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SCIENTIFIC ARTICLE

Chewing-like as a possible behavior of persistent orofacial pain in the experimental temporomandibular dysfunction*

Mastigação (chewing-like) como possível comportamento de dor orofacial persistente na disfunção temporomandibular experimental.

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SUMMARY

BACKGROUND AND OBJECTIVES: Stimulations with formalin in the orofacial region can be related to transient or subacute nociceptive activity and behavioral changes. The evaluation of behavioral changes induced by persistent or chronic irritating nociceptive substance has not yet been described.
METHOD: Complete Freund's Adjuvant (CFA) was injected in the temporomandibular joint (TMJ) region of rats and analyzed comparing it to the groups treated with saline and 2.5% formalin. In addition, behaviors such as grooming, freezing, rest/sleeping and chewing-like were electronically observed and quantified.
RESULTS: It was shown that the chewing-like behavior was significantly increased and that it was inhibited by indometacin (5 mg/kg) and morphine (4 mg/kg).
CONCLUSION: These results suggest that chewing-like may be a possible behavior of persistent or chronic orofacial pain, and may be a tool for clinical-pharmacological studies.
Keywords: chewing-like, formalin test, Freund's adjuvant, orofacial pain, nociception.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Estímulos com formalina na região orofacial podem estar relacionados com a atividade nociceptiva e as alterações comportamentais transitórias ou subagudas. A avaliação de comportamentos sob ação de substância irritante nociceptiva persistente e crônica ainda não foi descrita.
MÉTODO: Foi feita injeção de adjuvante completo de Freund (ACF) na região da articulação temporomandibular (ATM) de ratos e foi analisada comparando-a com os grupos tratados com salina e formalina a 2,5%. Além disso, foram observados e quantificados eletronicamente os comportamentos *grooming*, *freezing*, *rest*/*sleeping* e *chewing-like* (mastigação).
RESULTADOS: Observou-se que o comportamento mastigação (*chewing-like*) estava significativamente aumentado e que ele foi inibido pela indometacina (5 mg/kg) e morfina (4 mg/kg).
CONCLUSÃO: Esses resultados sugerem ser o *chewing-like* um possível comportamento de dor orofacial persistente, oferecendo-se como instrumento para análise clínico-farmacológica.
Descritores: adjuvante de Freund, dor, dor orofacial persistente, mastigação, nocicepção, teste da formalina, mastigação.

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INTRODUCTION

The temporomandibular dysfunction is an important clinical entity that is poorly studied, due in part, to limited experimental models developed to study pain in this region, as well as testing the pharmacologic effectiveness of drugs that could be used to treat this nociceptive orofacial disturbance[1].

Animal models of pain in the orofacial region through the induction of pro-inflammatory agents (CFA and formalin) leading to behavioral alterations as hyperalgesia and allodynia correlated with neural events in peripheral tissues and neurons of the CNS have been studied[2].

The association between orofacial pain and alterations of spontaneous behaviors and inflammatory stimulations, as Complete Freund's Adjuvant (CFA), still needs to be elucidated. Therefore, considering the CFA effect in the temporomandibular joint (TMJ) of rats in promoting a persistent inflammation and neuroplastic changes[3], we propose an inflammation model to analyze chronic behavioral alterations and its interrelation with orofacial pain, verifying if the behaviors are sensible to morphine, indometacine or lidocaine. The formalin, in turn, induces non-persistent transitory effects, serving for subacute or transitory models.

METHOD

For the experiment, 22 Wistar male rats weighting 180-300 g had been used. The animals were kept in plastic boxes (n = 6), with water and food *ad libitum*, and in conditions of ambient light. The observations were made between 08:00 and 17:00 hours and the study was lead in accordance with the animal ethical rules by Zimmermann[4] (1983), and approved by the UFC Ethic Committee.

TMJ inflammation induction

The animals got through a period of adaptation of 10 minutes in a wooden observatory box (100 cm x 50 cm x 50 cm) illuminated with a light bulb of 40 watts in one of its corners. The observatory box frontal wall was made of glass in order to allow the observer to watch the behavioral elements. The rats had no access to food or water during the experiment. After the period of adaptation, the animal was removed from the test box and lightly anesthetized with ethylic ether to allow the injection of 50 µl of CFA (n = 8), formalin 2.5% (n = 6) and saline solution (n = 8) in the TMJ.

The injection site in the TMJ region was identified by palpation of the zigomatic arc and condyle. A 30G needle was inserted in the immediately inferior point to the edge of the postero-inferior zigomatic arc; after the localization of the point, the solution was injected in accordance with each group[3].

Observation of spontaneous behaviors

Following the injection, the animals were returned to the observation box, following a behavioral protocol[5]. The behaviors chewing-like, grooming, freezing and rest/sleeping had been observed during 30 minutes and quantified and registered through a computer program (Comporta®), developed by Prof. Marcus Valley from UFC.

Pharmacologic tests

The animals were left during 10 minutes for adaptation to the environment and then it was initiated the drugs effect evaluation.

The tested drugs were indometacine (5 mg/kg), morphine (4 mg/kg) and the local anesthetic lidocaine with vasoconstrictor 2% (0.4 ml). Indometacine and morphine were administrated PO, by means of a orogastric probe, following the fasting protocol for 24 hours, thirty minutes before the behavior observation, with exception of lidocaine, which was injected IM immediately before the behavior observation without previous fasting period.

Statistics

The results were expressed as average ± average standard error. The averages were compared with the Student's t and the non-parametric Mann-Whitney tests for analysis of the behavioral data. The accepted minimum significance was $p < 0.05$.

Table 1 – Effect of the CFA injection it the TMJ region on the animals spontaneous behaviors at the 3rd post-injection day

Behavior	CFA*	Formalin*	Saline*	p
Chewing-like	142.25 ± 14.94	54.33 ± 11.52	68.75 ± 9.42	< 0.05
Rest/Sleeping	69.37 ± 33.62	317.83 ± 97.91	427.13 ± 141.46	< 0.05
Grooming	169.75 ± 20.22	116.50 ± 18.96	101.50 ± 17.57	< 0.05
Freezing	308.25 ± 46.59	121.17 ± 22.63	142.00 ± 44.62	< 0.05

*Time in seconds

Table 2 – Chewing-like behavior in the 3rd and 6th post-injection days of CFA, formalin and saline

Post-Injection Day	CFA*	Formalin*	Saline*	p
3rd	142.25 ± 14.94	54.33 ± 11.52	68.75 ± 9.42	< 0.005
6th	190.00 ± 33.30	59.62 ± 14.66	59.62 ± 14.66	< 0.005

*Time in seconds

Table 3 – Effects of morphine (4 mg/kg), indometacine (5 mg/kg) and lidocaine 2% on the chewing-like behavior induced by CFA in the 3rd post-injection day

Behavior	No Drug*	Morphine*	Indometacine*	Lidocaine*
Chewing-like	142.25 ± 14.94	31.00 ± 12.18	77.37 ± 18.00	124.17 ± 28.39
p	–	< 0.05	< 0.05	> 0.15

*Time in seconds

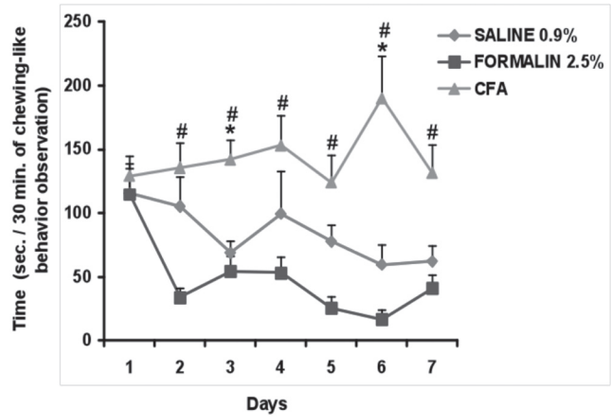

Figure 1 – Effect of saline 0.9%, formalin 2.5% and CFA in the hewing-like behavior. *Statistical significance in relation to saline 0.9% and #statistical significance in relation to formalin 2.5% with p < 0.05 in the Student-Newman-Keuls t and Mann-Whitney U tests.

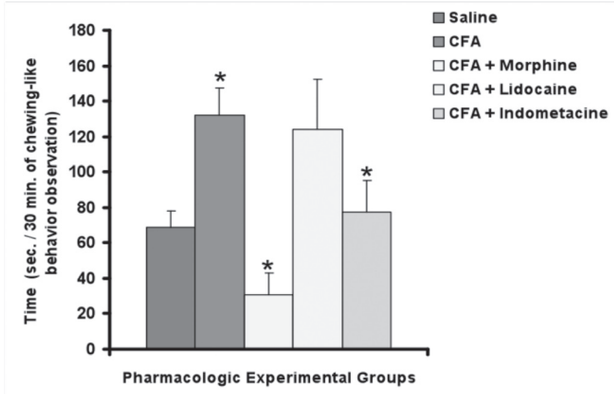

Figure 2 – Analgesic effect on the chewing-like behavior (CFA in the 3rd post-injection day). *Statistical significance p < 0.05 in the Student-Newman-Keuls t and Mann-Whitney U tests.

RESULTS

Effect of the CFA injection in the TMJ region on the animals spontaneous behaviors

The behaviors chewing-like (movement of rotation of the jaw or chew), rest/sleeping (to rest/to sleep), grooming (to clean itself) and freezing (to freeze itself) had shown significant alteration under the action of CFA in relation to the control and formalin groups (Table 1).

The chewing-like behavior demonstrated a superior time of execution in seconds in all the observations, mainly in the 3rd (142.25 ± 14.94) and 6th days (190.00 ± 33.30), where a significant difference was observed in relation to the control group in the 3rd (68.75 ± 9.42) and 6th days (59.62 ± 14.66), and to the formalin 2.5% group in the 3rd (54.33 ± 11.52) and 6th days (16.83 ± 7.49) (Figure 1; Table 2).

Effect of morphine (4 mg/kg), indometacine (5 mg/kg) and lidocaine 2% on the chewing-like behavior induced by CFA in the 3rd post-injection day

The PO administration of these analgesics in the group of animals in the CFA 3rd post-injection day, that is, in the day where this behavior was significantly distinguished in relation to the control and formalin 2.5% groups, presented significant reduction by morphine (31.00 ± 12.18) PO (4 mg/kg) and indometacine (77.37 ± 18.00) PO (5 mg/kg). This behavior was not reverted by lidocaine (124.17 ± 28.39) with vasoconstrictor 2% IM (0.4 mL) (Figure 2; Table 3).

DISCUSSION

The objective of the present work was to elaborate an inflammation model in the TMJ region of rats, correlating

it with modified behavioral patterns by CFA injection in the TMJ periarticular region.

Injury in the TMJ can cause inflammation in surrounding tissues and in the intracapsular region. This inflammatory process involves the mobilization of defense mechanisms, where local and systemic effects occur, and can include behavioral and functional changes for regeneration and repair of tissues[6].

Study in animal model of persistent pain at spinal level indicates that injuries in peripheral tissues induce a hyperexcitability state that participates in the development of the persistent pain and hyperalgesia. It has been demonstrated that persistent pain in the orofacial region leads to a similar state of hyperexcitability in the trigeminal system[7]. Inflammatory alterations in the TMJ lead to the production of substances as tachykinins and substance P that contribute for an increment of the activity of the nociceptive pathways and neurons excitability in the dorsal horn of the spinal cord, leading to what we call central sensitization[8].

In a polyarthritis model with CFA[5], there is a decrease in the time of execution of the behaviors rearing, running and climbing and an increase in the behaviors grooming, scratching, biting and freezing, what suggests that the scratching behavior may be a non-invasive marker of chronic pain.

In models using CFA[6,9] as pain induction agent in the TMJ, occurs an increase in the time of execution of this masticatory behavior, due to pain in the TMJ and orofacial regions during the chew. This pattern is observed with an increase in the time of execution of the chewing-like behavior after bilateral injection of CFA in the TMJ of male and female rats[10]. Clinical studies of juvenile rheumatic arthritis with symptoms in the TMJ reveal that the execution of the chewing-like behavior is supposedly a "defense" against pain[11].

Studies in animal model[12] demonstrate that the masticatory behavior can modulate the pain processing regarding to the sensory-motor integration through cortical mechanisms, where the trigeminal sensory system is somewhat involved in the modulation of the orofacial chronic pain. The exact neural pathway still needs to be elucidated.

Therefore, the jaw rotation movement, or chewing-like, evaluated in the experiments, showed significance from the 2nd post-injection day of CFA, but it was distinguished in the 3rd and 6th days comparing with the other experimental groups, what did not occur with the saline solution nor with the animals injected with formalin. These results demonstrate the CFA persistent irritating capacity, whereas the formalin did not. Episodes of this behavior in model with mustard oil are reported[13] relating this behavior to the hyperalgesic action of it applied in tissues of the TMJ. Our results oppose what it is demonstrated in the formaline model in the TMJ of rats where there is no difference in the time of execution of the jaw rotation movement (chewing-like) in relation to the control with saline solution[1].

Studies demonstrate that harmful stimulations applied to the TMJ region[14] can produce sustained excitement of some masticatory muscles, including masseter and digastric. These alterations at the craniofacial musculature level can contribute for the increase in the time of execution of this chew or jaw rotation behavior.

The reduction of this behavior through the COX administration inhibition, the indometacine (5 mg/Kg), suggests that this behavior can be related with the nociception that would be mediated by the arachidonic acid activity and its derivatives, produced by the CFA irritating action in the TMJ region of rats in the pain neurotransmission. Moreover, the chewing-like behavior showed sensitivity to the action of the morphine (4 mg/Kg), demonstrating the capacity of this opioid agonist in blocking the nociceptive pathway promoted by the CFA stimulation in the orofacial region[13] (Figure 2).

The inability of the lidocaine in blocking the chewing-like behavior may be due to its peripheral restricted effect, while this behavior may be related to changes in the spinal cord or CNS sensitization. Moreover, local anesthetic agents have an antinociceptive preferred action against thermal stimulation, and serve to differ thermal nociception from mechanic one[14].

CONCLUSION

The CFA is an important agent for induction of persistent inflammation, which modified, along one week, the chewing-like behavior, or the jaw rotation movement. The studied behavior presented sensitivity to the indometacine and morphine analgesic activity.

We suggest, thus, that the chewing-like behavior may serve as a non-invasive marker of persistent pain in the orofacial region, and may contribute to the understanding of the TMJ dysfunction or painful alterations. New studies are needed to evaluate which behavioral pattern is related to the CFA activity in the orofacial region of rats during bigger interval of behavioral observation, allowing the identification of other possible behaviors that can be related with the nociceptive activation by algogenic agents in this region.

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