NÁDIA CRISTINA FAVARO MOREIRA

β-BLOCKERS EFFECT IN THE TEMPOROMANDIBULAR JOINT PAIN IN RATS AND HUMANS AND ITS MODULATION BY GONADAL HORMONES

EFEITO DOS β-BLOQUEADORES NA DOR DA ARTICULAÇÃO TEMPOROMANDIBULAR DE RATOS E HUMANOS E SUA MODULAÇÃO PELOS HORMÔNIOS GONADAIS

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2014

Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

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Orientadora: Profa. Dra. Cláudia Herrera Tambeli

Este exemplar corresponde à versão final da tese defendida pela aluna Nádia Cristina Fávaro Moreira e orientada pela Profa. Dra. Cláudia Herrera Tambeli.

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ABSTRACT

Temporomandibular disorder (TMD) is a collective term embracing a number of clinical problems that involve the masticatory muscles, and the temporomandibular joint (TMJ), commonly associated with inflammation. Despite anti-inflammatory drugs (NSAIDs) are frequently used in the control of inflammatory pain, it is well known that inflammatory pain has a sympathetic component that might predominate in the cases less responsive to NSAIDs treatments. Therefore, the aims of this study were: (i) to evaluate whether gonadal hormones modulate the antinociceptive responsiveness to the blockade of β-adrenoreceptors (ARs) in the TMJ of rats, (ii) to evaluate whether one and three days of treatment with the nonselective β_1 and β_2-AR antagonist nadolol would reduce clinical pain symptoms in TMD patients significantly more than placebo, (iii) to evaluate whether women would experience relatively greater benefit from nadolol than men depending on their hormonal status, and (iv) compared the effect of nadolol with the effect of ibuprofen. The first aim was developed in male and female rats, intact or gonadectomized (with or without hormone replacement), by coadministration of formalin and specific antagonist for β_1, β_2 and β_3-ARs in the TMJ region. The nocieptive behavior was quantified and used for estatistical analysis. For the second aim Nadolol (40 mg/day), ibuprofen (400 mg/day) or placebo was administred in 27 women not using oral contraceptive (OC), 28 women using OC, and 29 men which met the Research Diagnostic Criteria for TMD. They completed a randomized, crossover, double–blind, placebo controlled study. Women participated for three months (during menstrual phase and during peri-ovulatory phase in women not using OC, and during menstrual phase and during OC using phase in women using OC) in a total of 6 stages of analysis (2 per month), and men participated for one month whith three stages of analysis and 6 days of wash out. Each stage consisted of a baseline evaluation and two evaluations during treatment, one on the first and the other on the third day of treatment. Clinical pain ratings were obtained by Visual Analog Scale (VAS) and comparisons were made across the pre-treatment (baseline), the first and the third

day of intervention (post-treatment). Blockade of β-ARs significantly reduce the TMJ nociception in male and female rats, but females are more responsive. The gonadal hormones reduce the responsiveness to the blockade of β-ARs in the TMJ of males and females rats. In the human study one and three days of treatment with nadolol or ibuprofen produces analgesia in TMD women and men significantly more than placebo, but women are more responsive independent of their hormonal status. In summary, these data demonstrate that gonadal hormones can modulate the analgesic effect of β-ARs blockers depending on the gonadal hormones serum levels, on the β-ARs activated subtype and on the dose of drug administred. The greater treatment efficacy in women is of clinical relevance since TMD is more prevalent and severe in women than in men.

Key Words: Temporomandibular Joint Disorders. Nociception. Pain. Analgesia. Beta Adrenergic Receptors. Adrenergic beta-Antagonists.

RESUMO

Disfunção temporomandibular (DTM) é um termo coletivo que abrange uma série de problemas clínicos que envolvem os músculos mastigatórios e a articulação temporomandibular (ATM) e está comumente associada à inflamação. Apesar de medicamentos anti-inflamatórios não esteróides (AINEs) serem frequentemente utilizados no controle de dor inflamatória, sabe-se que a dor inflamatória possui um componente simpático que pode predominar em casos menos responsivos a tratamentos com AINEs. Portanto, os objetivos deste estudo foram: (i) avaliar se os hormônios gonadais modulam a resposta antinociceptiva ao bloqueio dos β-adrenoreceptores (ARs) na ATM de ratos, (ii) avaliar se um e três dias de tratamento com o β-bloqueador não seletivo para AR β_1 e β_2 nadolol reduzem a dor em pacientes com DTM significativamente mais do que o placebo, (iii) avaliar se as mulheres experimentam relativamente maior benefício com o tratamento com nadolol que os homens dependendo do seu estado hormonal, e (iv) comparar o efeito do nadolol com o efeito do ibuprofeno. O primeiro objetivo foi desenvolvido em ratos machos e fêmeas, intactos ou gonadectomizados (com ou sem reposição hormonal) através da coadministração de formalina e antagonista específico para AR β_1, β_2 e β_3 na região da ATM. O comportamento nocieptive foi quantificado e utilizado para a análise estatística. Para o segundo objetivo Nadolol (40 mg/dia), ibuprofeno (400 mg/dia) ou placebo foi administrado em 27 mulheres que não usam contraceptivo oral (CO), 28 mulheres usando CO e 29 homens, que estavam de acordo com os Critérios Diagnósticos para Pesquisa (Research Diagnostic Chriteria) para DTM. Eles completaram um estudo cruzado, aleatorizado, duplo-cego e com controle placebo. As mulheres participaram durante três meses (durante a fase menstrual e durante a fase peri-ovulatória em mulheres que não usam CO, e durante a fase menstrual e durante a fase de uso do CO em mulheres usando CO), em um total de 6 etapas de análise (2 por mês), e homens participaram durante um mês com três etapas de análise com um período de 6 dias entre cada etapa. Cada etapa consistiu de uma avaliação de basal e duas avaliações durante o tratamento, uma no primeiro e o outra no

terceiro dia de tratamento. A dor foi avaliada por meio da utilização da escala visual analógica (VAS) e as comparações foram feitas através do pré-tratamento (basal), o primeiro e o terceiro dia de intervenção (pós-tratamento). O bloqueio dos ARs β reduz significativamente a nocicepção da ATM em ratos machos e fêmeas, mas as fêmeas são mais sensíveis. Os hormônios gonadais reduziram a resposta ao bloqueio de ARs β na ATM de machos e fêmeas. No estudo em humanos um e três dias de tratamento com nadolol ou ibuprofeno produz uma analgesia em mulheres e homens significativamente maior que placebo, mas as mulheres são mais sensíveis independente do estado hormonal. Em resumo, estes dados demonstram que os hormonios sexuais podem modular o efeito analgésico de bloqueadores de ARs β dependendo dos níveis séricos dos hormonios gonadais, do subtipo de ARs β ativado e da dose de droga administrada. A maior eficácia no tratamento da dor em mulheres é clinicamente relevante uma vez que a DTM é mais prevalente e mais severa em mulheres do que em homens.

Palavras-chave: Transtornos da Articulação Temporomandibular. Nocicepção. Dor. Analgesia. Receptores Adrenérgicos Beta. Antagonistas Adrenérgicos Beta.

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“Viver no coração daqueles que deixamos:
isso não é morrer”

Goeth

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“Contigo aprendi
Que existem novas e melhores emoções
Contigo aprendi
A conhecer um mundo novo de ilusões

Aprendi
Que a semana já tem mais de sete dias
Fazer maiores minhas poucas alegrias
E ser feliz eu contigo aprendi

Contigo aprendi
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Contigo aprendi
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INTRODUÇÃO

Disfunção temporomandibular (DTM) é um termo coletivo que abrange uma série de problemas clínicos que envolvem os músculos mastigatórios, a articulação temporomandibular (ATM) e estruturas associadas (Winocur et al., 2003). Afetam mais de 12% da população (Von Korff et al., 1988; Dworkin et al., 1990; Carlsson, 1999, Bender, 2014), 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).

A inflamação da ATM promove 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 aminoácidos 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). Nessas condições, o limiar nociceptivo diminui resultando em alodínia, ou seja, dor desencadeada pela aplicação de um estímulo não doloroso (Alstergren and Kopp, 2000) e hiperalgesia, ou seja, um aumento da dor já instalada em resposta à aplicação de um estímulo doloroso (De Laat et al., 1998). Alguns dos mediadores inflamatórios liberados no local da lesão que contribuem para a alodínia e hiperalgesia, 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 AINEs. Há alguns anos nosso laboratório tem se dedicado ao estudo do envolvimento dos β-adrenoreceptores (AR) 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 AR β_2 localizados nessa região, mas não de AR β_1 (Rodrigues et al., 2006). Em outro trabalho demonstramos que durante a inflamação na ATM de ratos a ativação de AR β_2, mas não de AR 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).

Estes resultados estão de acordo com dados publicados previamente (Nackley et al., 2007) que 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 AR β_2 e AR β_3, mas não de AR β_1. Esses dados, juntamente com dados previamente publicados (Khasar et al., 1999a; 1999b; Aley et al., 2001) indicam o envolvimento de AR β_2 em estados hiperalgésicos e demonstram pela primeira vez a participação dos AR β_3 na hiperalgesia.

Em seguida, demonstramos que o bloqueio do AR β_1, β_2 e β_3 na ATM de ratos reduz significativamente a nocicepção induzida por formalina na ATM de

maneira dependente da dose (Fávaro-Moreira et al., 2012). Corroborando com estes resultados estudos recentes demonstraram que de fato mulheres com DTM e fibromialgia frequentemente apresentam atividade β-adrenérgica desregulada o que contribui para a severidade clínica da dor. Neste estudo, o número de sítios dolorosos espalhados pelo corpo e a dor clínica de maneira geral diminuíram significativamente depois do tratamento intravenso com β-bloqueador propranolol indicando a participação dos AR β_1 e/ou β_2 na dor clínica em geral (Light et al., 2009). Mais especificamente com relação às DTMs outro estudo demonstra que ocorre uma redução na intensidade da dor relacionada à ATM em mulheres durante o tratamento com propranolol (Tchivileva et al., 2010).

Clinicamente, a utilização de β-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).

Os β-bloqueadores já são vastamente utilizados no tratamento da enxaqueca sendo que esta condição 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. Ainda, estudos sugerem que variações dos níveis dos hormônios sexuais associados ao ciclo menstrual podem modular a percepção dolorosa (Hapidou and Rollman, 1998; Riley et al., 1999).

Nesse aspecto, curiosamente a literatura vem sugerindo que, apesar de apresentar uma maior sensibilidade dolorosa (Martinez-Gomez et al., 1994; LeResche, 1997), o gênero 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 κ 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 κ (Gear et al., 1996a; Gear et al., 1996b) ou μ (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 κ, quando comparadas com machos. No entanto, se existe um dimorfismo sexual atuando sobre o efeito antinociceptivo de antagonistas seletivos de AR β_1, β_2 e β_3, este ainda permanece desconhecido.

Se as diferenças hormonais endógenas entre os gêneros parecem ter um papel importante na modulação da dor associada à DTM e à enxaqueca, presume-se então que hormônios reprodutivos exógenos (por exemplo, contraceptivos orais) também possam modular estas condições. A influência dos hormônios exógenos especificamente com relação à dor na ATM ainda é contraditória na literatura. Por um lado alguns estudos demonstram que o uso de CO parece estar associado ao desenvolvimento da DTM, levando à um aumento no número de casos em tratamento para dor relacionada à DTM (Abubaker, 1992; LeResche, 1997). No entanto, existem estudos demonstrando um baixo uso de CO em mulheres com DTM quando comparadas ao grupo controle (Marbach et al., 1988).

Portanto, é possível que o efeito dos β-bloqueadores no tratamento da dor da ATM também seja influenciado por variações hormonais endógenas ou

exógenas, além de apresentar um possível dimorfismo sexual, sugerindo que doses dos medicamentos β-bloqueadores talvez devam ser diferenciadas entre homens e mulheres, e entre mulheres em diferentes status hormonal, para obtenção do efeito analgésico mais eficiente entre os sexos.

Assim, os objetivos deste trabalho foram:

1 - Demonstrar a participação do componente simpático (β-AR) na modulação da nocicepção/dor na região da ATM de ratos e humanos;

2 - Verificar a presença de dimorfismo sexual no efeito do bloqueio dos β-AR na ATM;

3 - Verificar se os hormônios gonadais modulam o efeito do bloqueio dos β-AR na ATM.

Capítulo 1*

GONADAL HORMONES MODULATE THE ANTINOCICEPTION INDUCED BY BLOCKADE OF B-ADRENORECEPTORS IN THE TEMPOROMANDIBULAR JOINT OF RATS.

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Declaration of interests

The authors of the present study declare that there are no conflicts of interest in relation to this work.

Abstract

Background: We have previously demonstrated that blockade of β-adrenoreceptors (-AR) located in the temporomandibular joint (TMJ) of rats suppresses formalin-induced TMJ nociceptive behavior in both males and females, but females are more responsive. In this study we investigated whether gonadal hormones modulate the antinociceptive responsiveness to the blockade of β-AR in the TMJ of rats. Methods: Co-administration of each of the selective β_1 (Atenolol), β_2 (ICI 118.551) and β_3 (SR59230A) -AR antagonists with equinociceptive concentrations of formalin in the TMJ of intact, gonadectomized and hormone-treated gonadectomized male and female rats. Results: Atenolol, ICI 118.551 and SR59230A significantly reduced formalin-induced TMJ nociception in a dose response fashion in all groups tested. However, a lower dose of each β-AR antagonist was sufficient to significantly reduce nociceptive responses in gonadectomized but not in intact and testosterone-treated gonadectomized male rats. In the female groups a lower dose of β_1-AR antagonist was sufficient to significantly reduce nociceptive responses in gonadectomized but not in intact or gonadectomized rats treated with progesterone or a high dose of estradiol; a lower dose of β_2-AR antagonist was sufficient to significantly reduce nociceptive responses in gonadectomized but not in intact and gonadectomized rats treated with low or high dose estradiol. Conclusion: Gonadal hormones may reduce the responsiveness to the blockade of β-AR in the TMJ of male and female rats. Therefore, lower doses of β-blockers may be required to reduce TMJ pain in women of reproductive age during phases of low levels of gonadal hormones and in women at menopause.

Keywords: Temporomandibular Joint Disorders. Nociception. Beta Adrenergic Receptors. Adrenergic beta-Antagonists.

Introduction

Temporomandibular disorder (TMD) is a common pain condition of the temporomandibular joint (TMJ) and/or associated muscles (Von Korff et al., 1988; Dworkin et al., 1990; Carlsson, 1999). TMD pain has an important sympathetic component (Rodrigues et al., 2006; Pelegrini-da-Silva et al., 2008; Light et al., 2009; Tchivileva et al., 2010; Fávaro-Moreira et al., 2012) that may predominate in pain less sensitive to the analgesic effect of non-steroidal anti-inflammatory drugs (NSAID).

The TMJ receives rich sympathetic innervations arising from cells of the superior cervical ganglion (Widenfalk and Wiberg, 1990; Yoshino et al., 1998; Kido et al., 2001) that may release sympathetic amines. Sympathetic amines released in the TMJ region contribute to TMJ pain (Rodrigues et al., 2006; Fávaro-Moreira et al., 2012) through the activation of β-adrenoreceptors (β-AR) located into the TMJ region. For example, local blockade of β_1, β_2 or β_3-AR significantly reduces formalin-induced TMJ nociception (Fávaro-Moreira et al., 2012), and local blockade of β_2-AR significantly reduces carrageenan-induced hyperalgesia (Rodrigues et al., 2006) and nerve growth factor-induced nociception during TMJ inflammation (Pelegrini-da-Silva et al., 2008).

TMJ pain is significantly more prevalent in women than in men and represents 80% of the treated cases (Dworkin et al., 1990). We have recently demonstrated that blockade of β-AR in the TMJ suppresses formalin-induced TMJ nociceptive behavior in both males and females, but females are more responsive, suggesting that the use of β-blockers in the treatment of TMJ pain might be of benefit, especially in females. However, it is not known whether gonadal hormones modulate the antinociceptive effect induced by the blockade of β-AR in the TMJ, although they can modulate TMJ analgesia such as kappa-mediated TMJ antinociception (Clemente et al., 2009). Therefore, the aim of this study was to

determine whether gonadal hormones modulate the antinociceptive responsiveness to the blockade of β_1, β_2 or β_3-AR in the TMJ of rats.

Methods

Animals

This study was carried out in males and females Wistar rats (200-250g) housed in a temperature-controlled room (23±1°C) on a 12:12 light cycle (lights on at 6:00 A.M.) with food and water available *ad libitum*. Animals were handled at least 1 week prior to the experiments, which 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). Each rat was used only once.

Estrous phase determination

Estrous phase was determined by daily microscope examination of vaginal smears between 7 and 8 a.m. On 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. The 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 levels of ovarian hormones, respectively (Fischer et al., 2008).

Gonadectomy

Male and females rats (21 days old) were anesthetized with an intramuscular injection of a mixture of ketamine (55 mg/Kg) and xylazine (5.5 mg/kg). Ovariectomy (OVX) was achieved via bilateral flank incisions (Green et al., 1999). The ovarian bundles were ligated with silk sutures and removed, followed by the fascia and the skin separated suture (Green et al., 1999). Orchiectomy

(ORX) was achieved through a single scrotal incision; the testes were ligated with silk suture and removed followed by the skin suture.

The efficacy of ovariectomy was assessed by the absence of an estrous cycle verified by observation of vaginal smears for 10 days and by post-mortem examination of uterine atrophy in animals that did not received hormones. The efficacy of orchiectomy was verified by post-mortem examination of prostate and seminal vesicles which were atrophied in orchidectomized animals that did not received testosterone. The animals were used in experiments when they weighed 200-300g (Green et al., 1999).

Hormone treatment

When gonadectomized rats reached the required weight for experiments, they received gonadal steroid hormone through daily subcutaneous injection. Hormones were purchased from Sigma Chemicals, St. Louis, MO, USA, and dissolved in propylene glycol. Control groups received injections of propyleneglycol only.

Testosterone (ORX+T): ORX male rats were subcutaneously injected with testosterone propionate (100 µg/100 g) for 3 days and the experiment was performed on day 4 (Campos et al., 2003; Fischer et al., 2007).

Estradiol (OVX+LE_2 and OVX+HE_2): two protocols of 17β-estradiol-3-benzoate treatment were used as previously described (Okamoto et al., 2013). OVX female rats were subcutaneously injected with low (OVX+LE_2, 3 µg/Kg/day) or high (OVX+HE_2, 30 µg/Kg/day) doses of 17β-estradiol-3-benzoate for 3 days, and the experiment was performed on day 4.

Progesterone (OVX+P): OVX female rats were subcutaneously injected with a first dose of Progesterone (700 µg/250 g) followed 7 hours later by a second dose (350 µg/250 g) and the experiment was performed immediatly after the last injection (Kramer and Bellinger, 2009).

Drugs and doses

Formalin solutions were prepared from commercially available stock formalin (an aqueous solution of 37% of formaldehyde) further diluted in 0.9% NaCl (saline) to concentrations of 1.0 or 1.5%. Equi-nociceptive concentrations of formalin were used to compare β-blockers-mediated effects among groups. 1.0% formalin was used in diestrus, OVX and OVX+LE_2 females and 1.5% in proestrus, OVX+HE_2 and OVX+P females and in intact, ORX and ORX+T males. Equi-nociceptive concentrations of formalin were previously described (Clemente et al., 2004; Fisher et al., 2007; Clemente et al., 2009; Fávaro-Moreira et al., 2012) and confirmed in the present study.

Drugs used were atenolol ((RS)-4-[2-hydroxy-3-[(1methylethyl) amino] propoxy] benzeneacetamide), a selective β_1-AR antagonist (doses: 2, 6, 18, 54 and 162 µg/15 µL) (Rodrigues et al., 2006); ICI 118.551 ((±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-y) oxy]-3-[(1-methylethyl) amino]-2- butanol hydrochloride), a selective β_2-AR antagonist (doses: 0.1, 0.3 and 0.9 µg/15 µL) (Rodrigues et al., 2006) and SR59230A hydrochloride 1-(2-Ethylphenoxy)-3-[[(1S)-1,2,3,4-tetrahydro-1naphthalenyl]amino]-(2S)-2-propanol hydrochloride, a selective β_3-AR antagonist (doses: 0.1, 0.5, 1.5, 4.5 and 13.5 µg/15 µL) (Nackley et al., 2007). SR59230A hydrochloride was dissolved in dimethylsulfoxide (DMSO) and diluted in sterile 0.9% saline (1:4). All other drugs were dissolved in sterile saline (0.9% NaCl). SR59230A was obtained from Tocris Bioscience (Ellisville, MO) and all other drugs were obtained from Sigma–Aldrich (MO, USA).

TMJ Injections

Animals were briefly anesthetized by inhalation of isoflurane prior to the TMJ injection, which was performed with a 30-gauge needle connected to a 50 µl Hamilton syringe (Roveroni et al., 2001). Injection volumes were 15 µl per drug in all cases. Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber for counting nociceptive responses during the 45- min observation period.

Testing procedure for TMJ pain

Testing sessions took place during the light phase in a quiet room maintained at 23ºC (Rosland, 1991). Prior to the experiments, each animal was placed in the test chamber (30 cm x 30 cm x 30 cm mirror-wood chamber with a glass at the front side) for a 15-min habituation period. The nociceptive response was assessed by an observer blinded to the experimental manipulation. 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 s. The TMJ formalin nociceptive behaviors (flinching and rubbing) were summed and expressed in seconds as previously described (Roveroni et al., 2001).

After the conclusion of each experiment, animals were anesthetized with an intraperitoneal injection of a mixture of urethane (1 g/Kg) and α-chloralose (50 mg/Kg). Evans blue dye (0.1 %, 5 mg/Kg) was then intravenously administred in order to visualize formalin-induced plasma extravasation upon postmortem examination of the injected TMJs. This procedure also allowed confirmation that the plasma extravasation induced by the TMJ injection at the correct site was restricted to the immediate TMJ region.

Statistical analysis

The nociceptive behavior score, wich was obtained by summing the flinching and rubbing behaviors recorded during the entire duration of the experiment was used in statistical analysis. A One-way analysis of variance (ANOVA), 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 t-test analysis was used to determine if there were significant differences in nociceptive responses between control groups, that is, the groups receiving 0.9 % NaCl alone and the groups receiving the antagonists plus formalin vehicle. A probability level of p less than 0.05 was considered to indicate statistical significance. The data are plotted in figures as the mean±S.E.M..

Results

Effect of formalin on intact, gonadectomized and hormone-treated gonadectomized rats.

The nociceptive responses of diestrus, OVX and OVX+LE_2 females administred 1.0 % formalin were not significantly different from those of intact, ORX or ORX+T males and proestrus, OVX+HE_2 or OVX+P females administred 1.5 % formalin into the TMJ region (Fig. 1, $p>0.05$, One-way Anova and post hoc Tukey test). Therefore, these equinociceptive concentrations of TMJ formalin were used in the respective groups in subsequent experiments.

Figure 1

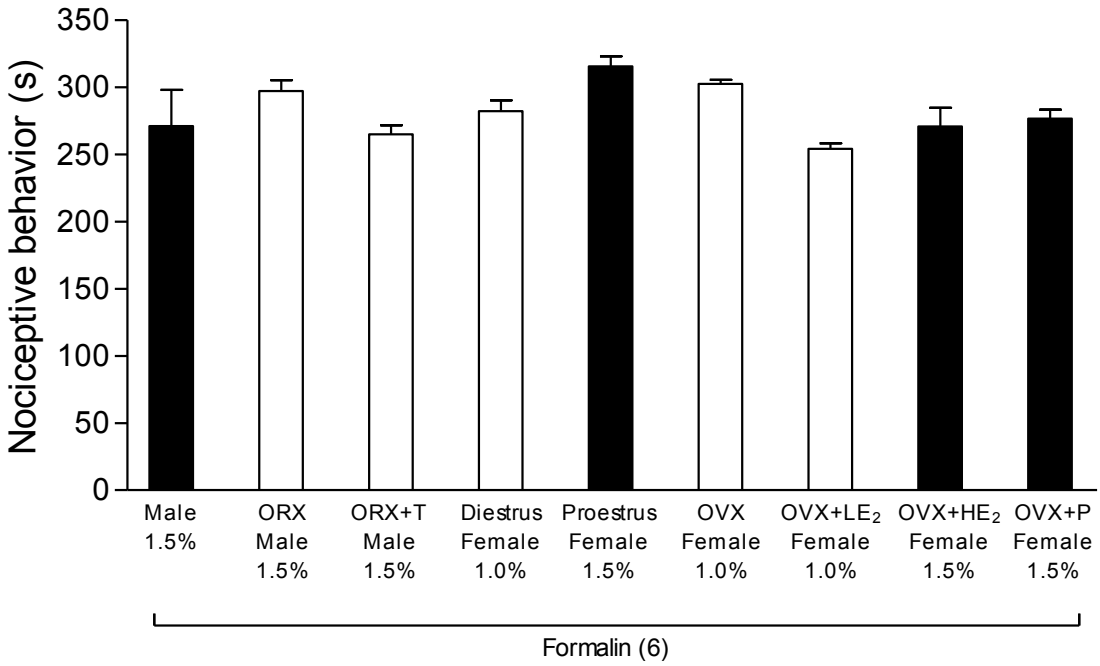


Fig. 1 – Effect of formalin on intact, gonadectomized and hormone-treated gonadectomized male and female rats.

The TMJ administration of 1.0 % formalin in diestrus, OVX and OVX+LE_2 females and of 1.5 % formalin in proestrus, OVX+HE_2 and OVX+P females and intact, ORX and ORX+T males induced nociceptive responses similar to each other. The number of rats used is between parentheses ($p>0.05$, ANOVA post hoc Tukey test).

Effect of testosterone on the antinociceptive response to the blockade of β-AR in the TMJ of male rats.

Co-administration of the selective antagonist for β_1 (Atenolol, Fig. 2), β_2 (ICI 118.551, Fig. 3) or β_3 (SR59230A, Fig. 4) -AR with 1.5 % formalin into the TMJ significantly reduced formalin-induced nociception in intact males (Fig. 2a, 3a and

4a), ORX males (Fig. 2b, 3b and 4b) and ORX+T males (Fig. 2c, 3c and 4c) in a dose related fashion ($p<0.05$, Tukey test).

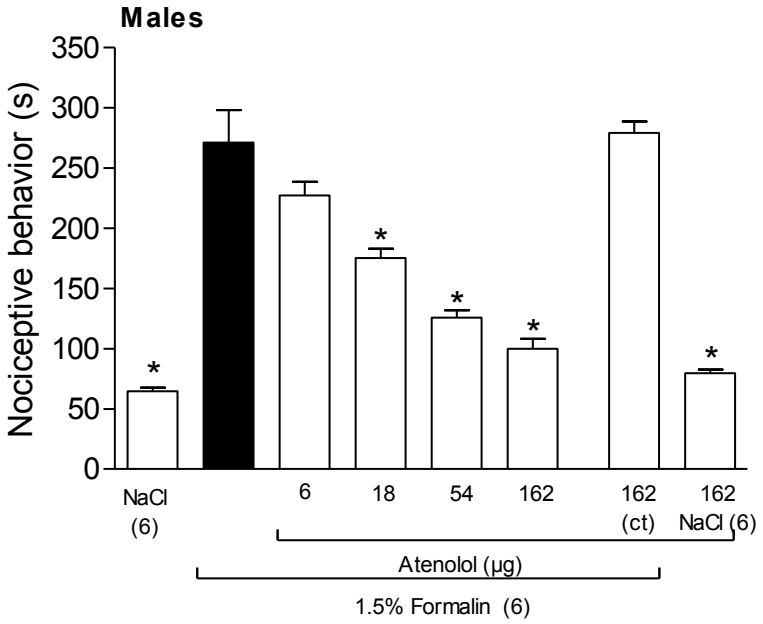
The lowest dose of Atenolol (6 µg, Fig. 2), ICI 118.551 (0.1 µg, Fig. 3) and SR59230A (0.1 µg, Fig. 4) significantly reduced ($p<0.05$, One-way Anova and post hoc Tukey test) nociceptive responses in the ORX groups (Fig. 2.b, 3b and 4b) but not in the intact (Fig. 2a, 3a and 4a) and ORX+T groups (Fig. 2c, 3c and 4c).

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

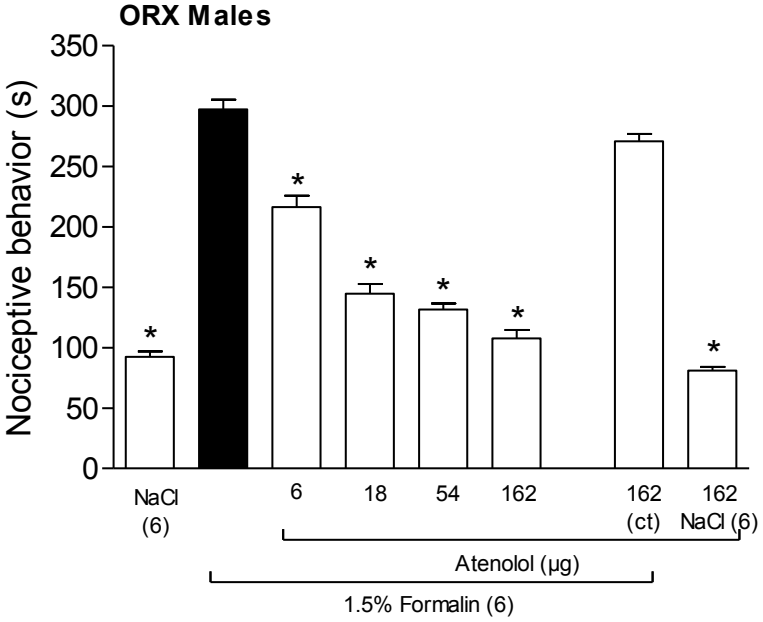
Taken together these findings indicate that TMJ formalin-induced nociceptive responses are significantly less responsive to β_1, β_2 and β_3-AR antagonists in the presence of testosterone.

Figure 2

2A



2B



2C

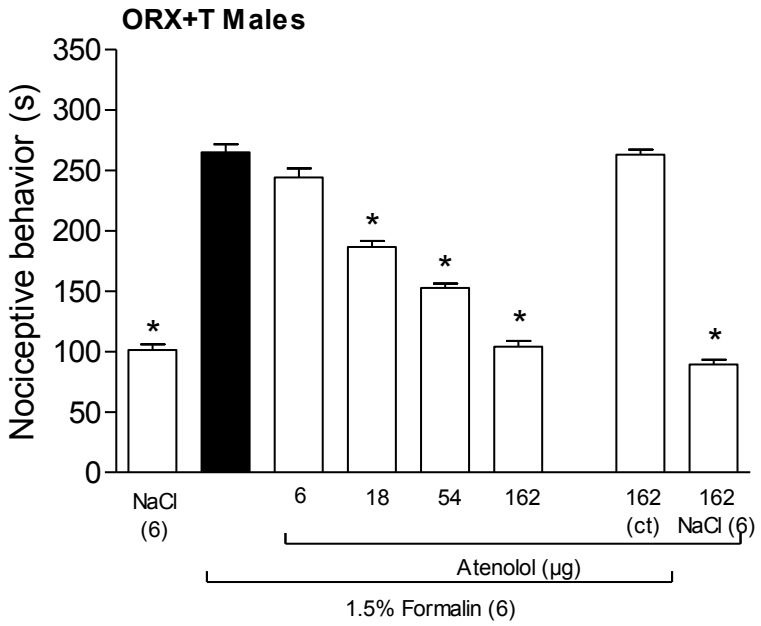
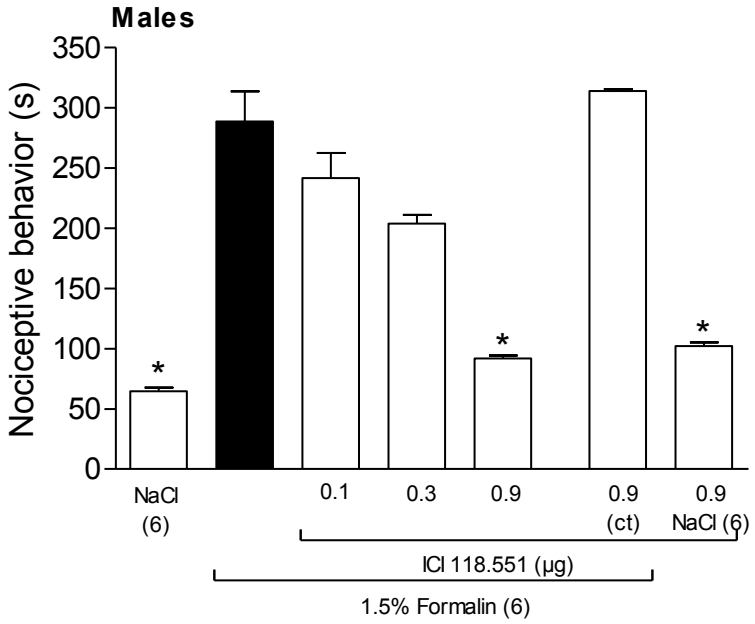


Fig.2 – Effect of testosterone on the antinociceptive response to the blockade of β_1-AR in the TMJ of male rats.

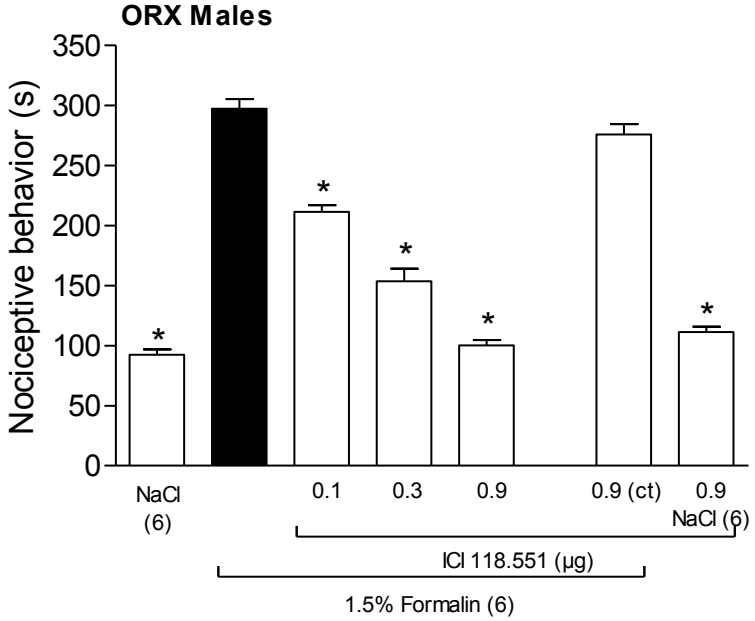
Co-administration of the selective antagonist for β_1-AR Atenolol with 1.5 % formalin into the TMJ significantly reduced formalin-induced nociception in intact males (Fig. 2a), ORX males (Fig. 2b) and ORX+T males (Fig. 2c) in a dose-related fashion. The lowest dose of Atenolol (6 µg) significantly reduced nociceptive responses in the ORX group (Fig. 2b) but not in the intact (Fig. 2a) and ORX+T (Fig. 2c) rats. The highest dose of the antagonist did not affect formalin-induced nociception when applied to the contralateral TMJ. Co-administration of the highest dose of the antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl. The symbol "*" indicates a response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Figure 3

3A



3B



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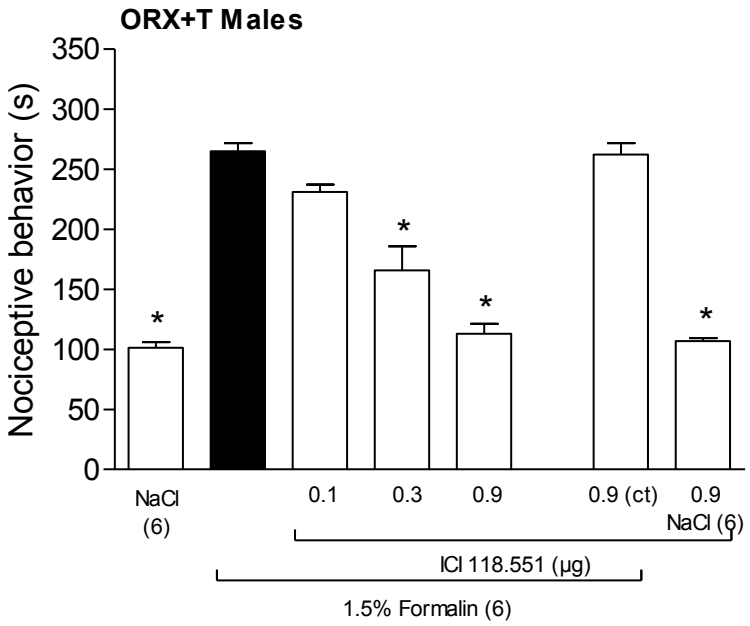
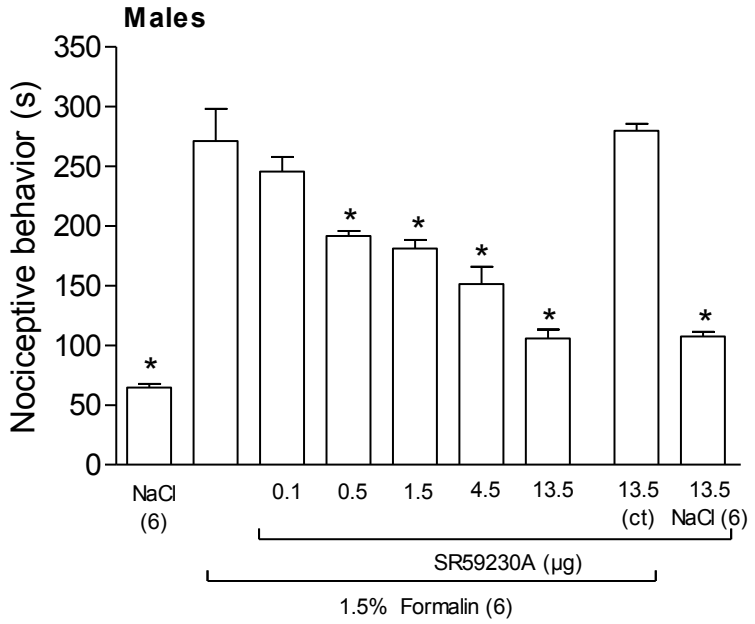


Fig.3 – Effect of testosterone on the antinociceptive response to the blockade of β_2-AR in the TMJ of male rats.

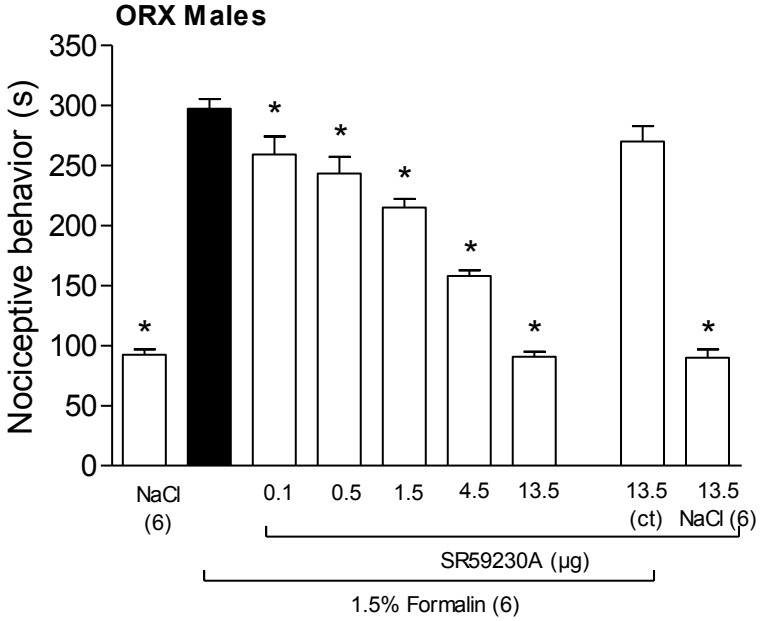
Co-administration of the selective antagonist for β_2-AR ICI 118.551 with 1.5 % formalin into the TMJ significantly reduced formalin-induced nociception in intact males (Fig. 3a), ORX males (Fig. 3b) and ORX+T males (Fig. 3c) in a dose related fashion. The lowest dose of ICI 118.551 (0.1 µg) significantly reduced nociceptive responses in the ORX group (Fig. 3b) but not in the intact (Fig. 3a) and ORX+T (Fig. 3c) rats. The highest dose of the antagonist did not affect formalin-induced nociception when applied to the contralateral TMJ. Co-administration of the highest dose of the antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl. The symbol "*" indicates a response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Figure 4

4A



4B



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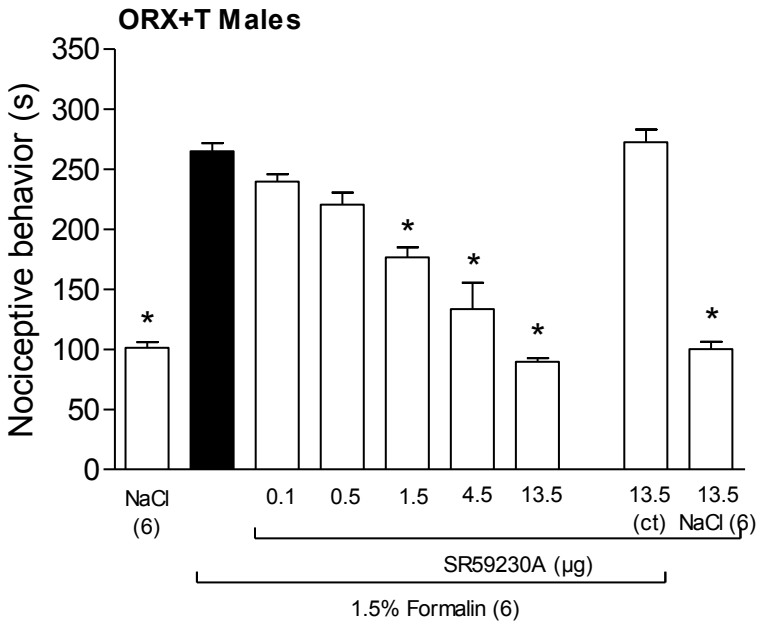


Fig.4 – Effect of testosterone on the antinociceptive response to the blockade of β_3-AR in the TMJ of male rats.

Co-administration of the selective antagonist for β_3-AR SR59230A with 1.5 % formalin into the TMJ significantly reduced formalin-induced nociception in intact males (Fig. 4a), ORX males (Fig. 4b) and ORX+T males (Fig. 4c) in a dose related fashion. The lowest dose of SR59230A (0.1 µg) significantly nociceptive responses in the ORX group (Fig. 4b) but not in the intact (Fig. 4a) and ORX+T (Fig. 4c) rats. The highest dose of the antagonist did not affect formalin-induced nociception when applied to the contralateral TMJ. Co-administration of the highest dose of the antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl. The symbol "*" indicates a response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Effect of estradiol and progesterone on the antinociceptive response to the blockade of β-AR in the TMJ of female rats.

Co-administration of the selective antagonist for β_1 (Atenolol, Fig. 5), β_2 (ICI 118.551, Fig. 6) or β_3 (SR59230A, Fig. 7) -AR with equinociceptive concentrations of formalin, that is, 1.5 % formalin into the TMJ of proestrus (Fig. 5a, 6a and 7a), OVX+HE_2 (Fig. 5e, 6e and 7e) and OVX+P females (Fig. 5f, 6f and 7f) and with 1.0 % formalin into the TMJ of diestrus (Fig. 5b, 6b and 7b), OVX (Fig. 5c, 6c and 7c) and OVX+LE_2 females (Fig. 5d, 6d and 7d) significantly reduced TMJ formalin-induced nociception in all groups in a dose related fashion ($p<0.05$, One-way Anova and post hoc Tukey test).

The lowest dose of Atenolol (2 µg, Fig. 5) significantly reduced ($p<0.05$, Tukey test) nociceptive responses in diestrus (Fig. 5b), OVX (Fig. 5c), and OVX+LE_2 (Fig. 5d) females. A higher dose was necessary to significantly reduce ($p<0.05$, One-way Anova and post hoc Tukey test) nociceptive responses in proestrus (Fig. 5a), OVX+HE_2 (Fig. 5e) and OVX+P (Fig. 5f) females, indicating that estradiol at higher levels and progesterone may reduce the antinociceptive response to the blockade of β_1-AR in the TMJ of females.

The lowest dose of ICI 118.551 (0.1 µg) significantly reduced ($p<0.05$, One-way Anova and post hoc Tukey test) nociceptive responses only in OVX (Fig. 6c) and OVX+P (Fig. 6f) females. A higher dose was necessary to significantly reduce ($p<0.05$, Tukey test) nociceptive responses in proestrus (Fig. 6a), diestrus (Fig. 6b), OVX+LE_2 (Fig. 6d), OVX+HE_2 (Fig. 6e) and OVX+P (Fig. 6f) females, indicating that estradiol at different plasma levels may reduce the antinociceptive response to the blockade of β_2-adrenoceptors in the TMJ of females.

The lowest dose of SR59230A (0.1 µg) induced significantly reduced ($p<0.05$, One-way Anova and post hoc Tukey test) nociceptive responses in all female groups (Fig. 7).

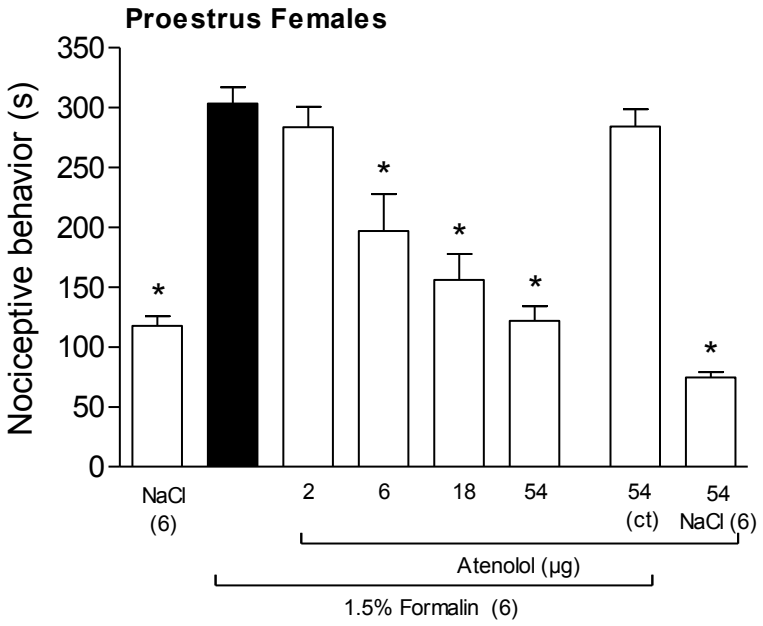
The highest doses of these antagonists did not affect formalin-induced nociception when applied to the contralateral TMJ (penultimate bar of each graphic of Fig. 5, 6 and 7, $p>0.05$, t test), confirming their local peripheral action. Co-

administration of the highest dose of each antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl (last bar of each graphic of Fig. 5, 6 and 7, $p>0.05$, t test).

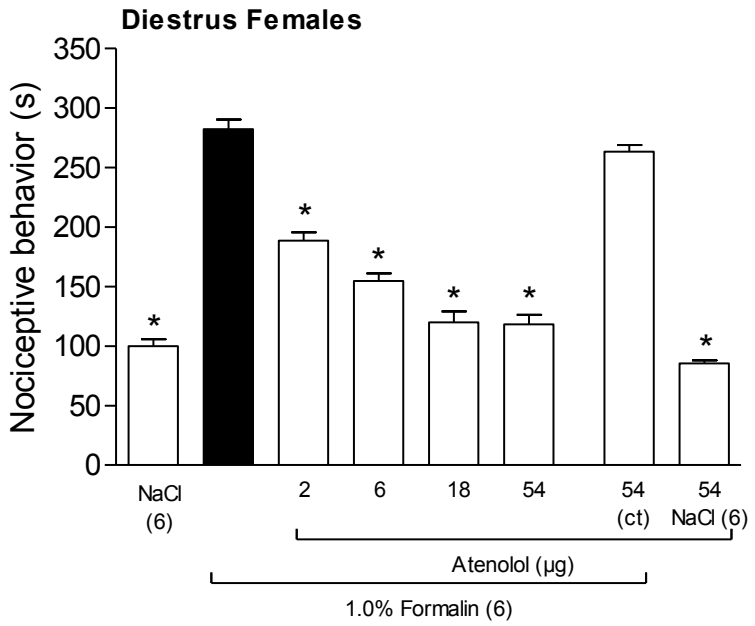
Taken together these findings indicate that TMJ formalin-induced nociceptive responses are significantly less responsive to the β_1-AR antagonist Atenolol in the presence of estradiol at higher levels and progesterone and to the β_2-AR antagonist ICI 118.551 in the presence of lower and higher estradiol levels.

Figure 5

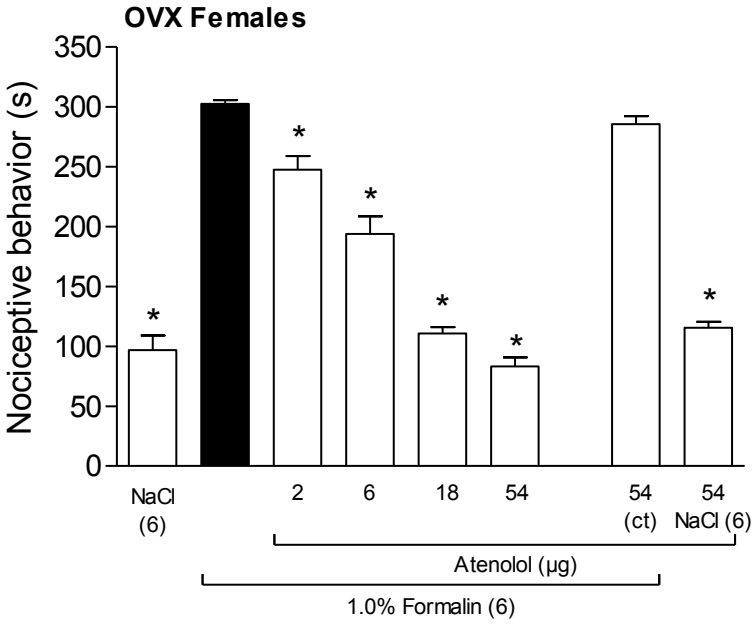
5A



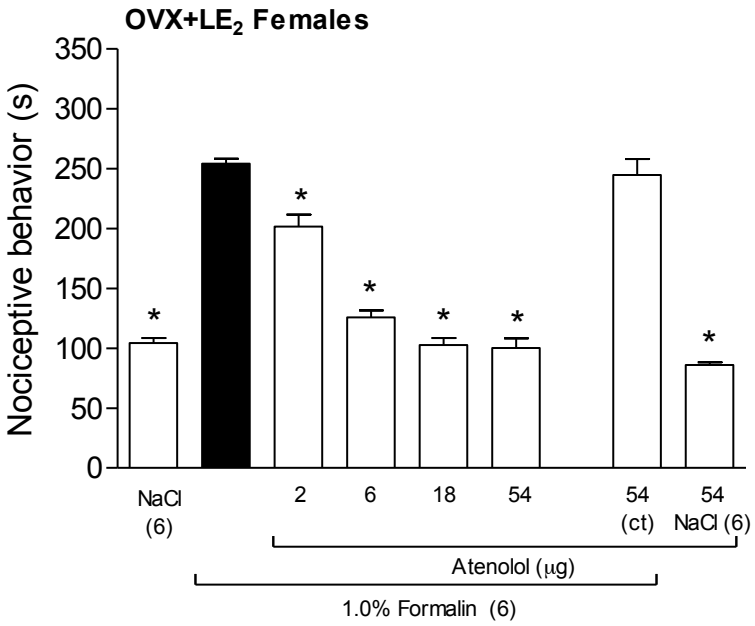
5B


Diestrus Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
2
6
18
54
54 (ct)
54 NaCl (6)
Atenolol (µg)
1.0% Formalin (6)

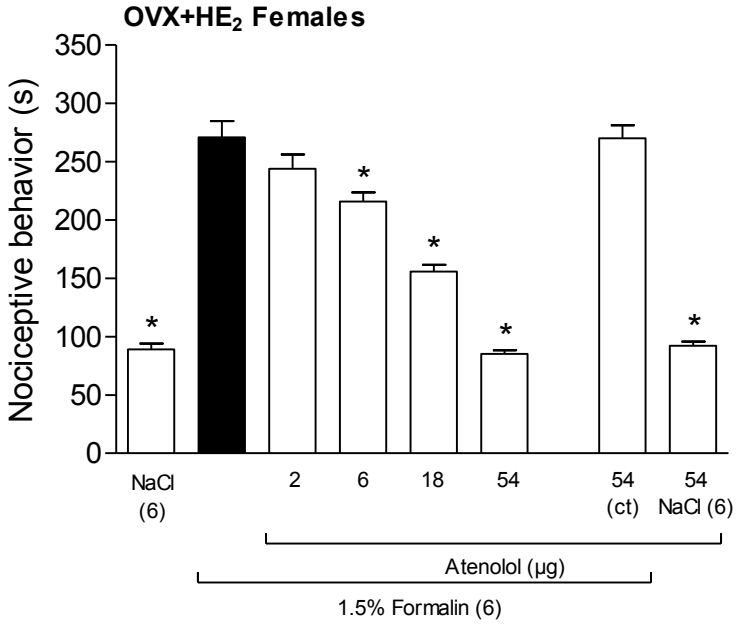
5C


OVX Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
2
6
18
54
54 (ct)
54 NaCl (6)
Atenolol (µg)
1.0% Formalin (6)

5D


OVX+LE2 Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
2
6
18
54
54 (ct)
54 NaCl (6)
Atenolol (μg)
1.0% Formalin (6)

5E


OVX+HE2 Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
2
6
18
54
54 (ct)
54 NaCl (6)
Atenolol (μg)
1.5% Formalin (6)

5F

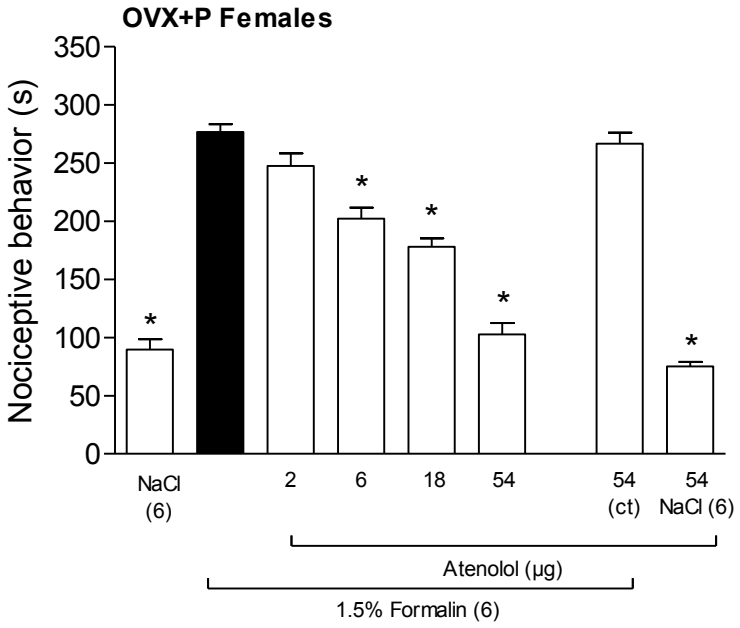


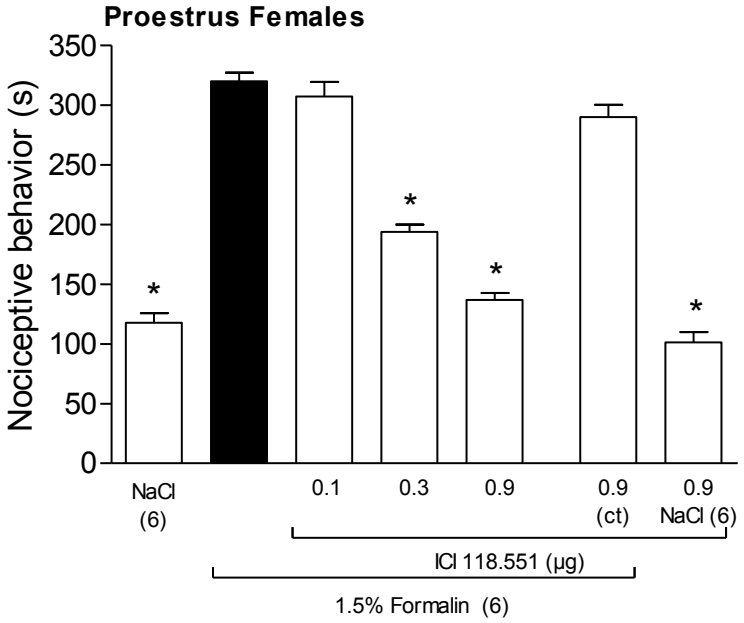
Fig.5 – Effect of estradiol and progesterone on the antinociceptive response to the blockade of β_1-AR in the TMJ of female rats.

Co-administration of the selective antagonist for β_1-AR Atenolol with equinociceptive concentrations of formalin, that is, 1.5 % formalin into the TMJ of proestrus (Fig. 5a), OVX+HE_2 (Fig. 5e) and OVX+P females (Fig. 5f) and with 1.0 % formalin into the TMJ of diestrus (Fig. 5b), OVX (Fig. 5c) and OVX+LE_2 females (Fig. 5d) significantly reduced TMJ formalin-induced nociception in all groups in a dose related fashion. The lowest dose of Atenolol (2 µg) significantly reduced nociceptive responses in the OVX (Fig. 5c), diestrus (Fig. 5b) and OVX+LE_2 (Fig. 5d) females. A higher dose was necessary to significantly reduce nociceptive responses in proestrus (Fig. 5a), OVX+HE_2 (Fig. 5e) and OVX+P (Fig. 5f) females. The highest doses of the antagonist did not affect formalin-induced nociception when applied to the contralateral TMJ. Co-administration of the highest dose of the antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl. The symbol "*" indicates a

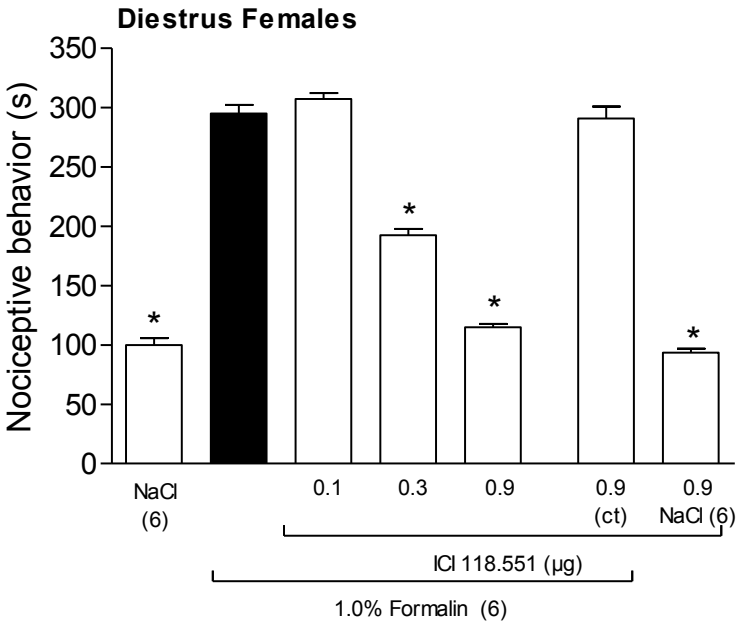
response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Figure 6

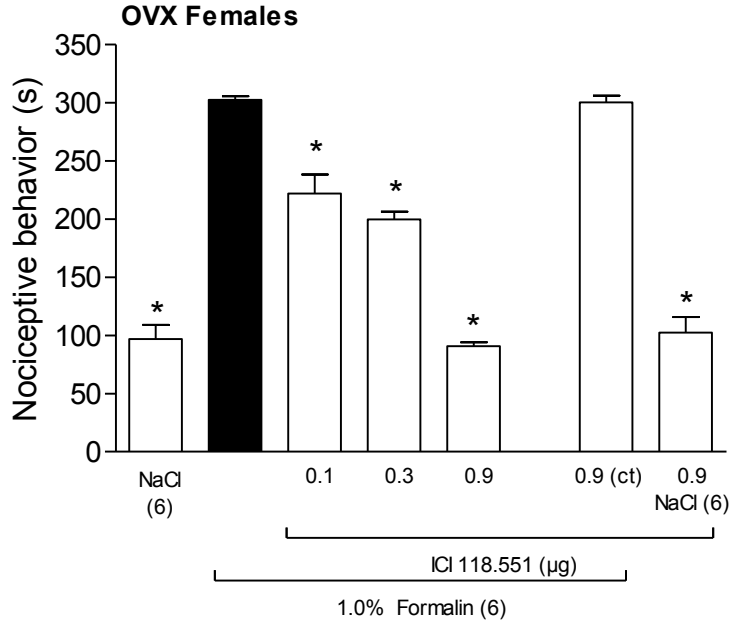
6A



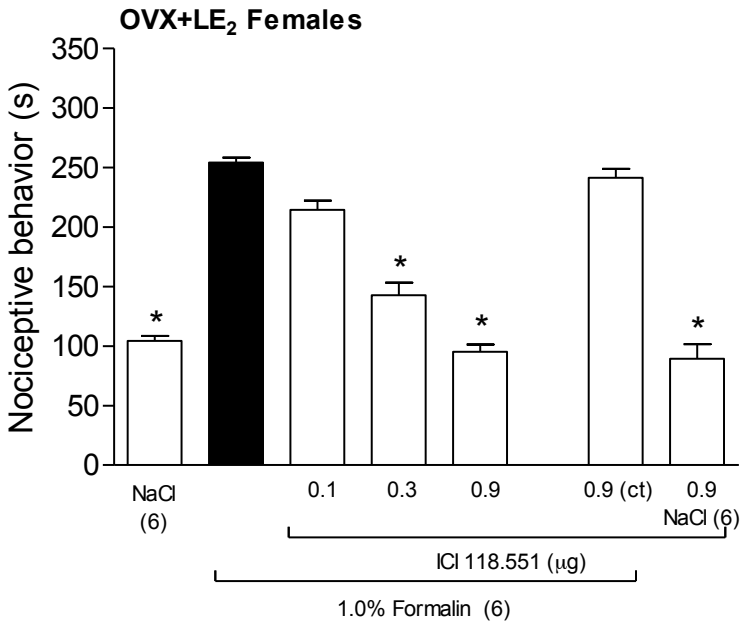
6B


Diestrus Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.3
0.9
0.9 (ct)
0.9 NaCl (6)
ICI 118.551 (µg)
1.0% Formalin (6)

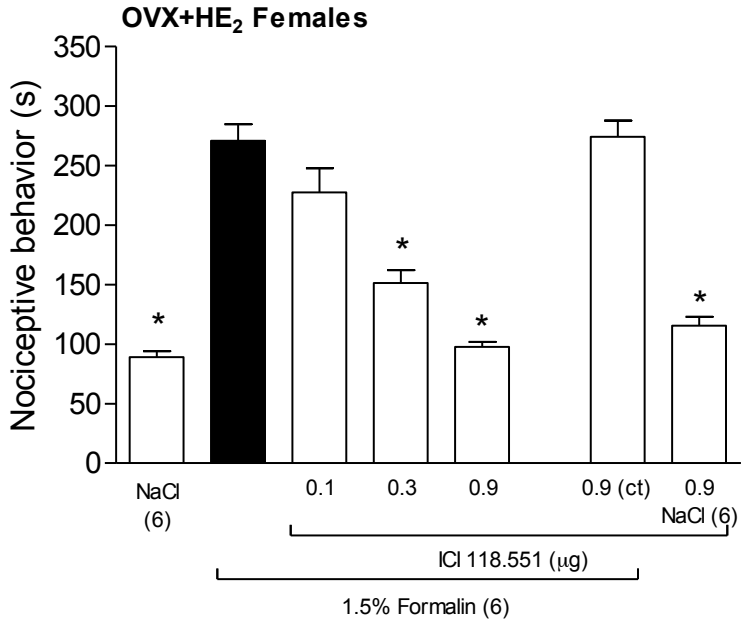
6C


OVX Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.3
0.9
0.9 (ct)
0.9 NaCl (6)
ICI 118.551 (µg)
1.0% Formalin (6)

6D


OVX+LE2 Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.3
0.9
0.9 (ct)
0.9 NaCl (6)
ICl 118.551 (μg)
1.0% Formalin (6)

6E


OVX+HE2 Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.3
0.9
0.9 (ct)
0.9 NaCl (6)
ICl 118.551 (μg)
1.5% Formalin (6)

6F

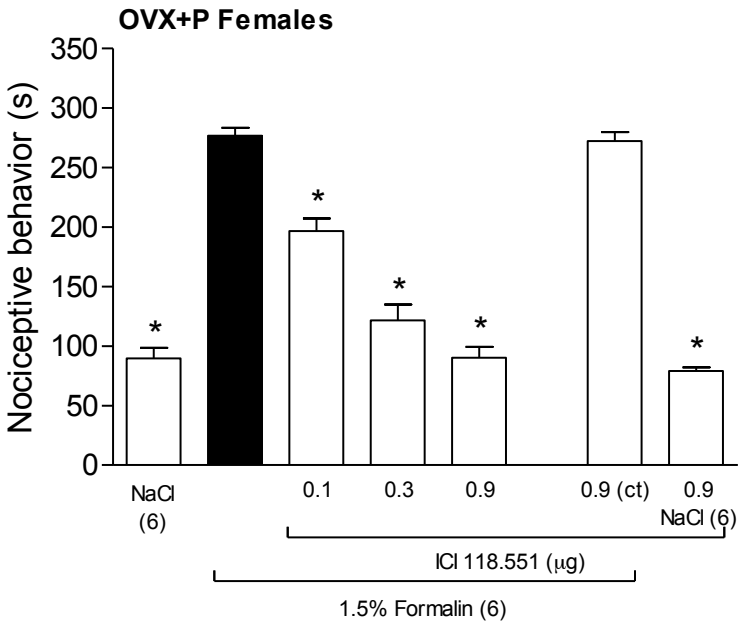


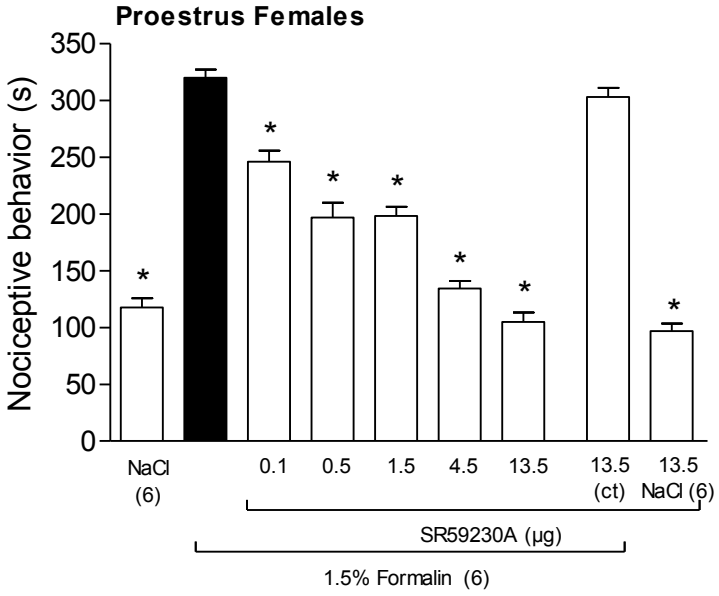
Fig. 6 – Effect of estradiol and progesterone on the antinociceptive response to the blockade of β_2-AR in the TMJ of female rats.

Co-administration of the selective antagonist for β_2-AR ICI 118.551 with equinociceptive concentrations of formalin, that is, 1.5 % formalin into the TMJ of proestrus (Fig. 6a), OVX+HE_2 (Fig. 6e) and OVX+P females (Fig. 6f) and with 1.0 % formalin into the TMJ of diestrus (Fig. 6b), OVX (Fig. 6c) and OVX+LE_2 females (Fig. 6d) significantly reduced TMJ formalin-induced nociception in all groups in a dose related fashion. The lowest dose of ICI 118.551 (0.1 μg) significantly reduced nociceptive responses only in OVX (Fig. 6b) and OVX+P (Fig. 6f) females. A higher dose was necessary to significantly reduce nociceptive responses in proestrus (Fig. 6a), diestrus (Fig. 6b), OVX+LE_2 (Fig. 6d) and OVX+HE_2 (Fig. 6e) females. The highest doses of these antagonists did not affect formalin-induced nociception when applied to the contralateral TMJ. Co-administration of the highest dose of each antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of

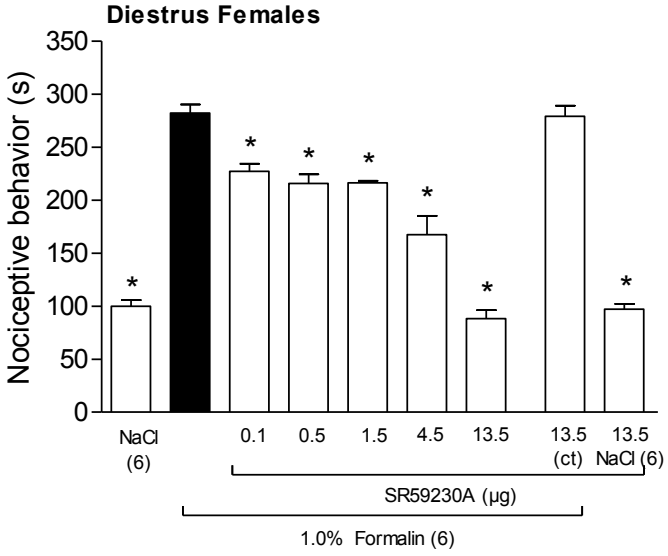
0.9 % NaCl. The symbol "*" indicates a response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Figure 7

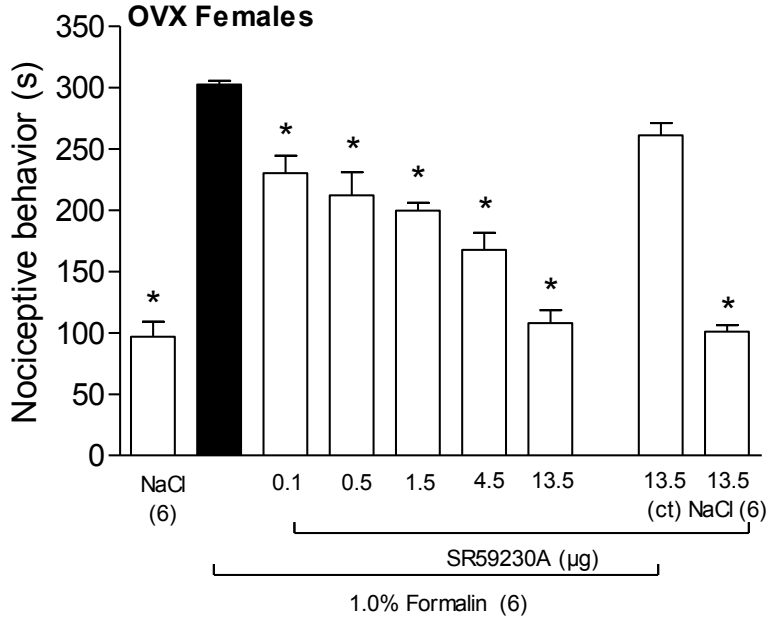
7A



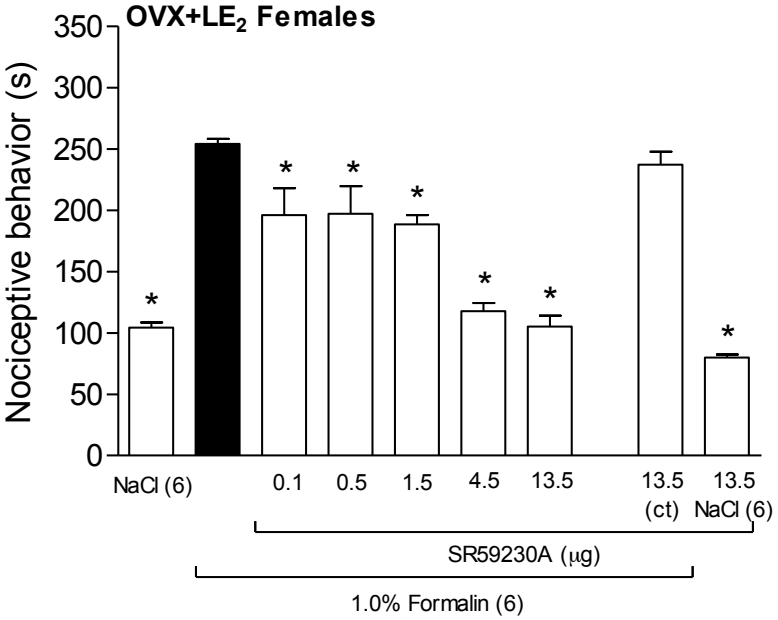
7B



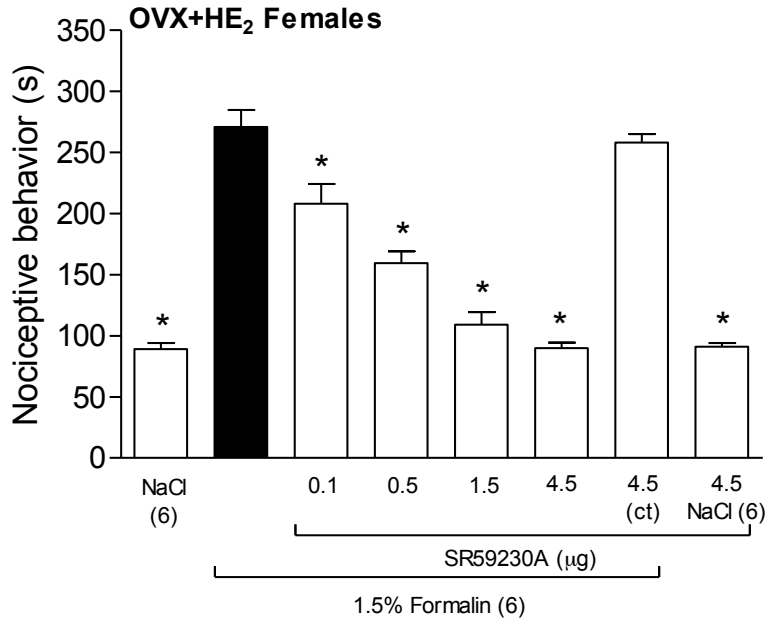
7C


OVX Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.5
1.5
4.5
13.5
13.5 (ct)
13.5 NaCl (6)
SR59230A (µg)
1.0% Formalin (6)

7D


OVX+LE2 Females
Nociceptive behavior (s)
350
300
250
200
150
100
50
0
NaCl (6)
0.1
0.5
1.5
4.5
13.5
13.5 (ct)
13.5 NaCl (6)
SR59230A (µg)
1.0% Formalin (6)

7E



7F

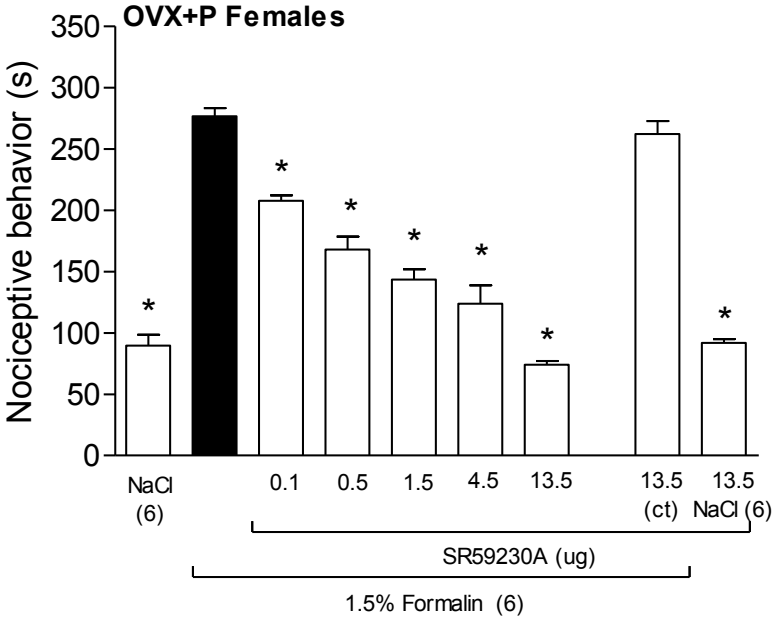


Fig. 7 – Effect of estradiol and progesterone on the antinociceptive response to the blockade of β_3-AR in the TMJ of female rats.

Co-administration of the selective antagonist for β_3-AR SR59230A with equinociceptive concentrations of formalin, that is, 1.5 % formalin into the TMJ of proestrus (Fig. 7a), OVX+HE_2 (Fig. 7e) and OVX+P females (Fig. 7f) and with 1.0 % formalin into the TMJ of diestrus (Fig. 7b), OVX (Fig. 7c) and OVX+LE_2 females (Fig. 7d) significantly reduced TMJ formalin-induced nociception in all groups in a dose related fashion. The lowest dose of SR59230A (0.1 µg) used significantly reduced nociceptive responses in all female groups. The highest doses of these antagonists did not affect formalin-induced nociception when applied on the contralateral TMJ. Co-administration of the highest dose of each antagonist with 0.9 % NaCl had no effect by itself, as the response was similar to that induced by the TMJ injection of 0.9 % NaCl. The symbol "*" indicates a response significantly lower than that of other groups ($p<0.05$, Tukey test). ct= contralateral.

Discussion

This study extends our previous findings that blockade of β-AR into the rat's TMJ significantly reduces formalin-induced TMJ nociception in a dose response fashion (Fávaro-Moreira et al., 2012) by showing that gonadal hormones may reduce the responsiveness to the blockade of β-AR in the TMJ of male and female rats. Specifically in males testosterone attenuated the responsiveness to the blockade of TMJ β-AR because a lower dose of the selective β_1 (Atenolol), β_2 (ICI 118.551) and β_3 (SR59230A) -AR antagonist was sufficient to significantly reduce nociceptive responses only in the absence of testosterone (ORX males) and a higher dose of each antagonist was necessary to significantly reduce nociceptive responses in the presence of testosterone (intact and ORX+T males). In females progesterone and high plasma levels of estradiol attenuated the responsiveness to the blockade of TMJ β_1-AR because a lower dose of Atenolol was sufficient to significantly reduce nociceptive responses only in the absence of gonadal hormones (OVX females) or in the presence of low levels of estradiol (diestrus and OVX+LE_2 females), and a higher dose of Atenolol was necessary to significantly

reduce nociceptive responses in the presence of progesterone (OVX+P females) and estradiol at higher levels (proestrus and OVX+HE_2 females). Additionaly, in females estradiol at low and high plasma levels, but not progesterone, attenuated the responsiveness to the blockade of TMJ β_2-AR because a lower dose of ICI 118.551 was sufficient to significantly reduce nociceptive responses only in the absence of gonadal hormones (OVX females) or in the presence of progesterone (OVX+P females), and a higher dose of ICI 118.551 was necessary to significantly reduce nociceptive responses in the presence of estradiol independent of its plasma level (proestrus, diestrus, OVX+LE_2 and OVX+HE_2).

The findings that the administration of the highest doses of each β-AR antagonist in the TMJ contralateral to that receiving formalin did not affect formalin-induced nociception in males and females confirmed the local action of the β-AR antagonists.

The mechanisms by which gonadal hormones reduce the responsiveness to the blockade of β-AR in the TMJ of rats are not known. One possibility is that gonadal hormones modulate the expression of β-AR expression in the TMJ. For example, gonadal hormones might up regulate β-AR expression in the TMJ region and as a consequence low doses of β-blockers may not be sufficient for blocking all expressed receptors. This could explain the requirement of higher doses of each of the β-blockers to significantly reduce formalin-induced TMJ nociception in the presence of testosterone and the requirement of higher doses of Atenolol in the presence of estradiol and progesterone in females and higher doses of ICI 118.551 in the presence of estradiol in females.

Consistent with the idea that gonadal hormones might up regulate β-AR expression in the TMJ region, it has been demonstrated in other tissues, such as the cardiac tissue, that testosterone treatment in gonadectomized rats increases the expression of β_2-AR (Sun et al., 2011) or β_1-AR mRNA levels (Golden et al; 2002).

In the TMJ region, gonadal hormones might act on inflammatory and/or resident cells to increase the expression of β-AR in these cells. Consistent with this

idea, TMJ formalin induces inflammatory cell migration (Chicre-Alcântara et al., 2012) and β-AR are expressed in neutrophils, macrophage, eosinophils, mast cells lymphocytes, (Barnes, 1993) and basophiles cells (Perper et al., 1972). Futhermore, gonadal hormones receptors are known to be expressed in inflammatory cells as demonstrated by the presence of androgen receptor in macrophages (Fang et al., 2013), progesterone receptors in T lymphocytes cells (Butts et al., 2008) and estradiol receptors in mast cells (Nicovani and Rudolph, 2002) macrophages (Capellino et al., 2006) and T-cells (Tornwall et al., 1999). Estradiol has also been shown to regulate proinflammatory cytokines in mast cells (Harnish et al., 2004), macrophages (Corcoran et al., 2010) and T-cells (Suzuki et al., 2008).

Gonadal hormones might also act on neuronal structures of the trigeminal ganglia and up-regulate expression of β-ARs on the nociceptive primary afferent fibers of the TMJ. Although β-AR RNA has not been detected in the dorsal root ganglion (Nicholson et al., 2005), we have recently demonstrated the presence of β-AR in trigeminal neurons of the trigeminal ganglia (Melo et al., 2012, unpublished data). In addition to β-AR, estradiol receptors (Puri et al., 2005a, 2005b) are also expressed within the trigeminal ganglia whereas no previous studies appear to have examined the presence of testosterone and progesterone receptors within this region.

In addition to the modulation of β-AR expression in the TMJ region, gonadal hormones might modulate the release of endogenous agonists of β-AR in the TMJ region. For example, gonadal hormones might increase the release of endogenous agonists of β-AR in the TMJ region, such as norepinephrine, and as a consequence, higher doses of β-blockers would be necessary to compete with norepinephrine for β-AR binding. This is another hypothesis that could explain the requirement of higher doses of each of the β-blockers to significantly reduce formalin-induced TMJ nociception in the presence of testosterone and the requirement of higher doses of Atenolol in the presence of estradiol and

progesterone in females and higher doses of ICI 118.551 in the presence of estradiol in females.

Although testosterone can influence norepinephrine metabolism, storage, and release (Lara et al., 1985), there is not a consensus about its effect on norepinephrine release. Testosterone has been reported to decrease norepinephrine release in some studies (Knoll et al., 2000) but to enhance renal norepinephrine release in others (Jones et al., 1998). Estradiol appears to generally decrease norepinephrine release in the cardiovascular system (Acs et al., 2001; Fukumoto et al., 2012) while progesterone does not influence the endogenous release of norepinephrine from the adrenal gland (Bukhari et al., 1981). It is important to note that the effect of gonadal hormones on the release of endogenous agonists of β-AR may depend on the tissue.

The effect of female gonadal hormones on the responsiveness to the blockade of β-AR in the TMJ of females depends on the β-AR subtype. We showed that in contrast to estradiol, which attenuated the responsiveness to the blockade of β_1-AR and β_2-AR in the TMJ of females, progesterone attenuated the responsiveness to the blockade only of β_1-AR. We also showed that both estradiol and progesterone did not affect the responsiveness to the blockade of β_3-AR in females. The lowest dose of the β_3-AR antagonist SR59230A used in the TMJ of females (0.1 μg) was chosen because it was ineffective in males. However, this dose was high enough to significantly reduce TMJ formalin-induce nociception in all females independent of their hormonal status and may have masked the effect of female gonadal hormones on the responsiveness to the blockade of β_3-AR in the TMJ of females. Therefore the effect of gonadal hormones on the responsiveness to the blockade of β-AR in the TMJ region may depend on the local dose of β-blockers.

The effect of estradiol on the responsiveness to the blockade of β-AR in the TMJ of females depends not only on the β-AR subtype but also on the estradiol plasma level. This is supported by the findings that estradiol at high (OVX+HE_2) but

not at low plasma levels (OVX+LE_2) reduced the antinociceptive response to the blockade of β_1-AR in the TMJ of females.

Conclusion

In summary, the present findings indicate that gonadal hormones may reduce the antinociceptive response to the blockade of β-AR in the TMJ of male and female rats. However their effect depends on their plasma level, on the subtype of β-AR and on the dose of β-blockers used. Furthermore, our findings suggest that lower doses of β-blockers may be required to reduce TMJ pain in women of reproductive age during phases of low levels of gonadal hormones and in women at menopause.

Acknowledgments

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Capítulo 2

Analgesic effect of Nadolol on Temporomandibular Disorders Pain.

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Abstract

We hypothesized that one and three days of treatment with the nonselective β_1 and β_2-adrenergic antagonist nadolol (40 mg once a day) would reduce clinical pain symptoms in TMD patients significantly more than placebo. We further hypothesized that women would experience relatively greater benefit from nadolol than men depending on their hormonal status and compared the effect of nadolol with the effect of ibuprofen (400 mg once a day). Nadolol, ibuprofen or placebo was administred in 27 women not using oral contraceptive (OC), 28 women using OC, and 29 men (all subjects between 18 and 48 years old) which met the Research Diagnostic Criteria for TMD. They completed a randomized, crossover, double–blind, placebo controlled study. Women participated for three months (during menstrual phase and during peri-ovulatory phase in women not using OC, and during menstrual phase and during OC using phase in women using OC) in a total of 6 stages of analysis (2 per month), and men participated for one month whith three stages of analysis and 6 days of wash out. Each period consisted of a baseline evaluation and two evaluations during treatment (nadolol, ibuprofen or placebo administration), one on the first day and the other on the third day of treatment. Clinical pain ratings were obtained by Visual Analog Scale (VAS) and comparisons were made across periods of pre-treatment (baseline), the first and the third day of intervention (post-treatment). Nadolol and ibuprofen significantly reduced pain associated with TMD in men and women when compared to placebo, but women were more responsive. Nadolol and ibuprofen also reduced pain in women using or not using OC, but their effect did not differ between menses and peri-ovulatory period in women not using OC, or between menses and the period using OC in women using OC. In all groups tested three days of drug intake induced a significantly higher analgesic effect than only one day of drug treatment. Taken together these findings indicate that one and three days of treatment with nadolol (40 mg once a day) or ibuprofen (400 mg once a day) produces analgesia in TMD women and men significantly more than placebo, but women are more responsive to all treatments independent of their hormonal status. The greater

treatment efficacy in women is of clinical relevance since TMD is more prevalent and severe in women than in men.

Keywords: Temporomandibular Joint Disorders. Pain. Beta Adrenergic Receptors. Adrenergic beta-Antagonists.

Introduction

Temporomandibular disorder (TMD) is a collective term embracing a number of clinical problems that involve the masticatory musculature, the temporomandibular joint (TMJ) and associated structures, or both (Winocur et al., 2003). Pain associated with TMD can be clinically expressed as masticatory muscle pain or TMJ pain.

Recently, animal and human studies have shown an important sympathetic component in TMJ pain which is consistent with the dense innervation of the TMJ 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 sympathetic amines may be released. Animal studies have demonstrated that sympathetic amines released in the TMJ region contribute to TMJ pain (Rodrigues et al., 2006, Fávaro-Moreira et al., 2012), through the activation of local β-adrenoceptors (β-ARs). Blockade of β-ARs located in the TMJ region suppresses formalin-induced TMJ nociception in both males and females rats (Fávaro-Moreira et al., 2012). Human studies have demonstrated that acute treatment with intravenous low-dose of the non-selective β-blocker propranolol produces improvement in clinical pain reported by women (Light et al., 2009) by a genetic dependent manner (Tchivileva et al., 2010).

Gender differences in the analgesic effect of drugs have been reported (Craft and Milholland, 1998). Regarding to the sympathetic component of TMJ pain, females appear to be more responsive to the antinociceptive effect of β-ARs blockade (Fávaro-Moreira et al., 2012) and gonadal hormones may reduce the responsiveness to the blockade of β-ARs in the TMJ of male and female rats

(unpublished data). However, whether the gonadal hormones modulate the effect of nadolol in women remains an open question.

Therefore, the aim of this randomized, double–blind, placebo controlled crossover study was to test the hypothesis that one and three days of treatment with the nonselective β_1 and β_2-ARs antagonist nadolol (40 mg once a day) would reduce clinical pain symptoms in TMD patients significantly more than placebo. We further hypothesized that women would experience relatively greater benefit from nadolol than men depending on their hormonal status. Finally, because nonsteroidal anti-inflammatory drugs (NSAIDs) do represent first-line drugs for many clinicians treating TMD pain (Hersh et al., 2008), we also compared the effect of nadolol with the effect of the NSAID ibuprofen.

Materials and Methods

Subjects

A total of 178 subjects were evaluated, but only 108 subjects were examinated through the RDC questionnaire. Final sample that reached both inclusion and exclusion criteria were 84 subjects, 29 men, 28 women using oral contraceptive (OC) and 27 women not using OC. This study was approved by the Research and Ethics Committee of Piracicaba Dental School (protocol number 089/2008), and all volunteers signed and informed, written consent to participate. Participants were recruited from patients who sought treatment at the Piracicaba Dental School.

Inclusion and Exclusion Criteria

Individuals were eligible for the study if they were between 18 and 48 years of age and met Research Diagnostic Criteria for TMD (RDC/TMD) (Dworkin et al., 1990). The RDC/TMD consists of a dual-axis approach (Axis I and II), established by a 30-item questionnaire and a physical examination. Axis I is used to stratify TMD into three groups: Group I, TMD with muscular disorders; Group II, TMD with temporomandibular joint (TMJ) disc displacement; and Group III, TMD with (a)

arthralgia, (b) osteoarthritis, or (c) osteoarthrosis of the TMJ. Axis II assesses TMD-related chronic pain, depression, nonspecific physical symptoms, and limitations in jaw function.

Women using or not OC and men were recruited. Exclusion criteria were self-reported osteoarthroses (RDC group IIIc), rheumatoid arthritis, fibromyalgia, trigeminal neuralgia, gynecological disorders (endometriosis), asthma, chronic lung disease, bronchospasm, renal, hepatic or cardiovascular diseases, nasal polyps, angioedema, reactivity to acetylsalicylic acid or other NSAIDs. Exclusion criteria also included unregularly menstrual cycles, use of hormonal replacement therapy, pregnancy or nursing, facial trauma or orofacial surgery within the last 6 weeks, orthodontic treatment, and major depression or other major psychiatric disorders, seizures, drug or alcohol abuse, current chemotherapy or radiation therapy; patients taking the following medications: drugs with central nervous system activity (e.g., opioids, benzodiazepines, nonbenzodiazepine sedative hypnotics, tricyclic antidepressants, monoamine oxidase inhibitors, and anticonvulsants) and β-blockers. The use of selective serotonin reuptake inhibitors was permitted only if the subject had already been taking it for at least 1 month before study enrollment. Medications for headaches (such as the triptans) and NSAIDs were not allowed during the last 2 days prior to the study visits.

To minimize the risk of an adverse hypotensive response to β-blockade, subjects with heart rate (HR) under 55 bpm and diastolic blood pressure below 50 mmHg were excluded from the study.

Study design

This study was a randomized, double–blind, placebo-controlled, crossover clinical trial. Patients (men, women using OC and women not using OC) were randomly allocated in one of six study arms (nodolol/ ibuprofen/placebo, nadolol/placebo/ibuprofen, ibuprofen/nadolol/placebo, ibuprofen/placebo/nadolol, placebo/nadolol/ibuprofen, placebo/ibuprofen/nadolol). During 3 days of treatment

period subjects received 40 mg of nadolol (Garcia-Monco et al., 2007), 400 mg of ibuprofen (Misra *et al.*, 2004) or placebo once a day.

The following protocols were used in normally cycling women (protocol 1), women using OC (protocol 2) and in men (protocol 3):

Protocol 1 (Figure 1A) - Normally cycling women were treated and evaluated during the menstrual and peri-ovulatory phases for 3 months (one month for each treatment). During the menstrual phase pain baseline assessement was performed on first day of bleeding and nadolol, ibuprofen or placebo was administred once a day for 3 days from the second to the fourth day. In this phase, post-treatment pain intensity rating was evaluated on the first and last day of treatment 3 hours after drug intake. During the peri-ovulatory phase pain intensity rating evaluation was based on menstrual cycle length. Pain baseline assessement was performed 3 days before the expected ovulation day and nadolol, ibuprofen or placebo was administred once a day for 3 days starting 2 days before the ovulation day. In this phase, post-treatment pain intensity rating was evaluated on the first and last day of treatment (ovulation day) 3 hours after drug intake. In face of a negative result for ovulation test, measurements were discarded and carried out in the following ovulatory menstrual cycle.

Protocol 2 (Figure 1B) - Women using OC were treated and evaluated during the menstrual and OC use phases for 3 months (one month for each treatment). During the menstrual phase the procedures were similar to those of protocol 1, that is, pain baseline assessement was performed on first day of bleeding and nadolol, ibuprofen or placebo was administred once a day for 3 days from the second to the fourth day. During the period of OC use pain baseline assessement was performed on day 6 after the initiation of OC use and nadolol, ibuprofen or placebo was administred once a day during the following 3 days. In both the menstrual and OC use phases, post-treatment pain intensity rating was evaluated on the first and last day of treatment 3 hours after drug intake.

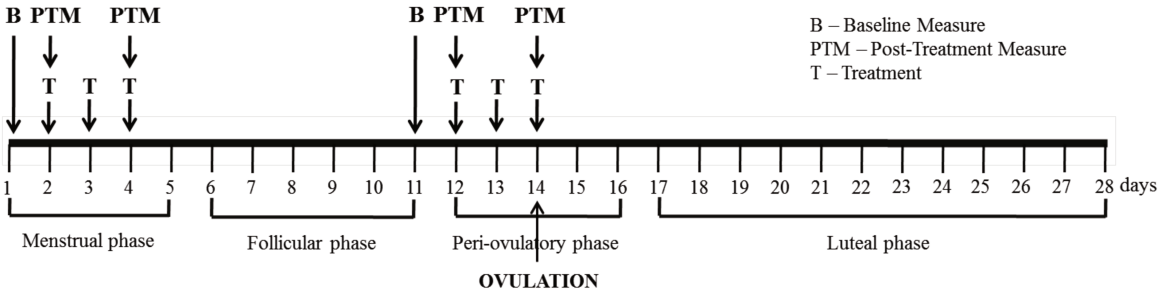
Protocol 3 (Figure 1C) – a protocol similar to that used in women was used in men except that men were treated and evaluated during only one month starting

promptly with a 6 days of wash out period among each stage of treatment (three stages, one for each treatment).

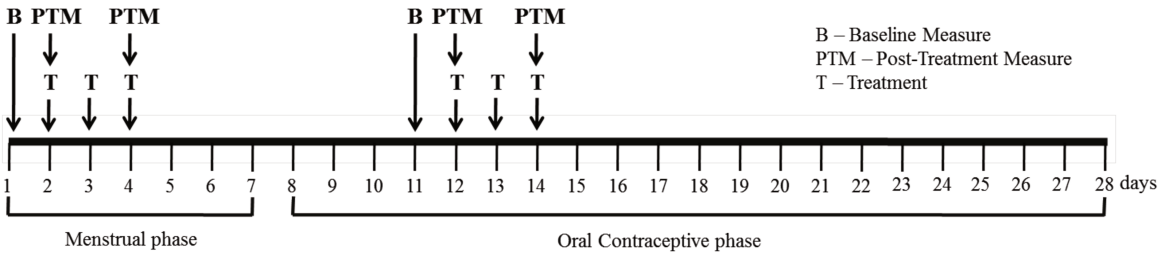
All evaluations were performed by a single researcher, scheduled for approximately the same time of the day, and consisted of 6 stages for women and 3 stages for men. Thus, all women were evaluated for 3 months and men for 1 month, totalizing 18 and 9 appointments, respectively.

Figure 1:

A - Ptotocol 1 - Normally cycling women



B - Ptotocol 2 - Women using OC



C - Ptotocol 3 - Men

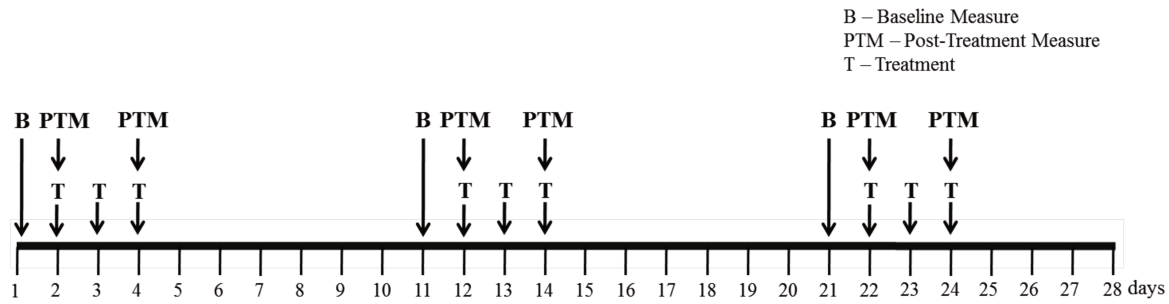


Fig.1 – Protocols used in normally cycling women, women using OC and in men.

Protocol 1 (used in normally cycling women, Fig. A) and protocol 2 (used in women using OC, Fig. B) demonstrate treatment and evaluation during the menstrual and peri-ovulatory phases for normally cycling women, and during the menstrual and OC use phases for women using OC (for 3 months - one month for each treatment). Protocol 3 (Fig. C) used in men demonstrates treatment and evaluation during only one month starting promptly with a 6 days of wash out period among each stage of treatment (three stages, one for each treatment). In summary, evaluations consisted of 6 stages for women and 3 stages for men. Thus, all women were evaluated for 3 months and men for 1 month, totalizing 18 and 9 appointments, respectively.

Placebo and ibuprofen pills were identical to nadolol pills to ensure subject and investigator blinding. The treatment sequence code for each subject was not disclosed to the project investigators untill completion of the entire research protocol. At initial screening, a RDC/TMD and intraoral examination was performed and medical history was recorded. At every subsequent study visit pain self-report (PSR) was administred.

Daily diary

Subjects were instructed to complete the diary every day while participating on this trial reporting the hour of drug intake, possible side effects, or whether any other drug intake, besides those used in this trail, was necessary. Women in all

groups reported whether they were having menstrual bleeding each day and women who were not on OCs also reported whether they had used the ovulation detection kit and, if so, whether results were negative or positive.

Menstrual cycle determination

All female subjects not using OC presented regular menstrual cycle and were observed during 2 months prior to the study to determine the length of the cycles, which remained between 28 and 32 days. Menstrual cycle was divided considering the date of last menstruation, ovulation period, and on the length of the cycle, into 4 phases (Janse de Jonge et al., 2001): (I) Menstrual: first day of bleeding, (II) Follicular: from 6th to 11th day; (III) Peri-ovulatory: from 12th to 16th day, determined by means of the ovulatory predictor test; and (IV) Luteal: stated between the 17th and the 28th day of the menstrual cycle. Menstrual phase was determined by self-report in the first day of bleeding. Based on the length of the cycle, subjects near to the expected time of ovulation were instructed to use Bioeasy ovulation prediction test (BioEasy Diagnóstica, Belo Horizonte, MG, Brazil) until identification of the peak of luteinizing hormone (LH), which occurs immediately before ovulation.

Outcome measure

Pain self-report (PSR) — Pain intensity rating was obtained via the PSR based on a ‘no pain at all’ and ‘the most intense pain imaginable’ scale. Subjects rated pain level in a paper Visual Analog Scale (VAS) once by each analyses time, used as primary outcome variable for this study (Wewers and Lowe, 1990).

Statistical analysis

Differences in age between treatments in the groups of men, women using OC and women not using OC were determined using one-way ANOVA.

The pain score obtained by rating the pain level on a 10-cm Visual Analog Scale (VAS) was used in statistical analysis. Baseline pain ratings represent absolute pain intensity scores obtained 1 day before treatment initiation. “Change in VAS pain rating” (cm) represents the value obtained by subtracting the baseline pain rating from the post-treatment pain rating of 1- and 3-day treatment.

The assumptions of equality of variances and normal distribution of errors were checked for all response variables tested.

For Table 1 a two-way analysis of variance (ANOVA) with two between-subjects factors, that is, sex (men and women using or not OC) and treatment (with three levels) was used to determine if there were significant sex differences in baseline pain ratings. A two-way ANOVA with one between-subjects (that is, treatment with three levels) and one within-subjects factor (that is, phase with two levels) was performed for each menstrual cycle phase of women not using OC (menses and peri-ovulatory menstrual cycle phases) and women using OC (menses and using OC phases) to determine if there were significant differences in baseline pain ratings among treatments.

For figure 2A a two-way ANOVA with two between-subjects factors, that is, sex (men and women not using OC) and treatment (with three levels) was used to determine if there were significant differences in “Change in VAS pain rating” among treatments considering the 1-day treatment. For figure 3A and 3B a two-way ANOVA with one between-subjects factors (that is, treatment with three levels) and one within-subjects factor (that is, phase with two levels, menses and peri-ovulatory menstrual cycle phases for women not using OC and menses and using OC phases for women using OC) was used to determine if there were significant differences in “Change in VAS pain rating” among treatments considering the 1-day treatment.

For figure 2B, 3C and 3D a pareaded Student´s t test was performed for each treatment to determine if there were significant differences in “Change in VAS pain rating” between One and Three Days post-treatment. A one-way ANOVA was performed to each post-treatment day (One Day and Three Days) to determine if

there were significant differences in “Change in VAS pain rating” among treatments.

Tukey posthoc tests were employed to determine the basis of significant differences. A probability level of 95% ($\alpha=0.05$) was considered to indicate statistical significance. Data are plotted in figures as mean±s.d.. The SAS software system (version 9.2; SAS Institute Inc., Cary, NC, USA) was used for statistical analysis.

Results

Characteristics of participants receiving Nadolol, Ibuprofen or Placebo.

Age was lower in women using OC group (27.71±4.93; max. = 41 and min. = 21) than in women not using OC (33.34±8.24; max. = 47 and min. = 21) or men (34.55±8.52; max. = 47 and min. = 19) groups (One-way Anova, Tukey post hoc test, $p<0.05$). Because the difference in age (approximately 6.2 years) was not considered to be a confounding variable, age was not included in the treatment analyses.

Baseline pain ratings were not significantly different between men and women considering the treatments (Two-way Anova, Tukey post hoc test, $p>0.05$). However, baseline pain ratings were significantly different among treatments in both phases of women not using OC (menses and peri-ovulatory menstrual cycle phases) and were significantly higher in the menses than in using OC phase in women using OC for each treatment (Two-way Anova, Tukey post hoc test, $p<0.0001$) (Table 1). The differences in baseline pain ratings between phases in women using OC indicate that women using OC experience greater pain during menses than during the phase using OC. Furthermore, because baseline pain rating differed in these groups, pain rating data are presented as ‘Change in VAS pain rating (cm)’ which represents the value obtained by subtracting the baseline pain rating from the post-treatment pain rating of 1- and 3-day treatment in order to normalize the differences in all figures, except Table 1.

Table 1 – Baseline VAS characteristics of participants receiving Nadolol, Ibuprofen or Placebo.

			Nadolol	Ibuprofeno	Placebo
Baseline VAS	Women not using OCs	Menses	7.77±1.94	7.34±1.70	7.55±1.57
		Peri-ovulatory	6.70±1.86	6.39±2.13	8.19±1.21
	Women using OCs	Menses *	8.06±1.46	7.42±1.96	7.01±1.75
		Using OC	6.24±2.03	5.60±2.18	5.61±1.77
	Men		7.36±1.60	7.01±1.57	7.73±1.62

Baseline pain ratings were not significantly different between men and women considering the treatments (Two-way Anova, Tukey post hoc test, $p>0.05$). However, baseline pain ratings were significantly different among treatments when considering the phases of women not using OC (menses and peri-ovulatory menstrual cycle phases) and were significantly higher in the menses phase when compared to using OC phase in women using OC considering the treatments. The symbol "*" indicates a Baseline pain rating significantly greater than that of the phase using OC in women using OC (Two-way Anova, Tukey post hoc test, $p<0.0001$). Values are means ± s.d..

Effect of nadolol, ibuprofen and placebo in women and men

Nadolol and ibuprofen produced a significantly produced a greater analgesia than placebo when considering 1-day treatment in both women and men but women were more responsive to treatments (Two-way Anova, Tukey post hoc test, $p=0.0449$) (Figure 2A). Three days of treatment produced a greater analgesia than only one day in both women and men (pareaded Student´s t test, for Nadolol and ibuprofen $p<0.0001$ and for placebo $p=0.0040$) (Figure 2B).

Figure 1

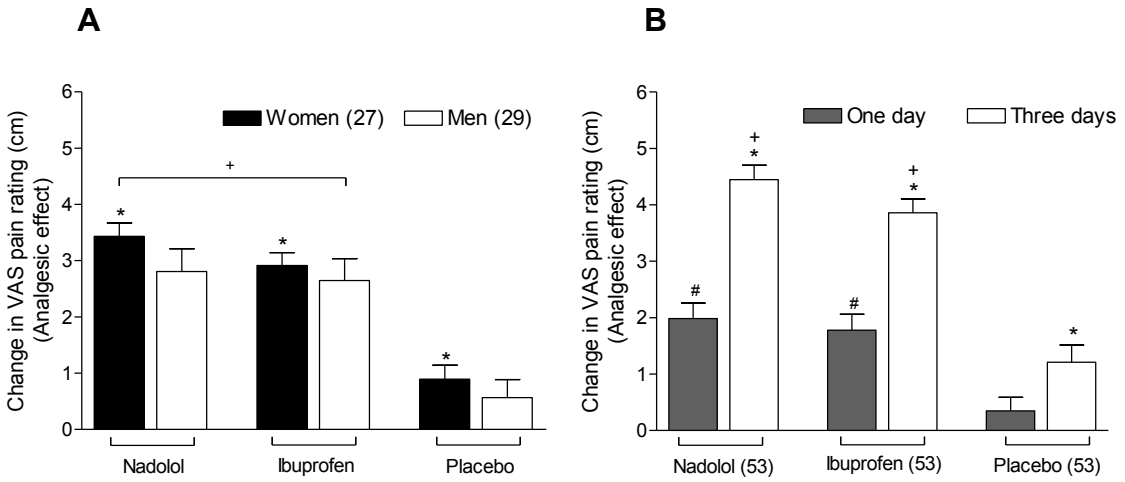


Fig.1 – Effect of nadolol, ibuprofen and placebo in women and men.

A- Nadolol and ibuprofen induced a significantly greater analgesic effect than placebo in women and men considering 1-day treatment. Women were more responsive to all treatments. The symbol "+" indicates an analgesic effect significantly greater than that of placebo and the symbol "*" indicates an analgesic effect significantly greater in women than in men (Two-way Anova, Tukey post hoc test, $p=0.0449$).

B- Three days of treatment induced a significantly greater analgesic effect than only one day, independently of gender. The symbol "*" indicates an analgesic effect significantly greater than that of one day treatment (pareaded Student´s t test, for Nadolol and ibuprofen $p<0.0001$ and for placebo $p=0.0040$), the symbols "#" and "+" indicate an analgesic effect significantly greater than that of one and three days of placebo treatment, respectively (One-way Anova, Tukey post hoc test, $p<0.05$).

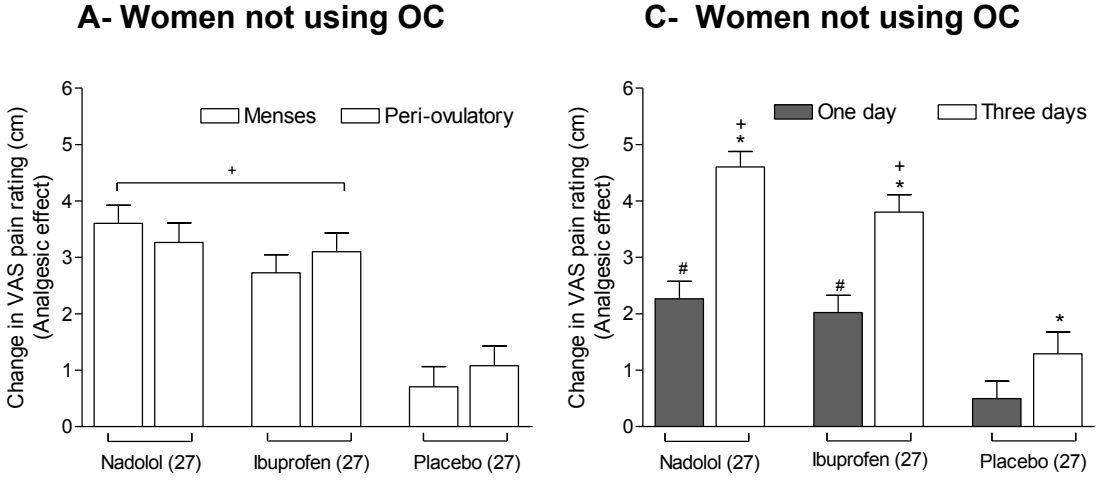
In this and in the subsequent figure the number between parenthesis indicates sample size number.

Effect of nadolol, ibuprofen and placebo in women in different hormonal status

Nadolol and ibuprofen produced a greater analgesia than placebo when considering 1-day treatment in both women using OC (Figure 3A) and women not using OC (Figure 3B) (Two-way Anova, Tukey post hoc test, $p<0.0001$). The analgesic effect of treatments was not significantly different between menses and peri-ovulatory menstrual cycle phases in women not using OC (Figure 3A) and between menses and OC use phases in women using OC (Figure 3B) (Two-way Anova, Tukey post hoc test, $p>0.05$).

Three days of treatment induced a greater analgesia than only one day in both women not using OC (Figure 3C) and women using OC (Figure 3D) (pareaded Student´s t test, $p<0.0001$).

Figure 2



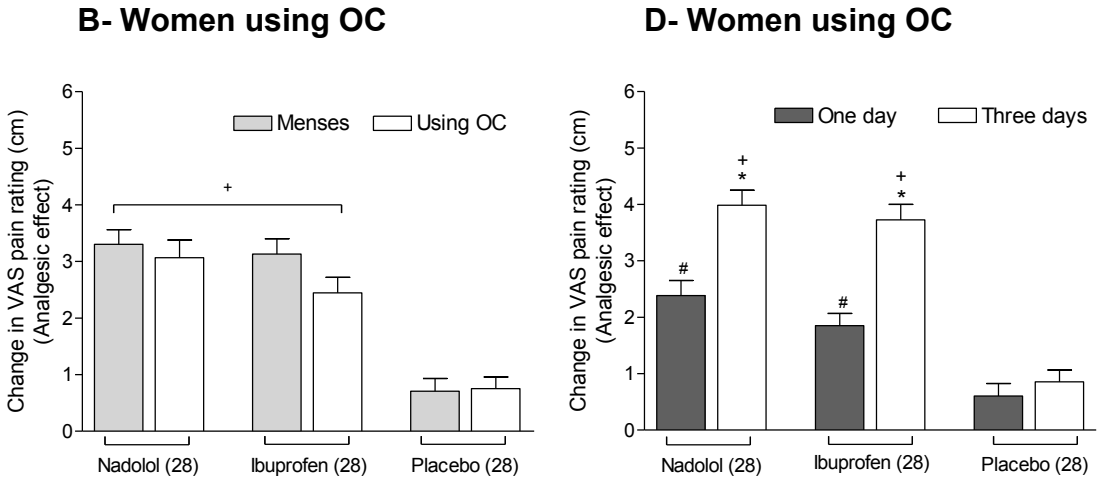


Fig.2 – Effect of nadolol, ibuprofen and placebo in women using or not OC.

A and B - Nadolol and ibuprofen induced a significantly greater analgesic effect than placebo considering 1-day treatment. Cycle phases did not affect treatments-induced analgesia. The symbol ""+" indicates a response significantly greater than that of placebo (Two-way Anova, Tukey post hoc test, $p<0.0001$).

C and D - Three days of treatment induced a significantly greater analgesic effect than only one day. The symbol "*" indicates an analgesic effect significantly greater than that of one day treatment (pareaded Student´s t test, $p<0.0001$), the symbols "#" and "+" indicate an analgesic effect significantly greater than that of one and three days of placebo treatment, respectively (One-way Anova, Tukey post hoc test, $p<0.05$).

Discussion

The results of this study indicate that one and three days of treatment with nadolol (40 mg once a day) or ibuprofen (400 mg once a day) produces analgesia in TMD women and men significantly more than placebo, but women are more responsive to treatments independent of their menstrual cycle phase. This human study is consistent with our previous animal studies showing that blockade of β-AR into the rat´s TMJ (Fávaro-Moreira et al., 2012) and local administration of NSAIDS (Oliveira-Fusaro et al., 2012) significantly reduces formalin-induced TMJ

nociception. They are also consistent with human studies showing that NSAIDS such as naproxen (Ta and Dionne, 2004) and ibuprofen are effective in reducing TMD pain compared with placebo (Marini et al., 2012) and with studies carried out only in women showing that propranolol significantly reduces the number of painful body sites and ratings of total clinical pain in women experiencing TMD and fibromyalgia (Light et al., 2009) and pain intensity rating in women experiencing TMD (Tchivileva et al., 2010).

To our knowledge, this is the first controlled study that evaluated gender and menstrual cycle phases differences in the effect of a β-blocker and of a NSAID on TMD pain. Our findings that nadolol and ibuprofen are more efficacious in women than in men are clinically relevant because TMD pain is more prevalent and severe in women (Dworkin et al., 1990). Furthermore, they are consistent with our previous findings that female rats are more responsive than male rats to the TMJ antinociception induced by β-blockers (Fávaro-Moreira et al., 2012).

The physiological basis for the sex-related difference in analgesic response to nadolol and ibuprofen treatment is not presently known. However, it is possible that a male-related hormone, such as testosterone, interacts negatively with nadolol and ibuprofen pathway, which is consistent with our previous findings that in male rats testosterone attenuates the responsiveness to the blockade of TMJ β-ARs (Fávaro-Moreira et al., 2012). Another possibility is that females-related hormones, such as progesterone and estradiol, potentiate the action of nadolol. However, this is unlikely because levels of female hormones vary during the menstrual cycle phases and there was not a significant difference in the analgesia induced by nadolol and ibuprofen between the menstrual and peri-ovulatory phases in normally cycling women (current findings). In addition, in female rats estradiol and progesterone appear to interact negatively with nadolol (unpublished data). Finally, it is possible that differences between males and females are not attributable to sex hormones but to genetic factors related to the sympathetic pathway. For example, common haplotypes in the gene encoding catechol-*O*-methyltransferase (COMT) have been associated with pain modulation and the risk

of developing TMD (Diatchenko et al., 2006, 2007). Extending this genetic-based approach, it has been demonstrated that individuals with COMT haplotypes coding for reduced enzyme activity experience relatively greater benefit from propranolol treatment than participants with other COMT haplotypes, suggesting that genetic factors may serve as a predictor of propranolol treatment outcome (Tchivileva et al., 2010).

Although the possibility that our results might be explained by differences in the pharmacokinetic mechanisms of nadolol and ibuprofen cannot be entirely excluded there are not studies that examined sex differences in the pharmacokinetics of nadolol and the avaiable study that examined the pharmacokinetic of ibuprofen does not support such an explanation (Knights et al., 1995).

The findings that the analgesic response to nadolol and ibuprofen did not differ between menses and peri-ovulatory period in women not using OC, or between menses and the period using OC in women using OC suggest that estradiol and progesterone do not moduate their analgesic effect. However, additional work is warranted with women in differents hormonal status, such as in women experiencing the menopause phase, to more precisely correlate female sex hormone levels with the analgesic response to nadolol and ibuprofen. Furthermore, the doses of nadolol and ibuprofen used in this study might be sufficient to mask a possible distinct response to the nadolol analgesic effect between the menstrual cycle phases.

It is well established that drugs pharmacokinetic, specifically the drug distribution, can be modified depending on a person's weight, which could represent a source of variation in this study. However, some drugs, including nadolol, have their doses adjusted during a period of time to define the individual dose depending on drug tolerance and on individual response but not on individual weight. Oral nadolol produces its antihypertensive effect in the wide dose range of 80 to 160 mg/day, and the greatest effect is obtained with the dose of 80 mg/day (Papadoyannis et al., 1982). Since the antihypertensive effect was an undesired

effect in this study and it has been demonstrated, after titration of the dose, that for prophylaxis treatment of migraine a single dose of 40 mg/day provides a good risk/benefit profile (Garcia-Monco et al., 2007), this dose was used in this study. Ibuprofen has been shown to be effective in relieving pain on a dose of 400 mg/day (Misra et al., 2004).

Despite of the doses used, nadolol and ibuprofen were effective to reduce TMJ associated pain in the first and in the third day of treatment, but third day was more effective. Therefore three days of nadolol and ibuprofen treatment produces a greater benefit in the treatment TMD pain.

In summary, we showed that one and three days of treatment with nadolol (40 mg once a day) or ibuprofen (400 mg once a day) produced greater analgesia in TMD women and men than placebo, but women were more responsive independent of their menstrual cycle phase. The analgesic efficacy of nadolol was similar to that of ibuprofen. Considering that many patients are intolerant to prolonged treatment with NSAIDs and that not all TMD pain patients respond to its effects, our findings point out nadolol as a great pharmacological option to treat TMD pain.

Conclusions

In summary, the present findings indicate that nadolol and ibuprofen reduce pain in the TMJ region in men and women since their analgesic effect was significantly more effective than placebo, but women are more responsive. In light of the evidence that clinical TMJ pain is more prevalent in women than in men, the use of β-blockers could be a pharmacological alternative in the treatment of TMJ pain, especially in women.

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CONCLUSÃO

Em resumo, os dados deste trabalho demonstram que os hormônios gonadais podem modular o efeito analgésico dos bloqueadores de β-AR dependendo dos níveis plasmáticos dos hormônios gonadais, do subtipo de β-AR ativado e da dose do β-bloqueador administrada.

Embora a prevalência de dor relacionada às DTMs seja maior em mulheres, elas também parecem ser mais sensíveis aos efeitos analgésicos dos medicamentos β-bloqueadores. E embora os hormônios femininos pareçam não participar da modulação do efeito analgésico dos β-bloqueadores nas mulheres durante o período reprodutivo, os dados apresentados sugerem que a ausência desses hormônios aumenta a responsividade ao efeito analgésico dos β-bloqueadores, sendo uma alternativa interessante para o tratamento da dor relacionada às DTMs em mulheres com baixos níveis hormonais como no período da menopausa.

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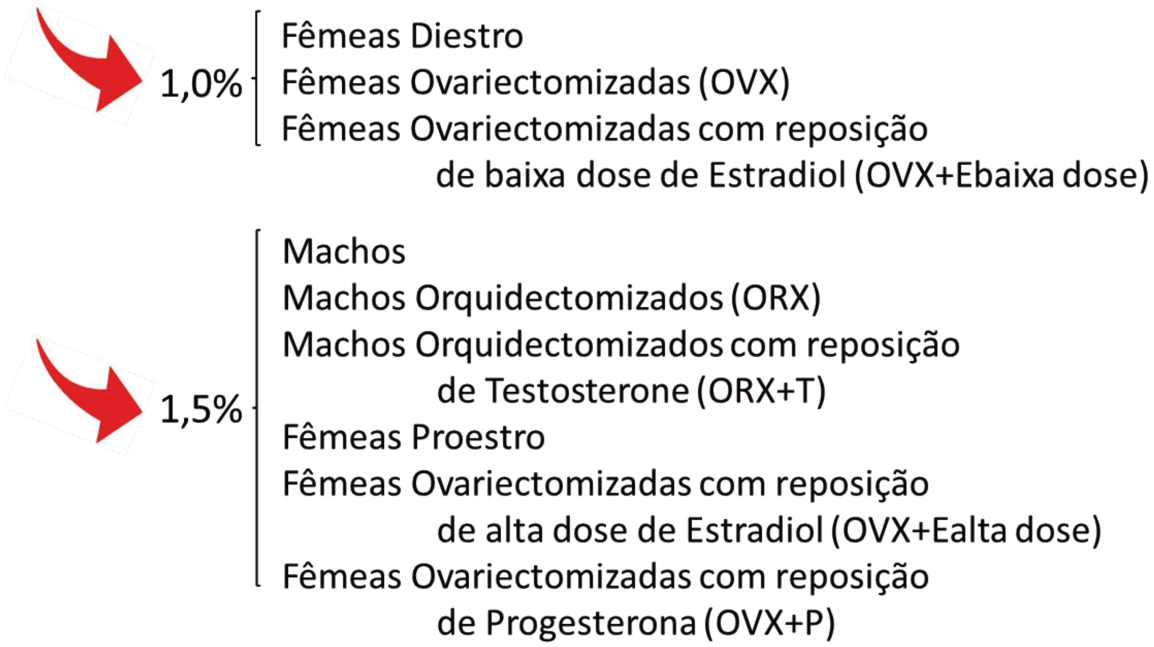
APÊNDICE 1 – Diagrama relacionado ao Artigo 1 – Resumo do Delineamento Experimental

1a: Doses equinociceptivas, ou seja, 1,0% ou 1,5% de formalina, nos grupos de animais machos e fêmeas.

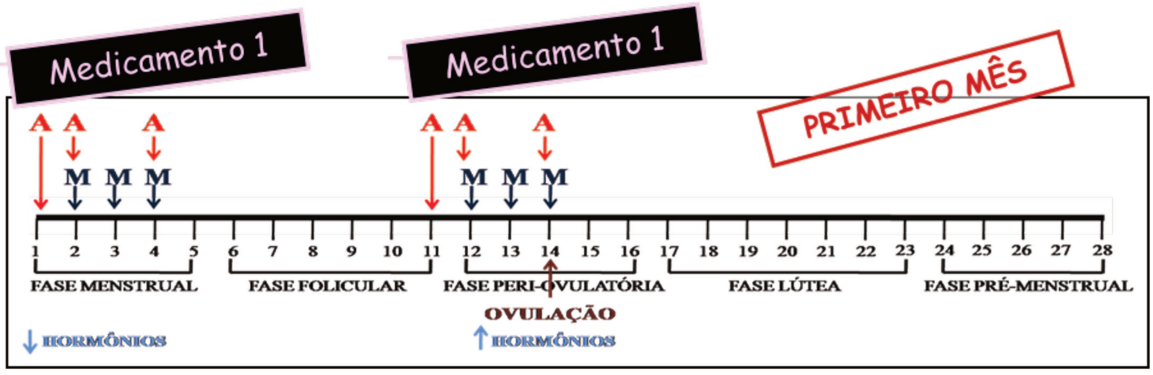


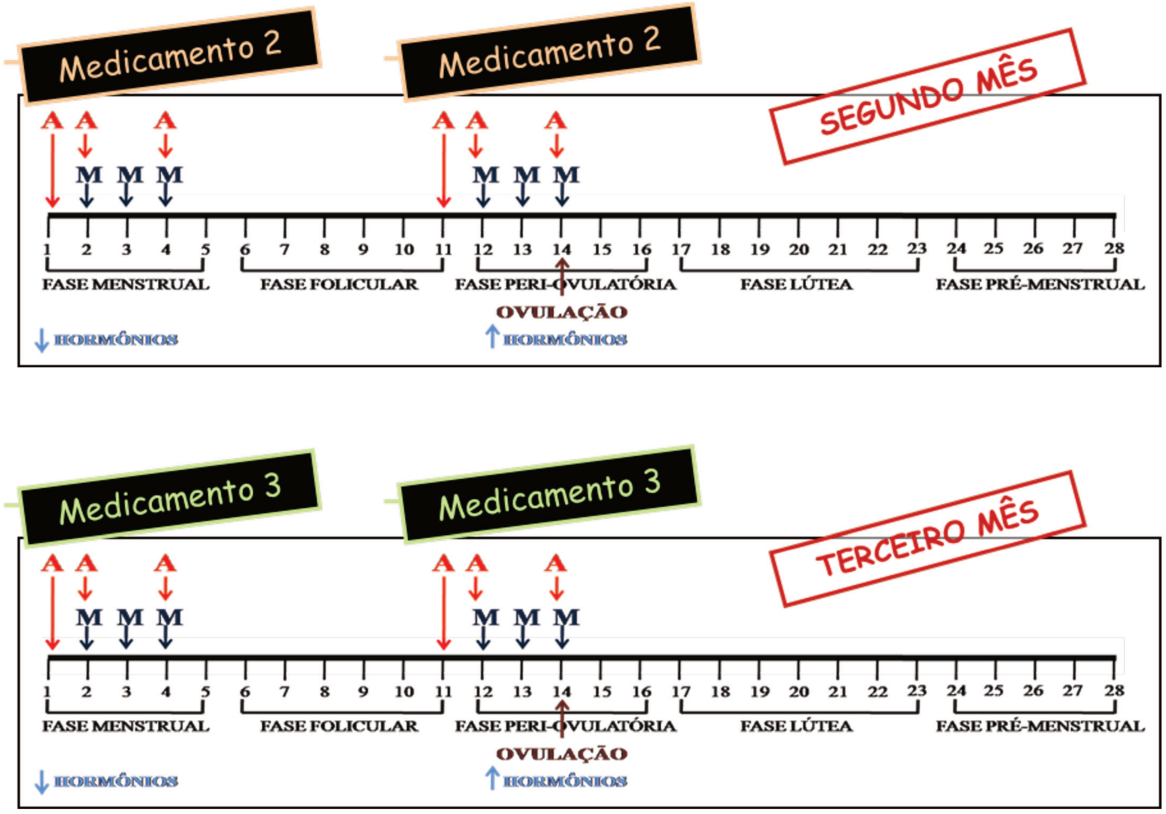
1b: Cada grupo de animal citado na figura 1a foi dividido nos seguintes subgrupos:

Subgrupo	Quantidade	Substâncias administradas ATM
1	6	NaCl 0,9%
2	6	Formalina 1,0% ou 1,5%
3	24	F + Atenolol 2µg, 6µg, 18µg, 54µg ou 162µg
4	18	F + ICI 0,1µg, 0,3µg e 0,9µg
5	30	F + SR 0,1µg, 0,5µg, 1,5µg, 4,5µg e 13,5µg
6	18	A ct, ICI ct e SR ct
7	18	NaCl 0,9% + A, + ICI, + SR

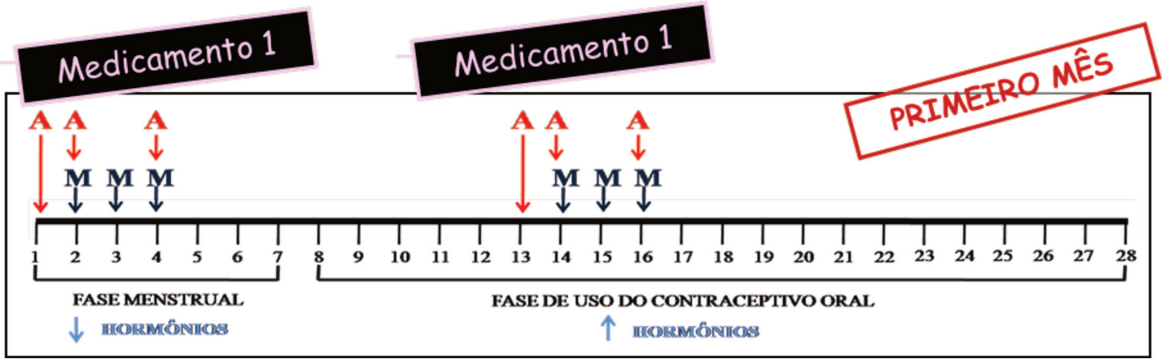
APÊNDICE 2 – Diagramas relacionados ao Artigo 2 referentes às análises e tratamentos para os voluntários.

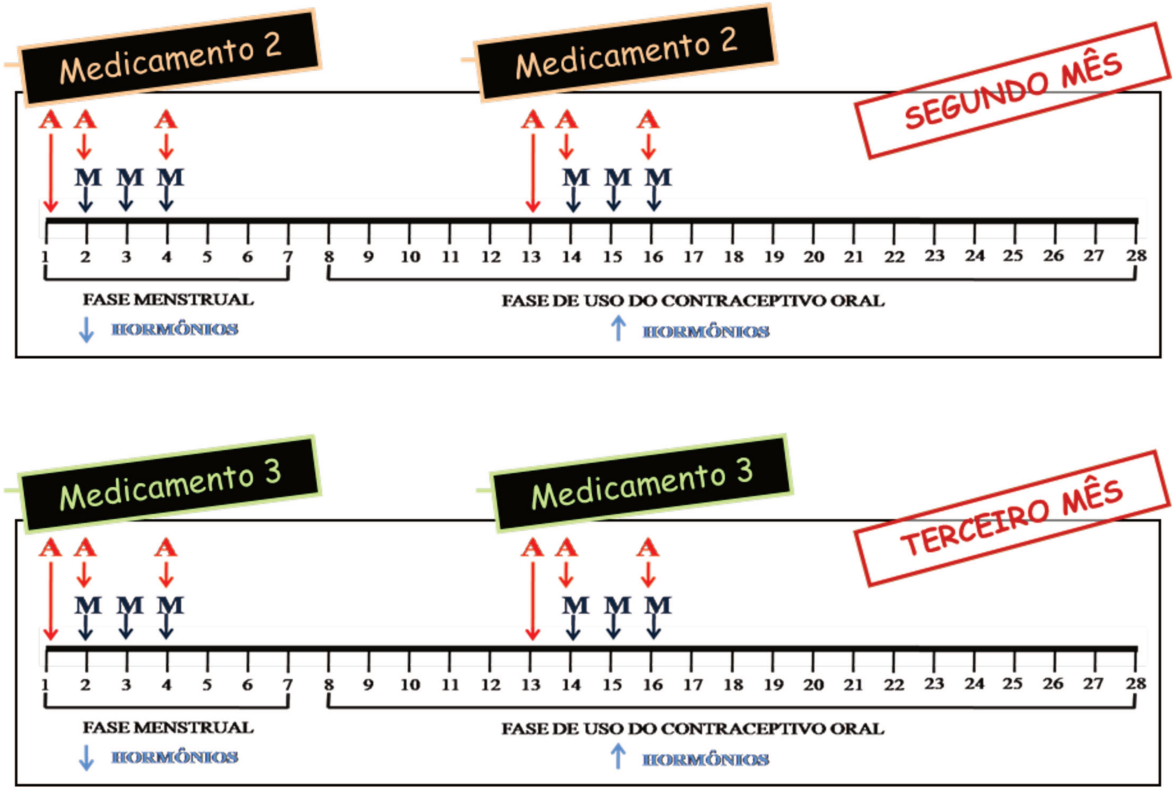
2a: Diagrama dos dias de Análise (A) e de Tratamento Medicamento (M) em Mulheres não usuárias de Contraceptivo Oral:



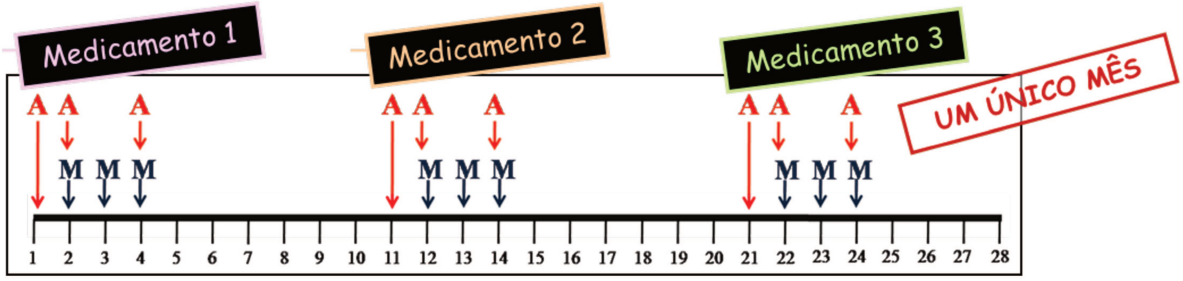


2b: Diagrama dos dias de Análise (A) e de Tratamento Medicamento (M) em Mulheres usuárias de Contraceptivo Oral:





2c: Diagrama dos dias de Análise (A) e de Tratamento Medicamento (M) em Homens:



ANEXO 1 - Certificado do Comitê de Ética em Pesquisa em Animais

UNICAMP

CEUA/Unicamp

Comissão de Ética no Uso de Animais
CEUA/Unicamp

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**.

Campinas, 27 de novembro de 2009.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
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Comissão de Ética no Uso de Animais
CEUA/Unicamp

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.

Profa. Dra. Ana Maria A. Guaraldo
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ANEXO 2 – Certificado do Comitê de Ética em Pesquisa em Humanos

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Estudo do efeito do nadolol na dor associada às disfunções temporomandibulares**", protocolo nº **089/2008**, dos pesquisadores **NáDIA CRISTINA FáVARO MOREIRA, CLAUDIA HERRERA TAMBELI, EDUARDO DIAS DE ANDRADE e RENATA CUNHA MATHEUS RODRIGUES GARCIA**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 30/09/2008.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Effect of nadolol on temporomandibular pain**", register number **089/2008**, of **NáDIA CRISTINA FáVARO MOREIRA, CLAUDIA HERRERA TAMBELI, EDUARDO DIAS DE ANDRADE and RENATA CUNHA MATHEUS RODRIGUES GARCIA**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 30/09/2008.

Prof. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 3 – Research Diagnostic Criteria for Temporomandibular Disorders (RDC-TMD)

RDC - TMD

Research Diagnostic Criteria for
Temporomandibular Disorders

Português – BRASIL

Nome	Prontuário / Matrícula n°	RDC n°
Examinador	Data _____/_____/_____	

HISTÓRIA - QUESTIONÁRIO

Por favor, leia cada pergunta e marque somente a resposta que achar mais correta.

1. Como você classifica sua saúde em geral?

1 Excelente
2 Muito boa
3 Boa
4 Razoável
5 Ruim

2. Como você classifica a saúde da sua boca?

1 Excelente
2 Muito boa
3 Boa
4 Razoável
5 Ruim

3. Você sentiu dor na face, em locais como na região das bochechas (maxilares), nos lados da cabeça, na frente do ouvido ou no ouvido, nas últimas 4 semanas?

0 Não
1 Sim

[Se sua resposta foi **não**, PULE para a **pergunta 14.a**]
[Se a sua resposta foi **sim**, PASSE para a **próxima pergunta**]

4. Há quanto tempo a sua dor na face começou pela primeira vez?

[Se começou **há um ano ou mais**, responda a **pergunta 4.a**]
[Se começou **há menos de um ano**, responda a **pergunta 4.b**]

4.a. Há quantos anos a sua dor na face começou pela primeira vez?

☐☐ Ano(s)

4.b. Há quantos meses a sua dor na face começou pela primeira vez?

☐☐ Mês(es)

5. A dor na face ocorre?

1 O tempo todo
2 Aparece e desaparece
3 Ocorreu somente uma vez

6. Você já procurou algum profissional de saúde (médico, cirurgião-dentista, fisioterapeuta, etc.) para tratar a sua dor na face?

1 Não
2 Sim, nos últimos seis meses.
3 Sim, há mais de seis meses.

7. Em uma escala de 0 a 10, se você tivesse que dar uma nota para sua dor na face agora, NESTE EXATO MOMENTO, que nota você daria, onde 0 é "nenhuma dor" e 10 é "a pior dor possível"?

NENHUMA DOR **0 1 2 3 4 5 6 7 8 9 10** A PIOR DOR POSSÍVEL

8. Pense na pior dor na face que você já sentiu nos últimos seis meses, dê uma nota pra ela de 0 a 10, onde 0 é "nenhuma dor" e 10 é "a pior dor possível"?

NENHUMA DOR **0 1 2 3 4 5 6 7 8 9 10** A PIOR DOR POSSÍVEL

9. Pense em todas as dores na face que você já sentiu nos últimos seis meses, qual o valor médio você daria para essas dores, utilizando uma escala de 0 a 10, onde 0 é "nenhuma dor" e 10 é "a pior dor possível"?

NENHUMA DOR **0 1 2 3 4 5 6 7 8 9 10** A PIOR DOR POSSÍVEL

10. Aproximadamente quantos dias nos últimos seis meses você esteve afastado de suas atividades diárias como: trabalho, escola e serviço doméstico, devido a sua dor na face?

☐☐ Dias

11. Nos últimos seis meses, o quanto esta dor na face interferiu nas suas atividades diárias utilizando uma escala de 0 a 10, onde 0 é "nenhuma interferência" e 10 é "incapaz de realizar qualquer atividade"?

NENHUMA INTERFERÊNCIA **0 1 2 3 4 5 6 7 8 9 10** INCAPAZ DE REALIZAR QUALQUER ATIVIDADE

12. Nos últimos seis meses, o quanto esta dor na face mudou a sua disposição de participar de atividades de lazer, sociais e familiares, onde 0 é "nenhuma mudança" e 10 é "mudança extrema"?

NENHUMA MUDANÇA **0 1 2 3 4 5 6 7 8 9 10** MUDANÇA EXTREMA

13. Nos últimos seis meses, o quanto esta dor na face mudou a sua capacidade de trabalhar (incluindo serviços domésticos) onde 0 é "nenhuma mudança" e 10 é "mudança extrema"?

NENHUMA MUDANÇA **0 1 2 3 4 5 6 7 8 9 10** MUDANÇA EXTREMA

14.a. Alguma vez sua mandíbula (boca) já ficou travada de forma que você não conseguiu abrir totalmente a boca?

☐ 0 Não

☐ 1 Sim

[Se você **nunca** teve travamento da mandíbula, PULE para a **pergunta 15.a**]
[Se **já teve** travamento da mandíbula, PASSE para a **próxima pergunta**]

14.b. Este travamento da mandíbula (boca) foi grave a ponto de interferir com a sua capacidade de mastigar?

☐ 0 Não

☐ 1 Sim

15.a. Você ouve estalos quando mastiga, abre ou fecha a boca?

☐ 0 Não

☐ 1 Sim

15.b. Quando você mastiga, abre ou fecha a boca, você ouve um barulho (rangido) na frente do ouvido como se fosse osso contra osso?

☐ 0 Não

☐ 1 Sim

15.c. Você já percebeu ou alguém falou que você range (ringi) ou aperta os seus dentes quando está dormindo?

0 Não

1 Sim

15.d. Durante o dia, você range (ringi) ou aperta os seus dentes?

0 Não

1 Sim

15.e. Você sente a sua mandíbula (boca) "cansada" ou dolorida quando você acorda pela manhã?

0 Não

1 Sim

15.f. Você ouve apitos ou zumbidos nos seus ouvidos?

0 Não

1 Sim

15.g. Você sente que a forma como os seus dentes se encostam é desconfortável ou diferente/ estranha?

0 Não

1 Sim

16.a. Você tem artrite reumatóide, lúpus, ou qualquer outra doença que afeta muitas articulações (juntas) do seu corpo?

0 Não

1 Sim

16.b. Você sabe se alguém na sua família, isto é seus avós, pais, irmãos, etc. já teve artrite reumatóide, lúpus, ou qualquer outra doença que afeta várias articulações (juntas) do corpo?

0 Não

1 Sim

16.c. Você já teve ou tem alguma articulação (junta) que fica dolorida ou incha sem ser a articulação (junta) perto do ouvido (ATM)?

0 Não

1 Sim

[Se você **não** teve dor ou inchaço, PULE para **a pergunta 17.a**.]
[Se você **já teve**, dor ou inchaço, PASSE para a **próxima pergunta**]

16.d. A dor ou inchaço que você sente nessa articulação (junta) apareceu várias vezes nos últimos 12 meses (1 ano)?

0 Não

1 Sim

17.a. Você teve recentemente alguma pancada ou trauma na face ou na mandíbula (queixo)?

0 Não

1 Sim

[Se sua resposta foi **não**, PULE para a **pergunta 18**]
[Se sua resposta foi **sim**, PASSE para a **próxima pergunta**]

17.b. A sua dor na face (em locais como a região das bochechas (maxilares), nos lados da cabeça, na frente do ouvido ou no ouvido) já existia antes da pancada ou trauma?

0 Não

1 Sim

18. Durante os últimos seis meses você tem tido problemas de dor de cabeça ou enxaquecas?

0 Não

1 Sim

19. Quais atividades a sua dor na face ou problema na mandíbula (queixo), impedem, limitam ou prejudicam?

	NÃO	SIM
a. Mastigar	0	1
b. Beber (tomar líquidos)	0	1
c. Fazer exercícios físicos ou ginástica	0	1
d. Comer alimentos duros	0	1
e. Comer alimentos moles	0	1
f. Sorrir/gargalhar	0	1
g. Atividade sexual	0	1
h. Limpar os dentes ou a face	0	1
i. Bocejar	0	1
j. Engolir	0	1
k. Conversar	0	1
l. Ficar com o rosto normal: sem a aparência de dor ou triste	0	1

20. Nas últimas quatro semanas, o quanto você tem estado angustiado ou preocupado:

	Nem um pouco	Um pouco	Moderadamente	Muito	Extremamente
a. Por sentir dores de cabeça	0	1	2	3	4
b. Pela perda de interesse ou prazer sexual	0	1	2	3	4
c. Por ter fraqueza ou tontura	0	1	2	3	4
d. Por sentir dor ou "aperto" no peito ou coração	0	1	2	3	4
e. Pela sensação de falta de energia ou lentidão	0	1	2	3	4
f. Por ter pensamentos sobre morte ou relacionados ao ato de morrer	0	1	2	3	4
g. Por ter falta de apetite	0	1	2	3	4
h. Por chorar facilmente	0	1	2	3	4
i. Por se culpar pelas coisas que acontecem ao seu redor	0	1	2	3	4
j. Por sentir dores na parte inferior das costas	0	1	2	3	4
k. Por se sentir só	0	1	2	3	4
l. Por se sentir triste	0	1	2	3	4
m. Por se preocupar muito com as coisas	0	1	2	3	4
n. Por não sentir interesse pelas coisas	0	1	2	3	4
o. Por ter enjôo ou problemas no estômago	0	1	2	3	4
p. Por ter músculos doloridos	0	1	2	3	4
q. Por ter dificuldade em adormecer	0	1	2	3	4
r. Por ter dificuldade em respirar	0	1	2	3	4
s. Por sentir de vez em quando calor ou frio	0	1	2	3	4
t. Por sentir dormência ou formigamento em partes do corpo	0	1	2	3	4
u. Por sentir um "nó na garganta"	0	1	2	3	4
v. Por se sentir desanimado sobre o futuro	0	1	2	3	4
w. Por se sentir fraco em partes do corpo	0	1	2	3	4
x. Pela sensação de peso nos braços ou pernas	0	1	2	3	4
y. Por ter pensamentos sobre acabar com a sua vida	0	1	2	3	4
z. Por comer demais	0	1	2	3	4
aa. Por acordar de madrugada	0	1	2	3	4
bb. Por ter sono agitado ou perturbado	0	1	2	3	4
cc. Pela sensação de que tudo é um esforço/sacrifício	0	1	2	3	4
dd. Por se sentir inútil	0	1	2	3	4
ee. Pela sensação de ser enganado ou iludido	0	1	2	3	4
ff. Por ter sentimentos de culpa	0	1	2	3	4

21. Como você classificaria os cuidados que tem tomado com a sua saúde de uma forma geral?

1 Excelente
2 Muito bom
3 Bom
4 Razoável
5 Ruim

22. Como você classificaria os cuidados que tem tomado com a saúde da sua boca?

1 Excelente
2 Muito bom
3 Bom
4 Razoável
5 Ruim

23. Qual a data do seu nascimento?

Dia ☐☐ Mês ☐☐ Ano ☐☐☐☐

24. Qual seu sexo?

1 Masculino
2 Feminino

25. Qual a sua cor ou raça?

1 Aleútas, Esquimó ou Índio Americano
2 Asiático ou Insulano Pacífico
3 Preta
4 Branca
5 Outra [Se sua resposta foi **outra**, PASSE para as **próximas alternativas** sobre sua cor ou raça]
6 Parda
7 Amarela
8 Indígena

26. Qual a sua origem ou de seus familiares?

1 Porto Riquenho
2 Cubano
3 Mexicano
4 Mexicano Americano
5 Chicano
6 Outro Latino Americano
7 Outro Espanhol
8 Nenhuma acima [Se sua resposta foi **nenhuma acima**, PASSE para as **próximas alternativas** sobre sua origem ou de seus familiares]
9 Índio
10 Português
11 Francês
12 Holandês
13 Espanhol
14 Africano
15 Italiano
16 Japonês
17 Alemão
18 Árabe
19 Outra, favor especificar _____
20 Não sabe especificar

27. Até que ano da escola / faculdade você freqüentou?

Nunca freqüentei a escola		0
Ensino fundamental (primário)	1ªSérie	1
	2ª Série	2
	3ª Série	3
	4ª Série	4
Ensino fundamental (ginásio)	5ª Série	5
	6ª Série	6
	7ª Série	7
	8ª Série	8
Ensino médio (científico)	1°ano	9
	2°ano	10
	3°ano	11
Ensino superior (faculdade ou pós-graduação)	1°ano	12
	2°ano	13
	3°ano	14
	4°ano	15
	5°ano	16
	6°ano	17

28a. Durante as 2 últimas semanas, você trabalhou no emprego ou em negócio pago ou não (não incluindo trabalho em casa)?

0 Não

1 Sim

[Se a sua resposta foi **sim**, PULE para a **pergunta 29**]
[Se a sua resposta foi **não**, PASSE para a **próxima pergunta**]

28b. Embora você não tenha trabalhado nas duas últimas semanas, você tinha um emprego ou negócio?

0 Não

1 Sim

[Se a sua resposta foi **sim**, PULE para a **pergunta 29**]
[Se a sua resposta foi **não**, PASSE para a **próxima pergunta**]

28c. Você estava procurando emprego ou afastado temporariamente do trabalho, durante as 2 últimas semanas?

1 Sim, procurando emprego

2 Sim, afastado temporariamente do trabalho

3 Sim, os dois, procurando emprego e afastado temporariamente do trabalho

4 Não

29. Qual o seu estado civil?

1 Casado (a) esposa (o) morando na mesma casa

2 Casado (a) esposa (o) não morando na mesma casa

3 Viúvo (a)

4 Divorciado (a)

5 Separado (a)

6 Nunca casei

7 Morando junto

30. Quanto você e sua família ganharam por mês durante os últimos 12 meses?
R$ ☐☐☐☐☐,☐☐

Não preencher. Deverá ser preenchido pelo profissional

- ☐ Até ¼ do salário mínimo
- ☐ De ¼ a ½ salário mínimo
- ☐ De ½ a 1 salário mínimo
- ☐ De 1 a 2 salários mínimos
- ☐ De 2 a 3 salários mínimos
- ☐ De 3 a 5 salários mínimos
- ☐ De 5 a 10 salários mínimos
- ☐ De 10 a 15 salários mínimos
- ☐ De 15 a 20 salários mínimos
- ☐ De 20 a 30 salários mínimos
- ☐ Mais de 30 salários mínimos
- ☐ Sem rendimento

31. Qual o seu CEP?
☐☐☐☐☐-☐☐☐

Muito Obrigado.
Agora veja se você deixou de responder alguma questão.

EXAME CLÍNICO

1. Você tem dor no lado direito da sua face, lado esquerdo ou ambos os lados?

0 Nenhum
1 Direito
2 Esquerdo
3 Ambos

2.Você poderia apontar as áreas aonde você sente dor ?

Direito	Esquerdo
0 Nenhuma	0 Nenhuma
1 Articulação	1 Articulação
2 Músculos	2 Músculos
3 Ambos	3 Ambos

3. Padrão de abertura:

0 Reto
1 Desvio lateral direito (não corrigido)
2 Desvio lateral direito corrigido (“S”)
3 Desvio lateral esquerdo (não corrigido)
4 Desvio lateral esquerdo corrigido (“S”)
5 Outro tipo _____
(Especifique)

4. Extensão de movimento vertical

Incisivo superior utilizado 11 21

a. Abertura sem auxílio sem dor ☐☐mm

b. Abertura máxima sem auxílio ☐☐mm

Dor Muscular	Dor Articular
0 Nenhuma	0 Nenhuma
1 Direito	1 Direito
2 Esquerdo	2 Esquerdo
3 Ambos	3 Ambos

c. Abertura máxima com auxílio ☐☐mm

Dor Muscular	Dor Articular
0 Nenhuma	0 Nenhuma
1 Direito	1 Direito
2 Esquerdo	2 Esquerdo
3 Ambos	3 Ambos

d. Trespasse incisal vertical ☐☐mm

5. Ruídos articulares (palpação)

a. abertura

Direito	Esquerdo
0 Nenhum	0 Nenhum
1 Estalido	1 Estalido
2 Crepitação grosseira	2 Crepitação grosseira
3 Crepitação fina	3 Crepitação fina
☐☐mm	☐☐mm
(Medida do estalido na abertura)	

b. Fechamento

Direito	Esquerdo
0 Nenhum	0 Nenhum
1 Estalido	1 Estalido
2 Crepitação grosseira	2 Crepitação grosseira
3 Crepitação fina	3 Crepitação fina
☐☐mm	☐☐mm
(Medida do estalido no fechamento)	

c. Estalido recíproco eliminado durante abertura protrusiva

Direito	Esquerdo
0 Não	0 Não
1 Sim	1 Sim
8 NA	8 NA
(NA: *Nenhuma das opções acima*)	

6. Excursões

a. Excursão lateral direita ☐☐mm

Dor Muscular	Dor Articular
0 Nenhuma	0 Nenhuma
1 Direito	1 Direito
2 Esquerdo	2 Esquerdo
3 Ambos	3 Ambos

b. Excursão lateral esquerda ☐☐mm

Dor Muscular	Dor Articular
0 Nenhuma	0 Nenhuma
1 Direito	1 Direito
2 Esquerdo	2 Esquerdo
3 Ambos	3 Ambos

c. Protrusão ☐☐mm

Dor Muscular	Dor Articular
0 Nenhuma	0 Nenhuma
1 Direito	1 Direito
2 Esquerdo	2 Esquerdo
3 Ambos	3 Ambos

d. Desvio de linha média ☐☐mm

1 Direito

2 Esquerdo

8 NA

(NA: *Nenhuma das opções acima*)

7. Ruídos articulares nas excursões

Ruídos direito

	Nenhum	Estalido	Crepitação grosseira	Crepitação fina
7.a Excursão Direita	0	1	2	3
7.b Excursão Esquerda	0	1	2	3
7.c Protrusão	0	1	2	3

Ruídos esquerdo

	Nenhum	Estalido	Crepitação grosseira	Crepitação fina
7.d Excursão Direita	0	1	2	3
7.e Excursão Esquerda	0	1	2	3
7.f Protrusão	0	1	2	3

INSTRUÇÕES, ÍTENS 8-10

O examinador irá palpar (tocando) diferentes áreas da sua face, cabeça e pescoço. Nós gostaríamos que você indicasse se você não sente dor ou apenas sente pressão (0), ou dor (1-3). Por favor, classifique o quanto de dor você sente para cada uma das palpações de acordo com a escala abaixo. Marque o número que corresponde a quantidade de dor que você sente. Nós gostaríamos que você fizesse uma classificação separada para as palpações direita e esquerda.

0 *= Somente pressão (sem dor)*
1 *= dor leve*
2 *= dor moderada*
3 *= dor severa*

8. Dor muscular extraoral com palpação	**Direita**				**Esquerda**			
a. Temporal posterior (1,0 Kg.) "Parte de trás da têmpora (atrás e imediatamente acima das orelhas)."	0	1	2	3	0	1	2	3
b. Temporal médio (1,0 Kg.) "Meio da têmpora (4 a 5 cm lateral à margem lateral das sobrancelhas)."	0	1	2	3	0	1	2	3
c. Temporal anterior (1,0 Kg.) "Parte anterior da têmpora (superior a fossa infratemporal e imediatamente acima do processo zigomático)."	0	1	2	3	0	1	2	3
d. Masseter superior (1,0 Kg.) "Bochecha/ abaixo do zigoma (comece 1 cm a frente da ATM e imediatamente abaixo do arco zigomático, palpando o músculo anteriormente)."	0	1	2	3	0	1	2	3
e. Masseter médio (1,0 Kg.) "Bochecha/ lado da face (palpe da borda anterior descendo até o ângulo da mandíbula)."	0	1	2	3	0	1	2	3
f. Masseter inferior (1,0 Kg.) "Bochecha/ linha da mandíbula (1 cm superior e anterior ao ângulo da mandíbula)."	0	1	2	3	0	1	2	3
g. Região mandibular posterior (estilo-hióideo/ região posterior do digástrico) (0,5 Kg.) "Mandíbula/ região da garganta (área entre a inserção do esternocleidomastóideo e borda posterior da mandíbula. Palpe imediatamente medial e posterior ao ângulo da mandíbula)."	0	1	2	3	0	1	2	3
h. Região submandibular (pterigóideo medial/ supra-hióideo/ região anterior do digástrico) (0,5 Kg.)"abaixo da mandíbula (2 cm a frente do ângulo da mandíbula)."	0	1	2	3	0	1	2	3
9. Dor articular com palpação								
a. Polo lateral (0,5 Kg.) "Por fora (anterior ao trago e sobre a ATM)."	0	1	2	3	0	1	2	3
b. Ligamento posterior (0,5 Kg.)"Dentro do ouvido (pressione o dedo na direção anterior e medial enquanto o paciente está com a boca fechada)."	0	1	2	3	0	1	2	3
10. Dor muscular intraoral com palpação								
a. Área do pterigóideo lateral (0,5 Kg.) "Atrás dos molares superiores (coloque o dedo mínimo na margem alveolar acima do último molar superior. Mova o dedo para distal, para cima e em seguida para medial para palpar)."	0	1	2	3	0	1	2	3
b. Tendão do temporal (0,5 Kg.) "Tendão (com o dedo sobre a borda anterior do processo coronóide, mova-o para cima. Palpe a área mais superior do processo)."	0	1	2	3	0	1	2	3

ANEXO 4 – Escala Visual Analógica