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Influence of Chronic Stress and Oclusal Interference on Masseter Muscle Pain in Rat

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A B S T R A C T

This study aimed to investigate the individual effects of chronic stress and occlusal interference, as well as their combined influence on masseter muscle pain. Experiments were performed on 28 male Wistar rats. Animals were submitted to chronic stress procedure, exposed to occlusal interference, or exposed to both mantioned procedures. At the end of the procedure animals were submitted to orofacial formalin test, and nociceptive behavioral response was evaluated. Statisticaly significant diference of nociceptive behavioral response in chronicaly stressed rats and in the animals with occlusal interference in comparation to the control group were not obtained ($p>0.05$). In contrast, nociceptive behavioral response was significantly increased in rats submitted to both of experimental procedures ($p<0.01$). These findings suggest that only combination of occlusal interference and chronic stress influence masseter muscle pain.

Key words: *chronic stress, masseter muscle, occlusal interference, pain, rat*

Introduction

Temporomandibular disorder (TMD) is a collective term that involves various clinical conditions of musculoskeletal and joint tissue. Disorder is characterized by pain in the prearicular area, temporomandibular joints (TMJ) and/or masticatory muscles, as well as by TMJ sounds during function, and limitation in mandibular movement[1].

The etiology of TMD is poorly understood, but it is recognized to be multifactorial. Role of psychosocial stressors, parafunctions, occlusal interference and other risk factors has been examined in numerous studies. Results of previous studies are contradictory. Some studies did not confirm the relationship between occlusal interference and TMD, or the connection was weak[2,3]**.** Other studies indicate that occlusal interference triggers masticatory muscle hyperactivity and bruxism, which can overload masticatory muscles causing tenderness, pain and TMJ sounds[4–6]. Some authors have found correlation between stress and pain and implicate that stress is the main factor responsible for TMD[7–10].

Pain in the masticatory muscles has long been recognized as a prominent symptom in TMD. According to literature, approximately 23% of TMD patients develop muscle disorders[11]. Since the results of studies investigating orofacial pain are contradictory, and because TMD patients report pain in the masticatory muscles as their major complaint, it is important to investigate the effect of physical and psychological factors on the muscle pain.

The purpose of this study was to investigate the effects of chronic stress, occlusal interference and their mutual effect on nociceptive behavioral response evoked by injection of formalin into the masseter muscle.

Materials and Methods

Animals

Experiments were performed on 28 male Wistar rats (weight 250–300 g at the beginning of experiment). Animals were maintained on a 12 h light–dark cycle and allowed free access to food and water. All experiments were performed between 10 a.m. and 2 p.m. in a silent room, at a temperature of 22°C–24°C. Adequate measures were taken to minimize pain or discomfort. All experimental procedures involving animals were performed in accordance with the regulations set by the Croatian related laws and rules (NN 19/99; NN 176/04) and with the

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guidelines set by the European Community Council Directive of 24 November 1986 (86/609/EEC). All experimental procedures were also approved by the Faculty ethical committee.

Animals were randomly divided into four experimental groups. The experimental groups were as follows: chronic stressed group; group with occlusal interference; group with both occlusal interference and chronic stress; and the control group free of occlusal interference and not submitted to restraint. Experimental procedures in all mentioned groups lasted for 56 days. The 48th experimental day all animals were anesthetized with chloral hydrate (360 mg/kg), and the 56th day they were submitted to the formalin test.

Chronic stress procedure

The animals were stressed by restrain 1 h/day, 5 days/week for 8 weeks[12,13]. Restraint was carried out by placing the animal in a 7×30 cm plastic tube. Tube was design with inner mobile wall, so it could be adjustable in size, depending on each animal, and could unable them to move. At the far end of the tube there was 1 cm hole for breathing.

Occlusal interference procedure

Occlusal interference procedure was performed in previously anesthetized animals on the 48th experimental day. Composite resin (Gradia direct, GC Dental products corp., Aichi, Japan) was build-up (height of 1.0 mm) on right upper molar teeth. Before placing the composite resin, teeth were etched with 37% phosphoric acid and washed with water and air-dried. To prevent reduction of material during function, right lower molar teeth were coated with fluid resin[14]. Occlusal interference was left in place for 8 days.

Combination of occlusal interference and chronic stress procedure

To evaluate the combination of both occlusal interference and chronic stress, animals were exposed to the chronic stress procedure, as described above, and occlusal interference was fitted on their right upper molar teeth in the 48th day from the beginning of the chronic stress procedure.

Testing procedure for the masseter muscle pain

The 56th experimental day each animal was submitted to the formalin test. Animals were first placed in the test chamber (plastic, transparent box, without access to food or water) for a 30 min habituation period[15]. A volume of 0.05 ml, 5% formalin in saline was injected into the mid-region of the right masseter muscle via 27 gauge needle[16]. After the formalin injection, rats were returned to the test chamber. The behavioral responses, characterized by rubbing orofacial region (amount of time) and flinching the head (number of head flinches), were quantified for 45 min period. Each flinch was expressed as 1s. Behavioral responses were evaluated together by their sum. Cumulative response time was analyzed[17]. The orofacial formalin test was done by investigator blinded to the rat group assignment.

Statistical analysis

The sum of rubbing and flinching behavior responses exhibited by each animal was computed. The comparison between groups was made by one-way ANOVA. *LSD post-hoc* test was used to determine the pairs of groups that were different. A probability level of less then 0.05 was considered to indicate statistical significance.

Results

Results of behavioral response alterations induced by occlusal interference, chronic stress and by their mutual effect are shown in Figure 1. ANOVA revealed significant interaction between groups [$F(3, 24)=4.5599$, $p=0.0115$]. Post-hoc test (LSD) indicated that exposure to chronic stress did not affect the nociceptive behavioral response evoked by formalin injected in the masseter muscle of rats, and occlusal interference also had no effect ($p>0.05$).

The increase of nociceptive behavioral response was highly statistically significant ($p=0.0011$, LSD) between control group and group with combination of both occlusal interference and chronic stress.

Discussion

The orofacial formalin test is a reliable model of nociception, used in studies examining deep pain conditions such as masticatory muscle and TMJ pain, indicating that nociceptive behavioral response characterized by head flinches and rubbing may be used as an objective index of pain[13,17,18].

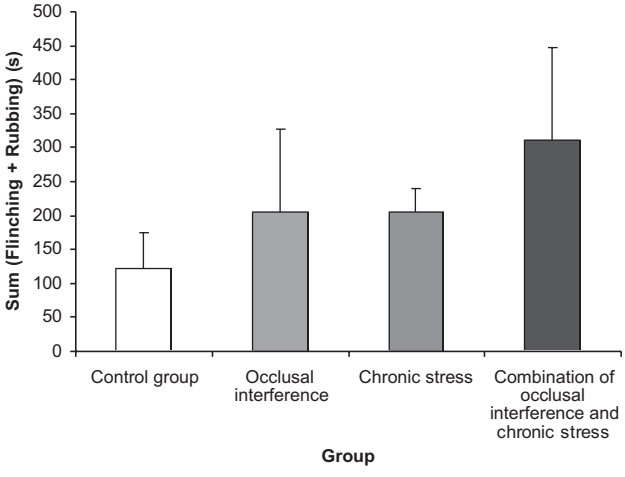

*Fig.1. Sum of nociceptive behavioral responses recorded in the animals of the control group and rats submitted to occlusal interference, chronic stress procedure, and to the combination of occlusal interference and chronic stress. Each column represents X±S.E. (n=7). * P<0.05; significantly different from the control group (ANOVA and LSD).*

Stress is considered as a possible etiological factor in many painful states including chronic orofacial pain. Literature suggests that chronic stress causes muscle hyperactivity and changes in opioid system leading to muscle tenderness and to microtraumatic damage to the TMJ, consequently inducing pain in the mentioned tissues[13,19]. According to these findings, we expected that chronic stress would increase nociceptive behavioral response. Discrepancy in results and disability of chronic stress to induce significant changes in pain response may be due to different formalin concentration used in this study. Study that confirmed hyperalgesic effect of chronic stress on TMJ pain used 2.5% formalin injected in temporomandibular joint[13]. We examined the effect of chronic stress on muscle pain induced by higher formalin concentrations (5%), according to studies that evaluated pain response after joint inflammation by injection of 5% formalin in the masseter muscle[16,18]. It has been suggested that excessive formalin concentrations may increase risk of peripheral fiber desensitization20. Possibly, lower formalin concentrations should be used to improve sensitivity to changes induced by chronic stress. Difference in results may also be due to discrepancy between the examined tissues. Although orofacial muscle pain and inflammation represent major health problems, there are only few studies of orofacial nociceptive behavioral activity evoked by nociceptive stimulation of the muscle tissue. Previous studies examined the effect of chronic stress procedure on nociceptive response induced by injection of formalin in TMJ. Muscle tissue reacts differently then joint tissue on painful stimulus. Joint inflammation activates stronger nociceptive response than muscle inflammation and noxious stimulus injected in the joint causes grater muscle activity than when injected directly in muscle[21–24].

Although occlusal interference has been considered a risk factor for TMD leading to masticatory muscle pain[5], results of our study show no statistically significant difference in nociceptive behavioral response in comparison with control group. There are few possible explanations for our findings. Our results are in agreement with studies that suggest that occlusal interference is not harmful in healthy individuals[3] showing that the period of tooth contact decreases after this type of interference[2]. Reduction of tooth contact period may be explained by avoidance of occlusal discomfort caused by interference. To exclude the possibility that the splint height reduction during mastication caused no alteration in pain response, lower molar teeth were coated with fluid composite resin. It has also been suggested that the masseter muscle can adapt to occlusal alterations by regeneration of myofibers. Masseter can develop normal histology, even seven days after increasing vertical dimension of occlusion[25].

In contrast to the lack of significant changes of pain response in chronically stressed animals and in the rats with occlusal interference, nociceptive behavioral response was significantly increased in group submitted to both of experimental procedures. Present findings indicate a major effect of occlusal interference and chronic stress on masseter muscle pain, but only when these two etiological factors act together, and almost no effect when applied individually. When subjects with occlusal interference are exposed to chronic stress, causing muscle hyperactivity, protective mechanism of avoiding tooth contact might be impaired and could cause a feeling of occlusal interference as a disturbing factor, subsequently increasing the pain in masticatory muscles. On the other hand, regenerative mechanisms may be insufficient and would not allow tissues to develop normal histology and function.

This study may give one of possible explanation why some patients with occlusal interference do not report signs and symptoms of TMD while other with just slight occlusal changes report great pain and other symptoms associated with TMD, but the underlying mechanism of pain is very complex and requires further studies. This animal model may serve as a useful tool for investigating the effect of both occlusal interference and chronic stress on pain response and pathohystological changes in masticatory muscles.

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REFERENCES

1. DWORKIN SF, LERESCH L, J Craniomandib Disord, 6 (1992) 301. — 2. MICHELOTTI A, FARELLA M, GALLO LM, VELTRI A, PALLA S, MARTINA R, J Dent Res, 84 (2005) 644. — 3. MICHELOTTI A, FARELLA M, STREENKS MH, GALLO LM, PALLA S, Eur J Oral Sci, 114 (2006) 167. — 4. CHEN CY, PALLA S, ERNI S, SIEBER M, GALLO LM, J Orofac Pain, 21 (2007) 185. — 5. CHRISTENSEN LV, RASSOULI NM, J Oral Rehab, 22 (1995) 515. — 6. CLARK GT, TSUKIYAMA Y, BABA K, WATANABE T, J Prosthet Dent, 82 (1999) 704. — 7. DE LEEUW JR, STEENKS MH, ROS WJ, LOBBEZOO-SCHOLTE AM, BOSMAN F, WINNUBST JA, J Oral Rehabil, 21 (1994) 501. — 8. GLAROS AG, WILIAMS K, LAUSTEN L, J Am Dent Assoc, 136 (2005) 451. — 9. UHAC I, KOVAC Z, MUHVIĆ-UREK M, KOVACEVIĆ D, FRANCISKOVIĆ T, SIMUNOVIĆ-SOSKIĆ M, Mil Med, 171 (2006) 1147. — 10. MUHVIĆ-UREK M, UHAC I, VUKSIĆ-MIHALJEVIĆ Z, LEOVIĆ D, BLECIĆ N, KOVAC Z, J Oral Rehabil, 34 (2007) 1. — 11. LERESCHE L, Epidemiology of orofacial pain. In: LUND JP, LAVIGNE GL, DUBNER R, SESSLE BJ Orofacial pain. From basic science to clinical management (Quintessence, Chicago, 2001). — 12. ELY DR, DAPPER V, MARASCA J, CORREA JB, GAMARO GD, XAVIER MH, MICHALOWSKI MB, Physiol Behav, 61 (1997) 395. — 13. GAMEIRO GH, ANDRADE DA S, DE CASTRO M, PEREIRA LF, TAMBELI CH, VEIGA MC, Pharmacol Biochem Behav, 82 (2005) 338. — 14. NISHIDE N, BABA S, HORI N, NISHIKAWA H, J Oral Rehabil, 28 (2001) 294. — 15. ABBOTT FV, FRANKLIN KBJ, CONNELL B, Eur J Pharmacol, 126 (1986) 141. — 16. OKAMOTO K, IMBE H, TASHIRO A, KUMABE S, SENBA E, Neurosci Lett, 367 (2004) 259. — 17. ROVERONI RC, PARADA CA, CECILIA M, VEIGA FA, TAMBELI CH, Pain, 94 (2001) 185. — 18. OKAMOTO K, IMBE H, TASHIRO A, KIMURA A, DONISHI T, TAMAI Y, SENBA E, Neuroscience, 30 (2005) 465. — 19. LASKIN DM, Myofascial pain disfunction syndrome: etiology. In: SARNET B, LASKIN DM, The temporomandibular

joint. A Biological Basis for Clinical practice (Charles C. Thomas, Springfield, 1980). — 20. PUIG S, SORKIN LS, Pain, 64 (1996) 345. — 21. BERBERICH P, HOHEISEL U, MENSE S, J Neurophysiol, 59 (1988) 1395. — 22. SCHAIBLE HG, SCHMIDT RF, J Neurophysiol, 54 (1985) 1109. — 23. RO JY, SVENSSON P, CAPRA N, Muscle Nerve, 25 (2002) 576. — 24. YU XM, SESSLE BJ, VERNON H, HU JW, Pain, 60 (1995) 143. — 25. AKAGAWA Y, NIKAI H, TSURU H, J Prosthet Dent, 50 (1983) 725.

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UČINAK KRONIČNOG STRESA I OKLUZALNE INTERFERENCE NA BOL MASETER MIŠIĆA U ŠTAKORA

S A Ž E T A K

Cilj istraživanja bio je istražiti učinak kroničnog stresa, okluzalne interference te njihov zajednički učinak na bol maseter mišića. Istraživanje je provedeno na 28 muških Wistar štakora. Životinje su podvrgnute kroničnom stresu, okluzalnoj interferenci ili su izložene objema spomenutim procedurama. Po završetku spomenutih procedura životinje su podvrgnute formalinskom testu, te je očitana bolnost masetera po opisanom modelu ponašanja. Razlika u ponašanju između kontrolne skupine i skupine s okluzalnom interferencom, te skupine izložene kroničnom stresu nije pokazala statističku značajnost ($p>0,05$). Naprotiv, bolni odgovor životinja podvrgnutih objema spomenutim procedurama bio je značajno povišen ($p<0,01$). Podatci dobiveni istraživanjem sugeriraju da jedino kombinacija okluzalne interference i kroničnog stresa utjeće na pojačanje boli maseter mišića.