Salivary opiorphin as a response to capsaicin stimulation - a comparison of burning mouth syndrome patients, temporomandibular disorders patients and controls

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INTRODUCTION

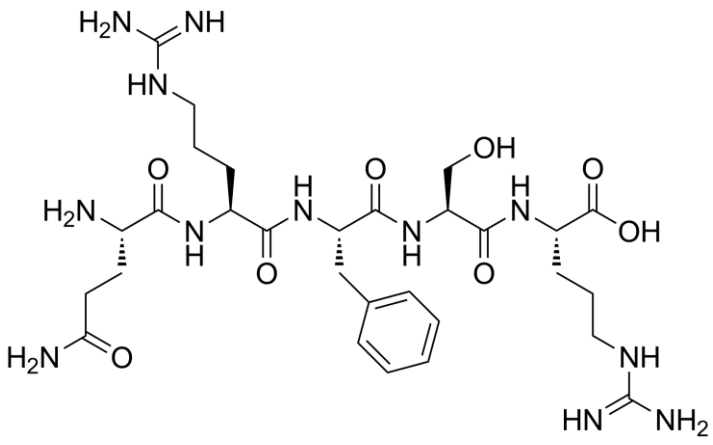
The mechanisms involved in the transition from acute to chronic pain involve structural neuroplastic changes in the brain. The physiological opioid pathways are predominant part of an endogenous nociceptive-modulating system that counterbalances the activity of pain transmission pathways. One of the potential mediators that intervene in the process of adaptation mediated by enkephalins is human opiorphin.

Opiorphin *- an analgesic peptide released by salivary glands*

Capsaicin *- an agonist of TRPV1 receptors eliciting burning sensations*

The primary objective of this study was to assess **opiorphin release after stimulation of the tongue by capsaicin (STC)**.

The secondary objectives were to **compare opiorphin release after STC** in 3 groups: **healthy (CTRL), Burning Mouth Syndrome (BMS), painful Temporomandibular disorders (TMDp)** and to compare **pain evoked by STC** in these 3 groups.



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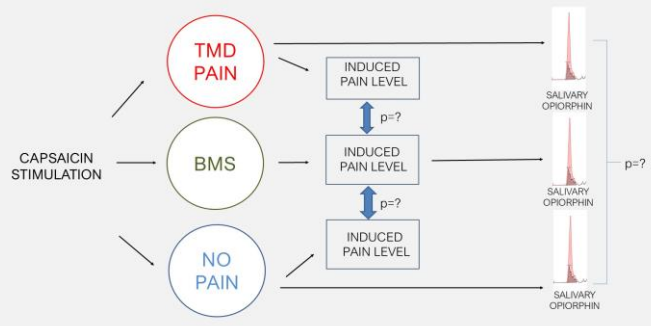


MATERIALS AND METHODS

Forty-eight subjects participated: 16 BMS (15F/1M, 48.5±10.89y) , 16 TMDp (14F/2M, 28.00±8.91y) and 16 CTRL (8F/8M, 25.62±9.03y).

Burning pain sensation was elicited by putting a series of 10 capsaicin-soaked disks (30µM) in contact with dorsal tongue (Figure 1), and the intensity was recorded with a Numerical Pain Rating Scale every minute during a 5-minute application period and every 2 minutes during next 20 minutes upon termination of capsaicin stimulation.

Saliva was collected three times, before capsaicin stimulation (1st sampling), immediately after the end of stimulation (2nd sampling) and 20 minutes after the end of stimulation (3rd sampling) (Figure 2).

Opiorphin levels were quantified by HPLC-MS /MS.

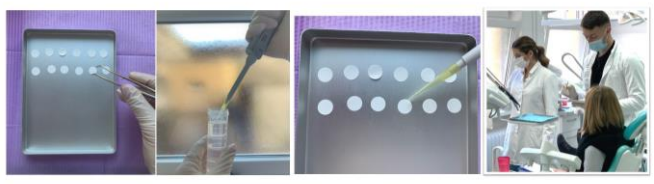


Figure 1. Disc preparation and application

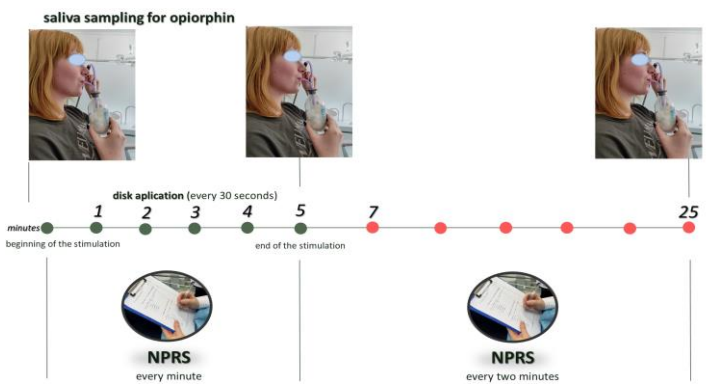


Figure 2. Course of the study

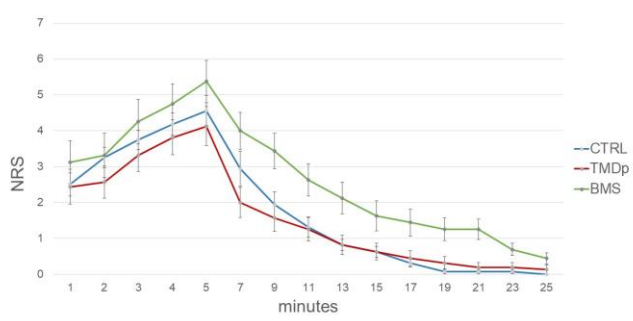
RESULTS

There was a higher representation of women in the TMDp and BMS groups compared to the CTRL group (87.5%, 93.8%, and 50%, respectively, p=0.006). The mean age of BMS patients was significantly higher than both TMDp patients (p=0.001) and CTRL (p<0.001).

The NRS scores for capsaicin-evoked burning sensation reached their maximum intensity at the 5th minute of measurement in all groups (Figure 3): CTRL=4.56±1.71; TMDp=4.13±2.24; BMS=5.37±2.44). From the 7th minute until the 23rd minute, the values of the experimentally induced burning pain were significantly higher in the BMS group compared to the TMDp group (p=0.01). From the 9th minute until the end of the measurement, burning pain scores were significantly higher in the BMS group compared to CTRL (p<0.05) group.

TMDp patients exhibited significantly higher levels of opiorphin compared to CTRL group in 1st and 3rd sampling.

BMS patients displayed statistically higher levels of opiorphin compared to CTRL group in 2nd and 3rd sampling. When comparing within individual, a statistically significant increase in opiorphin levels was observed only in the BMS group (p =0.019).



Statistical Analysis

Kruskal Wallis ANOVA and Mann Whitney U Test were used to test the differences between groups while Friedman ANOVA and post hoc Wilcoxon Matched Pairs Test were used for within-group analysis. The level of significance was set at 5 %.

Laboratory technique:

Originally developed and validated LC-MS/MS method was used for opiorphin quantification. Saliva (2ml) was collected with a vacuum collector in a tube containing 300 µl of TFA kept on ice. Samples were vortexed and left on ice for 20 min and centrifuged (20,000×g, 30 min, at 4ºC). Supernatant (800 µl) was placed into a separate tube and then freeze-dried. The residue was dissolved in 200 µl of 0.1% formic acid in water and an aliquot of 30 µl was analyzed by LC–MS/MS. Electrospray positive ionization-mass spectrometric multiple-reaction monitoring (ESI+/MRM) experiments were used for quantifying opiorphin.

DISCUSSION AND CONCLUSION

BMS patients experienced a **more intense pain sensation** after tongue stimulation by capsaicin than TMDp patients and healthy control subjects.

Opiorphin levels were **higher** in **orofacial pain patients** than in healthy subjects, and BMS patients displayed higher levels than TMDp patients. These results indicate a **specific regulation of opiorphin release** in patients with oral painful conditions.

Opiorphin kinetic should be explored in more details regarding the therapeutic potential of opiorphin and its analogues.

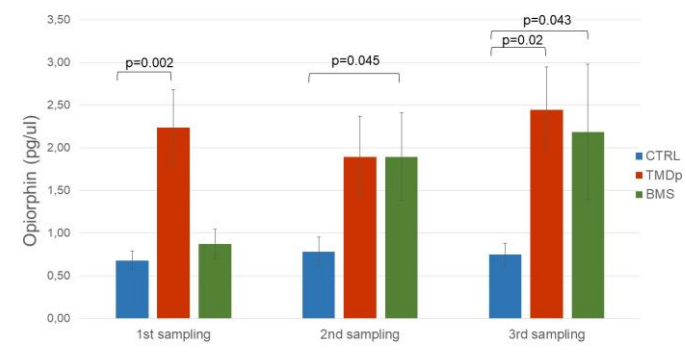


Figure 4. Comparison of opiorphin levels between groups

Opiorphin levels expressed in pg/ul (mean, range, and SD) in CTRL = healthy subjects, BMS = Burning Mouth Syndrome patients, and TMDp=TMD-pain patients (presence of myalgia, arthralgia, or both)

Figure 3. The intensity of burning pain sensation

Intensity of burning pain sensation self-recorded with a 0-10 Numeric Rating Scale (NRS), bars represent mean ± SD. CTRL = healthy subjects. BMS = Burning Mouth Syndrome patients. TMDp=TMD-pain patients - presence of myalgia, arthralgia, or both

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This work has been fully supported by Croatian Science Foundation under the project (IP-2019-04-6211). ClinicalTrials.gov Identifier NCT04694274. Registered on 01/04/2021