Relation between intra-articular temperature of the arthritic temporomandibular joint and presence of calcitonin gene-related peptide in the joint fluid

A clinical study

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Arthritic temporomandibular joints were investigated for intra-articular temperature and joint fluid content of calcitonin gene-related peptide. Eleven patients (16 joints) with rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, or chronic unspecific polyarthritis or monarthritis participated in the study. The intra-articular temperature varied between 35.5 and 37.5°C, with a mean of 36.5°C. The concentration of calcitonin gene-related peptide varied between 7.5 and 749.0 pmol/l, with a mean of 108.6 pmol/l. There was a positive correlation between the intra-articular temperature and the joint fluid concentration of calcitonin gene-related peptide. The plasma level of the peptide was on an average 5% of the joint fluid level. □ *Arthritis; inflammatory joint disease; rheumatoid arthritis; temporomandibular joint diseases*

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Like other joints of the body the temporomandibular joint (TMJ) can be affected by a wide variety of diseases (1). The most prevalent of the inflammatory joint diseases is rheumatoid arthritis (RA). The extent and severity of the inflammatory involvement of the TMJ vary considerably among individuals. About every second to third individual with RA can expect to have the TMJs involved (2). Nearly half of these individuals experience symptoms from the TMJ within 1 year of the onset of the general disease. The inflammatory joint disease may also appear as a generalized or local process of unspecific nature and unknown etiology. We have little knowledge about the factors that influence the development of the local inflammatory process—that is, the development of microcirculatory disturbances, pain, and tissue destruction. However, there is now reason to assume that neuropeptides contribute to the local development of joint inflammation.

The intra-articular temperature (IAT) of the TMJ in patients with RA is frequently abnormal (3). Decreased IAT suggests a decreased blood flow and metabolism in the TMJ or nearby muscles. A significant part of the IAT reduction that occurs in the arthritic TMJ has recently been shown to be due to release of neuropeptide Y (NPY) (4). Increased IAT, on the other hand, has been associated with increased blood flow and assumed to be a sign of 'active' disease (3). Calcitonin gene-related peptide (CGRP), which is found in nociceptive C-fibers together with substance P (SP) (5), has a strong vasodilatory effect in joints and muscles. In contrast to SP it does not increase the vascular permeability and has little or no ability to induce edema (6, 7). Clinically, CGRP has been found in higher concentrations in the knee joint of arthritic patients than in controls (8) and in concentrations greatly above plasma level in RA of the TMJ (9). Owing to its known physio-

logic effects on the microcirculation and the fact that it has been found in increased amounts in arthritic joints, we found it important to investigate its relationship to the microcirculation in the arthritic TMJ. The specific aim of this study was therefore to determine whether changes of IAT in arthritic TMJs are associated with changes of CGRP in the joint fluid.

Materials and methods

Patients

This study comprised 11 patients (16 joints), all women, with a mean age of 32.6 years and having signs and symptoms of TMJ arthritis. The primary inclusion criteria were local pain and palpatory tenderness laterally or posteriorly of the TMJ. The pain had to have been present for at least 6 weeks. The second criterion (except for the diagnosis of CUMA below) was the presence of inflammatory joint disease of systemic nature. Patients whose symptoms could be referred mainly to the muscular component or to disease in other components of the craniomandibular system were excluded. Four patients had RA (one positive for rheumatoid factor, two had ankylosing spondylitis (AS), one had psoriatic arthritis (PA), three had chronic unspecific polyarthritis (CUPA), and one had seronegative chronic unspecific monoarthritis (CUMA) (Table 1). The mean duration of disease was 3.1 years. Only painful and arthritic joints were punctured and examined, bilaterally in five patients and unilaterally in six. Examinations of the patients were made at two sessions, on an average of 2.1 months apart (range, 0.4–4.8; SD, 1.0 months).

Table 1. Age, sex, diagnosis, and duration of symptoms in 11 patients with temporomandibular joint arthritis

Patient	Age (years)	Sex	Diagnosis	Duration (years)
A	48	F	RA (RF-positive)	0.3
B	33	F	RA (RF-negative)	1.5
C	35	F	RA (RF-negative)	4.0
D	19	F	RA (RF-negative)	1.0
E	57	F	AS	3.0
F	30	F	AS	4.0
G	46	F	PA	6.0
H	29	F	CUPA	2.0
I	51	F	CUPA	6.0
J	37	F	CUPA	1.0
K	24	F	CUMA	5.0
Mean	32.6			3.1
SD	13.09			2.06

RA = rheumatoid arthritis; AS = ankylosing spondylitis; PA = psoriatic arthritis; CUPA = chronic unspecific polyarthritis; and CUMA = chronic unspecific monarthritis.

Temperature recordings

The IAT was measured to assess vascular (microcirculatory) changes associated with acute or chronic joint inflammation. The IAT of the TMJs was measured after 30 min of rest and before the aspiration of joint fluid or saline washing. The temperature measurement was made with a thin thermocouple probe (Exacon C-N5) through the same cannula as used for aspiration. The temperatures were recorded with a digital thermometer (Exacon MC 9200) with an accuracy of 0.1°C.

Table 2. Distribution of intra-articular temperature (IAT) (°C) at two sessions and their change in 11 patients with temporomandibular joint arthritis

		IAT		
Patient		Session 1	Session 2	Change 1 − 2
A	R	36.6	36.1	−0.5
B	R	37.0*	36.0	−1.0*
	L	36.8†	35.8†	−1.0*
C	R	36.1	37.0*	+0.9*
	L	36.2	37.5*	+1.3*
D	L	36.8†	35.5*†	−1.3*
E	R	36.2	35.7*†	−0.5
	L	35.7*	36.3†	+0.6*
F	L	37.2*	36.5	−0.7*
G	R	36.9	36.3	−0.6*
H	R	36.7	36.1†	−0.6*
	L	36.7	36.2	−0.5
I	R	37.3*	36.8	−0.5
	L	36.8	37.0*	+0.2
J	L	36.5†	35.6*†	−0.9*
K	L	37.3*	36.9	−0.4

R = right; L = left.
* Outside the normal IAT range (Åkerman & Kopp (13)).
† Joints aspirated without saline.

Joint fluid

An attempt was first made to aspirate undiluted joint fluid. If this was impossible, 1.0 ml saline was injected and aspirated after 20 sec. The samples were diluted in 0.25 ml heparin and 0.25 ml aprotinin (Trasylol®) and then immediately cold-centrifuged (800 *g* for 2 min) and frozen (−70°C).

CGRP-LI

The synovial fluid and blood plasma were analyzed for CGRP-like immunoreactivity (CGRP-LI). Samples were filtered, using a reverse-phase C18 cartridge (Sep Pak, Waters), and analyzed by means of a competitive radioimmunoassay (RIA) (10). CGRP-LI was analyzed with antiserum CGRPR8 raised against conjugated rat CGRP. High-performance liquid chromatography-purified ^{125}I-histidyl rat CGRP was used as radioligand, and rat CGRP as standard. The cross-reactivity of the assay to SP-LI, neurokinin A (NKA)-LI, neurokinin-B (NKB)-LI, neuropeptide K (NPK)-LI, gastrin, neurotensin, bombesin, NPY-LI, and calcitonin was less than 0.01%. Cross-reactivity towards CGRP alpha and beta was 93% and 24%, respectively, and towards rat CGRP alpha and beta 100% and 120%, respectively. Intra- and inter-assay coefficients of variation were 8% and 14%, respectively. Normal values for blood/plasma obtained with the RIA used in this study are <96 pmol/l for CGRP-LI (10–12).

Statistics

The correlation between changes in IAT and CGRP-LI was tested with Pearson's product-moment correlation coefficient. The differences in concentration of CGRP-LI, duration, and age were tested by the two-tailed *t* test. A probability level of $p < 0.05$ was considered significant.

Results

Joint fluid samples were aspirated from 11 patients (16 joints) (Table 1). The average volume of aspirated joint fluid was 0.8 ml.

Table 3. Concentration of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) in joint fluid aspirated with or without saline from 11 patients with arthritic temporomandibular joints at two sessions and their change

Patient		CGRP-LI (pmol/l) Session 1	Session 2	Relative change 2/1
A	R	115.9	92.0	0.79
B	R	372.4	95.2	0.26
	L	749.0*	324.9*	0.43
C	R	115.4	111.9	0.97
	L	104.8	90.8	0.87
D	L	55.6*	26.2*	0.47
E	R	41.4	30.7*	0.74
	L	67.2	72.7*	1.08
F	L	20.0	7.5	0.38
G	R	60.0	19.8	0.33
H	R	279.1	104.8*	0.38
	L	137.0	48.6	0.35
I	R	31.6	25.4	0.80
	L	26.4	32.0	1.21
J	L	32.9*	17.2*	0.52
K	L	120.1	46.3	0.39

R = right; L = left.
* Joint fluid aspirated without saline.

The IAT varied between 35.5°C and 37.5°C, with a mean of 36.5°C (Table 2). The concentration of CGRP-LI varied between 7.5 and 749.0 pmol/l, with a mean of 108.6 pmol/l (Table 3). The intra-individual absolute changes of IAT between sessions and the relative changes (ratio between sessions) of CGRP-LI showed a positive linear correlation ($r = 0.69$, $p < 0.03$, $Y = 0.69 + 0.31X$, $n = 10$ joints) (Fig. 1b) when saline aspirations were performed at both sessions. A similar but stronger positive linear correlation was found for undiluted joint fluid and for a combination of saline and joint fluid ($r = 0.88$, $p < 0.02$, $Y = 0.82 + 0.35X$, $n = 6$ joints) (Fig. 1a). For all 16 joints combined there was a positive linear correlation of intermediate strength ($r = 0.73$, $p < 0.001$, $Y = 0.73 + 0.30X$). The duration of TMJ symptoms was on an average 1.7 years (SD, 1.15) in the patients with an IAT of less than 36.0°C, 2.8 years (SD, 2.93) in the patients with an IAT of 36.0–36.9°C, and 4.1 years (SD, 1.67) in the patietns with IAT more than 36.9°C. The corresponding figures

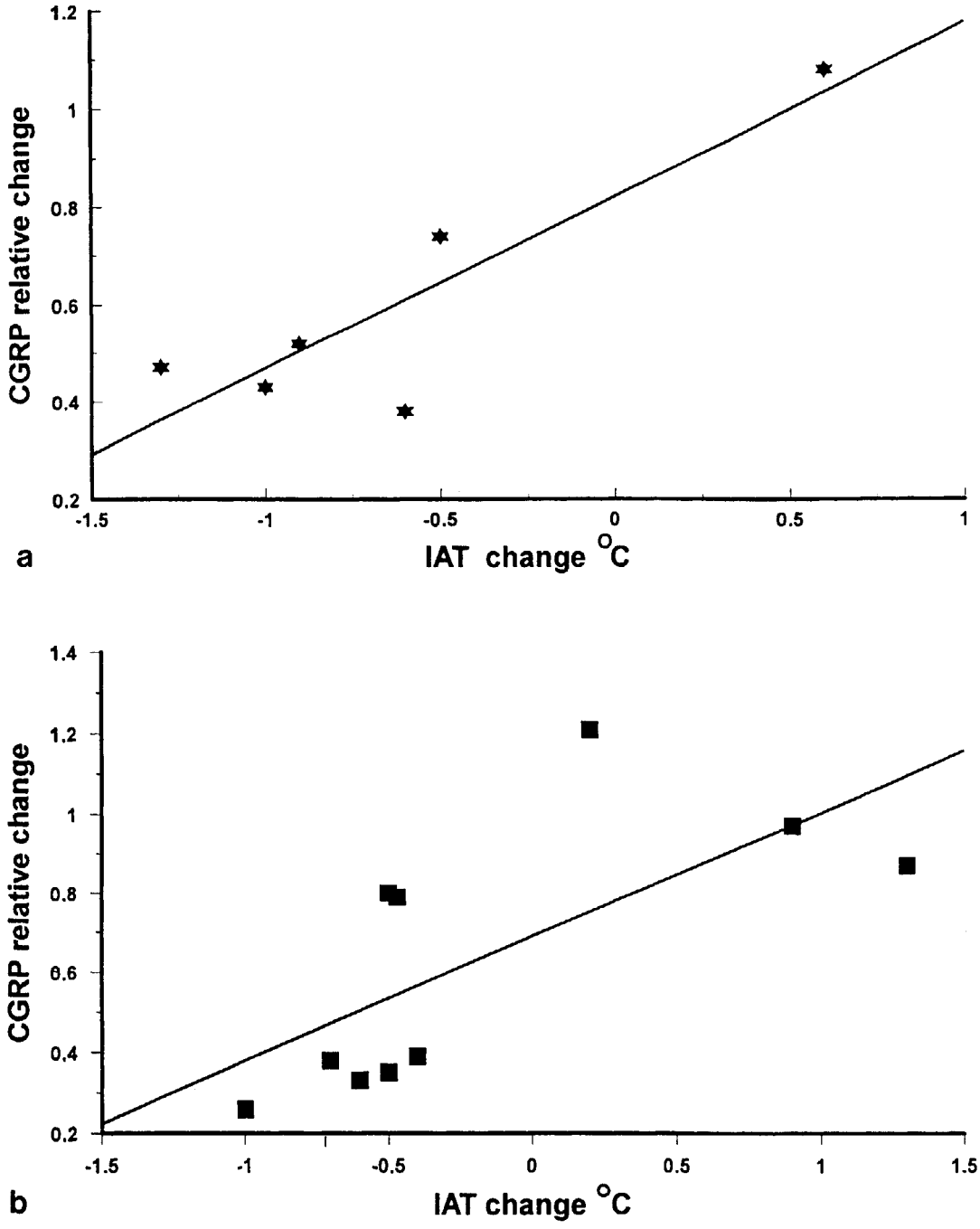

Fig. 1. Relation between changes of intra-articular temperature (IAT °C) and relative changes in calcitonin gene-related peptide (CGRP) in patients with arthritic temporomandibular joints. IAT change is the absolute difference in temperature between two sessions. 1a. Joint fluid or saline aspiration: $r = 0.88$, $p < 0.02$, $Y = 0.82 + 0.35X$, $n = 6$ joints. 1b. Saline aspirations: $r = 0.69$, $p < 0.03$, $Y = 0.69 + 0.31X$, $n = 10$ joints.

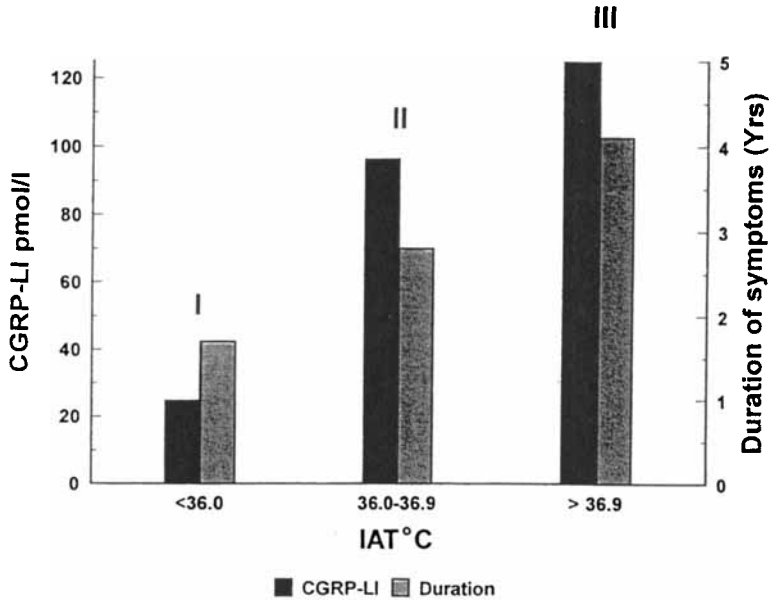

Fig. 2. Eleven patients with arthritic temporomandibular joints allocated to three groups on the basis of intra-articular temperature (IAT). Group I ($n = 3$) with IAT < 36.0°C, group II ($n = 3$) with IAT 36.0–36.9°C, and group III ($n = 5$) with IAT > 36.9°C. There was a significant difference between groups I + II and III with regard to calcitonin gene-related peptide-like immunoreactivity ($p < 0.04$).

for CGRP-LI were 24.7 pmol/l (SD, 6.87), 96.3 pmol/l (SD, 38.68), and 127.0 pmol/l (SD, 143.29) (Fig. 2). The difference in CGRP-LI between patients with low and high IAT was significant ($p < 0.04$), but not the difference in duration. The joint fluid concentration of CGRP-LI was not correlated to age.

The plasma concentration of CGRP-LI varied between 1.3 and 9.8 pmol/l, with a mean of 5.4 pmol/l (Table 4). The absolute changes between sessions were slight, with a mean of 2.0 pmol/l. The concentration of CGRP-LI in the joint fluid was always higher than in plasma. The plasma value was on an average 5% of the joint fluid value.

Discussion

Three of the patients in this study (27%) showed IAT within the normal range (36.0–36.9°C) modified after Åkerman & Kopp (13) at both visits. Three (27%) had hypothermia at one of the visits, whereas another five patients (45%) had intra-articular hyperthermia. The temperature distribution in this study deviates somewhat from that for patients with seropositive RA reported by Åkerman & Kopp 1988 (3). More patients in this study had hyperthermia. The differences between right and left joints were above the normal range (0–0.5°C) in eight of the patients (73%). Two of these patients belonged to a group with 'normal' temperatures, which probably means that circulatory disturbances occur also within this interval.

Decreased or increased IAT and ensuing

Table 4. Concentration of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) in blood plasma from 10 patients with arthritic temporomandibular joints at two sessions and its change

	CGRP-LI (pmol/l)	
Patient	Session 1	Session 2
A	3.2	4.8
B	—	—
C	—	9.7
D	6.6	5.5
E	7.9	6.0
F	3.2	3.6
G	3.8	3.2
H	6.5	2.8
I	9.8	1.3
J	9.2	7.0
K	4.7	2.9

temperature asymmetry in inflammatory joint disease of the TMJ have been assumed to be a consequence of microcirculatory changes in the synovial membrane or nearby muscles (14). The exact mechanism behind the circulatory changes is unknown, although factors such as inflammation, disuse atrophy, chronic muscle tension, and vasoconstriction due to sympathetic nerve activation have all been suggested (3). Attention has recently been focused on the role played by neuroendocrine peptides released from nerves in the synovial membrane of the joint, and NPY has recently been found to explain a significant part of the IAT reduction observed in the arthritic TMJ (4).

In this study an increase in joint fluid content of CGRP-LI between sessions was associated with a corresponding increase in IAT. This might be explained by the physiologic properties of CGRP, although it is impossible to draw any conclusions about cause and effect from this study. CGRP is probably one of several important mediators of vasodilation and hyperemia in the inflammatory process. The positive correlation was stronger for undiluted joint fluid than for joint fluid aspirated by saline, as was found earlier for the negative relationship between NPY-LI and IAT (4). The reason for this difference could be that saline washing results in an underestimation of the true concentration of CGRP-LI in the joint fluid, and there is probably a variation in the recovery rate of CGRP-LI between washings. Nevertheless, the regression lines are almost identical and thus show practically the same results. Another interesting finding is that the joint fluid of the diseased TMJ has been shown to contain significantly higher concentrations of CGRP than that of the arthritic knee joint (15).

When the patients were divided into groups with low/medium and high IAT there was also a significant difference in absolute values of CGRP-LI between the groups. The patients with high IAT had the highest joint fluid content of CGRP-LI and the longest duration of symptoms. The opposite relation was found between NPY-LI and the duration of symptoms; that is, NPY-LI decreased with duration (4).

CGRP-LI is released from sensory nerves whose distribution has been investigated in both normal and inflamed human synovial tissue (16). CGRP-LI immunostaining was predominantly found perivascularly. CGRP was not visible in the superficial tissues in the rheumatoid synovial membrane, and immunostaining was weaker in the diseased deeper tissue than in control patients. It was suggested that the reduced immunostaining might be caused by increased release of CGRP, reducing the stores in the nerves to undetectable levels. This is in agreement with our findings of CGRP-LI in the joint fluid of the TMJ, where the level of CGRP-LI greatly exceeded that of plasma despite the dilution in the saline washings. Therefore it can be concluded that CGRP is likely to be released locally in the joint.

In studies of the TMJ in the young rat, CGRP-LI has been found in the anterior margin of the disk, the fibrous tissue around the condyle, and the capsule (17). Similar to SP some of the CGRP-LI nerve fibers accompanied blood vessels, and some branched as free nerve endings. In blood vessels the fibers are localized in the periphery of the adventitia and have diameters varying from 200 to 500 nm (18). The CGRP-immunoreactive nerve fibers lacked synaptic contact with the aforementioned tissue components.

It was outside the scope of this study to determine why the IAT and the CGRP-LI decreased on an average basis between sessions. The changes might be explained in the future by the therapy administered during the period of study or by periodic change in disease activity. The patients in this study belonged to various diagnostic categories but were still in the group of inflammatory joint diseases. The patients can therefore be expected to have many aspects of the inflammatory process in common.

The results of this study indicate that a significant part of the increase of IAT occurring in arthritic TMJs can be attributed to an increase of CGRP in the joint fluid.

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References

1. Carlsson GE, Kopp S, Öberg T. Arthritis and allied diseases. In: Zarb GA, Carlsson GE, editors Temporomandibular joint. Function and dysfunction. Copenhagen: Munksgaard; St Louis [MO]: Mosby Co, 1979:269–320.
2. Tegelberg Å, Kopp S. Subjective symptoms from the stomatognathic system in individuals with rheumatoid arthritis and osteoarthrosis. Swed Dent J 1987;11:11–22.
3. Åkerman S, Kopp S. Intra-articular and skin surface temperature of the temporomandibular joint in patients with rheumatoid arthritis. Acta Odontol Scand 1988;46:41–8.
4. Appelgren A, Appelgren B, Kopp S, Lundeberg T, Theodorsson E. Relation between intra-articular temperature of the temporomandibular joint and presence of neuropeptide Y in the joint fluid. A clinical study. Acta Odontol Scand 1993;51:1–8.
5. Lundberg JM, Franco-Cereceda A, Hua X-Y, Hökfelt T, Fischer JA. Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. Eur J Pharmacol 1985;108:315–9.
6. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 1988;24:739–68.
7. Raud J, Lundeberg T, Brodda-Jansen G, Theodorsson E, Hedqvist P. Potent anti-inflammatory action of calcitonin gene-related peptide. Biochem Biophys Res Commun 1991;180:1429–35.
8. Larsson J, Ekblom A, Henriksson K, Lundeberg T, Theodorsson E. Concentration of substance P, neurokinin A, calcitonin gene-related peptide, neuropeptide Y and vasoactive intestinal polypeptide in synovial fluid from knee joints in patients suffering from rheumatoid arthritis. Scand J Rheumatol 1991;20:326–35.
9. Appelgren A, Appelgren B, Eriksson S, et al. Neuropeptides in temporomandibular joints with rheumatoid arthritis: a clinical study. Scand J Dent Res 1991;99:519–21.
10. Theodorsson-Norheim E, Hemsen A, Brodin E, Lundberg JM. Sample handling techniques when analyzing regulatory peptides. Life Sci 1987;41:845–8.
11. Theodorsson-Norheim E, Brodin E, Norheim I, Rosell S. Antisera raised against eledoisin and kassinin detect immunoreactive material in rat tissue extracts: tissue distribution and chromatographic characterization. Regul Peptides 1984;9:229–44.
12. Theodorsson-Norheim E, Hemsen A, Lundberg JM. Chromatographic characterization of immunoreactivity in plasma and tissue extracts. Scand J Clin Lab Invest 1985;45:355–66.
13. Åkerman S, Kopp S. Intra-articular and skin surface temperature of the human temporomandibular joint. Scand J Dent Res 1987;95:493–8.
14. Kopp S, Åkerman S, Nilner M. Short-term effects of intra-articular sodium hyaluronate, glucocorticoid, and saline injections on rheumatoid arthritis of the temporomandibular joint. J Craniomandib Disord Facial Oral Pain 1991;5:231–8.
15. Holmlund A, Ekblom A, Hansson P, Lind J, Lundeberg T, Theodorsson E. Concentrations of neuropeptides substance P, neurokinin A, calcitonin gene-related peptide, neuropeptide Y and vasoactive intestinal polypeptide in synovial fluid of the human temporomandibular joint. A correlation with symptoms, signs and arthroscopic findings. Int J Oral Maxillofac Surg 1991;20:228–31.
16. Mapp PI, Kidd BL, Gibson SJ, et al. Substance P-, calcitonin gene-related peptide- and c-flanking peptide of neuropeptide Y-immunoreactive fibers are present in normal synovium but depleted in patients with rheumatoid arthritis. Neuroscience 1990;37:143–53.
17. Ichikawa H, Wakisaka S, Matsuo S, Akai M. Peptidergic innervation of the temporomandibular disk in the rat. Experientia 1989;45:303–4.
18. Ichikawa H, Matsuo S, Wakisaka S, Akai M. Fine structure of calcitonin gene-related peptide-immunoreactive nerve fibres in the rat temporomandibular joint. Arch Oral Biol 1990;35:727–30.

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