Sensory nerves in the interface membrane of aseptic loose hip prostheses

M. Ahmed, J. Bergström, H. Lundblad, W. J. Gillespie, A. Kreicbergs

From the Karolinska Institute, Stockholm, Sweden

We studied the presence of sensory nerves by immunohistochemistry in the interface membranes of hip prostheses after aseptic loosening. Substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) were analysed as was protein gene product (PGP) 9.5, a general marker for nerve fibres.

We identified nerve fibres in all samples but differences in their density were found. SP- and NKA-positive fibres were predominantly non-vascular, forming varicose nerve terminals. CGRP-immunoreactive nerve fibres with varicose terminals were seen mostly close to blood vessels, but also as free nerve endings.

Sensory neuropeptides participate not only in nociception but also stimulate immune cells to release cytokines. The presence of sensory nerves in the interface membrane may reflect a pathophysiological response contributing to the aseptic loosening of hip prostheses.

Materials and Methods

We obtained nine interface membranes surrounding cemented femoral components from patients undergoing revision hip arthroplasty. There were four women and five men with a mean age of 70 years (60 to 86). The mean time to revision was 8 years (5 to 12). The primary hip arthroplasty (Charnley, Exeter or Howse) had been performed for osteoarthritis in seven patients and for rheumatoid arthritis in two. All had pain in the hip or thigh with radiological evidence of loosening at the time of revision. Preoperative cultures from the hip were all negative.

The membranes were rinsed in 0.01 mol/l of phosphate-buffered saline (PBS) at pH 7.3 and fixed with 4% paraformaldehyde in 0.2 mol/l of Sörensen phosphate buffer containing 0.2% picric acid at pH 7.3 for two days. They were soaked for at least two days in 20% sucrose in 0.1 mol/l of Sörensen phosphate buffer containing sodium azide and bacitracin at pH 7.2 (Sigma Chemicals, St Louis, Missouri).

Two samples of each specimen were included in the study. Four serial 15 μm sections were analysed from each membrane specimen for each peptide. The frozen sections were mounted directly on SuperFrost/Plus glass slides and immunostained according to the avidin-biotin complex
method. Briefly, the sections were rinsed for 5 × 2 minutes in PBS and incubated overnight in a humid atmosphere at 4°C with antiserum to substance P (SP), neurokinin A (NKA), calcitonin gene-related peptide (CGRP) (all Peninsula Laboratories Europe Ltd, St Helens, UK) and protein gene product 9.5 (PGP 9.5; UltraClone, Cambridge, UK). The characteristics of the primary antibodies used are shown in Table I.

The sections were then rinsed in PBS (5 × 2 min) and incubated with biotinylated goat anti-rabbit antibodies (1:250; Vector Laboratories, Burlingame, California) for 30 minutes at room temperature. Finally, fluorescein isothiocyanate (FITC)-conjugated avidin (1:500; Vector Laboratories, Burlingame, California) was used to visualise the immunoreaction. Control staining was performed by omitting the primary antiserum. Addition of 50 μl of the peptides (SP, NKA and CGRP) to the corresponding antiserum before application on tissue sections served as another control. A Nikon epifluorescence microscope (Eclipse E800; Nikon Corporation, Instruments Division, Yokohama, Japan) was used to analyse the sections. T-Max black-and-white film (Kodak, Rochester, Minnesota) was used for photography. We used a scale of one plus (+) to three plus (+++) for the semiquantitative assessment of nerve fibres identified in each tissue section; + indicated 1 to 3 nerve fibres per section in a ×10 magnification field, ++ 4 to 6, and +++ 6 and more nerve fibres.

We performed histological analysis of the interface membrane to assess inflammatory changes and to confirm the presence of blood vessels.

Results

We identified nerve fibres immunoreactive to SP, NKA, CGRP and PGP 9.5 in the interface membranes from all patients. There were clear differences in the density of nerve fibres in different areas of the sample and also in different individuals (Table II). Histological examination showed that the interface membrane was mainly composed of fibrovascular tissue with abundant fibroblasts, macrophages and foreign-body giant cells3,5 (Fig. 1).

Serial sections of the interface membranes were analysed by immunohistochemistry and haematoxylin and eosin staining to determine the relationship of the nerve fibres to blood vessels. The occurrence of nerve fibres in the interface membrane was established by positive staining with the general neuronal marker PGP 9.5 (Fig. 2A) in serial sections.

The sensory nerve fibres were not distributed uniformly. CGRP-positive fibres with varicose terminals were more numerous and were seen to be close to blood vessels. Immunoreactivity to CGRP was found in the nerve bundles and in single fibres (Fig. 2B). The single nerve fibres were arranged as free nerve endings between the cells. Serial sections of the membrane showed co-localisation of CGRP and PGP 9.5 in most of the nerve fibres.

SP and NKA-positive fibres were predominantly non-vascular and were present as thin fibres and formed varicose nerve terminals (Fig. 2C). They had an almost identical distribution and serial sections showed coexistence of SP and NKA as well as SP and CGRP fibres in most of the nerve fibres (Figs 2B and 2C).

Discussion

Our study has shown that the interface membrane in aseptic loose cemented hip prostheses is supplied by sensory nerve fibres immunoreactive to substance P, NKA and CGRP. These are synthesised in neurones of the dorsal root ganglia. SP and NKA belong to the tachykinin family and are
important modulators of nociceptive signals.\textsuperscript{15-17} They are mainly present in small-sized neurones (type B) of the dorsal root ganglia and their projections are unmyelinated C- and thinly myelinated A-type fibres, commonly known as nociceptive fibres.\textsuperscript{23-25} By contrast, a substantial proportion of CGRP occurs in large-sized (type A) neurones from which myelinated fibres arise.\textsuperscript{26,27} CGRP, often co-localised with SP in unmyelinated C fibres,\textsuperscript{26} facilitates the release of SP at the level of the spinal cord and delays SP degradation, thereby potentiating the nociceptive effects.\textsuperscript{28-30}

Patients with loose hip prostheses often have pain of varying intensity and distribution. The presence of different types of nerve fibre immunoreactive to SP, NKA and CGRP and also the difference in nerve density in the interface membrane may explain the different pain sensations and intensities. It is known that nociceptors respond to mechanical, thermal and chemical stimuli.\textsuperscript{27} In the interface membrane, increased levels of prostaglandin E\textsubscript{2}, one of the biochemical agents known to stimulate nociceptors, have been reported. The activation of nociceptive pathways by prostaglandins may be one of the many mechanisms involved in the transmission of pain from hips with loose prostheses.

The term neurogenic inflammation was coined when it was found that antidromic stimulation of primary afferent fibres elicited vasodilatation, increased capillary permeability, extravasation of plasma proteins and subsequently local oedema.\textsuperscript{18,19} Substance P and NKA have been shown to induce inflammatory signs along with the release of histamine from mast cells.\textsuperscript{32} Recently, activated mast cells have been identified in the interface membrane of aseptic loose hip prostheses.\textsuperscript{33} The inflammation induced by tachykinins is known to be inhibited by neurokinin receptor antagonists\textsuperscript{34} and also by pretreatment with capsaicin,\textsuperscript{19,35,36} which specifically depletes sensory neuropeptides. CGRP has been shown to enhance SP-induced oedema in the periphery.\textsuperscript{37} In patients with rheumatoid arthritis, and in rat adjuvant arthritis, increased expression of sensory neuropeptides has been reported in the synovial membrane and synovial fluid.\textsuperscript{38-41} Sensory neuropeptides have also been implicated in vasodilatation.\textsuperscript{32,42} In our study, CGRP-positive fibres were found mostly around the blood vessels. It may therefore be possible that sensory neuropeptides participate in the inflammatory events in the interface membrane.

Recently, it has been shown that implant wear particles induce local and systemic immune responses.\textsuperscript{44,45} Increased expression of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor-alpha (TNF-\alpha) has been reported in the macrophages and fibroblasts in the membrane\textsuperscript{6-10} and also in pelvic lymph nodes in patients with hip arthroplasties.\textsuperscript{22} These cytokines have been implicated in inflammation and periprosthetic bone resorption. A number of studies have suggested a functional link between the nervous and immune systems. Sensory nerve fibres have been demonstrated in lymphoid organs with a synapse-like contact to immune cells.\textsuperscript{46,47} Fibro-
blasts, lymphocytes and macrophages are equipped with SP and NKA receptors and SP has been shown to induce the release of IL-1, IL-6 and TNF-α from these cells. SP has also been reported to promote inflammatory cell chemotaxis, fibroblast proliferation and antibody formation. Furthermore, it has been shown that SP enhances the secretion of prostaglandin E2 and collagenase in synoviocytes and that CGRP stimulates lymphocyte proliferation. It is therefore possible that sensory neuropeptides may stimulate the different types of immune cells in a paracrine fashion to promote release of various proinflammatory mediators. Moreover, SP has recently been shown to induce bone resorption both in vitro and in vivo.

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