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ESTUDO DOS MECANISMOS ENVOLVIDOS NO EFEITO PROTETOR DA TESTOSTERONA SOBRE O DESENVOLVIMENTO DA DOR DA ATM EM RATOS

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

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"O mundo de dentro da gente é maior do que o mundo de fora da gente"...

(André Abujamra)

RESUMO

As disfunções temporomandibulares são mais prevalentes em mulheres que em homens, sendo o papel dos hormônios sexuais um dos fatores apontados como causa dessa diferença. Nesse contexto, a testosterona apresenta um efeito protetor ao diminuir o risco de ratos desenvolverem dor na articulação temporomandibular (ATM), já que a injeção de 0,5% de formalina na ATM não é capaz de induzir nocicepção em machos intactos, mas induz em machos gonadectomizados (Gx) e fêmeas. O objetivo deste trabalho foi investigar se o papel protetor da testosterona no desenvolvimento da dor da ATM em ratos: (a) depende da ação organizacional da testosterona durante o período de diferenciação sexual do sistema nervoso central; (b) é mediado diretamente pela ação andrógena ou pela ação do estrógeno sintetizado a partir da testosterona; (c) é mediado pela ativação do sistema opioide endógeno central; e (d) resulta de um menor risco de machos desenvolverem edema na ATM. A formalina foi utilizada como estímulo nociceptivo e inflamatório na ATM. Para testar se o efeito protetor da testosterona depende da sua ação organizacional no período de diferenciação sexual, ratos orquidectomizados com um dia de vida ou na fase adulta e fêmeas intactas receberam testosterona antes do teste da formalina na ATM. Para testar a ação direta da testosterona ou do estrógeno sintetizado a partir da testosterona, ratos machos receberam injeção subaracnoide, na região do núcleo sensorial trigeminal, de flutamide (antagonista de receptores androgênicos) ou de ICI 182 780 (antagonista de receptores estrogênicos), antes do teste da formalina na ATM. Para testar o envolvimento de um mecanismo neural central dependente da ativação do sistema opioide, ratos machos receberam injeção de naloxona (antagonista de receptores opioides), na região do núcleo sensorial trigeminal, antes do teste da formalina na ATM. Para verificar se a testosterona reduz o risco de desenvolvimento de edema na ATM, foi avaliado o extravasamento plasmático em ratos machos Gx ou sham Gx depois da injeção de formalina na ATM. A orquidectomia com um dia de vida ou na fase adulta, seguida de reposição hormonal antes do experimento comportamental, não afetou o comportamento nociceptivo dos ratos. A administração de testosterona em fêmeas intactas diminuiu o comportamento nociceptivo induzido pela injeção de formalina na ATM. Em machos sham Gx, a administração

subaracnoide de flutamide (120 μg) ou de naloxona (15 μg) aumentou o comportamento nociceptivo induzido pela injeção de formalina na ATM. Já a administração subaracnoide de ICI 182 780 não alterou o comportamento nociceptivo de ratos sham Gx. O extravasamento plasmático na região da ATM foi similar em machos sham Gx e em machos Gx que receberam injeção de formalina na ATM. Dessa forma, podemos concluir que o efeito protetor da testosterona no desenvolvimento da dor da ATM em ratos: (a) depende do efeito ativacional da testosterona, que medeia um mecanismo neural central dependente da ativação de receptores andrógenos e da liberação de peptídeos opioides e (b) não se deve a uma redução do risco de desenvolvimento de edema na região da ATM de ratos machos.

Palavras-chave: Testosterona, Articulação Temporomandibular, Nocicepção, Formalina, Dor, Edema.

ABSTRACT

Temporomandibular disorders are known to be around two times more prevalent in women than in men, and one of the factors pointed out as a possible cause for such a difference is the role of sexual hormones. In this context, testosterone presents a protective effect by diminishing the risk of rats developing temporomandibular joint (TMJ) pain, since 0.5% formalin injection does not induce nociception in naive males but it does in gonadectomized (Gx) male and female rats. The aim of this study was to investigate whether the protective role of testosterone in the development of TMJ pain in rats: (a) depends on the organizational action of testosterone during the sexual differentiation period of the central nervous system; (b) is mediated directly by androgens action or by the action of estrogens synthesized from testosterone; (c) is mediated by the activation of the endogenous opioid system; (d) results from a reduced risk of males developing TMJ edema. The nociceptive and inflammatory agent formalin was injected into the TMJ region of rats. To test whether the protective role of testosterone depends on its organizational effect at the critical period of sexual differentiation, one-day-old or six-week-old orchidectomized rats and naive females received testosterone before the formalin TMJ test. To test whether the effect of testosterone was direct or indirect via estrogen derived from its aromatization, male rats received a subarachnoid injection in the vicinities of the spinal trigeminal nucleus, respectively of flutamide (androgenic receptor antagonist) or of ICI 182 780 (estrogenic receptor antagonist), before the formalin TMJ test. To test the involvement of a central activation of the opioid system, male rats received a subarachnoid injection in the vicinities of the spinal trigeminal nucleus of naloxone (opioid receptor antagonist) before the formalin TMJ test. To test whether testosterone induces a lower risk of males developing TMJ edema, we evaluated plasma protein extravasation in sham Gx and Gx male rats, after the TMJ injection of formalin. Orchidectomy with one day of life or in adulthood followed by testosterone administration prior to the behavioral assay, did not affect nociceptive behavior in rats. Testosterone administration in naive females diminished the TMJ formalin-induced nociceptive behavior. In sham Gx male rats, subarachnoid administration

of flutamide (120 μg) or naloxone (15 μg) augmented the nociceptive behavior induced by TMJ formalin injection. On the other hand, subarachnoid injection of ICI 182 780 did not affect significantly the nociceptive behavior of sham Gx male rats. Plasma protein extravasation was similar in sham Gx and Gx male rats that received TMJ formalin injection. Therefore, we can conclude that the protective effect of testosterone on the development of TMJ pain in rats: (a) depends on testosterone activational effects which mediates a neural central mechanism dependent of the activation of androgen receptors and subsequent release of opioid peptides and (b) it is not due to a lower risk of male rats developing TMJ edema.

Key words: Testosterone, Temporomandibular Joint, Nociception, Formalin, Pain, Edema.

SUMÁRIO

1 - INTRODUÇÃO

Disfunção temporomandibular (DTM) é uma expressão usada para designar problemas envolvendo músculos mastigatórios, articulações temporomandibulares (ATMs) e estruturas relacionadas (LeResche, 1997; Cairns *et al.*, 2009; Leeuw, 2010) tendo como principal sintoma a queixa de dor nos músculos da mastigação e/ou articulação temporomandibular (ATM) (LeResche, 1997; Svensson *et al.*, 2000; Oral *et al.*, 2009). As DTMs apresentam baixa incidência, de 2 a 3 % ao ano (Von Korff *et al.*, 1988; Heikinheimo *et al.*, 1989; Kitai *et al.*, 1997), que associada a uma prevalência relativamente alta, explica o seu caráter crônico e o consequente prejuízo à vida social dos indivíduos que as apresentam (Von Korff *et al.*, 1988). Na região orofacial, correspondem à condição de dor crônica de maior prevalência, e são aproximadamente duas vezes mais comuns em mulheres que em homens (LeResche, 1997; Lund *et al.*, 2001; Leeuw, 2010), acometendo especialmente mulheres entre 35 e 45 anos de idade (LeResche, 1997). Esses dados sugerem que os hormônios sexuais atuam modulando a dor na ATM.

Estudos sobre a dor na ATM em modelos animais são essenciais para entender os mecanismos fisiológicos envolvidos na transmissão e modulação da informação nociceptiva. Vários pesquisadores estudaram a relação entre a dor na ATM e os hormônios sexuais femininos (Clemente *et al.*, 2004; Ribeiro-Dasilva *et al.*, 2009; Yu *et al.*, 2009), mas poucos estudaram o papel dos hormônios sexuais masculinos na dor da ATM. Em nosso laboratório de dor orofacial, o primeiro trabalho a abordar especificamente o tema em questão foi o de Fischer e colaboradores, em 2007. Dados obtidos neste estudo indicam que a testosterona apresenta um efeito protetor ao diminuir o risco de ratos desenvolverem dor na ATM (Fischer *et al.*, 2007). Nesse trabalho, por meio da utilização do teste da formalina na ATM (Roveroni *et al.*, 2001), foi demonstrado que a administração de formalina 0,5% não induz respostas nociceptivas comportamentais em machos intactos, mas as induz em machos gonadectomizados (Gx) e em fêmeas.

Os conceitos de “dor” e “nocicepção”, embora muitas vezes confundidos, são diferentes. Dor é definida como uma experiência sensorial e emocional desagradável

associada a dano tecidual real ou potencial, ou descrita como tal (Merskey, 1994). Nocicepção é a somatória de impulsos nervosos gerados por dano tecidual, ou seja, é apenas o componente sensorial da dor (Leeuw, 2010).

O mecanismo através do qual a testosterona reduz o risco de desenvolvimento de dor na ATM em machos não é conhecido, mas aparentemente é sexo-específico, ou seja, não está presente em fêmeas. As evidências para essa suposição partem de dois resultados experimentais previamente publicados por nosso grupo: (1) fêmeas intactas apresentam nocicepção semelhante à de machos Gx quando submetidas à injeção de formalina 0,5%, o que sugere que os níveis fisiológicos de testosterona, não induzem em fêmeas a mesma ação protetora que induzem em machos; e (2) a nocicepção de fêmeas Gx não é afetada pela administração supra fisiológica de testosterona, o que demonstra que em fêmeas a testosterona não tem efeito sobre a nocicepção (Fischer *et al.*, 2007).

Essa aparente sexo-especificidade do efeito protetor da testosterona sugere que esse efeito pode não depender somente da ação da testosterona durante a vida adulta, ou seja, pode depender também de sua ação durante um período crítico do desenvolvimento, quando ocorre a diferenciação sexual do sistema nervoso central. Tais diferenças abrangem desde características macroscópicas, como o volume do cérebro e comprimento da medula espinhal, até características funcionais, como a expressão de enzimas e mRNAs, e são mediadas pelos hormônios sexuais. De fato, a testosterona circulante durante os primeiros dias de vida medeia a diferenciação sexual do sistema nervoso, induzindo mudanças morfológicas permanentes que irão se refletir durante a vida adulta. Tal fenômeno é conhecido como efeito organizacional, e é diferente do efeito ativacional que, por sua vez, produz ações temporárias e reversíveis, desencadeando uma dada resposta somente na presença do hormônio apropriado (Kawata, 1995). Dessa forma, é possível que o efeito protetor da testosterona seja estabelecido durante o desenvolvimento do organismo masculino, promovendo efeitos organizacionais que irão determinar a sensibilidade desse mesmo organismo à presença da testosterona na vida adulta.

A testosterona pode ser convertida em estrógeno dentro das células neurais através do processo de aromatização, já que ocorre devido à ação da enzima aromatase. Este é um processo bastante estudado no que diz respeito à diferenciação sexual do sistema

nervoso central (Sakuma, 2009). Mais recentemente, a aromatização tem sido estudada e apontada também como mecanismo de modulação da dor, uma vez que a síntese de estrógenos a partir de andrógenos pela ação da enzima aromatase expressa nos neurônios do corno dorsal da medula espinhal modula a nocicepção (Evrard, 2006). Dessa forma, um dos objetivos deste trabalho foi investigar se o efeito protetor da testosterona poderia ser mediado pela ação da mesma nos receptores androgênicos ou através da ativação de receptores estrogênicos pelo estrógeno sintetizado a partir da testosterona, por meio da ação da enzima aromatase presente nos neurônios do complexo sensorial trigeminal. A resposta para esta questão é de extrema importância nos dias de hoje, já que tanto antagonistas de receptores estrogênicos quanto antagonistas de receptores androgênicos são amplamente utilizados em tratamentos de câncer de mama (Licznerska *et al.*, 2010) e próstata (Gao, 2010), respectivamente.

Além do efeito protetor da testosterona no desenvolvimento da dor da ATM, a administração supra fisiológica desse hormônio em machos Gx reduz a nocicepção induzida por uma dose maior (1,5%) de formalina (Fischer *et al.*, 2007). Dados preliminares obtidos em nosso laboratório sugerem que o efeito antinociceptivo da testosterona supra fisiológica é mediado pela ativação do sistema opioide endógeno, possivelmente na região do núcleo do trato espinhal trigeminal, uma vez que Fischer *et al.* (2009) conseguiram inibir o efeito antinociceptivo da testosterona por meio da injeção subaracnoide de naloxona (antagonista de receptores opioides) em ratos machos intactos. Sendo assim, também o efeito protetor da testosterona no desenvolvimento da dor na ATM em ratos poderia ser mediado pelo sistema opioide na região do núcleo espinhal trigeminal, mais especificamente no sub-núcleo caudal, que é o correspondente trigeminal do corno dorsal da medula espinhal.

Dados recentemente obtidos em nosso laboratório apontam para o fato de que a administração de testosterona supra fisiológica reduz o extravasamento de proteínas plasmáticas em ratos machos que receberam injeção de formalina 1,5% na ATM (Torres-Chávez *et al*., dados não publicados). Desse modo, formulamos a hipótese de que o efeito protetor da testosterona no desenvolvimento da dor na ATM poderia resultar de uma redução no risco de desenvolvimento do edema nesta região.

Em resumo, este estudo foi delineado para investigar os mecanismos envolvidos no efeito protetor da testosterona no desenvolvimento da dor na ATM de ratos. Baseados nas evidências citadas, investigamos se o efeito protetor da testosterona é: (a) dependente de efeitos organizacionais induzidos pela testosterona durante a diferenciação sexual do sistema nervoso central no período pós-natal, e não apenas dependente de efeitos ativacionais induzidos pela presença deste hormônio na vida adulta; (b) mediado diretamente pela ação de andrógenos, ou pela ação do estrógeno sintetizado através da aromatização da testosterona; (c) mediado por um mecanismo neural central de ativação do sistema opioide; e (d) mediado pela redução do risco de desenvolvimento de edema na ATM.

2 - PROPOSIÇÃO

Este trabalho teve por objetivo testar as hipóteses de que o efeito protetor da testosterona sobre o desenvolvimento da dor na ATM de ratos é:

1 - Estabelecido durante o período de diferenciação sexual do sistema nervoso pela ação organizacional da testosterona, e apenas mantido na vida adulta, pelos níveis fisiológicos deste hormônio.

2 - Mediado pela ação direta da testosterona ou pela ação do estrógeno derivado da aromatização da testosterona.

3 - Mediado pela ativação do sistema opioide endógeno, no sistema nervoso central.

4 - Mediado pela redução do risco de desenvolvimento de edema na ATM.

O presente estudo está apresentado em formato alternativo, conforme deliberação da Comissão Central de Pós-graduação (CCPG) da Universidade Estadual de Campinas (UNICAMP) nº 001/98

3 - CAPÍTULO

Mechanisms underlying testosterone protective effect on temporomandibular joint nociception development in rats.

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Original Article

Key words: Testosterone; TMJ; Nociception; Formalin; Pain; Edema.

Abstract

We have recently demonstrated that a 0.5% formalin injection into the TMJ induces a significant nociceptive behavior in Gx males and naive females but not in naive males, suggesting a testosterone protective effect on TMJ nociception. In this study, we investigated the mechanisms underlying this protective effect of testosterone. First, we asked if this protective effect depends on testosterone organizational actions during nervous system development. Second, we investigated whether it is mediated directly by androgens or by estrogens derived from testosterone aromatization, and by the opioid system. Finally, we investigated if testosterone reduces the development of TMJ edema in males. TMJ injection of 0.5% formalin was used as nociceptive and inflammatory stimulus. The protective effect of testosterone on TMJ nociception development was restored by testosterone replacement in either postnatal or adult Gx male rats. Testosterone administration in naive females increasing its serum levels to that present in naive males significantly reduced 0.5% formalin-induced TMJ nociception. Administration of the androgen receptor antagonist flutamide (120 μg) or of the opioid receptor antagonist naloxone (15 μg), but not of the estrogen receptor antagonist ICI 182 780 (60, 90 or 120 μg) into the medullary subarachnoid space, blocked testosterone protective effect. Finally, TMJ formalin-induced plasma protein extravasation was similar in sham and Gx males. Taken together, these findings indicate that testosterone protective effect on TMJ nociception development in rats depends on its activational actions on androgen receptors and subsequent release of endogenous opioids.

Introduction

Temporomandibular dysfunctions (TMD) are pain conditions of the masticatory muscles and temporomandibular joint (TMJ) with greater prevalence, severity and duration in women than in men (Anastassaki and Magnusson, 2004; LeResche, 1997). The higher prevalence and severity in women suggest that gonadal hormones may play a role in those pain conditions. Recently, we have demonstrated that 0.5% formalin injection into the temporomandibular joint induces a significant nociceptive behavior in gonadectomized

(Gx) males and in naive females but not in naive male rats, suggesting that testosterone protects naive males from developing TMJ nociception. However, the testosterone protective mechanism remains unclear. Although testosterone is also present in females, its plasma concentration is significantly lower than in males. Therefore, the specificity of the protective effect of endogenous testosterone in males could be explained by the high levels of the hormone inducing sex differentiation of the nervous system and determining structural changes (organizational effects) essentials for its action during male adulthood (activational effects) (Kawata, 1995). In addition to its protective effect on TMJ pain development, administration of supraphysiological testosterone doses decreases TMJ nociception (Fischer et al., 2007) and inflammation (Torres-Chávez et al., unpublished information) induced by a higher concentration of formalin (1.5%) in male rats. This antinociceptive effect of testosterone is mediated by the activation of endogenous opioid system in the trigeminal subnucleus caudalis region, also known as the medullary dorsal horn (Fischer et al., 2009). In the dorsal horn, the action of the enzyme aromatase converting testosterone into estrogen has been described as a new central mechanism of pain modulation (Evrard, 2006). Whether nociception can be modulated by the activation of estrogen or androgen receptors is of great importance nowadays since estrogen and androgen antagonists are widely used in breast cancer (Licznerska and Baer-Dubowska, 2010) and prostate cancer treatment (Gao, 2010), respectively.

In this study, we evaluated the mechanisms underlying testosterone protective effect. First, we asked if the protective effect of testosterone depends on its organizational effects during nervous system development and/or on its activational effect during adulthood. Second, we investigated if it is mediated by androgens or by estrogen derived from testosterone aromatization and by the opioid system. Finally, we investigated if testosterone reduces the risk of males developing TMJ inflammation. The TMJ injection of 0.5% formalin was used as nociceptive and inflammatory stimulus and the behavior and inflammatory responses of sham and gonadectomized animals were compared.

Materials and Methods

Animals

This study was carried out in male and females Wistar rats housed (5 per cage) in a temperature controlled room (23 ± 1°C) on a 12:12 light cycle, with food and water available *ad libitum*. The experiments were approved by the Committee on Animal Research of the University of Campinas and are in accordance with IASP guidelines for the study of pain in animals (Zimmermann, 1983).

Gonadectomy

Newborn or six-week-old male rats were gonadectomized. Six-week-old male rats were Gx (Ward and Abdel-Rahman, 2005) under anesthesia induced by an intramuscular injection of a mixture of ketamine (55 mg/kg) and xylazine (5.5 mg/kg). After a single scrotal incision, testes were ligated with 4-0 silk suture and removed, and the skin was closed with 5-0 silk suture. One-day-old male rats were Gx under continuous anesthesia induced by inhalation of a mixture of oxygen and halothane (2%). A single small skin incision was first made just below the abdomen. Afterwards, another small incision was made on the muscular tissue to expose the peritoneal cavity. The right and left tests were located inside the peritoneal cavity and then removed. The muscle tissue and the skin were closed with 6-0 silk sutures (Cicero et al., 2002). Sham operated animals underwent surgical procedure similar to that of Gx animals, except that the testes were not removed. The efficacy of orchidectomy was verified by *postmortem* examination of prostate and seminal vesicles atrophy in animals that did not receive hormone replacement.

Testosterone Replacement

Testosterone replacement therapy was initiated 3 days prior to nociceptive testing and consisted of three daily subcutaneous injections of testosterone (2mg/Kg of weight) (Banu et al., 2002; Liu et al., 2006). The last testosterone injection was performed 14 to 20 hours prior to nociceptive testing. Testosterone (17β-Hydroxy-3-oxo-4-androstene) was purchased from Sigma, São Paulo, SP, Brazil and diluted in propylene glycol.

Plasma Extravasation Measurement

Immediately after the TMJ injection, some rats (see in study design) received an injection of Evans blue dye (Sigma Chemicals; 50 mg/kg) (Haas et al., 1992), into the right femoral vein under anesthesia induced by an intramuscular injection of a mixture of ketamine (55 mg/kg) and xylazine (5.5 mg/kg). Evans blue dye binds to plasma protein extravasated in the inflammatory site, and therefore was used as a marker for plasma extravasation (Haas et al., 1992). Forty-five minutes after the TMJ injection, animals were perfused transcardiacally with saline (0.9% NaCl) to flush the dye from the vasculature. Joint tissues were dissected to a standardized size (30 ± 2 mg) and stored at -30^{o}C until analysis. The dye was extracted by immersing the samples into 1 ml of formamide at 60°C for 24 h. The amount of blue dye (μg) extracted from tissue sample was determined using a spectrophotometer set at 620 nm. The concentration of dye was then calculated per gram weight of tissue.

Subarachnoid Injection

In order to deliver drugs in the vicinities of the spinal trigeminal nuclei, subarachnoid injection technique was performed (Fischer et al., 2005). Rats were anesthetized by inhalation of a mixture of oxygen and halothane (2%) and a small area of skin overlying the high cervical region was shaved with an electric razor. With the animals dorsally positioned, a 30-gauge needle connected to a Hamilton syringe by a polyethylene cannula was inserted into the medullary subarachnoid space. A total of 10 μl volume was injected for all drugs used. Flutamide and naloxone were obtained from Sigma, São Paulo, SP, Brazil and ICI 182 780 was purchased from Tocris, St. Louis, MO, USA. Dimethyl Sulfoxide (DMSO) was obtained from Sigma, São Paulo, SP, Brazil. All injections were performed at a rate of 1 μl/s. Each animal regained consciousness approximately 30 s after discontinuing the anesthesia.

Nociceptive Assay

Behavior testing was performed in adults rats during the light phase (between 9:00 AM and 5:00 PM) in a quiet room maintained at 23°C (Rosland, 1991). On the day of

the experiment, each animal was individually placed in a test chamber (30 x 30 x 30 cm mirrored-wood chamber with a glass at the front side) for a 15-minutes habituation period to minimize stress. Some of the animals received a subarachnoid injection and returned to the test chamber for a recovery period of 10 minutes. Afterwards, animals were anesthetized by a briefly inhalation of halothane to allow the TMJ injection of 30 μl of formalin or its vehicle (0.9% NaCl). Formalin solution was prepared from commercially available (Sigma Chemicals) stock formalin diluted in 0.9% NaCl (saline) to the concentration of 0.5%. Each animal regained consciousness approximately 30 seconds after discontinuing the anesthetic and was returned to the test chamber for counting nociceptive responses during a 45-minutes observation period. The nociceptive response score was defined as the cumulative total number of seconds that the animal spent rubbing the orofacial region with the ipsilateral fore or hind paw plus the number of head flinches counted with a cell counter (Roveroni et al., 2001). Rats did not have access to food or water during the test, and each animal was used once. At the conclusion of the experiment (45 minutes after TMJ formalin injection), animals were anesthetized by an intra-peritoneal injection of a mixture of urethane (1g/Kg) and α-chloralose (50 mg/Kg), and a cardiac puncture was performed to allow the injection of Evans blue dye (1%) to visualize formalin-induced TMJ plasma extravasation on *postmortem* examination (Haas et al., 1992). This latter procedure allowed confirmation that the TMJ injection was restricted to the immediate TMJ region (Roveroni et al., 2001).

Study Design

To determine whether the protective effect of testosterone is dependent or not on its action during the sexual differentiation period, the nociceptive behavior induced by a TMJ injection of 0.5% formalin was compared between sham and newborn or six-week-old Gx rats receiving testosterone replacement or its vehicle and between naive females rats receiving testosterone replacement or its vehicle. To determine whether the protective effect of testosterone is mediated by estrogen converted from testosterone aromatization or by testosterone itself, the nociceptive behavior induced by a TMJ injection of 0.5% formalin was compared between sham and Gx rats receiving a subarachnoid injection of the

estrogen receptor antagonist ICI 182 780 (60, 90 or 120 μg) or the androgen receptor antagonist flutamide (60 or 120 μg), respectively. To determine whether the protective effect of testosterone is mediated by the activation of the opioid system, the nociceptive behavior induced by a TMJ injection of 0.5% formalin was compared between sham and Gx rats receiving a subarachnoid injection of naloxone (15 μg) or its vehicle. To determine whether testosterone reduces the risk of males developing TMJ inflammation, the intensity of plasma protein extravasation induced by a TMJ injection of 0.5% formalin was compared between sham and Gx rats.

Statistics

The nociceptive behavior score, obtained by summing the flinching and rubbing behaviors recorded during the entire duration of the experiment was used in statistical analysis in figures 1 to 3. The amount of Evans blue dye extravasated per gram of TMJ tissue was used in statistical analysis in figure 4. One-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used to determine if there were significant differences between groups. Data represent means ± SEM. Sigma Stat program was used for statistical analysis.

Results

As expected, TMJ injection of 0.5% formalin induced a significantly greater TMJ nociception in Gx than in sham Gx males, suggesting that testosterone protects males from developing TMJ nociception. Testosterone replacement reestablished the protective effect of this hormone on the development of TMJ nociception, in either post natal Gx male rats (first day of life, Figure 1 A) or adult Gx male rats (six-week-old, Figure 1 B). In adult female rats, testosterone administration significantly reduced the nociceptive behavior induced by the TMJ injection of 0.5% formalin (Figure 1 C). These findings suggest that the protective effect of testosterone on TMJ nociception development depends on activational rather than organizational actions. The total serum levels of testosterone evaluated 14 or 20hr after the last testosterone administration in testosterone replaced animals produced testosterone serum levels not significantly different from those of sham Gx or Gx male rats (Figure 1 D).

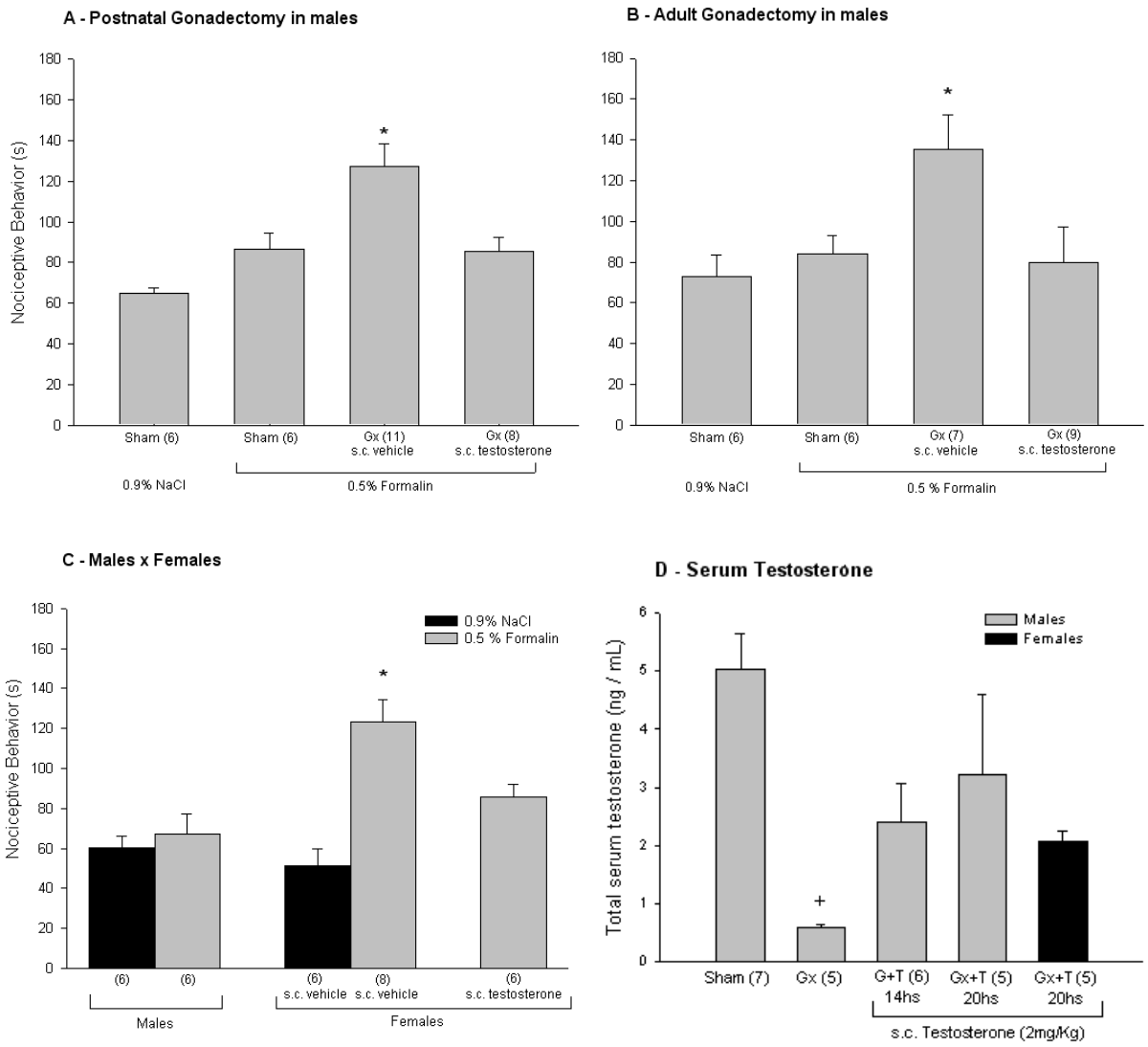

Figure 1. Effect of testosterone administration in post natal Gx or adult Gx males and in naive female rats. Testosterone replacement in post natal Gx male rats (**A)** and in adult Gx male rats (**B)** reestablished the protective effect of testosterone. Testosterone administration in female rats protected females from developing 0.5% formalin-induced TMJ nociception (C). The symbol "*" indicates a nociceptive behavior significantly greater than that of all other groups (Tukey test, $p<0.05$). **(D)** Testosterone replacement (2 mg/kg, s.c. per day, during 3 days) evaluated either 14 or 20 hrs after the last testosterone administration induced total serum testosterone levels not significantly different from those of sham Gx and Gx male rats. The symbol "+" indicates serum testosterone level significantly lower than that of sham Gx males (Tukey test, $p<0.05$). In this and the subsequent figures, data are plotted as mean $\pm$ SEM and group sample sizes are shown in

parentheses. See Materials and Methods for additional details regarding data presentation and analysis. Vehicle = propylene glycol; s.c. = subcutaneous; Gx = gonadectomized.

The effect induced by the administration of the estrogen receptor antagonist ICI 182 780 (60, 90 or 120 μg) into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin into the TMJ was similar to that induced by the administration of its vehicle into the medullary subarachnoid space of sham Gx males also receiving 0.5% formalin into the TMJ (Figure 2 A). Thus blockade of estrogen receptors into the medullary subarachnoid space of sham Gx animals did not affect the protective effect of testosterone. On the other hand, the administration of the androgen receptor antagonist flutamide (120 μg) into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin into the TMJ induced a behavioral response significantly greater than that induced by the administration of its vehicle into the medullary subarachnoid space of sham Gx males also receiving 0.5% formalin into the TMJ (Figure 2 A). The effect of the administration of flutamide (120 μg) into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin was similar to that of GX animals receiving its vehicle into the medullary subarachnoid space and 0.5% formalin into the TMJ. Thus, blockade of androgen receptors into the medullary subarachnoid space inhibited the protective effect of testosterone (Figure 2 B). Taken together these findings suggest that the protective effect of testosterone is mediated by androgens rather than by estrogen derived from testosterone aromatization.

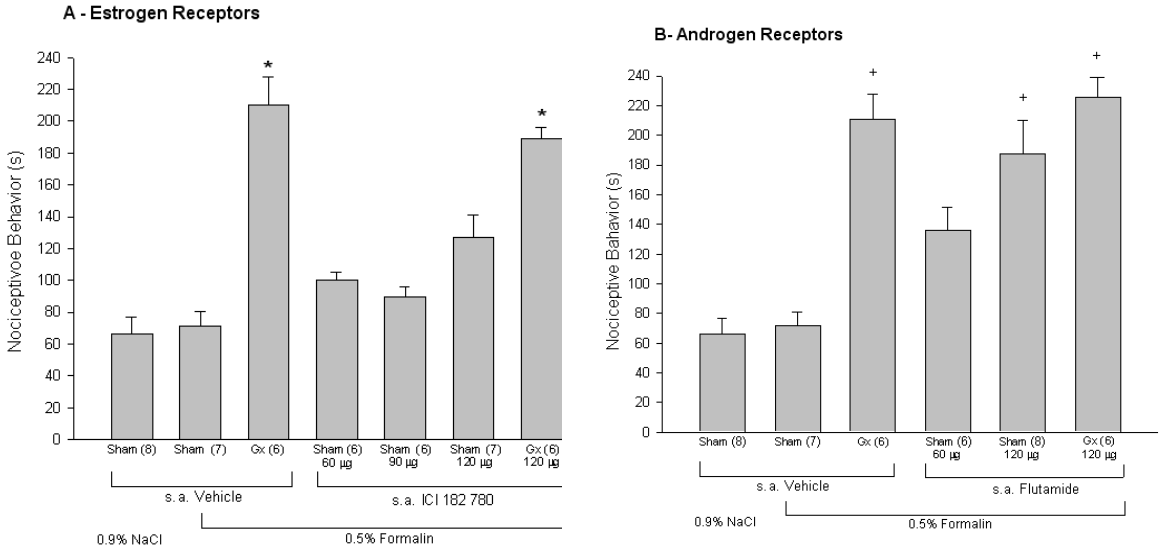

Figure 2. The role of estrogen and androgen receptors on the protective effect of testosterone. **A-** The administration of the estrogen receptor antagonist ICI 182 780 (60, 90 or 120µg) into the medullary subarachnoid space of sham Gx males receiving 0.5%formalin into the TMJ induced a behavioral response similar to that induced by its vehicle administration into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin into the TMJ. The symbol * indicates a nociceptive response significantly greater than that of all other groups (Tukey test, $p<0.05$). **B-** The administration of the androgen receptor antagonist flutamide (120 µg) into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin into the TMJ induced a behavioral response significantly greater than that induced by its vehicle administration into the medullary subarachnoid space of sham Gx males also receiving 0.5% formalin into the TMJ. The symbol + indicates a nociceptive response significantly greater than that of sham Gx male rats receiving a TMJ injection of 0.9 % NaCl or 0.5% formalin (first two bars; Tukey test, $p<0.05$). The administration of either ICI 182 780 or flutamide into the medullary subarachnoid space of Gx males receiving 0.5% formalin into the TMJ had no effect. Vehicle = DMSO; s.a. = subarachnoid.

The administration of the opioid receptor antagonist naloxone (15 µg) into the medullary subarachnoid space of sham Gx males receiving 0.5% formalin into the TMJ induced a behavioral response significantly greater than that induced by the administration of its vehicle (Figure 3). Thus blockade of opioid receptors into the medullary subarachnoid

space inhibited the protective effect of testosterone. This finding suggests that that the protective effect of testosterone is mediated by endogenous opioids.

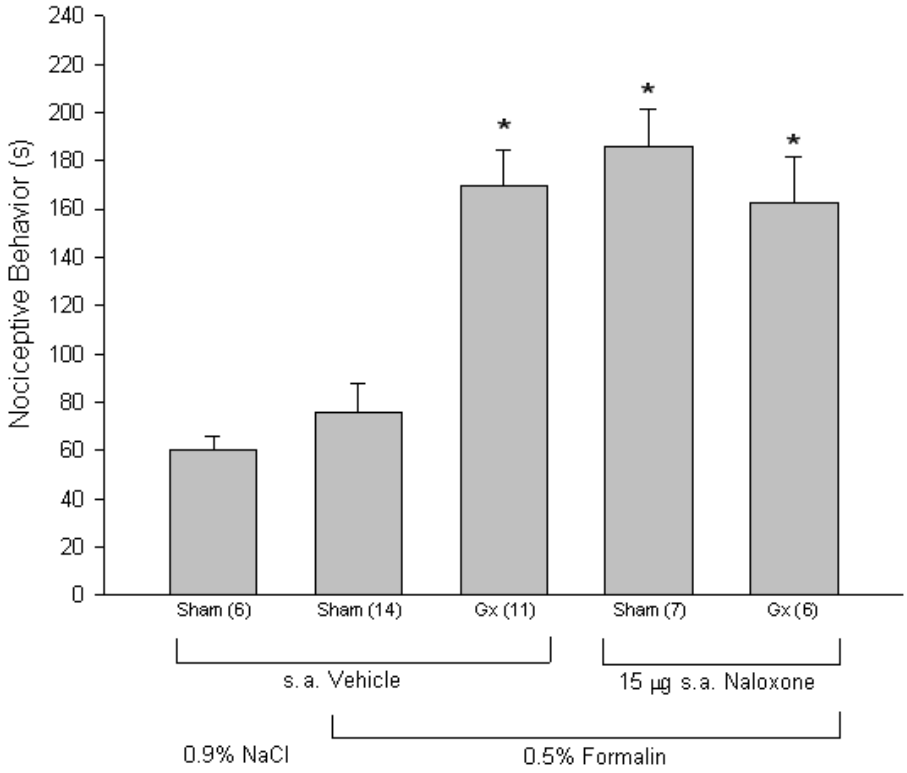

Figure 3. The role of endogenous opioids on the protective effect of testosterone. The administration of naloxone into the medullary subarachnoid space induced a behavioral response significantly greater than that induced by its vehicle administration into the medullary subarachnoid space of sham Gx males also receiving 0.5%formalin into the TMJ. The symbol * indicates a nociceptive response significantly greater than that of other groups (first two bars; Tukey test, $p<0.05$). The administration of naloxone into the medullary subarachnoid space of Gx males receiving 0.5% formalin into the TMJ had no effect. Vehicle = 0.9% NaCl.

Formalin-induced plasma protein extravasation was similar between sham and Gx males and was not significantly greater than the plasma protein extravasation induced by its vehicle (Figure 4). This finding suggests that the protective effect of testosterone is not mediated by a local reduction of edema.

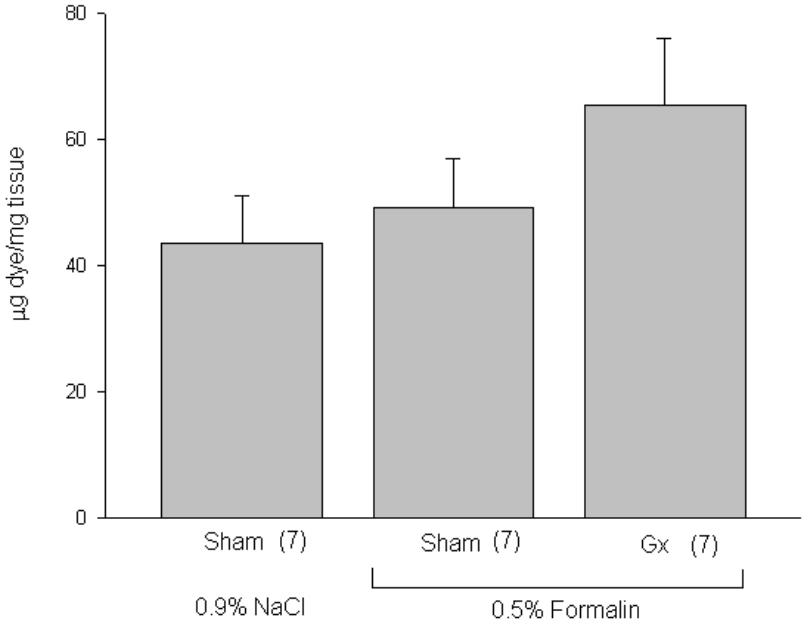

Figure 4. Plasma protein extravasation. There was no significantly difference between plasma protein extravasation of Sham Gx male rats receiving a TMJ injection of 0.9% NaCl or 0.5% formalin and Gx male rats receiving a TMJ injection of 0.5% formalin (Tukey test, $p>0.05$).

Discussion

In this study, we demonstrated that the protective effect of testosterone on TMJ pain development depends on its activational actions on androgen receptors during adulthood and on the subsequent release of endogenous opioids rather than on its organizational actions during central nervous system development. Furthermore, testosterone does not significantly affect the development of TMJ edema.

During post natal development, testosterone induces sex differentiation of the nervous system determining structural changes (organizational effects) (Kawata, 1995). However, the finding that testosterone replacement in postnatal Gx adult males for 3 days just before nociceptive testing restored the protective effect of testosterone on the development of 0.5% formalin-induced TMJ in these males, indicates that the presence of testosterone during post natal development is not necessary to the expression of its protective effect in adulthood. Rather, the presence of testosterone during adulthood, when its effects are activational, is sufficient to determine its protective effect on TMJ pain

development. Although gonadectomy on the first day of life is widely used to study organizational effects of testosterone, (Craft and Ulibarri, 2009; Handa et al., 1985; LaCroix-Fralish et al., 2005) testosterone production in male rats begins around day 14 of gestation (Breedlove et al., 1983) and the surge of testosterone occurs between embryonic days 17-18 (Corpéchot et al., 1981; Weisz and Ward, 1980). Therefore, animals are exposed to testosterone during their intrauterine life. While testosterone modulates some structural changes in the rat brain until the first 10 postnatal days or even later (Cooke et al., 1999; Davis et al., 1996; Sakuma, 2009) there are structures of the neural system that require prenatal testosterone exposure to present complete sexual differentiation, even though this differentiation is not detectable until after birth (Kawata, 1995). Therefore, a possible testosterone organizational effect in male rats during prenatal development could be sufficient to ensure its protective effect on the development of TMJ nociception during adulthood. However, this supposition is not correct, because when we raised testosterone serum level of naive females to a value similar to the physiological level present in males, testosterone significantly reduced 0.5% formalin-induced TMJ nociception in females. This interesting finding reinforces the idea that the protective effect of testosterone is activational, and indicates that is not sex specific as we first hypothesized. Rather, it is also present in females if the serum level of testosterone is raised.

The protective effect of testosterone on TMJ pain development is mediated by the activation of androgen but not estrogen receptors by estrogen converted from testosterone because blockade of androgen but not estrogen receptor in the surrounding of the medullary dorsal horn inhibited this effect. This finding is of interest, especially in view of recent data showing that dorsal horn is an important site to the antinociceptive effects of estrogens derived from testosterone aromatization (Evrard, 2006); indeed, many of the effects attributed to testosterone are in fact induced by estrogen (Kawata, 1995). Furthermore, this result opens an interesting researching field regarding the use of androgen antagonists on prostate cancer therapies (Gao, 2010): whether androgen receptor antagonists could significantly raise TMJ pain development on prostate cancer patients.

The protective effect of testosterone on TMJ pain development is also mediated by the activation of central endogenous opioid system, since administration of the opioid

receptor antagonist naloxone in the surrounding of the medullary dorsal horn blocked this effect. Our findings that the protective effect of testosterone on TMJ pain development depends on androgen as well as on opioid receptors significantly restrict the possible mechanisms involved. For example, it is important to point that both antagonists were administrated ten minutes before nociceptive testing and, therefore, blocked the protective effect of testosterone very quickly. Testosterone through its androgen receptors could activate the opioid system either by raising opioid receptor expression or opioid peptides release. In both cases, blockade of opioid receptors would block the protective effect of testosterone, as demonstrated. However, if the protective effect of testosterone depends on an increased expression of opioid receptors induced by androgen receptor activation, the blockade of these androgen receptors only 10 min before nociceptive testing would not affect the protective effect because endogenous testosterone effect prior to the androgen receptor blockade would have already increased the number of opioid receptors. Therefore, the finding that the administration of androgen receptor antagonist 10 min before nociceptive testing blocked the protective effect of testosterone strongly suggests that the protective effect of testosterone is due to a raise in endogenous opioid peptides release rather than due to an increased expression of opioid receptors.

Testosterone protective effect may be modulated by a neuronal circuit localized in the subnucleus caudalis. This is because the caudalis portion of the spinal trigeminal nucleus, also known as medullary dorsal horn, receives the majority of the sensory afferents of the orofacial region and has an important role in modulating the nociceptive information that ascends to higher brain levels (Amandusson et al., 1996). In accordance with this suggestion, androgen receptors immunoreactivity was detected in interneurons localized in the spinal trigeminal nucleus (Hamson et al., 2004) and the protective effect of testosterone depends on the activation of these receptors. However, because the antagonists injected into the subarachnoid space could diffuse through the cerebrospinal fluid to neighboring regions, the involvement of other regions can not be excluded.

Importantly, androgen and opioids receptors mediating the protective effect of testosterone may be localized in central neurons and not in the primary afferent nociceptors. This is because the mechanism underlying the protective effect of testosterone may depend

on the increased release of opioid peptides, and first-order neurons do not release opioids through their central terminal (Carlton and Coggeshall, 1997; Pohl et al., 1994).

The ability of the androgen receptor antagonist flutamide to block the protective effect of testosterone when administrated 10 min prior to nociceptive testing suggests that this effect is mediated by a non genomic mechanism, since the nociceptive assay lasts 45 minutes, a period of time incompatible with a genomic mechanism (McEwen, 2001). In fact, androgen receptors are located at the axons membrane and possibly mediate non-genomic effects, since it is unlikely that androgen receptors in axons represent a pool of receptors ready to translocate to the nucleus, because the receptors would have to be actively transported first down the axon and then back to the cell soma (DonCarlos et al., 2003).

Plasma protein extravasation is one of the important signs of acute inflammation (Green et al., 1999) and a valuable indirect measurement of edema. Therefore, the finding that 0.5% formalin-induced plasma protein extravasation in sham and in Gx rats was not significantly different from each other, suggests that endogenous testosterone does not protect the development of edema, but can not definitely exclude its influence on TMJ inflammation. While some researchers have suggested that physiologic testosterone does not affect TMJ plasma extravasation (Torres-Chávez et al., unpublished information), others affirm that gonadectomy has significantly diminished this possible inflammatory marker (Flake et al., 2006). For these reasons, we believe that further studies are warranted to better clarify the role of testosterone on the development of TMJ inflammation.

In summary, we provide evidence that testosterone reduces the risk of rats developing TMJ nociception through an activational action on androgen receptors during adulthood and subsequent release of endogenous opioids. This protective effect of testosterone is not due to a decreased risk of males developing TMJ edema. The complete knowledge of the mechanisms involved in the protective effect of testosterone is essential not only to understand the mechanisms involved in the lower prevalence of some pain conditions in males, but also to develop future therapeutic strategies that may interact with these mechanisms to result in a greater level of success in pain prophylaxis in both sexes.

Acknowledgments

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References

Amandusson A., Hermanson O. and Blomqvist A. Colocalization of oestrogen receptor immunoreactivity and preproenkephalin mRNA expression to neurons in the superficial laminae of the spinal and medullary dorsal horn of rats. Eur J Neurosci 1996;8:2440-5.

Anastassaki A. and Magnusson T. Patients referred to a specialist clinic because of suspected temporomandibular disorders: a survey of 3194 patients in respect of diagnosis, treatments and treatment outcome. Acta Odontol Scand 2004;62:183-92.

Banu S., Govindarajulu P. and Aruldhas M. Testosterone and estradiol up-regulate androgen and estrogen receptors in immature and adult rat thyroid glands in vivo. Steroids 2002;67:1007-14.

Breedlove S., Jordan C. and Arnold A. Neurogenesis of motoneurons in the sexually dimorphic spinal nucleus of the bulbocavernosus in rats. Brain Res 1983;285:39-43.

Carlton S. and Coggeshall R. Immunohistochemical localization of enkephalin in peripheral sensory axons in the rat. Neurosci Lett 1997;221:121-4.

Cicero T., Nock B., O'Connor L. and Meyer E. Role of steroids in sex differences in morphine-induced analgesia: activational and organizational effects. J Pharmacol Exp Ther 2002;300:695-701.

Cooke B., Tabibnia G. and Breedlove S. A brain sexual dimorphism controlled by adult circulating androgens. Proc Natl Acad Sci USA 1999;96:7538-7540.

Corpéchot C., Baulieu E. and Robel P. Testosterone, dihydrotestosterone and androstanediols in plasma, testes and prostates of rats during development. Acta Endocrinol (Copenh) 1981;96:127-35.

Craft R. and Ulibarri C. Sexual differentiation of rat reproductive versus opioid antinociceptive systems. Gend Med 2009;6:208-24.

Davis E., Popper P. and Gorski R. The role of apoptosis in sexual differentiation of the rat sexually dimorphic nucleus of the preoptic area. Brain Res 1996;734:10-18.

DonCarlos L., Garcia-Ovejero D., Sarkey S., Garcia-Segura L. and Azcoitia I. Androgen Receptor Immunoreactivity in Forebrain Axons and Dendrites in the Rat. Endocrinology 2003;144:3632-3638.

Evrard H. Estrogen synthesis in the spinal dorsal horn: a new central mechanism for the hormonal regulation of pain. Am J Physiol Regul Integr Comp Physiol 2006;291:R291-9.

Fischer L., Arthuri M., Torres-Chávez K. and Tambeli C. Contribution of endogenous opioids to gonadal hormones-induced temporomandibular joint antinociception. Behav Neurosci 2009;123:1129-40.

Fischer L., Clemente J. and Tambeli C. The protective role of testosterone in the development of temporomandibular joint pain. J Pain 2007;8:437-42.

Fischer L., Parada C. and Tambeli C. A novel method for subarachnoid delivery in the medullary region of rats. J Neurosci Methods 2005;148:108-12.

Flake N. M., Hermanstyne T. O. and Gold M. S. Testosterone and estrogen have opposing actions on inflammation-induced plasma extravasation in the rat temporomandibular joint. Am J Physiol Regul Integr Comp Physiol 2006;291:R343-8.

Gao W. Peptide antagonist of the androgen receptor. Curr Pharm Des 2010;16:1106-13.

Green P., Dahlqvist S., Isenberg W., Strausbaugh H., Miao F. and Levine J. Sex steroid regulation of the inflammatory response: sympathoadrenal dependence in the female rat. J Neurosci Methods 1999;19:4082-4089.

Haas D., Nakanishi O., MacMillan R., Jordan R. and Hu J. Development of an orofacial model of acute inflammation in the rat. Arch Oral Biol 1992;37:417-22.

Hamson D. K., Jones B. A. and Watson N. V. Distribution of androgen receptor immunoreactivity in the brainstem of male rats. Neuroscience 2004;127:797-803.

Handa R., Corbier P., Shryne J., Schoonmaker J. and Gorski R. Differential effects of the perinatal steroid environment on three sexually dimorphic parameters of the rat brain. Biol Reprod 1985;32:855-864.

Kawata M. Roles of steroid hormones and their receptors in structural organization in the nervous system. Neurosci Res 1995;24:1-46.

LaCroix-Fralish M., Tawfik V. and DeLeo J. The organizational and activational effects of sex hormones on tactile and thermal hypersensitivity following lumbar nerve root injury in male and female rats. Pain 2005;114:71-80.

LeResche L. Epidemiology of temporomandibular disorders: implications for the investigations of etiologic factors. Critic Rev Oral Biol Med 1997;8:291-305.

Licznerska B. and Baer-Dubowska W. [Estrogen intracrinology: therapy and chemoprevention of breast cancer]. Postepy Hig Med Dosw (Online) 2010;64:220-30.

Liu J., Tsang S. and Wong T. Testosterone is required for delayed cardioprotection and enhanced heat shock protein 70 expression induced by preconditioning. Endocrinology 2006;147:4569-77.

McEwen B. Estrogens effects on the brain: multiple sites and molecular mechanisms. J Appl Physiol 2001;91:2785-801.

Pohl M., Collin E., Conrath S. B. M., Benoliel J., Nevo I., Hamon M., Giraud P. and Cesselin F. Expression of preproenkephalin A gene and presence of Met-enkephalin in dorsal root ganglia of the adult rat. J Neurochem 1994;63:1226-34.

Rosland J. The formalin test in mice: the influence of ambient temperature. Pain 1991;45:211-216.

Roveroni R., Parada C., Cecília M., Veiga F. and Tambeli C. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. Pain 2001;94:185-91.

Sakuma Y. Gonadal steroid action and brain sex differentiation in the rat. Journal of Neuroendocrinology 2009;21:410-414.

Ward G. and Abdel-Rahman A. Effect of testosterone replacement or duration of castration on baroreflex bradycardia in conscious rats. BMC Pharmacol 2005;5:9.

Weisz J. and Ward I. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 1980;106:306-16.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109-110.

4 - CONCLUSÕES

De acordo com os resultados do presente trabalho, concluiu-se que a testosterona em níveis fisiológicos:

1. Reduz o risco de ratos machos desenvolverem dor na ATM através de seu efeito ativacional, mediado por receptores androgênicos presentes no sistema nervoso central e subseqüente liberação de opioides endógenos.
2. Não reduz o risco de desenvolvimento de edema na região da ATM de machos.

5 – REFERÊNCIAS*

Cairns BE, Gazerani P. Sex-related differences in pain. Maturitas. 2009 Aug 20; 63(4): 292-6.

Clemente JT, Parada CA, Veiga MC, Gear RW, Tambeli CH. Sexual dimorphism in the antinociception mediated by kappa opioid receptors in the rat temporomandibular joint. Neurosci Lett. 2004 Dec 6; 372(3): 250-5.

Evrard H. Estrogen synthesis in the spinal dorsal horn: a new central mechanism for the hormonal regulation of pain. Am J Physiol Regul Integr Comp Physiol. 2006; 291(2): R291-9.

Fischer L, Clemente J, Tambeli C. The protective role of testosterone in the development of temporomandibular joint pain. J Pain. 2007; 8(5): 437-42.

Gao W. Peptide antagonist of the androgen receptor. Curr Pharm Des. 2010; 16(9): 1106-13.

Heikinheimo K, Salmi K, Myllarniemi S, Kirveskari P. Symptoms of craniomandibular disorder in a sample of Finnish adolescents at the ages of 12 and 15 years. Eur J Orthod. 1989 Nov; 11(4): 325-31.

Kawata M. Roles of steroid hormones and their receptors in structural organization in the nervous system. Neurosci Res. 1995; 24(1): 1-46.

Kitai N, Takada K, Yasuda Y, Verdonck A, Carels C. Pain and other cardinal TMJ dysfunction symptoms: a longitudinal survey of Japanese female adolescents. J Oral Rehabil. 1997 Oct; 24(10): 741-8.

Leeuw Rd. Dor Orofacial: guia de avaliação, diagnóstico e tratamento. Quarta ed. São Paulo: Quintessence Editora LTDA; 2010.

LeResche L. Epidemiology of temporomandibular disorders: implications for the investigations of etiologic factors. Critic Rev Oral Biol Med. 1997; 8(3): 291-305.

Licznerska B, Baer-Dubowska W. [Estrogen intracrinology: therapy and chemoprevention of breast cancer]. Postepy Hig Med Dosw (Online). 2010; 64: 220-30.

Lund JP, Lavigne GJ, Dubner R, Sessle B. Orofacial Pain - From Basic Science to Clinical Management. Carol Stream: Quintessence Publishing Co; 2001.

Merskey H. Logic, truth and language in concepts of pain. Qual Life Res. 1994 Dec; 3 Suppl 1: S69-76.

Oral K, Küçük BB, Ebeoğlu B, Dinçer S. Etiology of temporomandibular disorder pain. Agri 2009; Jul;21(3): 89-94.

Ribeiro-Dasilva MC, Peres Line SR, Leme Godoy dos Santos MC, Arthuri MT, Hou W, Fillingim RB*, et al.* Estrogen receptor-alpha polymorphisms and predisposition to TMJ disorder. J Pain. 2009 May; 10(5): 527-33.

Roveroni R, Parada C, Cecília M, Veiga F, Tambeli C. Development of a behavioral model of TMJ pain in rats: the TMJ formalin test. Pain. 2001; 94(2): 185-91.

Sakuma Y. Gonadal steroid action and brain sex differentiation in the rat. Journal of Neuroendocrinology. 2009; 21(4): 410-4.

Svensson P, Arendt-Nielson L. Clinical and experimental aspects of temporomandibular disorders. Curr Rev Pain. 2000; 4(2): 158-65.

Von Korff M, Dworkin SF, Le Resche L, Kruger A. An epidemiologic comparison of pain complaints. Pain. 1988 Feb; 32(2): 173-83.

Yu S, Xing X, Liang S, Ma Z, Li F, Wang M*, et al.* Locally synthesized estrogen plays an important role in the development of TMD. Med Hypotheses. 2009 Jun; 72(6): 720-2.

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* De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

ANEXOS

Anexo 1

Certificado de aprovação pela Comissão de Ética na Experimentação Animal (CEEA) – UNICAMP.

UNICAMP

CEEA/Unicamp

Comissão de Ética na Experimentação Animal
CEEA-IB-UNICAMP

C E R T I F I C A D O

Certificamos que o Protocolo nº **1431-1**, sobre **"Estudo dos mecanismos envolvidos no efeito protetor da testosterona sobre o desenvolvimento da dor da ATM em ratos"** sob a responsabilidade de **Profa. Dra. Claudia Herrera Tambeli / Letícia Esmanhoto Fanton** 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 na Experimentação Animal (CEEA)-IB-UNICAMP em reunião de **19 de dezembro de 2007**.

C E R T I F I C A T E

We certify that the protocol nº **1431-1**, entitled **"Study of the mechanisms underlying the protective effect of testosterone on TMJ pain development in rats"**, 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 **december 19, 2007**.

Campinas, 19 de dezembro de 2007.

Prof. Dr. Stephen Hyslop
Presidente em exercício

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Anexo 2

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DECLARAÇÃO

As cópias de artigos de minha autoria ou de minha co-autoria, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, que constam da minha Dissertação de Mestrado, intitulada "ESTUDO DOS MECANISMOS ENVOLVIDOS NO EFEITO PROTETOR DA TESTOSTERONA SOBRE O DESENVOLVIMENTO DA DOR DA ATM EM RATOS", não infringem os dispositivos da Lei no. 9.610/98, nem o direito autoral de qualquer editora.

Piracicaba, 27 de agosto de 2010

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