Elevated Levels of β-Endorphin in Temporomandibular Joint Synovial Lavage Fluid of Patients with Closed Lock

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Aims: *To investigate the presence of endogenous β-endorphin, an opioid, in the synovial lavage fluid of the temporomandibular joint (TMJ), and to compare the concentration of β-endorphin in patients with closed lock with that in symptom-free subjects.* ***Methods:*** *Thirty-eight patients (38 joints) with closed lock diagnosed on the basis of the results of clinical examination and magnetic resonance imaging (MRI) and 11 healthy volunteers (19 joints) were examined. Samples of lavage fluid were obtained prior to arthrocentesis by washing the joint with saline. Samples were assayed for β-endorphin by an enzyme immunoassay, and concentrations of protein were measured by a bicinchoninic acid assay. Subjective pain was assessed by patients using a visual analog scale. Bone changes in the condyle were assessed by MRI, and synovitis was assessed on the basis of arthroscopic findings.* ***Results:*** *β-endorphin was present in the synovial fluid of the TMJ, and the concentration was significantly higher in patients with closed lock of the TMJ compared to symptom-free volunteers. The β-endorphin levels were not, however, significantly correlated with clinical parameters in the patients.* ***Conclusion:*** *The study results support recent findings that some opioids and their receptors exist not only within the central nervous system but also in the TMJ region, and that opioid concentrations are higher in patients with pain and dysfunction of the TMJ.* J OROFAC PAIN 2005;19:41–46

Key words: arthrocentesis, β-endorphin, synovial fluid, temporomandibular joint

Pain in the temporomandibular joint (TMJ) and/or orofacial muscles is a significant symptom in patients with temporomandibular disorders (TMD).[1–3] The mediators involved in peripheral mechanisms of pain and inflammation include neuropeptides, serotonin, cytokines, eicosanoids, and bradykinin. These have been investigated and have become useful as diagnostic tools or therapeutic targets.[3] For example, concentrations of neuropeptides in TMJ synovial fluid and tissues have been reported to be substantially higher than those observed in other articular joints.[1]

Opioids are neuropeptides that exert analgesic effects mediated by the central nervous system (CNS). Opiate effects have also been assumed to be mediated by opioid receptors whose presence on peripheral sensory nerve terminals has been documented.[4,5] β-endorphin, an endogenous opioid, has been detected in synovial fluid[6–8] and tissue[9] of patients with rheumatoid arthritis. Endogenous β-endorphin has also been found in peripherally inflamed tissues from patients undergoing arthroscopic knee surgery.[10]

Endogenous opioids are reportedly produced by nonneural inflammatory cells and interact with opioid receptors on peripheral sensory nerve terminals to inhibit inflammatory pain.[11,12] Opioids have been injected into the knees[13,14] or TMJ[15–17] synovial fluid of patients for treatment of pain or hyperalgesia, but the treatment effect is controversial. Sex differences in the effects of the peripheral opioid system on pain sensitivity and jaw muscle electromyographic activity have also been reported.[18,19]

No reports have described the existence of endogenous opioids in the TMJ synovial fluid, but based on other reports already noted, the authors hypothesized that opioids and their receptors exist not only within the CNS but also in the peripheral TMJ region. In order to investigate this hypothesis, concentrations of β-endorphin in synovial lavage fluid obtained from the TMJs of patients with closed lock and healthy volunteers by pumping before arthrocentesis were measured. Correlations between concentration of β-endorphin and clinical symptoms in patients with closed lock were also examined.

Materials and Methods

Subjects

The study sample comprised 38 patients (38 joints; 35 females and 3 males) with a median age of 40 years who had been diagnosed with closed lock based on the results of clinical examination and diagnostic magnetic resonance imaging (MRI) (Table 1) and 11 healthy volunteers (19 joints; 5 females [8 joints] and 6 males [11 joints]) with a median age of 26 years (Table 2). No patients were diagnosed with rheumatoid arthritis. Durations of disease and pain were more than 2 months in all patients. The healthy subjects, who had no subjective experience of TMD symptoms, were recruited from the staff of Hokkaido University Dental Hospital. The Ethics Committee of the Hokkaido University Dental Hospital approved the study protocol, and informed consent was obtained from each participant before the start of the study.

Samples and Assays

Samples of TMJ lavage fluid from both patients and healthy volunteers were collected using the methods described by Kubota et al.[20] Briefly, 1.5 mL of saline was pumped into the superior joint space and aspirated after administration of local anesthetic. This procedure was repeated at least 5 times, and the diluted synovial fluid was then collected. After pumping, arthrocentesis was performed on each patient. Samples were centrifuged at 3,000 g to remove cells and particulate matter. Then the samples were assayed for concentrations of β-endorphin by enzyme immunoassay kits (S-1134 β-Endorphin Human; Peninsula Laboratories; minimum detectable concentration = 0.03 ng/mL), and concentrations of protein were measured using bicinchoninic acid (BCA) assay reagent kits (23235 Micro BCA Protein Assay Reagent Kit; Pierce), according to the instructions given by the manufacturer.

Clinical Examination

Subjective pain during jaw opening and eating was rated by all 38 patients using a visual analog scale (VAS) of 0 (no pain) to 100 (maximum pain imaginable). The pain intensity upon jaw opening was assessed when patients actively opened their mouths just before arthrocentesis without assistance of the operator. Pain intensity during eating (especially biting) was also assessed immediately before arthrocentesis. Bone changes in the condyles of all patients were scored as 0 (no change), 1 (pathological change such as erosion, deformity, and/or marginal proliferation), or 2 (adaptive change such as flattening and/or sclerosis) from MRI scans[21] which were taken 2 weeks before the arthrocentesis. Synovitis in all patients was scored as 0 (no synovitis), 1 (slight synovitis with capillary hyperemia), or 2 (severe synovitis with hyperplasia of synovium) on the basis of arthroscopic findings[21] obtained at the arthrocentesis. Since only 1 patient was given a score of 0 for TMJ synovitis, patient scores were categorized as "0 or 1" or "2."

These clinical examinations, excluding bone change in the condyles, were not performed in healthy volunteers. Healthy volunteers were regarded as symptom-free subjects because they had not subjectively experienced pain in the TMJ and no MRI evidence of bony changes was found.

Statistical Analysis

Nonparametric tests were used in this study because the distribution of the concentration of β-endorphin in the patients was not normally distributed. The Mann-Whitney test was used for comparison of the concentrations of β-endorphin in patients and in symptom-free volunteers. The Spearman correlation coefficient was used to compare the VAS scores and the concentrations of β-endorphin in patients. The Kruskal-Wallis test was used for comparisons of the degree of bony changes of the condyle and the con-

Table 1 Characteristics of Patients with Closed Lock Involving the TMJ

Patient	Age (y)	Sex	Side	Bony changes on MRI	Synovitis on arthroscopy	VAS at jaw opening	VAS at biting	β-endorphin (ng/mg protein)
1	21	F	L	1	2	55	55	0.07
2	23	F	R	1	1	84	91	0.24
3	53	F	R	2	2	40	42	0.20
4	16	F	L	0	1	11	16	0.11
5	40	F	R	1	1	76	80	0.27
6	14	F	R	2	1	53	68	0.19
7	23	F	R	0	1	30	12	0.18
8	78	F	R	2	2	34	24	0.13
9	49	F	R	0	1	50	63	0.06
10	68	F	L	2	2	36	29	0.11
11	18	F	R	0	2	73	51	0.15
12	26	M	R	0	1	42	44	0.12
13	40	F	R	1	1	78	67	0.30
14	45	F	L	1	2	43	42	0.11
15	71	F	R	0	2	44	51	0.09
16	52	F	L	1	1	97	96	0.11
17	22	F	L	1	1	84	83	0.29
18	38	F	L	1	1	80	80	0.08
19	36	F	L	0	1	26	34	0.26
20	19	F	L	0	0	72	58	0.19
21	57	F	L	1	2	81	84	0.11
22	54	F	R	1	2	59	61	0.10
23	30	F	L	0	2	62	83	0.11
24	24	M	L	1	2	70	81	0.17
25	18	M	L	0	1	0	0	0.08
26	43	F	R	1	2	80	80	0.11
27	60	F	R	1	2	47	23	0.19
28	28	F	L	0	2	37	0	0.18
29	17	F	R	0	2	45	14	0.13
30	61	F	R	0	2	67	73	0.11
31	57	F	R	1	1	69	60	0.19
32	22	F	L	0	1	89	35	0.07
33	42	F	L	2	2	70	14	0.27
34	40	F	L	0	1	44	63	0.11
35	61	F	R	0	2	87	88	0.25
36	54	F	L	1	2	80	35	0.14
37	25	F	L	0	2	74	67	0.13
38	59	F	R	2	1	46	46	0.21

centration of β-endorphin in patients. The Mann-Whitney test was also used for comparisons between the degree of synovitis and the concentration of β-endorphin. These analyses were performed with the statistical package SPSS v 8.0. A probability level of .05 was considered statistically significant.

Results

Clinical Findings

Table 1 shows characteristics of the 38 joints in the 38 patients with closed lock involving the TMJ. Median VAS values measured during jaw opening and during biting were 61 and 57, respectively. Table 2 shows characteristics of the 19 joints in the 11 symptom-free subjects.

Presence of β-Endorphin in the Synovial Fluid

As shown Tables 1 and 2, endogenous β-endorphin was detected in TMJ synovial lavage fluid of all 38 patients and all 11 symptom-free subjects.

Figure 1 shows the results of box plots estimating the differences between concentrations of β-endorphin in TMJ lavage fluid from patients with closed lock and those from symptom-free subjects. Results are expressed as median values (25th percentile values, 75th percentile values). Concentrations of

Table 2 Characteristics of the 19 Joints of Healthy Subjects

Joint	Age (y)	Sex	Side	β-endorphin (ng/mg protein)
1	24	M	L	0.11
2	24	M	R	0.07
3	25	F	L	0.10
4	27	M	L	0.05
5	27	M	R	0.11
6	26	M	L	0.09
7	26	M	R	0.09
8	24	M	L	0.06
9	24	M	R	0.06
10	30	F	L	0.05
11	30	F	R	0.05
12	24	M	R	0.08
13	25	M	L	0.06
14	25	M	R	0.08
15	26	F	R	0.12
16	26	F	L	0.16
17	26	F	L	0.24
18	30	F	L	0.12
19	30	F	R	0.19

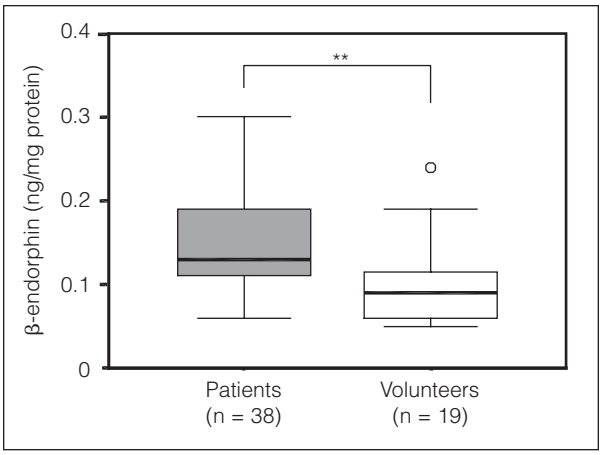

Fig 1 Box plots showing estimated differences between concentrations of β-endorphin in TMJ lavage fluid from patients with closed lock and from symptom-free subjects. Plots show lavage fluid concentrations of β-endorphin (median as well as 10th, 25th, 75th, and 90th percentiles). Concentration of β-endorphin in lavage fluid from patients was significantly higher than that from healthy subjects. $**P < .01$.

β-endorphin in the patients and symptom-free subjects were 0.13 ng/mg protein (0.11, 0.19) and 0.09 ng/mg protein (0.06, 0.11), respectively. The concentration of β-endorphin in lavage fluid from patients was significantly higher than that in fluid from symptom-free subjects ($P < .01$). Median values of protein concentration in patients and symptom-free subjects were 0.878 mg/mL and 0.674 mg/mL, respectively.

Correlation Between Clinical Symptoms and β-Endorphin Concentrations

The correlations between the VAS measured during jaw opening and during biting and the concentrations of β-endorphin in TMJ synovial fluid from patients with closed lock were not significant (jaw opening: r = 0.134, $P = .422$ and biting: r = 0.046, $P = .785$). The median concentrations (25th, 75th percentile values) of β-endorphin in patients with scores of 0, 1, and 2 for degree of condylar bone change were 0.12 ng/mg protein (0.11, 0.18), 0.14 ng/mg protein (0.11, 0.21), and 0.19 ng/mg protein (0.15, 0.20), respectively. The degree of bone change was not significantly associated with concentration of β-endorphin ($P = .201$).

The median concentration (25th, 75th percentile values) of β-endorphin in patients with synovitis scores of 0 or 1 was 0.18 ng/mg protein (0.11, 0.23), and in patients with a score of 2 was 0.13 ng/mg protein (0.11, 0.17). The degree of synovitis was not significantly related to the concentration of β-endorphin ($P = .393$).

Comparison of Concentrations of β-Endorphin in Females and Males

The authors also examined whether peripheral β-endorphin levels in TMJ lavage fluid are influenced by sex. The median concentrations (25th, 75th percentile values) of β-endorphin in all 43 females (female patients and female volunteers) and all 14 males were 0.13 ng/mg protein (0.11, 0.19) and 0.08 ng/mg protein (0.06, 0.10), respectively. The concentration of β-endorphin in lavage fluid from females was significantly higher than that from males ($P < .001$).

Discussion

The opioid β-endorphin was detected in TMJ synovial lavage fluid from both closed-lock patients and symptom-free volunteers in the present study. In general, opiate analgesia has been considered to be mediated within the CNS. However, recent studies[4,5,14,18,22,23] have demonstrated that opiate-related modulation can also occur in peripheral tissues. Hayashi et al[4] reported that the μ-opioid receptor, which binds to β-endorphin and met-enkephalin as specific ligands, can be detected in noninflamed synovial membranes of the rat TMJ. These various

findings indicate that some opioids exist not only within the CNS but also in the TMJ region.

In the present study, the concentration of β-endorphin in TMJ lavage fluid from patients was significantly higher than that from symptom-free volunteers. Takeba et al[12] reported that endorphin and enkephalin occur in increased concentrations in joints afflicted with rheumatoid arthritis. To explore the reason why β-endorphin levels in TMJ lavage fluid from closed-lock patients were also increased, correlations between concentrations of β-endorphin from patients and their clinical variables were examined in the present study. The durations of disease and pain were over 2 months in all patients, so pain reported by patients in this study was considered chronic, and VAS scores during maximal mouth opening and biting was reflective of the presence of chronic pain. However, β-endorphin levels were not significantly correlated with the VAS scores of the patients.

Although the degree of synovitis also was not significantly related to concentration of β-endorphin in the present study, Takeba et al have reported[12] that exogenous endorphin and enkephalin could inhibit the production of tumor necrosis factor-α and interleukin-1β, which take part in the mediation of acute and chronic inflammation, in synovial cells of patients with rheumatoid arthritis. Okeson reported[24] that histologic evidence of synovitis is usually absent even if the arthroscopic appearance seems consistent with synovitis. Peripheral β-endorphin levels may reflect not only their antinociceptive action, but also anti-inflammatory effects in the TMJ region, even in closed-lock patients.

The degree of bone change in the condyle was also not significantly associated with concentration of β-endorphin in TMJ lavage fluid. Prostaglandin E_2 is known to be involved in the development of pain and hyperalgesia in the TMJ and has been detected in TMJ synovial fluid of TMD patients.[25,26] Prostaglandin E_2 also inhibits bone formation and induces bone resorption in vitro.[27] Peripheral opioids may likewise be related to bone change or osteoarthritis.

Although it is unclear whether peripheral opiate levels are related to clinical parameters, the concentration of peripheral β-endorphin in TMJ lavage fluid from closed-lock patients was significantly higher than in that from symptom-free subjects. Recent studies[18,19,28,29] have suggested that gender is an important determinant of sensitivity to the antinociceptive effects of opioid compounds. In the present study, the gender ratio of joints in patients (female:male = 35:3) differed from that in symptom-free volunteers (8:11). Concentrations of β-endorphin in lavage fluid from all females (female patients and female volunteers) and from all males were therefore calculated, and the concentration of β-endorphin in lavage fluid from females was significantly higher than that from males. Peripheral β-endorphin levels in the TMJ may therefore be influenced by sex, and the significant difference between the concentration of β-endorphin in synovial fluid from patients and that in synovial fluid from volunteers seems to be related to the significant difference between concentrations of β-endorphin in the fluid from females and that from males in this study. The available animal and human data have indicated that sex may affect opioid analgesia but that the direction and magnitude of these differences depend on many interacting variables. It is possible that the higher level of β-endorphin in females in this study may be related to the higher prevalence of TMD in females, because sex hormones may modulate the function of the peripheral opioid system. In future studies, the gender ratio of the experimental group should be matched with that of the control group.

Acknowledgments

The authors would like to thank Prof Yasunori Totsuka, Prof Nobuo Inoue, Prof Kuniaki Suzuki, and Dr Mino Koshikawa-Matsuno (Graduate School of Dental Medicine, Hokkaido University) for their contributions to this study. This study was supported by grants-in-aid for scientific research (Nos. 14370685 and 14771171) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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