Distribution of somatostatin-immunoreactive nerve fibres in Peyer's patches

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Abstract

The distribution of somatostatin-immunoreactive nerve fibres in Peyer's patches of the cat was demonstrated by immunocytochemical techniques. A large number of immunoreactive nerve fibres was observed in the tela submucosa very close to the Peyer's patches. Some immunoreactive nerve cell bodies were also found in this layer. The immunoreactive nerve terminals ran around the margin of the follicles and only a few nerve fibres were observed in the centre of follicles. Electron-microscopic investigation showed that these immunoreactive nerve terminals were in very close contact with lymphocytes and plasma cells, where no Schwann cell sheath was interposed. The gap between the nerve processes and the lymphocytes and plasma cells was about 20–200 nm, and occasionally less. These results provide morphological evidence consistent with the view that somatostatin has a neuroimmunomodulatory action.

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It has been shown that autonomic nerve fibres are present in the different lymphoid organs.^{1–7} The postganglionic sympathetic noradrenergic nerves are thought to be the functional link between the immune system and the nervous system^{8,9} and are proposed to act as neuro-immunomodulators.¹⁰ It is thought that some neuropeptides also modulate the immune function.^{11–16}

Several studies in the rabbit have shown that noradrenergic sympathetic fibres innervate the gut associated lymphatic tissue – that is, Peyer's patches and the appendix.^{1,17} Some experimental observations have suggested a possible involvement of somatostatin in immunomodulatory actions.^{18–20} This study aimed to determine the distribution of somatostatin-immunoreactive nerve fibres, and in particular their relation to immune cells, in the innervation of Peyer's patches of the cat, by use of immunocytochemistry at both light and electron microscope levels.

Methods

Young cats ($n=5$) (1.5–2 kg) of either sex, kept under normal laboratory conditions, were used in this study. Before they were killed the animals were starved overnight and then anaesthetised with an intraperitoneal injection of sodium pentobarbitone (20 mg/kg body weight). The animals were perfused transcardially with 37°C phosphate buffered saline (PBS) at pH 7.3, followed by a 4°C fixative containing 2% paraformaldehyde, 0.1% glutaraldehyde, and 150 ml saturated picric acid in 1000 ml of Sörensen buffer (pH 7.3). After dissection, pieces of ileum were immersed in glutaraldehyde free fixative for an additional 24 hours, then rinsed for 24 hours in 0.1 M phosphate buffer, pH 7.3. The pieces were then frozen in liquid nitrogen and thawed in phosphate buffer at room temperature. Sections (40 μ m) were cut on a vibratome and processed according to the peroxidase-antiperoxidase technique of Sternberger *et al.*²¹ Briefly, sections were washed in PBS and exposed to normal goat serum diluted 1:50 in PBS containing 0.1% sodium azide for one hour at room temperature. They were then rinsed in PBS and subsequently incubated for 48 hours at 4°C with an antiserum to synthetic somatostatin conjugated to keyhole limpet haemocyanin (RIA, Amersham, UK) raised in rabbit, at a 1:1000 dilution.

Subsequent steps were performed on sections at room temperature as follows: sections were washed in PBS, exposed for 1.5 hours to goat anti-rabbit immunoglobulin G (IgG) serum diluted 1:50 in PBS containing 0.01% sodium azide, washed in TRIS buffer (0.1 M; pH 7.5), and exposed for 1.5 hours to rabbit peroxidase-antiperoxidase complex diluted 1:100 in the same TRIS buffer. After reaction with a diaminobenzidine solution (15 mg diaminobenzidine and 165 μ l 0.3% H₂O₂ in 25 ml 0.05 M TRIS-HCl buffer, pH 7.5; 7–8 min at 25°C), the tissue was osmicated, dehydrated and embedded in epoxy resin (Epon). Ribbons of sections were stained with uranyl acetate and lead citrate and

Figure 1: Somatostatin-immunoreactive nerve fibres (arrows) around the lymph follicle (LF). Dotted arrow shows a somatostatin-immunoreactive nerve cell body in the tela submucosa. (Original magnification $\times 500$; bar = 100 μ m.)

Figure 2: Somatostatin-immunoreactive nerve terminals (arrow) surrounding the lymph follicle. (Original magnification $\times 800$; bar = $50\ \mu\text{m}$.)

Figure 3: Somatostatin-immunoreactive nerve process (arrow), with no Schwann cell sheath, facing a lymphocyte (LC). (Original magnification $\times 32\,000$; bar = $1\ \mu\text{m}$.)

Figure 4: Somatostatin-immunoreactive nerve terminal (arrow) lying close to the lymphocyte membrane (LC). (Original magnification $\times 32\,000$; bar = $1\ \mu\text{m}$.)

examined with a Tesla BS 300 electron microscope.

For controls, the specificity of the immunostaining was tested by preabsorption of the antiserum with synthetic somatostatin ($10\ \text{nmol/ml}$) for 12 hours. All specific immunostaining was blocked.

Results

Systematic light microscopic examination of serial vibratome sections showed that somatostatin-immunoreactive nerve cell bodies were located close to the Peyer's patches in the tela submucosa (Fig 1). The immunoreactive nerve fibres were observed in all layers of the ileum, not only in the region of Peyer's patches, but also in the surrounding areas, both in nerve fibres and cell bodies. A large number of them were located in close proximity to, and often surrounding, the follicles (Fig 2). These fibres were occasionally present within the follicle. However, they were numerous around the vessels at the adventitial-medial border.

When viewed with the electron microscope, the immunoreactive nerve fibres displayed homogeneous labelling. Most vesicles in the profiles were small ($30\text{--}40\ \text{nm}$ in diameter) but a few large ($80\text{--}120\ \text{nm}$ in diameter) dense-cored vesicles were observed among them. Most of the nerve fibres were found in very close proximity to the blood and lymph vessels. In addition they occurred as non-vascular fibres around the follicle, where they were in close association with lymphoid cells (Figs 3 and 4). In most cases the immunoreactive nerve fibres were free of Schwann cell cytoplasm, and were located close to the lymphoid cells. Sometimes they were found in direct contact with the lymphocytes and plasma cells (Fig 5). The gap between the membranes of immunoreactive nerve terminals and immunocells was $20\text{--}200\ \text{nm}$, or in a few cases even less (Fig 6).

Discussion

Strong evidence has accumulated over the past decade of environmental and psychosocial factors which can influence immune functions through the central nervous system that then communicates with the immune system via autonomic nerves.²² In the present study, this view has been supported by electron microscopy which demonstrated somatostatin-immunoreactive nerve terminals in close contact with immunocompetent cells in Peyer's patches of the cat ileum. Close contact between nerves and immune cells has been previously demonstrated by Felten *et al*²⁸ in the spleen and by Novotny and Kliche²³ in rat lymph nodes. Felten *et al*⁸ have also shown (by double-labelled immunocytochemistry for tyrosine-hydroxylase and specific markers for cells of the immune system) that nerve fibres lie directly adjacent to both helper and suppressor T cells.

In vitro studies have shown that neuropeptides can modulate the function of immunocompetent cells in many ways – for example the sensory peptides substance P and calcitonin gene-related peptide enhance the proliferation of

Figure 5: Somatostatin-immunoreactive nerve terminals (arrows) lie close to the lymphocyte (LC) and in direct contact with the plasma cell (PC). (Original magnification $\times 20\,000$; bar = $1\,\mu\text{m}$.)

T lymphocytes, whereas vasoactive intestinal polypeptide has opposite effects.²⁴ Opiate alkaloids as well as endogenous opioid peptides modify the reactions of all lymphoid cell types when administered systemically or in vitro.^{25, 26} It has also been shown that somatostatin produces a noticeable inflammatory cell reaction^{20, 27} as well as enhanced formation of the leukocyte

Figure 6: Arrow shows a somatostatin-immunoreactive nerve terminal in very close contact with a plasma cell (PC) in the mucous membrane. LV, lymph vessel; E, epithelial cell. (Original magnification $\times 18\,000$; bar = $1\,\mu\text{m}$.)

migration inhibiting factor.¹⁹ It is known that either lymphocytes or plasma cells produce immunoglobulins of a single class and with unique antigenic specificity. These antibodies are released at an early stage of the response and at the very site of antigen stimulation. Therefore, it is tempting to speculate that somatostatin has similar effects when released from terminal nerve endings. This innervation may provide one channel of communication among the many neurohormonal modulations and also a direct morphological link between the nervous and immune systems.

The codistribution of somatostatin and noradrenaline in the mesenteric-coeliac ganglia²⁸ indicates that somatostatin innervation of Peyer's patches, like other tissues, may be sympathetic. On the other hand, somatostatin has been reported in enteric neurones,^{29, 30} so some of the immunoreactive processes observed in this study may originate from that source.

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