Acta Orthop Scand *56*, 484–486, 1985

Innervation of synovial membrane and meniscus

Substance P-immunofluorescent nerves, which are closely connected to pain transmission, were shown in human knee synovial membrane and menisci. Both tissues also contained enkephalin-immunofluorescent nerves, which are probably involved in the modulation of pain transmission. Previous suggestions on the presence of nociceptive receptors in these non-cartilaginous joint structures, made on a histological basis, are thus confirmed by a specific immunohistochemical method.

Mats Grönblad
Olli Korkala
Päivi Liesi[1]
Erkki Karaharju

Department of Orthopaedics and Traumatology, University Central Hospital, and [1]Department of Virology, University of Helsinki, Topeliuksenkatu 5, 00260 Helsinki 26, Finland

In clinical practice, the fibrous capsule, ligaments and periosteum are known to react by releasing a strong nociceptive neural input to a traumatic stimulus. The presence of rich somatic and sympathetic innervation has been verified in these tissues (Kellgren 1939, Kellgren & Samuel 1950, Kennedy et al. 1982).

Substance P is a neuropeptide involved in the mediation of pain (Jessell 1982, Liesi et al. 1983, Pernow 1983, Grönblad et al. 1984a & b, Basbaum 1984). Substance P probably plays a "trigger role" in the initiation of the pain signal (Henry 1982). Thus, the presence of substance P-immunofluorescent nerves in the synovial and meniscal tissues would strongly suggest a nociceptive innervation of these tissues. The opioid peptides (leucine and methionine enkephalins) are intimately involved in the regulation of nociceptive information (Jessell 1982). Substance P and enkephalin containing nerves were traced in these non-cartilaginous joint structures by specific immunohistochemical methods.

Material and methods

Preparation of tissue

and from arthritic hip and wrist joints. Five samples were taken during meniscectomy operations from the posterior horns of the medial or lateral menisci of separate knee joints. The samples were trimmed into small 1–2 mm^3 pieces and then fixed by immersion overnight in 3.5% ice-cold paraformaldehyde in phosphate-buffered saline (PBS). On the next morning the tissue samples were rinsed in PBS and cut into 10-μm-thick sections by a cryostat.

Immunohistochemistry

The sections were processed for immunohistochemistry to substance P and leucine (leu-) or methionine (met-) enkephalins (Grönblad et al. 1984a). All the antibodies were kindly donated by Dr. Julia Polak of Hammersmith Hospital, London; their specificities have previously been confirmed (Wharton et al. 1979, 1980). To diminish the background fluorescence the sections were incubated in normal sheep serum, diluted 1:30. The primary antibodies were then raised in rabbits and applied in dilutions of 1:500, the sections being exposed to the primary antibodies overnight at room temperature. After a rinse in PBS, the sections were incubated with sheep anti-rabbit immunoglobulins coupled to fluorescein-isothiocyanate (FITC) (Wellcome Co., UK) diluted 1:20 for 2 h at room temperature. The sections were mounted in 1:1 PBS-glycerol and viewed with a Leitz Dialux 20 EB microscope using epi-illumination. Phase contrast microscopy was used in parallel for the identification of immunofluorescent structures (Liesi et al. 1983).

Figure 1. Fine varicotic nerve (bars) with substance P-immunoreactivity, in the synovial membrane. ×280.

Figure 2. A glomerular nerve terminal (bar) with substance P-immunofluorescence in the hip joint synovial membrane. ×280.

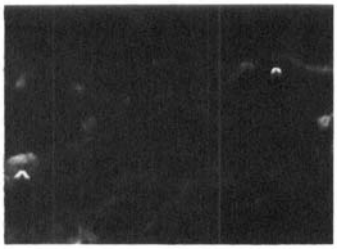

Figure 3. Leu-enkephalin-immunofluorescent nerves (arrows) in the knee synovial membrane. ×300.

The immunocytochemical controls included omission of one of the three antibodies or incubation with anti-leu- or anti-met-enkephalins pre-absorbed with 1 mg/ml of the corresponding antigen (Sigma, St. Louis, MO) (Grönblad et al. 1984b).

Results

Intensely fluorescent fine varicotic nerves exhibiting an immunofluorescence specific for substance P were seen in the joint synovia. Similar nerves were seen in the synovial membrane taken from normal and arthritic joints, there being no marked differences in the morphology or distribution of these nerves. The immunopositive nerve profiles were easily distinguished from the diffuse background fluorescence of the collagenous tissue. Substance P-immunofluorescent nerve terminals were also occasionally observed (Figures 1 and 2). There were few enkephalin immunofluorescent nerves, but they were occasionally seen (Figure 3).

Both substance P-immunofluorescent and enkephalin-immunofluorescent nerves were observed in the menisci. However, only fine nerve fibres were seen, without evidence of large corpuscular or glomerular nerve terminals in any of the meniscal samples (Figures 4–6).

Discussion

It has been shown that the meniscal horns are much more intensely innervated than the meniscal bodies, the central thirds of which are totally devoid of innervation (Wilson et al. 1969, O'Connor & McConnaughey 1978). Innervation of the menisci has been suggested to play a sensory role, as the menisci possess some receptor-like endings with typical sensory characteristics (Wilson et al. 1969, Kennedy et al. 1982).

Relief of pain after meniscectomy is a common finding, although the meniscal tear may be a small one; the presence of nociceptive

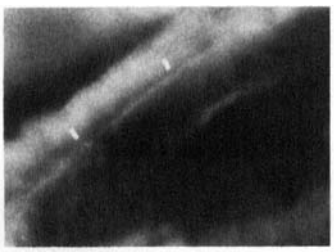

Figure 4. A substance P-immunofluorescent nerve (bars) in lateral knee meniscus. ×220.

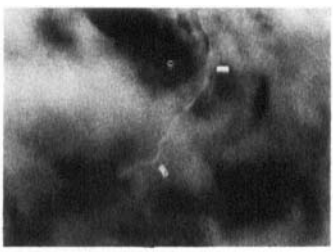

Figure 5. Intensely fluorescent met-enkephalin-immunofluorescent nerve (bars) in the posterior horn of the medial knee meniscus. ×350.

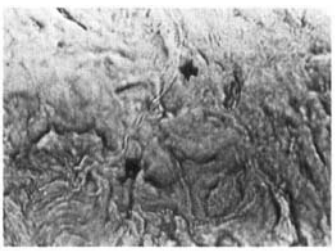

Figure 6. The same view as in Figure 5, in phase contrast microscopy. ×350.

fibres in the menisci may explain the pain relief as a denervation effect. The peptidergic nerve terminals of the synovial membrane are very much like those of the posterior longitudinal ligament of the human spine (Korkala et al. 1985); both fine nerve fibres and glomerula-like nerve terminals were observed. The menisci contained only fine nerve fibres, but no glomerular-like nerve terminals. It seems reasonable to suggest that the glomerular-like terminals of the synovium, like those of the spine ligament (Liesi et al. 1983, Korkala et al. 1985), may be the real nociceptors, whereas the substance P-immunofluorescent fine nerve fibres may be more concerned with the mechanoreceptor or slow pain modalities. The opioid peptides seem to be connected with the regulation of sensory pain input (Jessel 1982), perhaps being linked to extreme pain sensitivity (Grönblad et al. 1984a). In this respect it was interesting to observe enkephalin-immunoreactive nerves in both the meniscal and synovial samples.

References

Basbaum, A. I. (1984) Anatomical substrates of pain and pain modulation and their relationship to analgesic drug action. In: *Analgesics: neurochemical, behavioral and clinical perspectives.* (Eds. Kuhar, M. & Pasternack, G.), pp. 97–123. Raven Press, New York.

Grönblad, M., Liesi, P. & Munck, A. M. (1984a) Peptidergic nerves in human tooth pulp. *Scand. J. Dent. Res.* **92**, 319–324.

Grönblad, M., Liesi, P., Korkala, O., Karaharju, E. & Polak, J. (1984b) Innervation of human bone periosteum by peptidergic nerves. *Anat. Rec.* **209**, 297–299.

Henry, J. L. (1982) Relation of substance P to pain transmission: neurophysiological evidence. In: *Substance P* (Eds. Porter, R. & O'Connor, M.), pp. 206–217. Ciba Found. Symp. 91, Pitman, London.

Jessel, T. M. (1982) Pain. *Lancet* **ii**, 1084–1087.

Kellgren, J. H. (1939) Some painful joint conditions and their relation to osteoarthritis. *Clin. Sci.* **4**, 193–205.

Kellgren, J. H. & Samuel, E. P. (1950) The sensitivity and innervation of the articular capsule. *J. Bone Joint Surg.* **32**-B, 84–92.

Kennedy, J. C., Alexander, I. J. & Hayes, K. C. (1982) Nerve supply of the human knee and its functional importance. *Am. J. Sports Med.* **10**, 329–335.

Korkala, O., Grönblad, M., Liesi, P. & Karaharju, E. (1985) Immunohistochemical demonstration of nociceptors in the ligamentous structures of the lumbar spine. *Spine* **10**, 156–157.

Liesi, P., Grönblad, M., Korkala, O., Karaharju, E. & Rusanen, M. (1983) Substance P: a neuropeptide involved in low back pain? *Lancet* **i**, 1328–1329.

O'Connor, B. L. & McConnaughey, J. S. (1978) The structure and innervation of cat knee menisci, and their relation to a "sensory hypothesis" of meniscal function. *Am. J. Anat.* **153**, 431–442.

Pernow, B. (1983) Substance P. *Pharmacol. Rev.* **35**, 85–141.

Wharton, J., Polak, J. M., Bloom, S. R., Will, J. A., Brown, M. R. & Paerse, A. G. E. (1979) Substance P-like immunoreactive nerves in mammalian lung. *Invest. Cell Pathol.* **2**, 3.

Wharton, J., Polak, J. M., Pearse, A. G. E., McGregor, G. P., Bryant, M. G., Emson, P. C., Bisgard, G. E. & Will, J. A. (1980) Enkephalin-vasoactive intestinal polypeptide (VIP-) and substance P-like immunoreactivity in the carotid body. *Nature* **284**, 269–271.

Wilson, A. S., Legg, P. G. & McNeur, J. C. (1969) Studies on the innervation of the medial meniscus in the human knee joint. *Anat. Rec.* **165**, 485–492.