Somatostatin-like immunoreactive primary sensory neurons supplying the porcine adrenal glands in physiological conditions and after adrenalectomy

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Abstract

Retrograde neuronal tracing, using fast blue, in combination with a single-labelling immunofluorescence technique, was applied to determine whether somatostatin (SOM) participates in sensory innervating of the porcine adrenal glands in physiological conditions and after adrenalectomy. In control animals, SOM-like immunoreactive neurons comprised 7.0 ± 0.7% of adrenal gland-projecting cells in dorsal root ganglia (DRG) at neuromeres Th6-7 and 6.5 ± 1.2% at neuromeres Th12-14. After adrenalectomy the percentage of SOM-positive DRG cells considerably increased and attained the level of 44.7 ± 2.5% at neuromeres Th6-7 and 36.6 ± 1.7% at neuromeres Th12-14. The obtained results demonstrate that SOM is not only a neuromediator within sensory neurones supplying the porcine adrenal glands, but also suggest the role of this substance during repairing processes within the nervous system after adrenalectomy.

Keywords: swine, adrenal glands, somatostatin, dorsal root ganglia, adrenalectomy.

Introduction

Somatostatin (SOM) is a tetradecapeptide (14 amino acids) which was isolated for the first time in 1973 from the ovine hypothalamus and was demonstrated to inhibit growth hormone secretion (6). This substance is an active factor widely distributed in the body of all vertebrates and some invertebrate species, which can be produced by neurons, neuroendocrine, inflammatory and immune cells (18). Functions of somatostatin are mediated by five types of receptors (sst1-5), all of which are members of the G protein coupled receptor superfamily (8). In spite of the fact that physiological functions of SOM are relatively well established, some aspects of its action remain not clear. One of them is the participation of somatostatin in sensory innervation of the adrenal glands.

SOM is an important regulatory peptide in the central and peripheral nervous system (2). Nevertheless, it is well known as an active substance within the autonomic nervous system (11, 15, 24), it may also take part in the transmission of sensory and nociceptive information (3, 20). It is known that primary sensory neurons are located in sensory nuclei of cranial nerves, as well as in dorsal root ganglia (DRG). Neurons in the DRG are bipolar cells, which have perikarya within ganglia and send their endings centrally to the dorsal horn of the spinal cord and peripherally to the skin and internal organs. Until now, both somatostatin and its receptors have been described in the DRG of various species, including humans (3, 14, 23). The presence of SOM has been mainly observed in small–cell–diameter neurons of the DRG, and prevalent types of somatostatin receptors in these ganglia are two variant forms of sst2 (sst2A and sst2B) (23).

Nowadays, somatostatin is considered to be a strong antinociceptive factor (12, 13), which inhibits the conduction of sensory neurons and may be used as an analgesic factor during various pathological processes. On the other hand, some investigations have shown pro-nociceptive action of intrathecally administered somatostatin (22).
Antinociceptive actions of somatostatin suggest that this substance plays important roles during pathological processes. Moreover, SOM is a well-known anti-inflammatory agent, which down-regulates lymphocyte proliferation, reduces immunoglobulin production, and inhibits the release of pro-inflammatory cytokines (25). However, up till now, functions of SOM in the sensory and nociceptive conducting neurons, especially during pathological states connected with damage of nervous fibers and adrenal glands remain unknown. Therefore, the aim of the present study was the description of SOM–like immunoreactive (SOM-LI) neurons of the DRG supplying the porcine adrenal glands under physiological conditions and after adrenalectomy. It should be noted that pig becomes the most popular experimental animal because of considerable similarities to human in anatomical, physiological, and pathological characteristics (26).

Material and Methods

The present study was performed on ten immature sows of the Large White Polish breed (12-18 kg b.w., approximately 8 weeks old). The animals were kept in standard laboratory conditions. All experimental operations were conducted in compliance with the instructions of the Local Ethical Committee in Olsztyn.

The pigs were divided into two groups: control (group C; n = 5) and experimental (group A after adrenalectomy, n = 5). All animals were pre-treated with atropine (Polfa, Poland, 0.4 mg/kg, s.c.) and propionyl promasine (Stresnil, Janssen, Belgium, 0.8 mg/kg, i.m.) 15 min before application of the main anaesthetic - sodium thiopental (Thiopental; Sandoz, Austria; 20 mg/kg i.v.), and were subjected to median laparotomy. The left adrenal gland was injected with 20 μL of 5% aqueous solution of fast blue (FB, Dr. K. Illing GmbH&KG, Germany; four injections, 5 μL each) using a Hamilton syringe equipped with 26 gauge needle. A great attention was paid to avoiding any contamination of the surrounding tissues with FB due to the hydrostatic leakage from the injection canal.

After convalescence period of three weeks, animals of group A were subjected to reoperation, when, after laparotomy, the adrenalectomy of the left adrenal gland was performed. One week after the adrenalectomy, all animals (groups C and A) were reanaesthetised, and then euthanized by an overdose of sodium thiopental. Afterwards, they were perfused transcardially with 4% buffered paraformaldehyde (pH 7.4) prepared ex tempore.

Left dorsal root ganglia from neuromeres Th6-7 and Th12-13 (previous investigations (9) demonstrated that the major number of sensory neurones supplying the left porcine adrenal gland are localised in these segments) were taken from all animals. The ganglia were rinsed in a 0.1 M buffer solution, pH 7.4, for 72 h at 4°C and then kept at 4°C in a buffered 18% sucrose solution. Finally, the ganglia were cut with a cryostat (at -22°C) into 10 μm serial sections. These sections were examined under a fluorescence Olympus BX51 microscope equipped with an appropriate filter set to localise all FB + neurons. The diameter of perikaryon of each FB – positive cell (in this section, where the nucleus was clearly visible) was measured by means of image analysis software (AnalySIS version 3.0, Soft Imaging System GmbH, Germany). The neurons were divided into three size –classes: small (diameter up to 30 μm), medium (diameter 31-50 μm), and large (diameter >51 μm).

All FB - labelled neurons (at least 80 cells per each experimental animal) were processed for the routine single labelling immunofluorescence method (10), using primary SOM antibody (monoclonal rat antiserum from Chemicon Int. Inc., USA, catalogue No. MAB354, work dilution: 1:50). After air-drying at room temperature for 45 min, the sections were incubated with solution containing 10% of goat serum, 0.1% of bovine serum albumin, 0.01% of NaN3, Triton X-100, and thimerozol in PBS for 1 h at room temperature, then incubated with the SOM antiserum (overnight; at room temperature), and further incubated with goat anti-rat FITC conjugated IgG (ICN Biomedicals, USA, dilution 1:400) (1 h, at room temperature). Each step of immunolabelling was followed by rising the sections with PBS (3 × 10 min, pH 7.4). Standard controls, i.e. pre-absorption of the primary antibody with appropriate antigen and omission of replacement of primary antibody by non-immune serum were performed to test antibody and specificity of the method. The pre-absorption test was performed as follows: sections of DRG were incubated with “working” dilution of anti-somatostatin antibody, which was pre-absorbed for 18 h at 37°C with 20 μg of human SOM (Hölzel Diagnostika GmbH, Germany).

The labelled sections were analysed using Olympus fluorescence microscope equipped with epillumination and appropriate filter sets for FITC and FB. Relationships between immunohistochemical staining and FB distribution were examined by interchanging filters. Finally, the obtained data was pooled, expressed as means ±SEM and statistically analysed using GraphPad Prism 5 software (graphPad Software, USA). The differences were considered statistically significant at P ≤ 0.05.

Results

SOM-positive neurones supplying the adrenal glands were observed in DRG of control pigs, as well as in animals after adrenalectomy (Fig. 1, Table 1).
Table 1. The percentage of somatostatin – like immunoreactive neurons supplying porcine adrenal glands in dorsal root ganglia under physiological conditions (group C) and after adrenalectomy (group A)

<table>
<thead>
<tr>
<th>Neuronomes</th>
<th>Group C</th>
<th>Group A</th>
</tr>
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<tbody>
<tr>
<td>Th 6-7</td>
<td>7.07 ± 0.7a</td>
<td>44.7 ± 2.5b</td>
</tr>
<tr>
<td>Th 12-13</td>
<td>6.5 ± 1.2a</td>
<td>36.6 ± 1.7c</td>
</tr>
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Statistically significant data (P ≤ 0.05) is marked by different letters, and not significant data is marked by the same letter.

Fig. 1. Somatostatin – like immunoreactive neurons supplying the adrenal glands (arrows) in dorsal root ganglia within neuronomes Th6-7 (A) and Th12-13 (B) under physiological conditions (1) and after adrenalectomy (2). Scale bar 20 μm.
Under physiological conditions, only small percentage of FB-positive cells revealed the presence of the SOM. These values amounted to 7.0 ± 0.7% in segments Th6-7 and 6.5 ± 1.2% in neuromeres Th12-13, and did not display statistically significant differences between groups of neuromeres (Table 1).

Moreover, in control animals, the SOM was visualised in all size – classes of FB-positive neurons (Table 2), without considerable quantitative differences between them. The percentage of SOM-LI cells amounted to 6.4 ± 1.0%, 6.5 ± 0.3%, and 7.7 ± 0.3% in small, medium, and large FB+ cells, respectively.

After adrenalectomy, the number of FB – positive neurons immunoreactive to SOM rapidly increased within both groups of neuromeres. Changes were more visible in segments Th6-7, where the percentage of SOM-LI cells containing FB amounted to 44.7 ± 2.5%. In segments Th12-13 this value was lower and amounted to 36.6 ± 1.7% (Table 1). In contrast to control animals, after adrenalectomy, statistically significant differences in the percentage of SOM-LI neurons were observed between Th6-7 and Th12-13 segments. In experimental animals, the expression of SOM increased especially in small and middle-size cells, where the percentage of SOM-positive neurons was 59.2 ± 1.2% and 46.1 ± 1.0%, respectively (Table 2). In large perikarya, these changes were less visible and were expressed by an increase in the percentage of SOM-LI cells to 16.7 ± 1.5%. Moreover, after adrenalectomy statistically significant differences in the percentage of SOM-positive cells were observed between particular size-classes of neurons.

Table 2. The percentage of somatostatin – like immunoreactive neurons supplying porcine adrenal glands in dorsal root ganglia under physiological conditions (group C) and after adrenalectomy (group A) in particular size – classes of cells

<table>
<thead>
<tr>
<th>Diameter of neurons (μm)</th>
<th>Group C</th>
<th>Group A</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>6.4 ± 1.0a</td>
<td>59.2 ± 1.2a</td>
</tr>
<tr>
<td>31-50</td>
<td>6.5 ± 0.3a</td>
<td>46.1 ± 1.0a</td>
</tr>
<tr>
<td>&gt;51</td>
<td>7.7 ± 0.3a</td>
<td>16.7 ± 1.5a</td>
</tr>
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Statistically significant data (P ≤ 0.05) is marked by different letters, and not significant data is marked by the same letters.

Discussion

The results obtained show that the SOM takes part in sensory innervating of porcine adrenal glands. Under physiological conditions, the participation of this peptide in conducting of sensory stimuli from adrenal glands seems to be minor, but during pathological states the significance of the SOM in these processes clearly increases.

In previous studies, SOM-positive DRG neurons, as well as somatostatin receptors have been observed in various species, including humans (3, 14, 15, 24). On the other hand, SOM has not been reported in DRG cells supplying the adrenal glands in rodents (14), but it should be pointed out that data concerning the chemical coding of sensory neurons supplying these glands is scant and fragmentary, and limited to typical sensory neuromediator such as substance P (29).

During the study, the percentage of SOM-positive neurons supplying the adrenal glands was not high in control animals. It is in agreement with previous studies on pig, where the number of SOM-LI neuronal cells within DRG has fluctuated around 1% (3). On the other hand, till now somatostatin has been observed only in small neurons of DRG (16), while in the present study, various classes of somatostatin-positive cells were noted, and differences in expression of SOM in particular size-classes of cells have not been visible under physiological conditions.

It should be pointed out that at present, not only the functions of SOM within the adrenal glands, but also roles of all sensory innervation of these organs are not known. Some studies suggest that sensory neurons supplying the adrenal glands, beside conduction of pain stimuli during pathological processes, may send information connected with baro- and/or chemoreflexes (19) and, following a change of blood pressure, affect the secretion of catecholamins by the adrenal medulla. Additionally, colocalisation of SOM with substance P, nitric oxide synthase, calcitonin gene related peptide, and galanin (14) in intra-adrenal nerves fibres, which partially can be the peripheral endings of DRG neurons, suggest the similar functions of the mentioned substances, i.e. effect on secretion of adrenaline, noradrenaline, and steroid hormones (17, 28).

The functions of somatostatin in conducting of sensory and pain stimuli are discussed, but still not clear. It is known that this substance may act mainly as an anti-nociceptive factor (12, 13), but some investigations have demonstrated pro-nociceptive properties of this peptide (22). Previous studies have demonstrated that somatostatin and its analogue - octreotide (OCT) exhibit anti-nociceptive and anti-inflammatory properties and can evoke the analgesic effects during different types of pathological pain (7, 21). Moreover, previous investigations have also revealed inhibitory effects of somatostatin on sensory neurons within dorsal horn of the spinal cord (7).

A dramatic post-adrenalectomy increase in SOM-positive sensory neurons, supplying the porcine adrenal glands, observed in the present investigation is not completely in agreement with previous studies. Till now, this fact was not observed in other species, in which, in contrast to present results, the reduction of SOM-positive sensory neurons has been described after axotomy (27). It is possible that an increase in the number of SOM-positive sensory neurons in the DRG after injury of their peripheral endings is characteristic of the pig. Some investigations concerning this species, in spite of their fragmentary character, seem to confirm
the mentioned argument. Namely, post-axotomical augmentation of SOM-like immunoreactivity has been described in the porcine autonomic nervous system (15). Moreover, scanty information concerning SOM-positive sensory neurons in porcine DRG revealed that the percentage of such cells supplying the urinary bladder increases under the influence of selected toxins (4, 5).

An increase in the number of SOM-LI neurons of dorsal root ganglia during pathological processes, observed both in previous studies (4, 5, 15) and in the present study, may arise from the augmentation of SOM synthesis as an adaptive process within the nervous system, but it could also indicate an inhibition of the SOM release from nerve endings. The augmentation of SOM synthesis, which on the grounds of well-known anti-nociceptive actions of this peptide (12, 13) is more probable, can be connected with altered activity of the sensory neurons under noxious factors. Nonetheless, these mechanisms are very difficult to explain. Thus, the changes observed in the present study may be due to primary (damage of nervous endings) or secondary (pain) results of adrenalectomy, and can be a reflection of changes in the transcriptional, translational, or metabolic level.

The differences in the reaction to adrenalectomy between neurons within neuromeres Th6-7 and TH2-14 are also worth to be noted. More intensive increase in the number of SOM-LI FB-labelled neurons located at neuromeres Th6-7 may suggest that SOM-positive cells of these segments play a more important role in the regulation of sensory and/or pain conductivity from the adrenal glands during pathological processes. Moreover, neurons in neuromeres Th6-7 can respond more rapidly to damage of their peripheral endings, which may indicate their greater dependence on trophic factors produced by tissues innervated by them.

At present, sensory functions of SOM after axotomy, and especially after adrenalectomy, are not known. They can be only generally elucidated by repairing processes in destroyed cells. It is possible, that SOM in the porcine axotomised neurons take control of the functions of nerve growth factors, which are necessary for regeneration of destroyed fibres. It is also known that some growth factors, such as glial-cell-line-derived neurotrophic factor (GDNF), which is in charge of repairing processes, modulate the activity and induce the release of SOM from both central and peripheral terminals of adult sensory neurons (16). Neuroprotective and/or neurodegenerative functions of SOM are more probable. It is well-known that an injury to neurons causes an increase in the expression of neurotransmitters, which promote the regeneration of injured cells, at least in in vitro experiments (1). Moreover, some actions of SOM after adrenalectomy may be due to relatively well-known anti-nociceptive and anti-inflammatory properties of this peptide (21).

To sum up, results obtained in the study show that somatostatin takes part in the sensory innervation of porcine adrenal glands, and is involved in response to the damaging factors within these organs. Nevertheless, the exact roles of somatostatin within sensory nervous system under physiological conditions and during pathological processes are not fully explained and require further investigations.

References