISA. The assays revealed that all the examined neuropeptides were present in the tissues of the experimental and control animals. The most statistically significant differences were those concerning the concentration of GAL in IMG, DRG, and colon, those concerning the concentration of VIP in CSMG and IMG, concentration of SOM in the ileum, IMG, DRG and CSMG, concentration of NPY in CSMG, DRG and IMG, as well as concentration of SP in IMG.

Key words: pig, neuropeptides, dysentery, intestines, ganglia.

During the last years, tremendous progress may be noted in the research of the role of neuropeptides in pathological processes in humans and animals. Many studies are focused on the role of these substances in different intestinal diseases, including intestinal inflammation. In the great majority of the studies, the rat was used as a model animal, in which the inflammation was experimentally induced with chemical compounds. The substance most often used for the induction of these conditions was trinitrobenzene-sulfonic acid (TNBS) (1, 15, 19).

Experiments performed with TNBS allowed to study the behaviour of neuropeptides in neurons supplying pathologically changed segments of intestines, as well as in neurons located in their wall. These results proved that the changes in the neuropeptide contents occurred not only in organs affected by the pathological process, but also in the neuronal perikarya and nerve processes.

The mentioned below results concern substances whose content was investigated in efferent and afferent neurons supplying intestines, as well as in the enteric intramural plexuses of laboratory animals.

It was found that vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and somatostatin (SOM) are potential anti-inflammatory agents (1, 9, 14, 22, 27, 31), while substance P (SP) is a pro-inflammatory factor (11, 15, 23, 32). It was also found that other neuropeptides, such as pituitary adenylate cyclase-activating peptide (PACAP) (31) or neurokinins A and B (10, 14, 17) and galanin (GAL) (6, 16, 24, 29) play the role in the inflammatory processes. Recently, it was demonstrated that also neuropeptide Y (NPY) is involved in the inflammatory processes (13). Another atypical substance involved in the inflammatory processes is nitric oxide (NO) (19). Changes in the expression of neuropeptides were found in the intestinal wall during the ulcerative colitis and Crohn’s disease in humans (4, 26, 30, 32), in the inferior mesenteric ganglion (IMG) and dorsal root ganglia (DRG) supplying the porcine colon, as well as in the intrinsic colonic neurons during the course of the porcine adenomatosis (12, 13).

As it can be deduced from the above-mentioned results, substances involved in the inflammatory processes are VIP, CGRP, SOM, SP, PACAP, GAL, neurokinins and NO. However, the data on the involvement of neuropeptides in the inflammatory conditions in domestic animals are lacking, as are the data on participation of these substances in other spontaneously-occurring pathological conditions in these animal species.

The present study was designed to determine tissue concentration of GAL, VIP, NPY, SOM, CGRP and SP in the colon and ileum, as well as in IMG,
coeliac-superior mesenteric ganglion (CSMG) and DRG of pigs suffering from swine dysentery in comparison with that found in control animals.

Material and Methods

The study was performed on 10 female, 4 to 5-month-old pigs of the Large White Polish race. The animals were divided into two groups. Control group (n=5) consisted of clinically healthy animals. Animals of the experimental group (n=5) were kept for one week in animal quarters. Then, they were infected with Brachyspira hyodysenteriae via the catheter introduced transorally into the stomach. Cultures of the microbes were obtained from the National Veterinary Research Institute in Pulawy (Poland). Bacteria were grown on the culturing media (agar with sheep blood) on Petri dishes under anaerobic conditions. Each animal received content of two Petri dishes dispersed in phosphate buffer (0.1 M, pH 7.4).

First symptoms of the disease appeared in experimental animals approx. one week after infection (partial anorexia, soft faeces, elevated body temperature). One to two weeks later, all the symptoms of the disease were clearly visible. They consisted of mucoid diarrhoea with flecks of blood and mucus progressing through a watery muco-haemorrhagic form to that of brown faeces containing flecks of fibrin and necrotic tissues, dehydration, weakness, gauntness and emaciation. Experimental animals were sacrificed in this stage of the disease. First, they were praemedicated with propionylpromazine (Combelen, Bayer, Germany) at a dose of 0.4 mg/kg of body weight, (i.m.). After 30 min the animals were killed with an overdose of sodium pentobarbital (Vetbutal, Biowet, Poland) at a dose of 25 mg/kg of body weight, (i.v.). The control animals were sacrificed following the same procedure as applied in the case of experimental animals.

From all the animals the samples of the ileum and colon, as well as ganglia - inferior mesenteric (IMG), coeliac-superior mesenteric (CSMG) and spinal (DRG), were collected, weighed, and snap-frozen in liquid nitrogen for storage. The samples were then taken out of the liquid N2 and homogenized with a homogenizer (UltraTurrax, Germany) in phosphate-buffered saline (PBS) containing phenyl-methyl-sulphonyl-fluoride (Sigma-Aldrich, USA) as a protease inhibitor. The homogenates were centrifuged for 15 min at 10 000 g at 4°C and supernatants were collected and frozen at −70°C. Tissue concentrations of NPY, VIP, GAL, SOM, SP and CGRP were determined with ELISA (Peninsula Laboratories, USA) according to the manufacturer instructions. ELISA plates were read with a Dynex MRX (Dynex Technologies, USA) immunoplate reader equipped with a 450 nm filter. Ten-point standard curve was and absorbances were converted to peptide concentrations. The results were recalculated for 1 g of fresh tissue. The data were statistically analysed with a Student t test using GraphPad PRISM 3.0 software. The differences were considered statistically significant at P<0.05.

Results

The assays revealed that all the studied neuropeptides were present in the examined tissues of the experimental and control animals. The most statistically significant differences were those concerning the concentration of GAL in IMG, DRG and colon, those concerning the concentration of VIP in CSMG and IMG, concentration of SOM in the ileum, IMG, DRG and CSMG, concentration of NPY in CSMG, DRG and IMG, as well as concentration of SP in IMG.

All the results are presented in Table 1.

Discussion

The results presented in this paper show that the concentration of GAL increased significantly in the porcine colon during swine dysentery in comparison with that found in control animals. The present results confirm data reported by other investigators who found that GAL is involved in the inflammatory processes in the paw skin (16) and arthritis (24) in the rat.

The present results show that in animals suffering from dysentery, concentration of GAL increases clearly in DRG. Other papers report that the axotomy of, for example, the sciatic nerve, causes a drastic increase in the concentration of GAL in DRG of the rat (29). It was established that this neuropeptide played a significant role in the transmission of the inflammatory pain (33). Similarly, very high increase in GAL concentration was found in the dysenteric pigs in IMG being the main source of efferent nerve fibers to the porcine colon (21). This suggests that the inflammation of peripheral tissues results also in changes of the neurochemical coding of neurons responsible for their innervation.

VIP is widely accepted as an anti-inflammatory factor. The highest increase in VIP concentration was found in IMG and CSMG of dysenteric animals. Distinct changes in VIP contents were found in human intestines during inflammation, or in Crohn’s disease (11). VIP is the neuropeptide expressed not only by neurons, but also by lymphocytes, hence it probably modulates innate and induced immunity, which suggests that, in case of, for example, Crohn’s disease, or experimental colitis, it can be of therapeutic use (1).

The present study has not revealed significant alterations of VIP contents in the ileum and colon in comparison with the control animals, however, clear changes in VIP content were detected in sympathetic ganglia (IMG, CSMG). This phenomenon was not described before in laboratory animals.

Another accepted anti-inflammatory and anti-nociceptive neuropeptide is SOM. Also in case of SOM, the statistically significant increase in the neuropeptide concentration was detected in all the ganglia studied (IMG, CSMG, DRG) and ileum.
Table 1
Results of the quantitative analysis of the content of neuropeptides in the ganglia and gut of the control (con) and dysenteric (exp) pigs. Results are displayed as mean±SEM

<table>
<thead>
<tr>
<th></th>
<th>CSMG</th>
<th>CON</th>
<th>EXP</th>
<th>IMG</th>
<th>CON</th>
<th>EXP</th>
<th>DRG</th>
<th>CON</th>
<th>EXP</th>
<th>ILEUM</th>
<th>CON</th>
<th>EXP</th>
<th>COLON</th>
<th>CON</th>
<th>EXP</th>
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<tr>
<td>GAL</td>
<td>14.86 ± 4.236</td>
<td>30.60 ± 18.16</td>
<td>4.072 ± 1.186</td>
<td>31.03 ± 9.426***</td>
<td>4.371 ± 0.6775</td>
<td>16.45 ± 1.709***</td>
<td>113.4 ± 44.55</td>
<td>217.5 ± 43.23</td>
<td>15.97 ± 7.334</td>
<td>119.7 ± 11.41***</td>
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<tr>
<td>VIP</td>
<td>14.03 ± 3.073</td>
<td>100.5 ± 19.22***</td>
<td>51.34 ± 6.380</td>
<td>87.91 ± 13.89*</td>
<td>4.514 ± 0.3968</td>
<td>8.436 ± 3.214*</td>
<td>17.03 ± 5.352</td>
<td>42.24 ± 11.26</td>
<td>8.914 ± 1.029</td>
<td>10.19 ± 3.912</td>
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<tr>
<td>SOM</td>
<td>7.243 ± 2.365</td>
<td>101.0 ± 11.08***</td>
<td>10.22 ± 1.650</td>
<td>86.13 ± 23.51***</td>
<td>1.670 ± 0.2918</td>
<td>8.728 ± 2.809***</td>
<td>13.80 ± 2.741</td>
<td>36.24 ± 6.547**</td>
<td>3.288 ± 0.6506</td>
<td>7.777 ± 2.921</td>
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<tr>
<td>NPY</td>
<td>89.94 ± 41.22</td>
<td>431.3 ± 50.71***</td>
<td>432.4 ± 112.2</td>
<td>971.9 ± 69.61**</td>
<td>4.678 ± 1.085</td>
<td>28.33 ± 7.521**</td>
<td>5.279 ± 0.8267</td>
<td>25.38 ± 13.89</td>
<td>2.108 ± 0.3287</td>
<td>13.43 ± 7.111</td>
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<tr>
<td>CGRP</td>
<td>6.397 ± 1.003</td>
<td>5.130 ± 1.666</td>
<td>9.258 ± 1.295</td>
<td>10.57 ± 3.007</td>
<td>2.866 ± 0.3103</td>
<td>3.084 ± 0.1433</td>
<td>8.274 ± 1.786</td>
<td>11.27 ± 1.570</td>
<td>3.736 ± 0.9656</td>
<td>5.158 ± 1.240</td>
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<tr>
<td>SP</td>
<td>11.50 ± 3.556</td>
<td>11.04 ± 2.898</td>
<td>1.263 ± 0.1696</td>
<td>2.160 ± 0.4365</td>
<td>5.804 ± 1.478</td>
<td>6.836 ± 1.568</td>
<td>14.06 ± 1.920</td>
<td>17.57 ± 2.275</td>
<td>5.427 ± 0.8977</td>
<td>6.164 ± 1.151</td>
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Description of the results of statistical analysis:
* significant
** highly significant
*** very highly significant
Morphological studies performed on the identical biological material aimed at comparing of the number of perikarya in three particular layers of the ileum and colon revealed that in the myenteric plexus (MP) and outer submucous plexus (OSP) of these segments of intestines the number of neuronal cell bodies increased clearly in dysenteric animals (28).

Some years ago, it was found that SOM may be actively involved in the pathophysiological processes accompanying the inflammation of the gut, and, maybe, of other organs (27). Recently, it was documented that exogenous SOM exhibited systemic anti-inflammatory and anti-nociceptive properties (2, 22). The role of the neurogenic SOM in sensory neurons in inflammatory states remains unclear. It may be supposed that the expression of SOM occurs mainly in small neurons in DRG (2). The changes in SOM concentration in DRG reported here may indirectly confirm this hypothesis.

Significant changes in SOM concentration in the ileum of dysenteric pigs may indicate that the inflammatory process developing in the intestine damages intestinal tissues, including nerve components and affects both DRG neurons being source of afferent nerve fibers and IMG and CSMG supplying efferent nerve fibers to the gut. In the pigs suffering from the proliferative enteropathy (PE), in which the pathological changes were located mainly in the colon, the number of SOM-positive perikarya in IMG was lower than in the control animals, but the number of SOM-positive nerve fibers was higher (18). These results do not allow for a detailed and accurate comparison with results of the present study. However, it may be stated that different aetiological factors may evoke similar reactions resulting, for example in infections, in neuropeptide expression in the tissues involved in the inflammatory process.

The available literature lacks almost entirely any data regarding the involvement of NPY in the inflammation of intestines or other organs and tissues. Recent studies (13) have reported the increased number of NPY-positive perikarya in three nerve plexuses of the wall of the porcine colon in animals suffering from adenomatosis, as compared to control pigs. The statistically significant increase in the tissue concentration of this neuropeptide in dysenteric pigs is found in IMG, CSMG and DRG, but not in the ileum. These data suggest that NPY may be an active factor influencing the intestinal inflammation. The role of NPY in these conditions requires further studies.

Neuropeptides regarded as the pro-inflammatory factors are SP and CGRP. Statistically significant differences regarding the concentration of SP were found in DRG and IMG. Results similar to these described in the present paper were reported in rats in which experimental cystitis was induced (6). In dysenteric pigs no statistically significant differences in SP content were detected in the intestinal wall, however, such changes were observed in rats with experimentally-induced colitis (15). It is supposed that SP exerts very strong immunomodulatory effect coming from the fact that both production of SP peptide and SP receptor expression are characteristic for “mucosal immunity” and SP may act as the modulator of the inflammation in the normal gut by acting through its specific receptor on T lymphocytes in an autocrine and/or paracrine mode (23). Other reports confirm the fact that SP plays an extremely important role in the pathogenesis of intestinal inflammatory states (32).

The present paper reported the differences in CGRP concentration between control and experimental (dysenteric) pigs, but these differences were found to be statistically insignificant. CGRP and SP are involved in the inflammation of many organs and tissues, such as the skin, joints and oral cavity (2). It was also showed that CGRP was associated with inflammations of the gut (14) and urinary bladder (6).

Every inflammatory process is an extremely complex condition and bowel inflammations are associated with a massive infiltration of neutrophils and macrophages producing large amounts of pro-inflammatory cytokines, such as TNF, IL-β or IL-6, which may be observed in the early period of inflammation. Later, the tissues are infiltrated with T-cells producing interferon (7) and the synthesis of IL-4 is reduced (1). The changes in the neuropeptide contents are observed in both the extrinsic and intrinsic innervation of tissues and organs undergoing inflammatory changes. The mechanisms of reciprocal interactions of neuropeptides and the above-mentioned substances are not fully understood. It was proved that, e. g. VIP is the peptide present both in the nerve tissue and lymphocytes and via its three G protein-coupled receptors it modulates the innate and acquired immunity (1), causing a release of histamine (8), arrest of the production of pro-inflammatory cytokines and chemokines (9), etc. SOM can also induce the histamine release (8) and exerts anti-nociceptive effects (2). SP is probably released from the extrinsic sensory nerve fibers of the gut during the inflammation; it regulates the blood flow in the intestinal mucosa (25) and may play the role in tissue repair (11). CGRP affects gastrointestinal tract functions via its CGRP1 and CGRP2 receptors (14) and GAL regulates not only the contractility of intestines but can also modulate the ion transport in the epithelium (3).

In addition to the mentioned neuropeptides, the role of which in the inflammation is well known, also other peptides, such as neurokinins (10, 17) and NPY (13) were found to be involved in these processes. Other substances, namely NO (19), nerve growth factor (3) and cytokines (7, 20) are also involved.

The results of the studies performed so far suggest that some neuropeptides may be applied therapeutically. It was documented that VIP might be used as a therapeutic and prophylactic factor in the experimental colitis (1, 9). Other studies have shown that tachykinins and CGRP antagonists may be also applied as therapeutics (10) due to their spasmylytic, anti-diarrhoel, anti-inflammatory and anti-nociceptive functions (14).

The present results, together with the results of other authors working on the behaviour of neuropeptides in the porcine adenomatosis (5, 12, 13, 18), show unanimously that the inflammation changes the
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References


