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ACTIVATION OF PERIPHERAL κ/δ OPIOID RECEPTORS MEDIATES 15-DEOXY-$^{\Delta 12,14}$-PROSTAGLANDIN J_2 INDUCED-ANTINOCICEPTION IN RAT TEMPOROMANDIBULAR JOINT

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Abstract—This study assessed the effect of the agonist 15d-PGJ_2 administered into the rat temporomandibular joint (TMJ) on nociceptive behavioral and the anti-inflammatory potential of this prostaglandin on TMJ. It was observed that 15-deoxy-$^{\Delta 12,14}$-prostaglandin J_2 (15d-PGJ_2) significantly reduced formalin-induced nociceptive behavior in a dose dependent manner, however injection of 15d-PGJ_2 into the contralateral TMJ failed to reduce such effects. This antinociceptive effect is dependent on peroxisome proliferator-activated receptors-γ (PPAR-γ) since pre-treatment with GW9662 (PPAR-γ receptor antagonist) blocked the antinociceptive effect of 15d-PGJ_2 in the TMJ. In addition, the antinociceptive effect of 15d-PGJ_2 was also blocked by naloxone suggesting the involvement of peripheral opioids in the process. Confirming this hypothesis pre-treatment with κ, δ, but not μ receptor antagonists significantly reduced the antinociceptive effect of 15d-PGJ_2 in the TMJ. Similarly to opioid agonists, the 15d-PGJ_2 antinociceptive action depends on the nitric oxide (NO)/guanilate cyclase (cGMP)/ATP-sensitive potassium channel blocker(K^+_{ATP}) channel pathway since it was prevented by the pre-treatment with the inhibitors of nitric oxide synthase (NOS; aminoguanidine), cGMP (ODQ), or the K^+_{ATP} (glibenclamide). In addition, 15d-PGJ_2 (100 ng/TMJ) inhibits 5-HT-induced TMJ hypernociception. Besides, TMJ treated with 15d-PGJ_2 showed lower vascular permeability, assessed by Evan's Blue extravasation, and also lower neutrophil migration induced by carrageenan administration. Taken together, these results demonstrate that 15d-PGJ_2 has a potential peripheral antinociceptive and anti-inflammatory effect in the TMJ via PPAR-γ activation. The results also suggest that 15d-PGJ_2 induced-peripheral antinociceptive response in the TMJ is mediated by κ/δ opioid receptors by the activation of the intracellular L-arginine/NO/cGMP/K^+_{ATP} channel pathway. The pharmacological properties of the peripheral administration of 15d-PGJ_2 highlight the potential use of this PPAR-γ agonist on TMJ inflammatory pain conditions. © 2009 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: TMJ, pain, PGJ2, 15d-PGJ_2, PPAR-gamma, opioid receptors.

There is consensus in the literature that inflammatory hypernociception occurs, at least in part, as a consequence of the sensitization of primary afferent nociceptors. This phenomenon has been attributed to the direct action of hypernociceptive inflammatory mediators (mainly prostaglandins and sympathetic amine) on their receptors present in the nociceptor membrane (Khasar et al., 1999). Temporomandibular joint (TMJ) pain is a significant part of symptoms in patients with temporomandibular disorders (TMD) and a common source of chronic orofacial pain (Alstergren et al., 1999). A considerable amount of evidence suggests that TMJ pain may result from an inflammatory episode (Kopp, 2001). Although the mechanism underlying TMJ pain conditions is not completely known, it has been shown that inflammatory mediators such as prostaglandin E_2, 5-HT and pro-inflammatory cytokines (tumoral necrosis factor alpha [TNF-α], interleukin-1β [IL-1β]) are highly present in the synovial fluid of patients with TMD (Kopp, 2001). In addition, recently it has been demonstrated that sympathomimetic amines also contribute to the inflammatory TMJ hyperalgesia by activating β2-adrenoceptors (Rodrigues et al., 2006).

Several lines of evidence support the concept that endogenous formation of cyclooxygenase (COX)-derived electrophilic lipid oxidation products may play an anti-inflammatory role (Jiang et al., 1998; Ricote et al., 1998; Zingarelli and Cook, 2005; Napimoga et al., 2008a). The 15-deoxy-$^{\Delta 12,14}$-prostaglandin J_2 (15d-PGJ_2), a potent lipid mediator derived from the prostaglandin D_2 *in vivo* by dehydration (Yu et al., 1995), is a natural ligand for peroxisome proliferator activated receptors (PPAR-γ) (Schoonjans et al., 1997; Ricote et al., 1998). 15d-PGJ_2, is abundantly produced by mast cells, platelets, alveolar macrophages and has been proposed as a key immunoregulatory lipid mediator (Straus and Glass, 2001; Zingarelli and Cook, 2005). In addition to its anti-inflammatory effects, we have previously demonstrated for the first time that 15d-PGJ_2 inhibits carrageenan-induced mechanical inflammatory hypernociception in rat paws via PPAR-γ

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Abbreviations: cGMP, guanilate cyclase; CTOP, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2; GW9662, 2-chloro-5-nitro-N-phenylbenzamide; ICI 174,864, N, N-diallyl-Tyr-Aib-Aib-PheLeu; K^+_{ATP}, ATP-sensitive potassium channel blocker; MPO, myeloperoxidase; NOS, nitric oxide synthase; Nor-BNI, nor-binaltorphimine dihydrochloride; ODQ, 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxaline-1-one; PPAR-γ, peroxisome proliferators activated receptors-γ; TMD, temporomandibular disorders; TMJ, temporomandibular joint; 15d-PGJ_2, 15-deoxy-$^{\Delta 12,14}$-prostaglandin J_2.

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activation that is dependent on local macrophages and endogenous opioids production (Napimoga et al., 2008b). However 15d-PGJ_2 did not inhibit formalin-induce nociception in the rat paws while it blocked formalin-induced nociception in the TMJ of rats (Napimoga et al., 2008b). These findings led us to investigate the potential anti-hypernociceptive and anti-inflammatory properties of exogenous administration of these lipids into TMJ joint of rats. The possible cellular mechanisms involved in the antinociceptive effect of 15d-PGJ_2 were also addressed.

EXPERIMENTAL PROCEDURES

Animals

This study was carried out with male Wistar rats (150–250 g) maintained in a temperature-controlled room (23±1 °C) with a 12 h light/dark cycle. All experiments were conducted in accordance to the IASP guidelines on using laboratory animals for investigations of experimental pain in conscious animals (Zimmermann, 1983). All animal experimental procedures and protocols were approved by the Committee on Animal Research of the University of Uberaba. The animals' suffering and number per group were kept at a minimum and each animal was used once.

Drugs

15d-PGJ_2 and 2-chloro-5-nitro-N-phenylbenzamide (GW9662) from Calbiochem, San Diego, CA, USA; naloxone, aminoguanidine, glibenclamide, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP), Nor-Binaltorphimine (Nor-BNI) and an aqueous solution of 37% of formaldehyde were obtained from Sigma, St. Louis, MO, USA; 1H-(1,2,4)-oxadiazolo(4,2-a)quinoxalin-1-one (ODQ) and N, N-diallyl-Tyr-Aib-Aib-Phe-Leu (ICI 174,864) were obtained from Tocris Cookson, Ballwin, MO, USA. Formalin solution were prepared from commercially stock formalin (an aqueous solution of 37% of formaldehyde) and further diluted in 0.9% NaCl. The ATP-sensitive potassium channels blocker glibenclamide was dissolved in 2% Tween 80 and re-suspended in saline. Naloxone and aminoguanidine were dissolved in saline. GW9662 and ODQ were dissolved in dimethyl sulfoxide (DMSO) (Sigma, St. Louis, MO, USA) and re-suspended in saline to minimize the final concentration of DMSO (max. 0.5%).

Testing procedure for TMJ pain

Testing sessions took place during light phase (between 9:00 AM and 5:00 PM) in a quiet room maintained at 23 °C. Each animal was manipulated for 7 days to be habituated to the experimental manipulation. After this period, the animal was placed in a test chamber (30×30×30 cm^3 mirrored wood chamber with a glass at the front side) for 15 min habituation period to minimize stress. Animals were briefly anesthetized by inhalation of halothane to allow the TMJ injection, which was performed with a 30-gauge needle connected to a 50 μl-Hamilton syringe (Roveroni et al., 2001). Each animal regained consciousness approximately 30 s after discontinuing the anesthetic and was returned to the test chamber for counting nociceptive responses. The nociceptive response score was defined as the cumulative total number of seconds that the animal spent rubbing the orofacial region asymmetrically with the ipsilateral fore or hind paw plus the number of head flinches counted during the observation period as described previously. Since head flinches followed a uniform pattern of 1 s of duration, each flinch was expressed as 1 s. Results are expressed as the duration time of nociceptive behavior (Roveroni et al., 2001; Clemente et al., 2004). At the conclusion of the experiment, animals were anesthetized by an i.p. injection of a mixture of urethane (1 g/kg) and α-chloralose (50 mg/kg), followed by i.v. administration of Evan's Blue dye (1%; 5 mg/kg), to visualize formalin-induced plasma extravasation upon post-mortem examination of injected TMJs (Haas et al., 1992). This procedure also confirmed that the plasma extravasation induced by TMJ injection at the correct site was restricted to the immediate TMJ region. A different investigator performed each test, prepared the solution, and administered the TMJ injections. All animals received a final volume of 45 μl into TMJ as previously described (Clemente et al., 2004). All experiments were conducted in a double blind fashion in which the person who injected the solutions was different of the one who made the behavioral assessment.

Experimental protocols

Effect of 15d-PGJ_2 on formalin-induced TMJ nociception. Rats were pretreated (15 min) with an intra-TMJ injection of 15d-PGJ_2 (1, 10 or 100 ng; $n=6$; 15 μl/TMJ; Napimoga et al., 2008b) followed by ipsilateral intra-TMJ injection of 1.5% formalin in a final volume of 45 μl. Behavioral nociception response was evaluated for a 45 min observation period. In order to confirm the peripheral 15d-PGJ_2-mediated antinociception, the highest dose of 15d-PGJ_2 was also injected in the contralateral TMJ that received injection of 1.5% formalin.

Effect of PPAR-γ receptor antagonist on 15d-PGJ_2-induced antinociception. Rats were pretreated (15 min) with an intra-TMJ injection of PPAR-γ receptor antagonist GW9662 (0.3, 1 or 3 ng/15 μl/TMJ; $n=6$; Napimoga et al., 2008b) followed by 15 d-PGJ_2 (100 ng/15 μl/TMJ) 15 min prior ipsilateral intra-TMJ injection of 1.5% formalin (15 μl/TMJ). Behavioral nociception response was evaluated for 45 min observation period. In order to confirm the peripheral effect of GW9662, the highest dose of 15d-PGJ_2 was also injected into the contralateral TMJ that received pretreatment of 15d-PGJ_2 and 1.5% formalin. All animals received a final volume of 45 μl of solutions into TMJ.

Effect of the nonselective opioid receptor antagonist naloxone on 15d-PGJ_2-induced antinociception. Rats were pretreated (15 min) with an intra-TMJ injection of naloxone (10 μg/15 μl/TMJ; $n=6$; Eisenberg et al., 1996) followed by 15d-PGJ_2 (100 ng/15 μl/TMJ) 15 min prior ipsilateral intra-TMJ injection of 1.5% formalin (15 μl/TMJ). Behavioral nociception response was evaluated for 45 min observation period. All animals received a final volume of 45 μl of solutions into TMJ.

Role of NO/cGMP/ATP-sensitive potassium channel blocker (K_{ATP}^+) channel pathway on 15d-PGJ_2-induced antinociception. Rats were divided in groups of six animals, and each group was pretreated (15 min) with an intra-TMJ injection of a nonselective inhibitor of nitric-oxide synthase (NOS) aminoguanidine (0.1 mol/15 μl/TMJ; Sachs et al., 2004) or inhibitor of soluble cGMP enzyme ODQ (8 μg/15 μl/TMJ; Napimoga et al., 2008b) or the ATP-potassium sensitive channel blocker glibenclamide (160 μg/15 μl/TMJ; Napimoga et al., 2008b) followed by 15d-PGJ_2 (100 ng/15 μl/TMJ; $n=6$) 15 min prior ipsilateral intra-TMJ injection of 1.5% formalin (15 μl/TMJ). Behavioral nociception response was evaluated for 45 min period observation. All animals received a final volume of 45 μl of solutions into TMJ.

Role of μ, δ and κ-opioid receptors on 15d-PGJ_2-induced antinociception. Rats were divided in groups of six animals, and each group was pretreated (15 min) with an intra-TMJ injection of a specific inhibitor of μ-opioid receptor CTOP (20 or 60 μg/15μl/TMJ; Picolo et al., 2000) or the inhibitor of δ-opioid receptor ICI 174,864 (10 or 30 μg/15 μl/TMJ; Picolo et al., 2000) followed by 15d-PGJ_2 (100 ng/15 μl/TMJ; $n=6$) 15 min prior ipsilateral intra-TMJ injection of 1.5% formalin (15 μl/TMJ). The selective κ-opioid receptor antagonist Nor-BNI (200 μg/15 μl/TMJ; Clemente et al., 2004) was injected 24 h prior to ipsilateral 15d-PGJ_2