Short Communications

Peptidergic innervation of the temporomandibular disk in the rat

H. Ichikawa, S. Wakisaka, S. Matsuo and M. Akai

First Department of Oral Anatomy, Faculty of Dentistry, Osaka University, 1–8 Yamadaoka, Suita, Osaka 565 (Japan)
Received 12 September 1988; accepted 2 November 1988

Summary. The peptidergic innervation of the temporomandibular disk was investigated in the postnatal young rat by using an indirect immunofluorescence method. Calcitonin gene-related peptide-containing nerve fibers were located around the blood vessels and terminated as free nerve endings in the disk. These nerve fibers may be of a sensory nature.
Key words. Calcitonin gene-related peptide; immunohistochemistry; temporomandibular disk; rat.

Some previous studies on laboratory animals and man have shown certain differences in innervation of the temporomandibular disk at early and later ages[1–3]. A number of nerve fibers were observed within the disk at early ages. In the older animals, however, nerves were found only in the periphery of the disk, at its border with the capsule. The functional significance of these nerve fibers in the disk was unknown. Recently, several neuropeptides have been demonstrated in the central and peripheral nervous systems by immunohistochemistry. However, little is known about the peptidergic innervation of the disk and other soft tissues in the temporomandibular joint (TMJ). The elucidation of the distribution of peptidergic innervation of the disk in comparison with other TMJ soft tissues may facilitate the understanding of the functional significance of nerve fibers in the disk.

In the present study the innervation of the disk and other TMJ soft tissues at postnatal early ages was investigated by the use of indirect immunohistochemistry with antisera against calcitonin gene-related peptide (CGRP), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP).

Materials and methods. Wistar strain rats on postnatal days 0–10 were used. Three animals of each age were anesthetized with 5% urethane, and perfused through the heart with Zamboni fixative[4]. Large blocks of TMJ containing mandibular condyle, temporal bone and environmental soft tissues were removed, immersed at 4 °C in the same fixative for 12 h and then placed in phosphate buffered saline containing 30% sucrose for 24 h. Subsequently the blocks were cut into sections 30 µm thick on a cryostat. The sections were processed for the immunohistochemical demonstration of CGRP, NPY and VIP using the indirect immunofluorescence method of Coons[5]. The first layer of antibodies was rabbit anti-CGRP serum (dilution 1:2000, MILAB Co., Sweden), rabbit anti-NPY serum (dilution 1:800, MILAB Co., Sweden) or rabbit anti-VIP serum (dilution 1:1000, INRC, USA). The second layer of antibody for all sections was goat anti-rabbit immunoglobulin labelled with fluorescein isothiocyanate (dilution 1:1000, Dakopatts Co., Denmark). Control sections were processed in parallel and in the same manner, except that they were incubated with primary antisera preabsorbed with the appropriate synthetic neuropeptide (100 µg/ml) or normal rabbit serum instead of primary antisera. No immunoreactivity was observed in control sections.

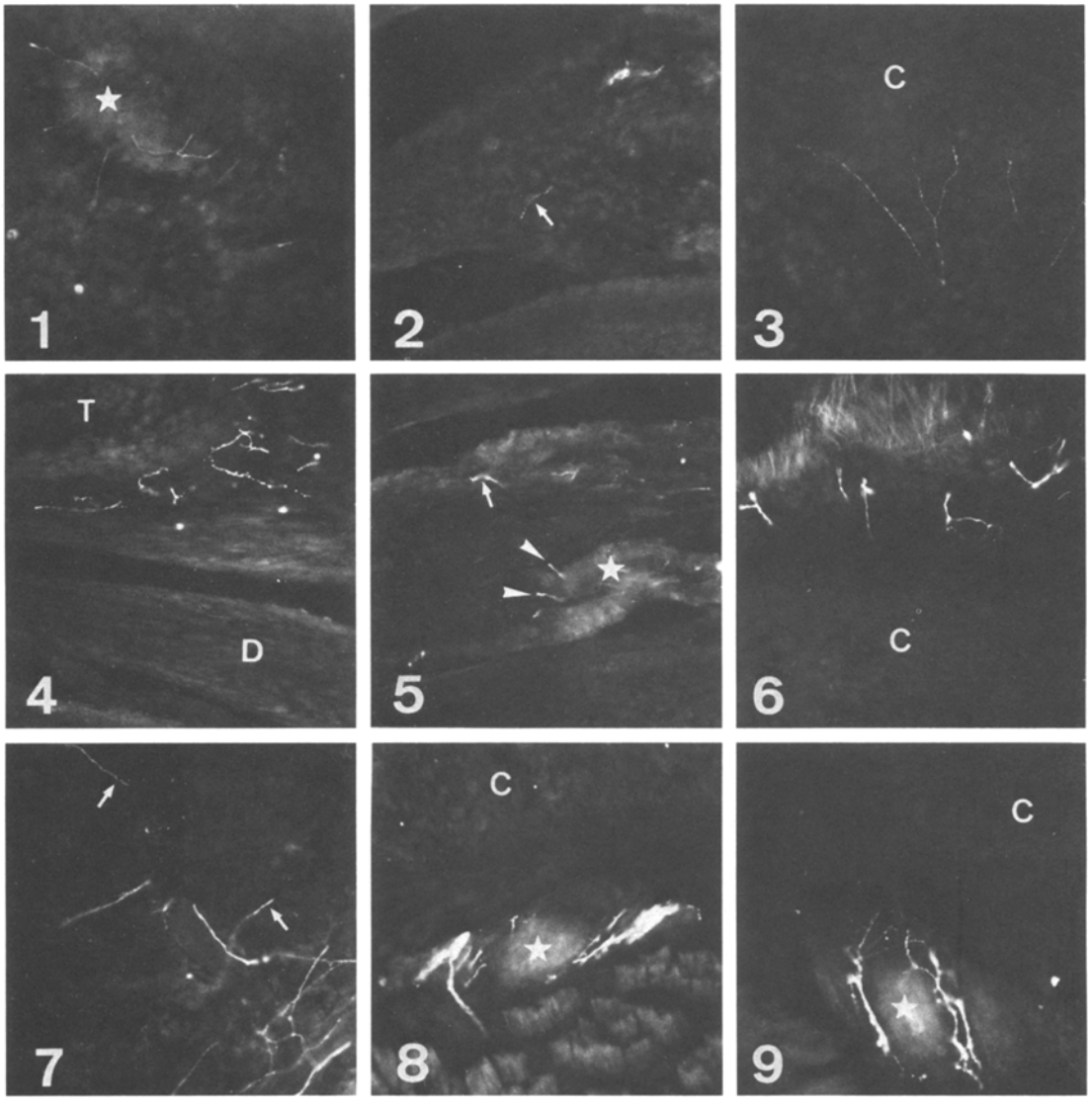
Results and discussion. On postnatal days 0–10, the temporal bone covered the mandibular condyle which was composed of hyaline cartilage. The fibrous articular disk intervened between the articular surface of the temporal bone and the condyle and divided the articular space into the superior and inferior compartments. The disk was united with the articular capsule and condyle at its margins. The fibrous connective tissue encased the articular surface of the temporal bone and the entire condyle. Some blood vessels and nerve bundles were distributed in the capsule.

CGRP-like immunoreactivity. At birth CGRP-like immunoreactive (CGRP-LI) nerve fibers were seen within some nerve bundles and around the blood vessels in the capsule (fig. 1). No CGRP-LI nerve fibers were observed in the disk at birth. At postnatal day 3 some CGRP-LI nerve fibers were distributed in the capsule accompanying blood vessels, approaching the anterior marginal region of the disk band and entering the disk as free nerve endings (fig. 2). CGRP-LI nerve fibers were also found in the fibrous tissue around the condyle as free nerve endings (fig. 3). At postnatal day 5, in addition to the CGRP-LI nerve fibers described above, CGRP-LI nerve fibers were seen in the fibrous tissue around the temporal bone as free nerve endings (fig. 4). At postnatal day 10 the number of CGRP-LI nerve fibers appeared to increase in the disk as well as in the fibrous tissue around the condyle (figs 5, 6). Many CGRP-LI nerve fibers were seen entering the disk at its anterior attachment margin and proceeding, with blood vessels, to the thin central portion. Some of these CGRP-LI nerve fibers were separated from the blood vessels and branched as free nerve endings within the disk (fig. 5). Many CGRP-LI nerve fibers were observed not only around blood vessels but also as free nerve endings in the anterior part of the capsule (fig. 7).

NPY- and VIP-like immunoreactivities. At birth NPY- and VIP-like immunoreactive (NPY- and VIP-LI) nerve fibers were seen within some nerve bundles and around the blood vessels in the capsule (figs 8, 9). These distribution patterns of NPY- and VIP-LI nerve fibers did not change thereafter. No immunoreactive nerve fibers were observed in the disk at any age.

The present study demonstrates the existence of CGRP-containing nerve fibers in the temporomandibular disk. The innervation of the disk has already been the subject of some studies[1–3]. At early ages the disk was shown to be penetrated by numerous nerve fibers, but at later ages these nerve fibers were thought to degenerate and persist only in the capsule. The CGRP-containing nerve fibers in the disk which are described here may degenerate at later ages. The functional significance of CGRP-containing nerve fibers in the disk is unclear, but CGRP-containing fibers are known to be one of the largest subpopulations of peptidergic primary sensory neurons[6]. Thus, CGRP-containing nerve fibers in the disk as well as in other TMJ soft tissues may be of a sensory nature. Previous physiological studies suggested that sensory nerve fibers in the TMJ mediate pain and proprioceptive impulses[7,8]. CGRP-containing nerve fibers in the disk and other TMJ soft tissues may be associated with the mediation of pain or/and proprioceptive sensations.

The present study also demonstrates the presence of NPY- and VIP-containing nerve fibers around blood vessels in the capsule. NPY occurs in peripheral noradrenergic neurons, and a further study has shown that NPY and noradrenaline coexist in sympathetic peripheral nerve fibers[9]. VIP has been demonstrated in cholinergic nerve cells innervating blood vessels[10]. Our observations suggest that many blood vessels



Immunofluorescent photomicrographs of TMJ sections incubated with CGRP (figs 1–7), NPY (fig. 8) and VIP (fig. 9) antisera. ×70. Figure 1. At birth. Some CGRP-LI nerve fibers are seen around the blood vessel (asterisk) in the capsule. Figure 2. Postnatal day 3. A CGRP-LI nerve fiber in the marginal region of the anterior disk attachment is seen to enter the disk as a free nerve ending (arrow). Figure 3. Postnatal day 3. Some CGRP-LI nerve fibers are seen in the fibrous connective tissue around the condyle (C) as free nerve endings. Figure 4. Postnatal day 5. Numerous CGRP-LI nerve fibers are seen in the connective tissue at the articular surface of the temporal bone (T). D, articular disk. Figure 5. Postnatal day 10. Some CGRP-LI nerve fibers (arrowheads) in the disk are seen around the blood vessel (asterisk). Another nerve fiber is seen as a free nerve ending (arrow). Figure 6. Postnatal day 10. Some CGRP-LI nerve fibers are seen as free nerve endings in the fibrous connective tissue close to the condyle (C). Figure 7. Postnatal day 10. Some CGRP-LI nerve fibers are seen as free nerve endings (arrows) in the anterior part of the capsule. Figure 8. At birth. Numerous NPY-LI nerve fibers are seen around the blood vessel (asterisk) in the capsule. C, condyle. Figure 9. At birth. Numerous VIP-LI nerve fibers are seen around the blood vessel (asterisk) in the capsule. C, condyle.

in the TMJ are innervated by numerous noradrenergic/NPY-containing and cholinergic/VIP-containing autonomic nerve fibers. These peptidergic nerve fibers may be involved in the regulation of local blood flow in the TMJ region.

1 Bernick, S., Oral Surg. *15* (1962) 488.
2 Dixon, A. D., Oral Surg. *15* (1962) 48.
3 Thilander, B., Acta odontol. scand. *22* (1964) 151.
4 Zamboni, L., and De Martino, C., J. Cell Biol. *35* (1967) 148 A.
5 Coons, A. H., in: General Cytochemical Methods, p. 399. Academic Press, New York 1958.
6 Uddman, R., Grunditz, T., and Sundler, F., Scand. J. dent. Res. *94* (1966) 219.
7 Clark, R. K. F., and Wyke, B. D., J. Dent. *2* (1973) 121.
8 Kawamura, Y., and Majima, T., J. dent. Res. *43* (1964) 150.
9 Lundberg, J. M., Terenius, L., Hökfelt, T., Martling, C. R., Tatemoto, K., Mutt, V., Polak, J., Bloom, S., and Goldstein, M., Acta physiol. scand. *116* (1981) 477.
10 Lundberg, J. M., Hökfelt, T., Schultzberg, M., Uvans-Wallensten, K., Kohler, C., and Said, S., Neuroscience *4* (1979) 1539.

0014-4754/89/030303-02$1.50 + 0.20/0