Relation between the intra-articular temperature of the temporomandibular joint and the presence of neuropeptide Y-like immunoreactivity in the joint fluid

A clinical study

Anna Appelgren, Björn Appelgren, Sigvard Kopp, Thomas Lundeberg and Elvar Theodorsson

Departments of Clinical Oral Physiology and Physiology I and II, Karolinska Institutet, Huddinge, and Department of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden

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Arthritic temporomandibular joints were examined for the joint fluid content of neuropeptide Y-like immunoreactivity (NPY-LI) and the intra-articular temperature at two separate sessions. Sixteen patients (23 joints) with rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and unspecific polyarthritis or monarthritis were investigated in this study. The intra-articular temperature ranged between 35.6 and 37.5 °C. The concentration of NPY-LI ranged between 72.1 and 4466.0 pmol/l and was above the normal plasma level in all patients. The intra-articular temperature was negatively correlated with the joint fluid concentration of NPY-LI. Moreover, patients with low intra-articular temperature and high concentration of NPY-LI had a shorter duration of TMJ symptoms than those with high intra-articular temperature and low concentration of NPY-LI. □ *Arthritis; inflammatory joint disease; neuropeptide Y; rheumatoid arthritis; temperature; temporomandibular joint*

Anna Appelgren, Department of Clinical Oral Physiology, School of Dentistry, Karolinska Institutet, Box 4064, S-141 04 Huddinge, Sweden

The intra-articular temperature (IAT) of the temporomandibular joint (TMJ) in patients with rheumatoid arthritis (RA) is frequently abnormal (1, 2). Temperature reduction has been found to correlate with joint/muscle pain and tenderness (2). Furthermore, patients with unilateral joint involvement have a temperature reduction on the symptomatic side resulting in a temperature asymmetry between sides. This asymmetry was associated with joint crepitus, and thus with structural damage of the joint (3). Decreased temperature has been assumed to suggest a chronic or late phase of the disease with low or no inflammatory activity, which is associated with a decrease in both blood flow and metabolism in the TMJ or nearby mandibular muscles. The diminished blood flow might be caused by microcirculatory failure (4), sympathetic vasoconstriction (5), increased intra-articular pressure (6, 7), atrophy (8), muscle fibrosis or, more likely, by increased muscle tone. Among these factors sympathetic vasoconstriction is worth investigation, since the TMJ pain seems to be associated with a decreased blood circulation.

Neuropeptide Y (NPY), which is produced together with norepinephrine in certain efferent sympathetic nerve fibers (9), has a strong and a long-lasting vasoconstrictive effect in both arterial and venous blood vessels. In the TMJ of the rat, this neuropeptide was found to be located around blood vessels in the capsule of the TMJ but not in the disk or joint surfaces (10). Intrasynovial release of this peptide might therefore be an explanation of

decreased IAT in the TMJ. NPY also played an important role as a regulator of the severity of joint inflammation, as did substance P in experimentally induced adjuvant arthritis in rats (11). Clinically, NPY has been found in significantly higher concentrations in the synovial fluid of patients with arthritis of the knee than in controls (12, 13) and in concentrations higher than the plasma level in the joint fluid of the TMJ from patients with RA (14). These findings suggest that NPY acts as a modulator of IAT and joint inflammation also in humans.

The aim of this study was to determine whether changes in IAT observed in arthritic TMJs are associated with changes of NPY in the joint fluid.

Patients and methods

Patients

This study comprised 23 TMJs in 16 patients, 15 women and 1 man, with a mean age of 38.3 years, who had signs and symptoms of TMJ arthritis—that is, local pain and tenderness to palpation of the joint. Six of the patients had rheumatoid arthritis (two RF-positive), two ankylosing spondylitis (AS), one psoriatic arthritis (PA), five seronegative chronic unspecific polyarthritis (CUPA), and two chronic unspecific monarthritis (CUMA) (Table 1). The mean duration of disease was 2.4 years. Temperature recordings of the patients were made at two sessions on an average 2.5 months apart (range, 0.5–8.0; SD, 2.22).

Arthrocentesis

The TMJ was punctured with a standard disposable needle with a diameter of 0.65 mm and a length of 30 mm. The needle was inserted into the posterior part of the upper joint compartment, if existing, or into

Table 1. Age, sex, diagnosis, and duration of symptoms in 16 patients with temporomandibular joint arthritis

Patient	Age, years	Sex	Diagnosis	Duration, years
A	23	F	RA (RF+)	0.25
B	48	F	RA (RF+)	0.25
C	33	F	RA (RF−)	1.5
D	35	F	RA (RF−)	4.0
E	75	F	RA (RF−)	1.0
F	19	F	RA (RF−)	1.0
G	57	F	AS	3.0
H	30	F	AS	4.0
I	46	F	PA	6.0
J	29	F	CUPA	2.0
K	27	F	CUPA	2.0
L	51	F	CUPA	6.0
M	37	F	CUPA	1.0
N	27	M	CUPA	0.3
O	23	F	CUMA	5.0
P	53	F	CUMA	1.5
Mean	38.3			2.4
SD	15.32			1.99

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

Table 2. Distribution of intra-articular temperature (IAT) at two sessions and its change in 16 patients with temporomandibular joint arthritis

Patient		IAT, °C Session 1	Session 2	Change 1–2
A	L	36.9	35.9	−1.0
B	R	36.6	36.1	−0.5
C	R	37.0	36.0	−1.0
	L	36.4	35.8	−0.6
D	R	36.1	37.0	+0.9
	L	36.2	37.5	+1.3
E	R	35.9	36.2	+0.3
F	R	37.2	36.1	−1.1
	L	36.8	35.5	−1.3
G	R	35.7	36.5	+0.8
	L	36.3	36.6	+0.3
H	R	36.9	36.6	−0.3
	L	37.2	36.6	−0.6
I	R	36.9	36.3	−0.6
J	R	36.7	36.0	−0.7
	L	36.6	35.8	−0.8
K	L	35.7	36.7	+1.0
L	R	37.3	36.8	−0.5
	L	36.8	37.0	+0.2
M	L	35.6	36.5	+0.9
N	R	35.9	36.8	+0.9
O	L	37.3	36.9	−0.4
P	R	37.0	36.1	−0.9
Mean		36.6	36.4	−0.1
SD		0.55	0.48	0.81
SEM		0.12	0.10	0.17

R = right; L = left; SD = standard deviation; and SEM = standard error of the mean.

pannus tissue filling this part of the joint space.

Temperature recordings

The IAT was measured to assess any vascular changes associated with acute or chronic joint inflammation. The IAT of the TMJs was measured after 30 min of rest and before 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, at a depth of 20–25 mm beneath the skin surface. The temperature was recorded by a digital thermometer (Exacon MC 9200) with an accuracy of 0.1 °C.

Joint fluid

First we attempted to aspirate undiluted joint fluid. If this was impossible, 1.0 ml saline was injected slowly and aspirated after 20 sec. The samples were diluted in 0.25 ml of 5000 IE/ml heparin sodium (Heparin®) and 0.25 ml 10,000 KIE/ml aprotinin (Trasylol®), and then immediately cold-centrifuged (800 *g* for 2 min) and frozen (−70 °C).

NPY-LI

The aspirated fluid was analyzed for NPY-like immunoreactivity (NPY-LI). Samples were filtered by means of a reverse-phase C18 cartridge (Sep Pak, Waters) and analyzed with a competitive radioimmunoassay

Table 3. Concentration of neuropeptide Y-like immunoreactivity (NPY-LI) in joint fluid aspirated with or without saline from 16 patients with arthritic temporomandibular joints at two sessions and their change

		NPY-LI			
Patient		Session 1, pmol/l	Session 2, pmol/l	Change, pmol/l	Relative change
A	L	1089.0	4466.0	+3377.0	4.1
B	R	175.4	152.2	−23.2	0.9
C	R	165.6	248.8	+83.2	1.5
	L	80.1*	668.7*	+588.6	8.4
D	R	226.8	344.4	+117.6	1.5
	L	140.7	348.2	+207.5	2.5
E	R	534.6	1138.8*	+604.2	2.1
F	R	241.2*	1076.4*	+835.2	4.5
	L	298.8*	1219.8	+921.0	4.1
G	R	1275.6	355.2	−920.4	0.3
	L	738.0*	562.4	−175.6	0.8
H	R	233.0	572.7	+339.7	2.5
	L	301.5	2460.3	+2158.0	8.2
I	R	2300.0	2504.0	+204.0	1.1
J	R	449.3	1189.8	+740.5	2.6
	L	403.0*	1004.4	+601.4	2.5
K	L	395.4	72.1*	−323.3	0.2
L	R	103.2	175.2	+72.0	1.7
	L	467.2	276.9	−190.3	0.6
M	L	207.4*	116.8*	−90.6	0.6
N	R	1311.6	229.1	−1082.5	0.2
O	L	1357.2	1930.8	+573.6	1.4
P	R	213.2	852.8	+639.6	4.0
Mean		574.0	968.1	+394.0	2.18
SD		556.68	1063.43	941.87	1.90
SEM		118.68	226.73	200.81	0.41

R = right; L = left; SD = standard deviation; and SEM = standard error of the mean.

* Joint fluid aspirated without saline.

(14). NPY-LI was analyzed using antiserum N1, which crossreacts 1.0% with avian pancreatic polypeptide but not with other peptides (16). Intra- and inter-assay coefficients of variation were 7% and 14%, respectively. The normal value for blood plasma obtained with this radioimmunoassay is <50 pmol/l (15–17).

Statistics

The correlation between changes in IAT and NPY-LI was tested by Pearson's product-moment correlation coefficient (r). The difference in duration of TMJ symptoms and NPY-LI between patients with high and low IAT was tested for statistical significance by analysis of variance (ANOVA) for independent samples. The level of statistical significance (p value) is given when $p < 0.05$.

Results

The distribution of IAT is shown in Table 2, and the distribution of NPY-LI in Table 3.

The IAT ranged between 35.6°C and 37.5°C, with a mean of 36.5°C. NPY-LI ranged between 72.1 and 4466.0 pmol/l, with a mean of 771.0. All NPY values were above normal plasma level, irrespective of whether aspiration was made with saline.

The intra-individual changes between sessions in IAT and relative NPY-LI when

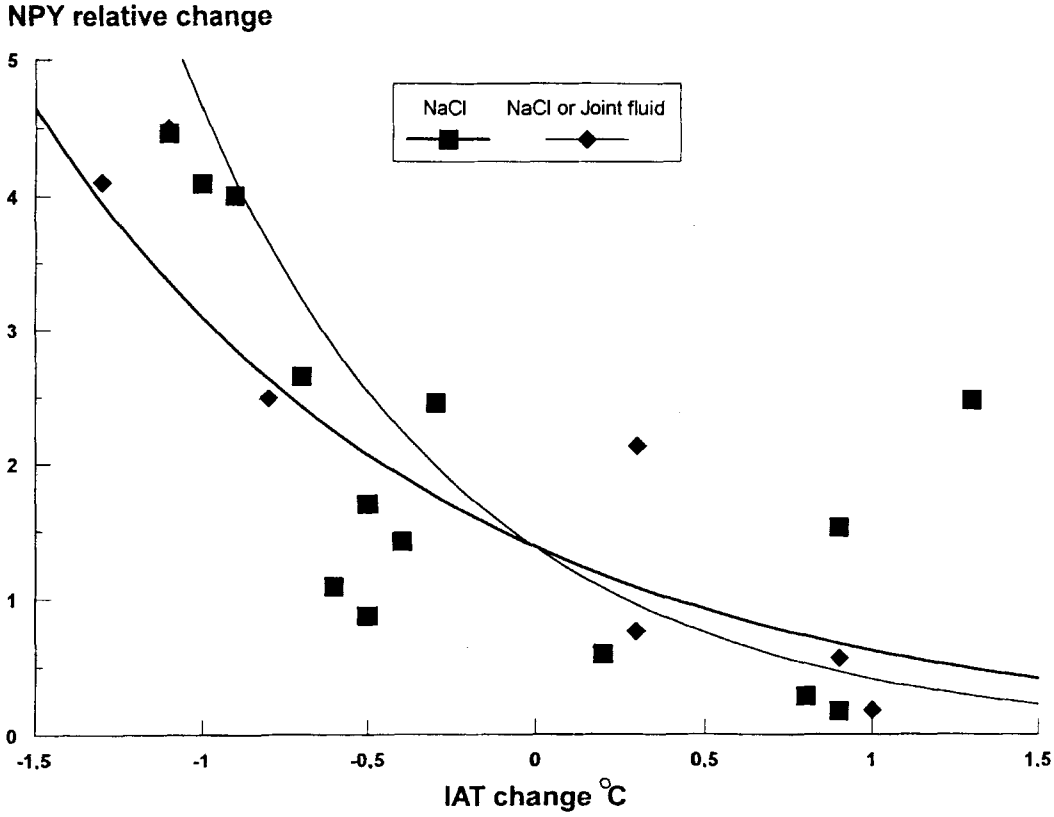

Fig. 1. Relation between changes in intra-articular temperature (IAT °C) and relative changes in neuropeptide Y (NPY) in 16 patients with arthritic temporomandibular joints. IAT change is the absolute difference in temperature between the two sessions. NPY relative change is the ratio between values obtained at session 2 and session 1. Saline aspirations: $r = -0.60$, $p < 0.02$, $Y = 1.38 \times e^{-0.81X}$, $n = 15$ joints. Joint fluid or saline aspiration: $r = -0.86$, $p < 0.01$, $Y = 1.38 \times e^{-1.22X}$, $n = 8$ joints.

saline aspirations were performed at both sessions showed a negative exponential correlation ($r = -0.60$, $p < 0.02$, $Y = 1.38 \times e^{-0.81X}$, $n = 15$ joints) (Fig. 1). A similar negative exponential correlation was found when undiluted joint fluid was aspirated at both sessions or a combination of saline and joint fluid aspirations was performed ($r = -0.86$, $p < 0.01$, $Y = 1.38 \times e^{-1.22X}$, $n = 8$ joints) (Fig. 1). The duration of TMJ symptoms was on an average 1.4 years in the patients with IAT less than 36.0°C, 3.1 years in the patients with IAT 36.0–36.9°C, and 3.3 years in the patients with IAT more than 36.9°C. The corresponding figures for NPY-LI were 1231.5, 722.8, and 372.4 pmol/l (Fig. 2). The differences in duration of TMJ symptoms and NPY-LI between patients with low and high IAT were significant ($p = 0.03$ and $p = 0.04$, respectively).

The individual changes in IAT and NPY-LI between the two sessions for one of the patients (J) are depicted in Fig. 3.

Discussion

Increased or decreased IAT and ensuing temperature asymmetry in inflammatory joint disease has been assumed to be a consequence of microcirculatory changes in the synovial membrane or nearby muscles (3). Acidosis has been found in severely destroyed knee joints of individuals with RA, which indicates an impairment of the synovial microcirculation in severe forms of this

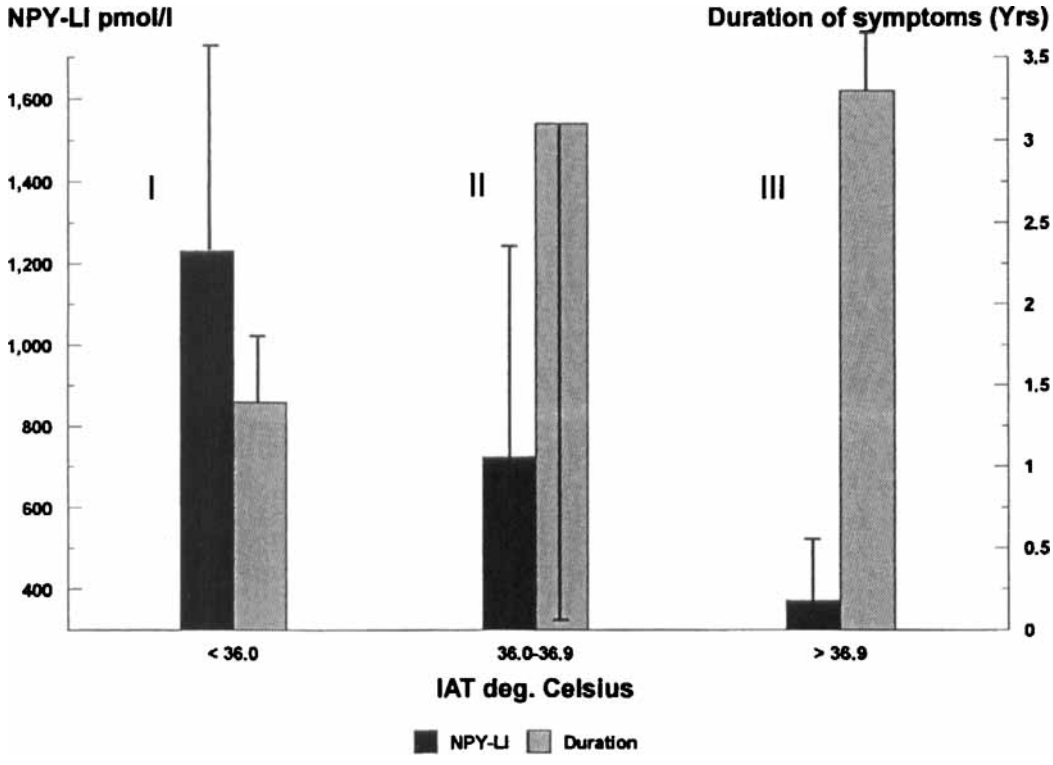

Fig. 2. Sixteen patients with arthritic temporomandibular joints allocated to three groups in accordance with intra-articular temperature (IAT). Group I ($n = 7$) had IAT < 36.0°C; group II ($n = 2$) had IAT 36.0–36.9°C; and group III ($n = 7$) had IAT > 36.9°C. There were significant differences between groups I and III with regard to NPY-LI ($p = 0.03$) and duration of TMJ symptoms ($p = 0.04$). The black bars on top of the columns denote the standard error of the mean (SEM).

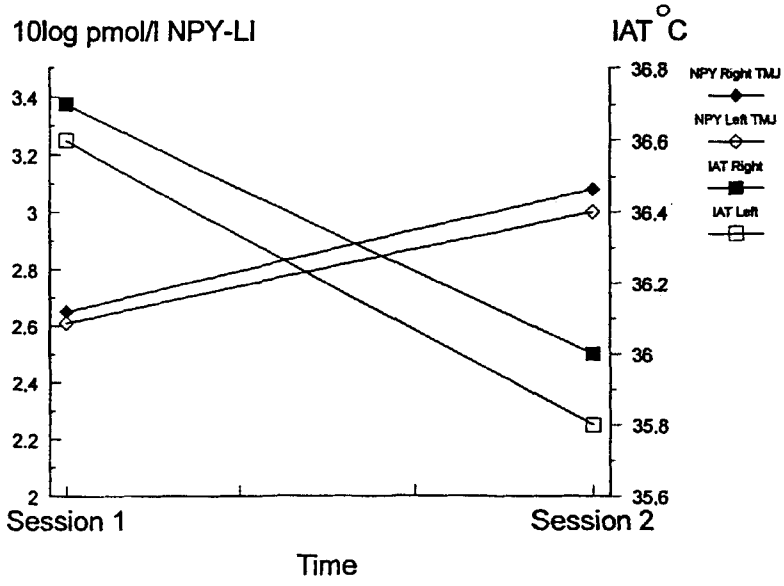

Fig. 3. The changes of intra-articular temperature (IAT°C) and neuropeptide Y-like immunoreactivity (NPY-LI) between the two sessions for one patient (J), 5 months apart. TMJ = temporomandibular joint.

disease (7). The exact mechanisms behind these circulatory changes are unknown, although factors such as inflammation, disuse atrophy, chronic muscle tension, and vasoconstriction due to sympathetic nerve activation have been suggested (2).

In this study a decrease in IAT was associated with increase in joint fluid content of NPY-LI, as can be expected since NPY is a strong vasoconstrictor. It is remarkable, however, that the decrease in IAT occurred in an early phase of the disease, which is in complete contradiction to earlier beliefs. It has been assumed that the decrease in IAT is a late event in the chronic phase of the disease (2, 3). Nevertheless, the present results show that the decrease in IAT is an early sign of pathophysiology in the TMJ and that it might be caused by intrasynovial release of NPY. This mechanism could explain the earlier finding that the skin surface temperature over the TMJ increased with duration of RA from subnormal levels towards normal (18). It was recently reported that IAT asymmetry of the TMJ, owing to unilateral reduction of IAT, decreased with duration of RA (3), which also is in concordance with the results of this study. This study thus indicates that a neural mechanism (sympathetic intrasynovial release of NPY) is the cause of a significant part of the temperature reduction reported previously in TMJs with RA (2, 18). Future studies will investigate whether NPY-LI also is correlated to joint/muscle pain and joint destruction. The mechanisms behind NPY release in joints are unknown, but substance P is known to stimulate NPY release, and pain is known to activate the sympathetic nervous system (19). In the human synovial membrane NPY is located predominantly in perivascular sympathetic nerves (20, 21).

The change in IAT and the relative change (ratio) in NPY-LI correlated significantly between sessions, and when the patients were allocated into groups with low, medium, and high IAT, a significant difference between groups in absolute values of NPY-LI was obtained. The clinical significance of the absolute values of NPY-LI is at present uncertain. The method of saline washing results in an underestimation of the true concentration of the peptide in the joint fluid, since only a fraction is extracted. The recovery rate of NPY-LI from the joint fluid that is obtained with saline washing is at present unknown. The method of saline washing was used of necessity owing to difficulty in obtaining joint fluid samples from all patients. The method, however, seems to

be validated by the ability to give a reliable relative estimate of the amount of NPY-LI, since the exponential curves of changes between sessions were similar for saline washings and undiluted joint fluid. The relative change in NPY-LI was in five instances based on values from undiluted joint fluid and saline washing in combination. These values did not deviate from the curve of correlation to change in IAT. As can be expected, the saline washing estimates demonstrated a greater deviation from the curve, as indicated by the lower value of the correlation coefficient. Nevertheless, the results of this study indicate that saline washing may be used to estimate relative changes in the joint fluid content of NPY-LI.

It was outside the scope of this study to determine why the IAT decreased and NPY-LI increased on an average basis between sessions, but the changes might be explained by therapy administered during the period of study or periodic change in disease activity.

The results of this study indicate that a significant part of the observed changes in IAT of the arthritic TMJ can be explained by release of NPY in the synovial membrane. It was also found that the decrease in IAT combined with increase in joint fluid NPY occurs early in the disease.

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