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Estudo do dimorfismo sexual na participação de adrenoceptores beta na nocicepção induzida por formalina na ATM de ratos.

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, Universidade Estadual de Campinas, para obtenção do título de Mestre em Odontologia, Área de Concentração em Fisiologia Oral.

Orientadora: Profa. Dra. Cláudia Herrera Tambeli

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"Há algo mais interessante que as mais belas descobertas: é o conhecimento da maneira pela qual são feitas."

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RESUMO

As disfunções temporomandibulares (DTM) são condições dolorosas que envolvem a articulação temporomandibular (ATM) e os músculos mastigatórios, apresentam maior prevalência, severidade e duração no sexo feminino e são comumente associadas à inflamação. Apesar das drogas antiinflamatórias não esteroidais (AINEs) serem freqüentemente utilizadas no controle de dores inflamatórias, muitos pacientes podem apresentar intolerância ao tratamento prolongado e nem todos os pacientes com dor na ATM respondem aos efeitos de tais medicamentos. Sabe-se que a dor inflamatória possui um componente simpático que pode predominar em casos com menor sensibilidade aos AINEs. Assim, o objetivo deste trabalho foi avaliar a contribuição desse componente simpático na dor instalada da ATM. Para isso (1) Investigamos a participação de adrenoceptores β_1, β_2 e β_3 na nocicepção induzida por formalina na ATM de ratos e (2) Verificamos se existe um dimorfismo sexual no efeito desses β-bloqueadores sobre a nocicepção. De acordo com o objetivo (1): A co-administração de formalina com os antagonistas de receptores β_1, β_2 e β_3, Atenolol, ICI 118.551 e SR59230A respectivamente, reduziu significativamente a nocicepção induzida por formalina na ATM de ratos. De acordo com o objetivo (2): A co-administração de doses baixas do antagonista de adrenoceptores β_1 (6µg) ou β_2 (0,1µg) com formalina apresentou um efeito antinociceptivo em fêmeas mas não em machos, indicando a existência de um dimorfismo sexual sobre o efeito destes antagonistas. Esses resultados sugerem que o bloqueio de adrenoceptores beta, mais especificamente β_1, β_2 e β_3, diminui a dor da ATM. Além disso, demonstram que as fêmeas são mais sensíveis aos efeitos antinociceptivos dos antagonistas β-adrenérgicos do tipo 1 e 2.

Palavras-chave: Articulação Temporomandibular, nocicepção, adrenoceptores, beta-bloqueador, antiinflamatório não-esteroidal.

ABSTRACT

Temporomandibular disorders (TMD) are pain conditions that affect the temporomandibular joint (TMJ) and masticatory muscles. These conditions present higher prevalence, severity and duration in females and appear to be associated with inflammation. Although non-steroidal anti-inflammatory drugs have been frequently used in the control of inflammatory pains, many patients may be intolerant to the prolonged treatment and some of them may not respond to the effect of these medications. It is already known that inflammatory pain has a sympathetic component that may predominate in the cases less sensitive to the non-steroidal anti-inflammatory drugs. Therefore, in this study we investigated the contribution of this sympathetic component in TMJ´ pain. Specifically we investigated (1) the participation of β_1, β_2 and β_3 adrenoceptors in the rat´s TMJ nociception induced by formalin and (2) the existence of sexual dimorphism in the effect of β-blockers in the TMJ nociception. Co-administration of formalin with β_1, β_2 and β_3 adrenoceptors antagonists, Atenolol, ICI 118.551 and SR59230A respectively, significantly reduced formalin-induced TMJ nociception. Co-administration of a low dose of β_1 (6μg) or β_2 (0,1μg) adrenoceptors antagonists with formalin significantly reduced formalin-induced TMJ nociception in females but not in males, indicating the existence of a sexual dimorphism in the effect of these antagonists. The findings of this study indicate that beta adrenoceptors, specifically β_1, β_2 and β_3 adrenoceptors significantly reduce TMJ pain. Furthermore, females are more sensitive than males to the antinociceptive effect of β_1 and β_2 adrenoceptor antagonists.

Keywords: Temporomandibular Joint, nociception, adrenoceptors, beta-blockers, non-steroidal anti-inflammatory drugs.

SUMÁRIO

I – INTRODUÇÃO

As disfunções temporomandibulares (DTMs) são condições dolorosas que envolvem a articulação temporomandibular (ATM) e os músculos mastigatórios, e afetam mais de 12% da população (Von Korff et al., 1988; Dworkin et al., 1990; Carlsson, 1999), com maior prevalência, severidade e duração no sexo feminino (Riley and Gilbert, 2001). Essas condições resultam principalmente de trauma agudo, desarranjo interno ou artrites, e são comumente associadas à inflamação aguda ou crônica (Alstergren and Kopp, 2000; Suzuki et al., 2003).

As condições inflamatórias da ATM promovem a sensibilização de nociceptores periféricos desta região (Raja et al., 1988; Alstergren and Kopp, 2000; Nordahl et al., 2000; Kopp, 2001; Oliveira et al., 2005) e de neurônios nociceptivos centrais do complexo sensório-nuclear trigeminal do tronco encefálico (Iwata et al., 1999; Sessle, 2000; Dubner and Ren, 2004). Tanto a sensibilização periférica como a central são caracterizadas por aumento da excitabilidade da membrana neuronal causada por mediadores inflamatórios liberados no local da lesão (Alstergren and Kopp, 2000; Kopp, 2001; Suzuki et al., 2003) e por neuropeptídeos e amino-ácidos excitatórios liberados no complexo sensório-nuclear trigeminal do tronco encefálico, respectivamente (Bereiter and Benetti, 1996; Yu et al., 1996; Bakke et al., 1998; Cairns et al., 2001). Essa sensibilização neuronal é a base da hiperalgesia ou alodinia. Alguns dos mediadores inflamatórios liberados no local da lesão que contribuem para isso, incluindo as prostaglandinas E_2, estão presentes em alta concentração no fluido sinovial de pacientes que apresentam dor na ATM (Kopp, 2001). Drogas antiinflamatórias não esteroidais (AINEs) são freqüentemente utilizadas no controle de dores inflamatórias (Dionne, 1997; List et al., 2003; Ta and Dionne, 2004). A ação analgésica dessas drogas resulta do bloqueio da síntese das prostaglandinas, prevenindo assim a sensibilização periférica dos nociceptores (Ferreira, 1972; Ferreira, 2002). No entanto, muitos pacientes podem apresentar intolerância ao tratamento prolongado com AINEs e nem todos os pacientes com dor inflamatória na ATM respondem aos efeitos de tais medicamentos (Ta and Dionne, 2004).

Sabe-se que a dor inflamatória possui um componente simpático (Levine et al., 1986; Nakamura and Ferreira, 1987) que pode predominar em casos com menor sensibilidade aos antiinflamatórios não esteroidais (AINEs).

Dados publicados recentemente (Nackley et al., 2007), demonstram que a inibição da enzima catecol-o-metil-transferase (COMT), que metaboliza as catecolaminas, induz hiperalgesia mecânica e térmica na pata de ratos semelhante à induzida pela administração do agente inflamatório carragenina. Esse efeito induzido pela inibição da COMT foi bloqueado pela administração conjunta de antagonista de receptor adrenérgico β_2 e β_3, mas não de β_1. Esses dados, juntamente com dados previamente publicados (Khasar et al., 1999a; Khasar et al., 1999b; Aley et al., 2001) indicam o envolvimento do adrenoceptor β_2 em estados hiperalgésicos e demonstram pela primeira vez a participação dos receptores β_3 na hiperalgesia.

Há alguns anos nosso laboratório tem se dedicado ao estudo do envolvimento dos β-adrenoceptores na hiperalgesia da ATM. Por exemplo, demonstramos que as aminas simpatomiméticas são liberadas no local da lesão articular onde contribuem com o desenvolvimento de hiperalgesia na ATM de ratos através da ativação de adrenoceptores β_2 localizados nessa região, mas não de adrenoceptores β_1 (Rodrigues et al., 2006). Em outro trabalho demonstramos que durante a inflamação na ATM de ratos a ativação de adrenoceptores β_2, mas não β_1, localizados na região da ATM, induz a sensibilização necessária para ocorrência de dor pelo fator de crescimento neural (Pelegrini-da-Silva et al., 2008). No entanto, não se sabe se os β-adrenoceptores contribuem com a dor instalada da ATM, como, por exemplo, a nocicepção induzida pela administração de formalina na ATM de ratos.

Clinicamente, a utilização de beta bloqueadores no tratamento da dor na ATM é um alvo em potencial, uma vez que a ATM possui rica inervação simpática (Widenfalk and Wiberg, 1990; Yoshino et al., 1998; Kido et al., 2001), e a modulação da dor por esta via iria contribuir para o tratamento de pacientes que apresentam dor inflamatória nessa região e que não respondem bem ao uso dos AINEs (Ta and Dionne, 2004). Recentemente, um estudo clínico demonstrou que mulheres com DTM ou fibromialgia podem apresentar

função adrenérgica desregulada, o que contribui com a severidade de dor corporal. Essas mulheres com DTM ou fibromialgia apresentaram uma diminuição do número de sítios dolorosos espalhados pelo corpo, uma diminuição da dor proveniente dessas regiões e uma diminuição da dor induzida por isquemia do braço após a administração endovenosa de beta-bloqueador não seletivo propranolol indicando a participação dos adrenoceptores β_1 e/ou β_2 na dor clínica em geral (Light et al., 2009). No entanto, o estudo não avaliou se o propranolol reduz especificamente a dor da ATM.

Os β-bloqueadores já são vastamente utilizados no tratamento da enxaqueca e a enxaqueca e a dor da ATM apresentam algumas características em comum. Por exemplo, tanto a enxaqueca (O'Brien et al., 1994; Stewart et al., 1994; Rasmussen, 1995) quanto à dor da ATM (Dworkin et al., 1990; LeResche, 1997) apresentam maior prevalência, severidade e duração em mulheres, durante o período reprodutivo (Stewart et al., 1992), o que sugere que essas duas condições dolorosas são moduladas por fatores hormonais.

Curiosamente, a literatura vem sugerindo que, apesar de apresentar uma maior sensibilidade dolorosa (Martinez-Gomez et al., 1994; LeResche, 1997), o sexo feminino parece ser mais sensível aos efeitos analgésicos e colaterais decorrentes da administração sistêmica de medicamentos analgésicos. Por exemplo, tem sido demonstrado que a administração sistêmica de drogas colinérgicas (Chiari et al., 1999; Lhomme et al., 1999), canabinóides (Tseng and Craft, 2001) ou nicotínicas (Craft and Milholland, 1998) é mais eficaz na redução da resposta nociceptiva induzida experimentalmente em fêmeas. Resultados semelhantes têm sido observados após o uso sistêmico de opióides. Em animais experimentais, os agonistas dos receptores opióides capa induzem um efeito antinociceptivo significativamente maior em fêmeas quando comparadas com machos (Binder et al., 2000; Tershner et al., 2000). Similarmente, em humanos, a administração sistêmica de morfina (Larijani et al., 2004), ou de agonistas seletivos para os receptores opióides capa (Gear et al., 1996a; Gear et al., 1996b) ou mu (Gordon et al., 1995), tem proporcionado uma maior analgesia pós-operatória no sexo feminino.

Com relação à dor na ATM, dados obtidos em nosso laboratório (Clemente et al., 2004) demonstram que fêmeas são mais sensíveis ao efeito antinociceptivo desencadeado pela administração local do agonista do receptor opióide capa, quando comparadas com

machos. Portanto, é possível que exista também um dimorfismo sexual envolvido na ação de β-bloqueadores com relação à dor da ATM.

II – PROPOSIÇÃO

Os objetivos do presente trabalho foram avaliar a participação de receptores β-adrenérgicos 1, 2 e 3 na nocicepção induzida por formalina na ATM de ratos e verificar se existe dimorfismo sexual no efeito dos β bloqueadores 1, 2 e 3 neste mesmo modelo.

O presente estudo está apresentado em formato alternativo, conforme deliberação da Comissão Central de Pós-Graduação (CCPG) da Universidade Estadual de Campinas (UNICAMP) n° 001/98.

III – CAPÍTULO

O presente estudo será submetido ao periódico "The Journal of Pain".

Sex differences in the antinociception mediated by blockade of beta adrenoceptors in the rat temporomandibular joint.

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Abstract

Temporomandibular joint receives rich sympathetic innervations. However, whether the blockade of beta adrenoceptors reduces temporomandibular joint pain remains unclear. In this study we investigated whether beta adrenoceptors in the rat temporomandibular joint modulates temporomandibular joint nociception resulting from formalin injection into this joint. We also evaluated sex differences by comparing the responses of females in different phases of the estrous cycle and males. Co-administration of the selective adrenoceptors antagonists β_1, β_2 and β_3, Atenolol, ICI 118.551 and SR59230A respectively, with equinociceptive concentrations of formalin significantly reduced formalin-induced temporomandibular joint nociception in a dose response fashion in males and proestrus and diestrus females. However, a low dose of Atenolol (6μg) or ICI 118.551 (0.3μg) significantly attenuated nociceptive responses in both groups of females but not in males. These findings indicate that beta adrenoceptors in the temporomandibular joint region modulate temporomandibular joint pain in both males and females. However, formalin-induced nociceptive responses are significantly more responsive to beta 1 and 2 adrenoceptor-antagonists in females than in males.

Keywords: Temporomandibular joint, nociception, adrenoceptors, beta-blockers, non-steroidal anti-inflammatory drugs, sexual dimorphism.

Introduction

Temporomandibular joint (TMJ) disorders, especially those associated with acute trauma, internal derangement, or arthritis are commonly associated with acute or chronic inflammation (Alstergren and Kopp, 2000; Suzuki et al., 2003). They represent a group of chronic painful conditions involving the muscles of mastication and the TMJ with prevalence in the general population up to 12% (Von Korff et al., 1988; Dworkin et al., 1990; Carlsson, 1999).

Non-steroidal anti-inflammatory drugs have been frequently used in the control of inflammatory pain (Dionne, 1997; List et al., 2003; Ta and Dionne, 2004). However, many patients are intolerant to prolonged treatment with NSAIDs and not all patients with TMJ inflammatory pain respond to its effects (Ta and Dionne, 2004).

It is well known that inflammatory pain has a sympathetic component (Levine et al., 1986; Nakamura and Ferreira, 1987) that may predominate in pain less sensitive to NSAIDs. Since the TMJ receives a rich sympathetic innervation (Widenfalk and Wiberg, 1990; Yoshino et al., 1998; Kido et al., 2001), the clinical use of beta blockers in the treatment of TMJ pain might be of benefit, especially in those patients that do not respond to the NSAIDs. Recently, it has been demonstrated that women with TMJ pain have adrenergic dysregulation and greater clinical whole-body pain that are improved by acute treatment with low dose of the non-selective beta blocker propranolol (Light et al., 2009). However, whether the blockade of beta1, 2 or 3 adrenoceptors in the TMJ region reduce specifically TMJ pain remains an open question. Sex differences in the analgesic effect of drugs have been reported (Craft and Milholland, 1998). For example, in the rat TMJ application of morphine induces greater antinociception in males (Cai et al., 2001), while application of the selective kappa opioid agonist U50488 (Clemente et al., 2004) induces greater analgesia in females. These findings, taken together with evidence that TMJ pain is significantly more prevalent in women, (Riley and Gilbert, 2001) highlight the potential clinical relevance of beta blockers-mediated analgesia in the treatment of TMJ pain. The aim of the current study was to determine if beta adrenoceptors in the rat TMJ play a role in modulating inflammatory pain resulting from formalin injection. We also evaluated sex

differences by comparing the responses of females in different phases of the estrous cycle and males.

Experimental procedures

Materials and Methods

Animals

Males and females Wistar rats (200-250g) obtained from the Multidisciplinary Center for Biological Research (CEMIB) – University of Campinas, were used in this study. The animals were housed in plastic cages with soft bedding (five/cage) on a 12:12 light cycle (lights on at 06:00 A.M.) with food and water available *ad libitum*. They were maintained in temperature-controlled room (±23°C) and handled at least 1 week prior to the experiments. Experimental protocols were approved by the Committee on Animal Research of the University of Campinas (protocols number: 2014-1 and 2015-1) and conformed to IASP guidelines for the study of pain in animals (Zimmermann, 1983). Animal suffering and the number of rats per group were kept at the minimum.

Drugs and doses

The following drugs were used: Formalin (aqueous solution of 37% of formaldehyde) 1.0% (diestrus females) and 1.5% (proestrus females and males) (Clemente et al., 2004); ((RS)-4-[2-hydroxy-3-[(1methylethyl) amino] propoxy] benzeneacetamide): antagonist of β_1 adrenoreceptors Atenolol 6µg, 18µg, 54µg e 162µg/15uL (Rodrigues et al., 2006); ((±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-y) oxy]-3-[(1-methylethyl) amino]-2-butanol hydrochloride): selective antagonist of β_2 adrenoreceptors ICI 118.551 0.1µg, 0.3µg e 0.9µg/15uL (Rodrigues et al., 2006); 1-(2-Ethylphenoxy)-3-[[(1S)-1,2,3,4-tetrahydro-1naphth alenyl]amino]-(2S)-2-propanol hydrochloride: antagonist of β3 adrenoreceptors SR59230A hydrochloride 1.5µg, 4.5µg e 13.5µg/15uL (Nackley et al., 2007), prepared in DMSO. SR59230A was obtained from Tocris Bioscience (Ellisville, MO) and all other drugs were obtained from Sigma–Aldrich (MO, USA). All drugs were dissolved in sterile saline (0.9% NaCl).

Estrous phase determination

Estrous phase was determined by daily microscope examination of vaginal smears between 7 and 8 a.m. In the day of the experiment, estrous phase was confirmed before and immediately after each experiment to ensure that the rats remained in the same phase. Proestrus phase and the initial phase of diestrus (first 4 h) were identified by the predominance (>70%) of nucleated epithelial cells and leukocytes, respectively (Butcher et al., 1974) in rats with at least two consecutive regular 4-5 day cycles. These phases were chosen because they represent phases of high and low ovarian hormonal level, respectively (Butcher et al., 1974).

TMJ Injections

The animals were briefly anesthetized by inhalation of Isoflurane to allow the TMJ injection; the posteroinferior border of the zygomatic arch was palpated. A needle was inserted immediately inferior to this point and was advanced in the anterior direction until reaching the posterolateral aspect of the condyle. TMJ injections were performed with a 30-gauge needle introduced into the TMJ at the moment of injection. A cannula consisting of a polyethylene tube was connected to the needle and also to a Hamilton syringe (50µl) (Roveroni et al., 2001). Each animal regained consciousness approximately 30 seconds after discontinuing the anesthetic. The volume injection was 15µl per drug.

After the conclusion of each experiment, animals were anesthetized with an intraperitoneal injection of a mixture of urethane (1g/Kg) and α-chloralose (50mg/Kg). Evans blue dye (0.1%, 5mg/Kg) was then administered systemically to visualize the inflammation-induced plasma extravasation of Evans blue dye bound to plasma protein upon postmortem examination of the injected TMJs. The correct site of injection was indicated by the TMJ injections was restricted to the TMJ region.

Testing procedure for TMJ pain

Behavior test was performed during light phase (between 09:00 AM and 5:00 PM) in a quiet room maintained at ±23ºC (Rosland, 1991). Rats did not have access to food or water during the test and each animal was used once. The nociceptive response was

assessed by an observer blinded to the experimental manipulation. Before the experiments, each animal was manipulated for 7 days in the test room (handled for approximately one minute) to be habituated to the experimental manipulation. On the day of the experiment, each animal was individually placed in a test chamber (30 x 30 x 30 cm mirrored-wood chamber with a glass at the front side) for a 10 minutes habituation period to minimize stress. After the TMJ injection, the animal was returned to the test chamber for counting two types of nociceptive behavior, rubbing the orofacial region asymmetrically with the ipsilateral fore or hind paw and flinching the head in an intermittent and reflexive way characterized by high frequency shakes of the head. These behaviors were quantified in blocks of 5 minutes for 45 minutes. For each block of 5 min, the behavior characterized by rubbing the orofacial region was quantified by a chronometer that recorded the amount of time that the animal exhibited it and the behavior characterized by flinching the head was quantified by a hand tally counter that recorded its occurrence. Considering that the flinching head behavior followed a uniform pattern of 1 second in duration, each flinching was expressed as 1 second. The TMJ formalin nociceptive behaviors (flinching and rubbing) were summed and expressed in seconds as previously described (Roveroni et al., 2001).

Statiscal analysis

The nociceptive behavior score, obtained by summing the flinching and rubbing behaviors recorded during the entire duration of the experiment was used in statistical analysis. For Fig. 1, 2 and 3 a t-test or a one-way analysis of variance, as appropriate, was used to determine if there were significant differences in nociceptive responses among the groups. Tukey post hoc tests were employed to determine the basis of significant differences. A probability level of p less than 0.05 was considered to indicate statistical significance. Data are plotted in figures as mean±S.E.M..

Results

Effect of formalin on males and females

The nociceptive responses of diestrus females administered 1% formalin were not significantly different from those of males and proestrus females administered 1.5% formalin (Fig. 1, $p>0.05$, Tukey test). Therefore, these equinociceptive concentrations of formalin were used in the respective groups in subsequent experiments as previously described (Clemente et al., 2004).

Effect of beta1, 2 and 3-adrenoceptors antagonists on formalin-induced nociceptive behavior

To verify whether beta 1, 2 and 3-adrenoceptors contribute to formalin-induced nociception, selective antagonists for beta 1, 2 or 3 adrenoceptors were co-administred with equinociceptive concentrations of formalin (1.5% in males and proestrus females, and 1.0% in diestrus females) in the rat´s TMJ.

All beta-adrenoceptors antagonists tested significantly reduced formalin-induced nociception in males and females in a dose related fashion ($p< 0.05$, Tukey test, Fig. 2, 3 and 4).

The highest doses of these antagonists did not affect formalin-induced nociception when applied on the contralateral TMJ (Fig. 2, 3 and 4, $p>0.05$, t test), confirming their local peripheral action. Co-administration with the highest dose of each antagonist with 0.9% NaCl had no effect by itself since the response was similar to that induced by the TMJ injection of 0.9% NaCl plus vehicle (Fig. 2, 3, and 4, $p>0.05$, t test).

The lowest dose of Atenolol (6µg) significantly attenuated nociceptive responses in both groups of females but not in males (Fig. 2), indicating that formalin-induced nociceptive responses are significantly more responsive to beta 1 adrenoceptor-antagonists in females than in males. Similarly, a low dose of ICI 118.551 (0.3µg) significantly attenuated nociceptive responses in both groups of females but not in males (Fig. 3), indicating that formalin-induced nociceptive responses are also significantly more responsive to beta 2 adrenoceptor-antagonists in females than in males. In contrast, the

lowest dose of SR59230A tested (1.5µg) significantly attenuated nociceptive responses in both groups of females and also in males (Fig. 4).

Discussion

In this study, we demonstrated that blockade of Beta 1, 2 or 3 adrenoceptors in the rat TMJ attenuates inflammatory pain resulting from injection of equinociceptive concentrations of formalin in males and females. However, formalin-induced nociceptive responses were significantly more responsive to β_1 and β_2 adrenoceptor-antagonists in females than in males.

The findings that blockade of articular β_1, β_2 and β_3-adrenoceptors significantly attenuated TMJ nociception indicate that catecholamines contribute to the development of TMJ nociception, by activating these adrenoceptors located in the TMJ region. This is consistent with the dense innervation of the temporomandibular joint by sympathetic fibers arising from cells of the superior cervical ganglion (Widenfalk and Wiberg, 1990; Yoshino et al., 1998; Kido et al., 2001) from where catecholamines may be released. This sympathetic component of TMJ inflammatory pain may predominate in some patients that suffer from TMJ pain and may explain why some of these patients are unresponsive to the analgesic effect of NSAIDs.

New evidence suggests the existence of a genetic basis for the effects that we observed. In a prospective study of TMD development, 3 genetic variants (haplotypes) for the gene-encoding catecholamine-O-methyltransferase (COMT), an enzyme that catalyses the O-methylation of all catechol compounds including dopamine, Norepinephrine and Epinephrine were examined. The haplotype linked to the lowest COMT activity was associated with the highest pain sensitivity and with the greatest risk for TMJ pain development (Diatchenko et al., 2006). Furthermore, it has been suggested that either positive or negative imbalances in adrenergic receptor β_2 function increase the vulnerability to develop TMJ pain (Diatchenko et al., 2007).

Two basic physiological properties are described in primary afferent neurons: pain transmission (nociception) and sensitization (hyperalgesia). While nociception is essentially an ionotropic event, sensitization is a metabolic phenomenon that facilitates and increases the nociception.

It has been demonstrated that COMT inhibition induces mechanical and thermal hyperalgesia in rat´s paw that is blocked by the co-administration of the beta (2 and 3 but

no 1)-adrenoceptors antagonists (Nackley et al., 2007). In the TMJ region, we have recently shown that blockade of β_2 but not β_1 adrenoceptor by the selective adrenoceptor antagonists ICI 118.55 and Atenolol, respectively, significantly reduces carrageenan-induced TMJ hyperalgesia (Rodrigues et al., 2006). These findings taken together with our current findings that blockade of both β_1, and β_2 adrenoceptors significantly attenuated TMJ nociception support the suggestion that the mechanisms mediating hyperalgesia can be separate and distinct from those mediating nociception (Taiwo and Levine, 1991; Waldron and Sawynok, 2004; Oliveira et al., 2007), although common mechanisms may be involved. Furthermore, these findings also suggest that the lack of effect of Atenolol in TMJ hyperalgesia is not due to the inexistence of functional β_1 adrenoceptor in the TMJ region.

The mechanisms underlying the contribution of catecholamines to formalin-induced TMJ nociception via Beta 1, 2 and 3-adrenoceptor activation is presently unknown. However, it might result from a direct or indirect action of these amines on the primary afferent nociceptive terminals. Although β-adrenoceptor RNA has not been detected in the dorsal root ganglion (Nicholson et al., 2005) no previous studies appear to have examined the presence of β-adrenoceptor RNA or binding sites within nociceptive trigeminal neurons. While the presence of β-adrenoceptor RNA or binding sites within nociceptive trigeminal neurons would support the hypothesis that catecholamines contribute to formalin-induced TMJ nociception by a direct action of these amines on the primary afferent nociceptive terminals, the expression of beta-adrenoceptors in macrophage, eosinophils, mast cells, lymphocytes, neutrophils (Barnes, 1993) and basophiles cells (Perper et al., 1972) support an indirect action of these amines on the primary afferent nociceptive terminals.

The present data indicate that females are more responsive to the antinociceptive effect of β_1 or β_2 but not β_3 adrenoceptor antagonists than males. This finding is consistent with a previous report that peripherally acting kappa opioid receptor agonists induce greater antinociception in female than male rats (Binder et al., 2000; Clemente et al., 2004; Clemente-Napimoga et al., 2009). On the other hand, morphine, which acts predominantly at mu opioid receptors (Kitanaka et al., 1998), administered into the rat TMJ induces greater antinociception in males (Cai et al., 2001). These findings, taken together with the

current findings suggest that sex differences depend, at least in part, on the particular receptor type under study.

The physiological basis for the sex-related difference in Beta 1 and 2-adrenoceptor blockade-mediated TMJ antinociception is not presently known. Our findings that females are more responsive to the antinociceptive effect of Beta 1 or 2-adrenoceptor antagonists than males suggest that testosterone might attenuate the antinociceptive effect induced by these antagonists. Therefore, additional work is warranted with gonadectomized rats to more precisely determine how sex hormones modulate the antinociceptive effects of β_1 and β_2 adrenoceptor antagonists.

In summary, the present findings suggest that $\beta_{1,\ 2}$ and $_3$ adrenoceptors in the rat TMJ play a role in modulating inflammatory pain resulting from formalin injection. Blockade of these receptors suppresses formalin-induced TMJ nociceptive behavior in both males and females but females are more responsive. In light of the evidence that clinical TMJ pain is more prevalent in women than in men (Dworkin et al., 1990), these findings suggest that beta adrenoceptor antagonists could be of benefit in the treatment of TMJ pain, especially in women. Furthermore, since TMJ pain is higher in women than in men, the present findings warrant further studies to better evaluate the effects of β-adrenergic antagonists on the treatment of TMJ pain in women and men.

Figures and legends

Figure 1

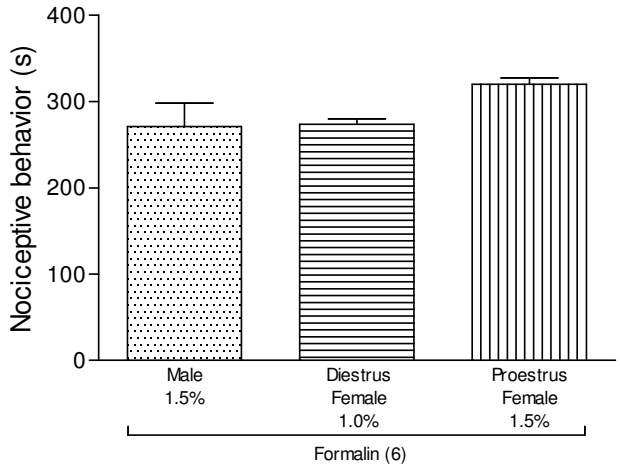
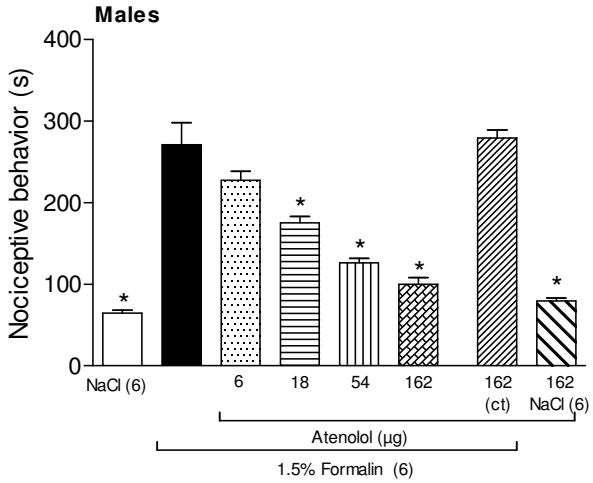


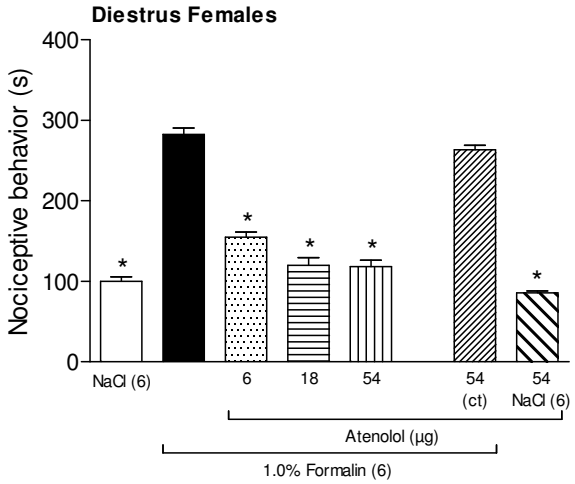
Fig. 1 – Effect of different concentrations of formalin in rats of both sexes.
Administration of formalin 1.0% in diestrus females and 1.5% in proestrus females and males into the rat TMJ induced nociceptive responses similar to each other ($p>0.05$, Tukey test). The number of rats used is between parentheses.

Figure 2

A


Males
Nociceptive behavior (s)
400
300
200
100
0
NaCl (6)
6
18
54
162
162 (ct)
162 NaCl (6)
Atenolol (µg)
1.5% Formalin (6)

B


Diestrus Females
Nociceptive behavior (s)
400
300
200
100
0
NaCl (6)
6
18
54
54 (ct)
54 NaCl (6)
Atenolol (µg)
1.0% Formalin (6)

C

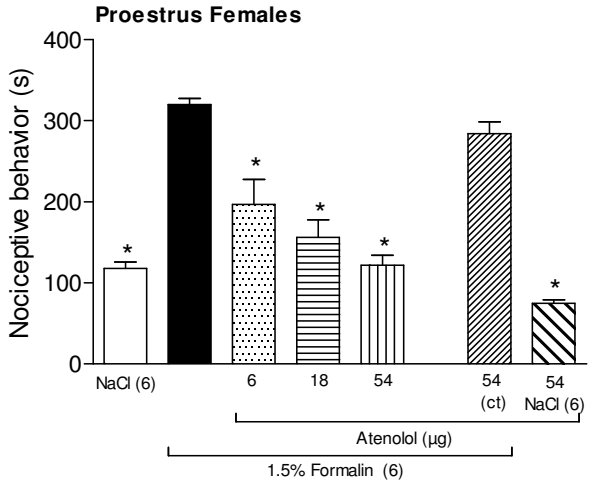
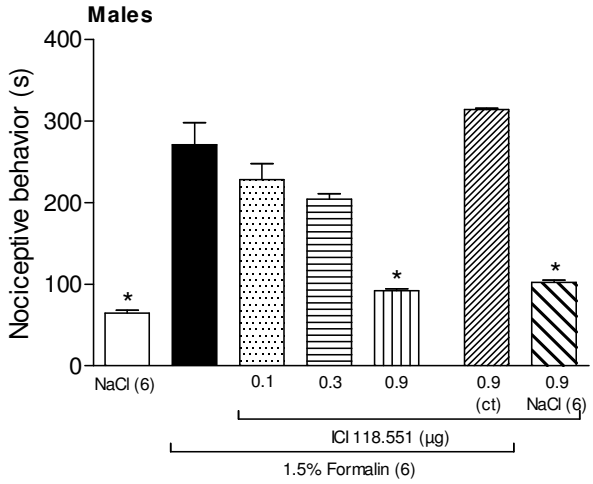


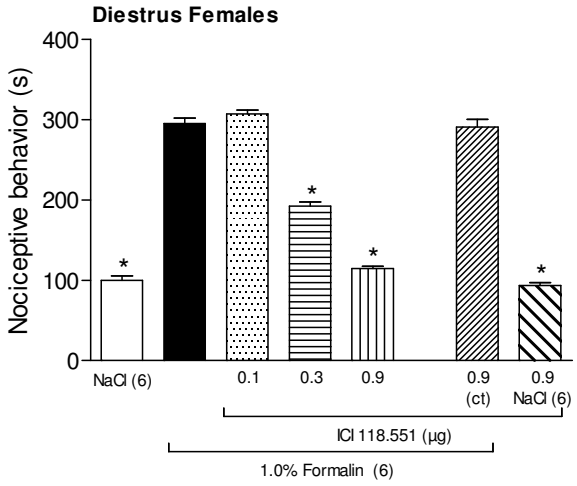
Fig.2 – Effect of locally administered β1 adrenoreceptor antagonist Atenolol on TMJ formalin-induced nociceptive behavior in males, diestrus females and proestrus females.
Co-administration of Atenolol with equinociceptive concentrations of formalin significantly reduced formalin-induced nociceptive behavior in males (A), diestrus females (B) and proestrus females (C). However, at 6µg Atenolol significantly reduced formalin-induced nociceptive behavior only in females. The symbol "*" indicates a response significantly lower than that of formalin in males, diestrus and proestrus females ($p<0.05$, ANOVA post hoc Tukey test). Co-administration of Atenolol (54µg or 162µg) with 0.9% NaCl had no effect by itself ($p>0.05$, t test). Administration of Atenolol (54µg or 162µg) into contralateral (ct) TMJ did not affect formalin-induced nociceptive behavior ($p>0.05$, t test).

Figure 3

A


Males
Nociceptive behavior (s)
400
300
200
100
0
*
*
*
NaCl (6)
0.1
0.3
0.9
0.9
(ct)
0.9
NaCl (6)
ICI 118.551 (µg)
1.5% Formalin (6)

B


Diestrus Females
Nociceptive behavior (s)
400
300
200
100
0
*
*
*
*
NaCl (6)
0.1
0.3
0.9
0.9
(ct)
0.9
NaCl (6)
ICI 118.551 (µg)
1.0% Formalin (6)

C

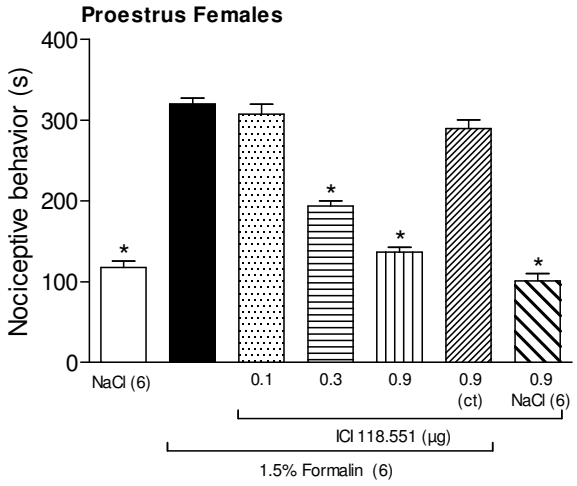
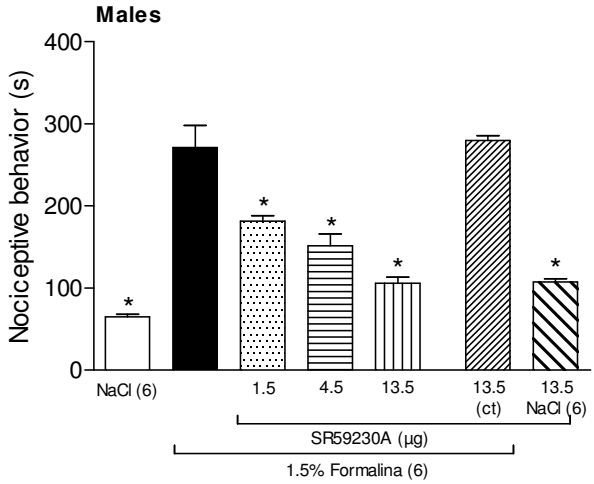


Fig.3 – Effect of locally administered β2 adrenoreceptor antagonist ICI 118.551 on TMJ formalin-induced nociceptive behavior in males, diestrus females and proestrus females.

Co-administration of ICI 118.551 with equinociceptive concentrations of formalin significantly reduced formalin-induced nociceptive behavior in males (A), diestrus females (B) and proestrus females (C). However, at 0.3µg ICI 118.551 significantly reduced formalin-induced nociceptive behavior only in females. The symbol "*" indicates a response significantly lower than that of formalin in males, diestrus and proestrus females ($p<0.05$, ANOVA post hoc Tukey test). Co-administration of ICI 118.551 (0.9µg) with NaCl had no effect by itself ($p>0.05$, t test). Administration of ICI 118.551 (0.9µg) into contralateral (ct) TMJ did not affect formalin-induced nociceptive behavior ($p>0.05$, t test).

Figure 4

A


Males
Nociceptive behavior (s)
400
300
200
100
0
NaCl (6)
1.5
4.5
13.5
13.5 (ct)
13.5 NaCl (6)
SR59230A (µg)
1.5% Formalina (6)

B


Diestrus Females
Nociceptive behavior (s)
400
300
200
100
0
NaCl (6)
1.5
4.5
13.5
13.5 (ct)
13.5 NaCl (6)
SR59230A (µg)
1.0% Formalin (6)

C

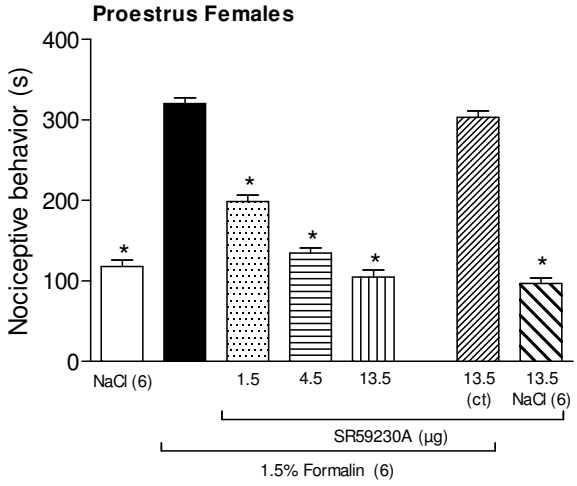


Fig.4 – Effect of locally administered β3 adrenoreceptor antagonist SR59230A *on TMJ formalin-induced nociceptive behavior in males, diestrus females and proestrus females.*

Co-administration of SR59230A with equinociceptive concentrations of formalin significantly reduced formalin-induced nociceptive behavior in males (A), diestrus females (B) and proestrus females (C). The symbol "*" indicates a response significantly lower than that of formalin in males, diestrus and proestrus females ($p<0.05$, ANOVA post hoc Tukey test). Co-administration of SR59230A (13.5µg) with NaCl had no effect by itself ($p>0.05$, t test). Administration of SR59230A (13.5µg) into contralateral (ct) TMJ did not affect formalin-induced nociceptive behavior ($p>0.05$, t test).

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IV – CONCLUSÕES

O presente trabalho demonstrou que β-adrenoceptors 1, 2 e 3 participam da nocicepção induzida por formalina na ATM de ratos e que existe um dimorfismo sexual modulando o efeito antinociceptivo de antagonistas beta-adrenérgicos 1 e 2, mas não antagonista de adrenoceptores beta 3. Os dados obtidos sugerem que os beta-bloqueadores podem ser uma alternativa interessante no tratamento de pacientes com dor da ATM que apresentam intolerância ao tratamento prolongado com antiinflamatórios não esteroidais ou que simplesmente não respondem aos seus efeitos. Além disso, o dimorfismo sexual observado sugere que doses dos medicamentos beta-bloqueadores devem ser diferenciadas entre homens e mulheres para obtenção do efeito analgésico mais eficiente entre os sexos, uma hipótese que ainda deve ser mais estudada para se elucidar os mecanismos envolvidos neste dimorfismo.

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VI – ANEXOS

Anexo 1

UNICAMP

CEUA/Unicamp

Comissão de Ética no Uso de Animais
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CERTIFICADO

Certificamos que o Protocolo nº **2014-1**, sobre "**Papel dos adrenoceptores beta na nocicepção induzida por formalina na ATM de ratos**", sob a responsabilidade de **Profa. Dra. Claudia Herrera Tambeli / Nádia Cristina Fávaro Moreira**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética no Uso de Animais – CEUA/Unicamp em **27 de novembro de 2009**.

CERTIFICATE

We certify that the protocol nº **2014-1**, entitled "**Role of beta adrenoceptores in the nociception induced by formalin in rats'TMJ**", is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on **November 27, 2009**.

Campinas, 27 de novembro de 2009.

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Anexo 2

UNICAMP

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Comissão de Ética no Uso de Animais
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CERTIFICADO

Certificamos que o Protocolo nº **2015-1**, sobre **"Papel dos adrenoceptores beta na nocicepção induzida por formalina na ATM de ratas em diferentes status hormonal"**, sob a responsabilidade de **Profa. Dra. Claudia Herrera Tambeli / Nádia Cristina Fávaro Moreira**, está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética no Uso de Animais – CEUA/Unicamp em **27 de novembro de 2009**.

CERTIFICATE

We certify that the protocol nº **2015-1**, entitled **"Role of beta adrenoceptores in the nociception induced by formalin in the TMJ of females rats with different hormonal status"**, is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - Unicamp) on **November 27, 2009**.

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Anexo 3

Anexo 3 **UNIVERSIDADE ESTADUAL DE CAMPINAS**

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Piracicaba, 15 de Março de 2010.

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