15d-Prostaglandin J$_2$ Inhibits Inflammatory Hypernociception: Involvement of Peripheral Opioid Receptor


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Received May 21, 2007; accepted October 9, 2007

ABSTRACT

The 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$ (15d-PGJ$_2$) is an endogenous ligand of peroxisome proliferator-activated receptors-γ (PPAR-γ) and is now recognized as a potent anti-inflammatory mediator. However, information regarding the influence of 15d-PGJ$_2$ on inflammatory pain is still unknown. In this study, we evaluated the effect of 15d-PGJ$_2$ upon inflammatory hypernociception and the mechanisms involved in this effect. We observed that intraplantar administration of 15d-PGJ$_2$ (30–300 ng/paw) inhibits the mechanical hypernociception induced by both carrageenan (100 μg/paw) and the directly acting hypernociceptive mediator, prostaglandin E$_2$ (PGE$_2$). Moreover, 15d-PGJ$_2$ [100 ng/temporomandibular joint (TMJ)] inhibits formalin-induced TMJ hypernociception. On the other hand, the direct administration of 15d-PGJ$_2$ into the dorsal root ganglion was ineffective in blocking PGE$_2$-induced hypernociception. In addition, the 15d-PGJ$_2$ antinociceptive effect was enhanced by the increase of macrophage population in paw tissue due to local injection of thioglycollate, suggesting the involvement of these cells on the 15d-PGJ$_2$-antinociceptive effect. Moreover, the antinociceptive effect of 15d-PGJ$_2$ was also blocked by naloxone and by the PPAR-γ antagonist 2-chloro-5-nitro-N-phenylbenzamide (GW9662), suggesting the involvement of peripheral opioids and PPAR-γ receptor in the process. Similar to opioids, the 15d-PGJ$_2$ antinociceptive action depends on the nitric oxide/cGMP/protein kinase G (PKG)/KATP Channel pathway because it was prevented by the pretreatment with the inhibitors of nitric-oxide synthase ($\text{N}^\rho$-monomethyl-$\text{L}$-arginine acetate), guanylate cyclase $\text{J}1\text{H}-(1,2,4)$-oxadiazolo(4,2-$\alpha$)-quinoxalin-1-one, PKG [indolo[2,3-α]pyrrolo[3,4-c]carbazole aglycone (KT5823)], or with the ATP-sensitive potassium channel blocker glibenclamide. Taken together, these results demonstrate for the first time that 15d-PGJ$_2$ inhibits inflammatory hypernociception via PPAR-γ activation. This effect seems to be dependent on endogenous opioids and local macrophages.

Pain is one of the classic signals of the inflammatory process. It is now accepted that the sensitization of primary sensory neurons is essential to inflammatory pain. In humans, this nociceptor sensitization usually leads to clinical conditions known as hyperalgesia (an increased response to a stimulus that is normally painful) or allodynia (pain due to a stimulus that does not normally provoke pain). In the present study, we are using the term “hypernociception” to designate this decrease in the nociceptive threshold response in experimental animals.

The mechanisms involved in the sensitization of primary sensory neurons and, consequently, in the establishment of inflammatory hypernociception may be divided into two phases. The first phase is the non-neuronal events; the resident and migrated immune cells produce a sequence of hypernociceptive inflammatory mediators initiated by tumor necrosis factor α (TNF-α), which triggers the release of interleukin-1β and chemokines that in turn stimulate the release of directly acting hypernociceptive mediators (Verri et al., 2006). The most well known directly acting hypernociceptive mediators are prostaglandins. These mediators are consid-

ABBREVIATIONS: TNF-α, tumor necrosis factor α; NSAID, nonsteroidal anti-inflammatory drug; PGE$_2$, prostaglandin E$_2$; 15d-PGJ$_2$, 15-deoxy-$\Delta^{12,14}$-prostaglandin J$_2$; PPAR-γ, peroxisome proliferator-activated receptor γ; COX, cyclooxygenase; GW9662, 2-chloro-5-nitro-N-phenylbenzamide; KT5823, indolo[2,3-α]pyrrolo[3,4-c]carbazole aglycone; L-NMMA, $\text{N}^\rho$-monomethyl-$\text{L}$-arginine acetate; ODQ, $\text{J}1\text{H}-(1,2,4)$-oxadiazolo(4,2-$\alpha$)-quinoxalin-1-one; DRG, dorsal root ganglion; TMJ, temporomandibular joint; PKG, protein kinase G; i.pl., intraplantar; NAG, N-acetylglucosaminidase; ANOVA, one-way analysis of variance; NOS, nitric-oxide synthase.
ered to be directly acting because they activate the specific receptors present on the nociceptive neuron membrane. The second phase includes the neuronal events: activation of receptors on primary nociceptive neurons that trigger intracellular signaling pathways, such as cAMP, protein kinase A, and protein kinase C (Aley and Levine, 1999; Khasar et al., 1999). These signaling pathways resulted in subsequent phosphorylation of the voltage-dependent sodium channels (Gold et al., 1998) and inhibition of the voltage-dependent potassium channels (Evans et al., 1999). Consequently, the nociceptor threshold was lowered and ultimately led to an enhancement of neuron excitability.

Experimentally, the peripheral pharmacological control of inflammatory pain is based on two main strategies. The first is the use of drugs that prevent the nociceptor sensitization, such as nonsteroidal anti-inflammatory drugs (NSAIDs) (aspirin and aspirin-like drugs) that inhibit prostaglandin synthesis (Ferreira, 1972) and, therefore, prevent the development of hypernociception. The second strategy is the direct blockade of the ongoing nociceptor sensitization, which can be achieved by the use of peripheral morphine (opioids), dipyrone, and diclofenac (Lorenzetti and Ferreira, 1985; Ferreira et al., 1991). In fact, these drugs reverse the already established hypernociception induced by prostaglandin E2 (PGE2) in the rat hind paws. In addition, several studies support the fact that their antinociceptive activities are due to the activation of the l-arginine/NO/cGMP/protein kinase G (PKG) channel pathway (Ferreira et al., 1991; Sachs et al., 2004).

The 15-deoxy-Δ12,14-PGJ2 (15d-PGJ2) is one of the derivatives of the prostaglandin D2 metabolism pathway and is a natural ligand for peroxisome proliferator-activated receptors γ (PPAR-γ) (Schoonjans et al., 1997; Ricote et al., 1998). It has been demonstrated that fluctuation of 15d-PGJ2 levels is associated with the inflammatory process (Ricote et al., 1998; Willoughby et al., 2000), suggesting that it may play an important role in the regulation of the inflammatory reaction in vivo. Several in vitro studies demonstrated that pharmacological activation of PPAR-γ by 15d-PGJ2 produces anti-inflammatory effects, such as repression of the expression of several inflammatory response genes in activated macrophages, including the genes encoding TNF-α, gelatinase B, and cyclooxygenase (COX)-2 (Jiang et al., 1998; Ricote et al., 1998). In line with these findings, in vivo treatment with PPAR-γ agonists has been reported to attenuate several experimental inflammatory diseases, such as colitis in mice (Su et al., 1999; Desreumaux et al., 2001), adjuvant-induced arthritis in rats, and reduction of systemic inflammation in polymicrobial sepsis (Kawahito et al., 2000; Zingarelli et al., 2003). Thus, PPAR-γ activation may have beneficial effects that control inflammation. However, whether this treatment approach has beneficial effects on inflammatory pain remains unclear. Therefore, in the present study, we investigated the effect of 15d-PGJ2 on the inflammatory hypernociception in rats and also the cellular mechanisms involved in this effect.

Materials and Methods

Animals. The behavioral experiments were performed on male Wistar rats weighing 180 to 200 g and housed in the animal care facility of the School of Medicine of Ribeirão Preto. Rats were then taken to the testing room at least 1 h before the experiments. Food and water were available ad libitum. All behavioral tests were performed between 9:00 AM and 5:00 PM, and the animals were used only once. Animal care and handling procedures were in accordance with the guidelines of the International Association for the Study of Pain on the use of animals in pain research. All efforts were made to minimize the number of animals used and their discomfort.

Drugs. The drugs used in this study were as follows. 15d-PGJ2, 2-chloro-5-nitro-N-phenylbenzamide (GW9662), and indolo[2,3-a]pyrrolo[3,4-c]carbazole aglycone (KT5823) were obtained from Calbiochem (San Diego, CA). Naloxone, glibenclamide, and formalin solution were prepared from commercially stock formalin (an aqueous solution containing 37% formaldehyde) and further diluted in 0.9% NaCl (saline). The maximal concentration of ethanol was 1%. The ATP-sensitive potassium channel blocker (Alves and Duarte, 2002) glibenclamide was dissolved in 2% Tween 80 and resuspended in saline. Naloxone and l-NNMA were dissolved in saline. GW9662, ODQ, and KT5823 were dissolved in dimethyl sulfoxide (Sigma) and resuspended in saline to minimize the final concentration of dimethyl sulfoxide (maximum, 0.5%). Rat TNF-α was a gift from Dr. Stephen Poole (National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK).

Intraplantar Drug Administration. A hypodermic 26-gauge needle was inserted s.c. into the plantar surface of the rat’s hind paw between the five distal footpads. At the same place where mechanical stimulus was applied, the drugs were administered in a volume of 50 µl (Vivancos et al., 2003).

Intranganglion Drug Administration. Briefly, after shaving the fur over the lower back, rats were lightly anesthetized and placed over a small cylinder to elevate the lumbar region. The intranganglionic injection was performed using a 30-cm PE-10 catheter (Intramedic; Clay Adams, Parsippany, NJ; internal diameter 0.28 mm and external diameter 0.61 mm), calibrated in such a way that 25 µl corresponded to an injected volume of 1 µl. The needle injection was 1.5 cm laterally from the vertebral column, approximately 0.5 cm caudal from a virtual line passing over the rostral borders of the iliac crests. Delicate movements of the needle were made until the bone resistance was diminished and a paw flinch reflex was observed. The paw flinch reflex was used as a sign that the needle tip penetrated the dorsal root ganglion (DRG) of the fifth lumbar spinal nerve located underneath the transversal process of the fifth lumbar vertebra. After the needle reached the ganglion, 5 µl of solution was injected (Ferrari et al., 2007).

Evaluation of Mechanical Hypernociception. Hypernociceptive mechanical threshold was measured by the electronic von Frey method as described. In a quiet room, rats were placed in acrylic cages (12 × 20 × 17 cm) with wire grid floors, 15 to 30 min before the start of testing. During this adaptation period, the paws were tested (probed) three times. The test consisted of evoking a hind paw flexion reflex with a hand-held force transducer adapted with a 0.7-mm2 polypropylene tip (electronic von Frey hair; IITC Life Science, Woodland Hills, CA). A tilted mirror placed under the grid provided a clear view of the rat hind paw. The investigator was trained to apply the tip in between the five distal footpads with a gradual increase in pressure. The stimulus was automatically discontinued, and its intensity was recorded when the paw was withdrawn. The maximal force applied was 80 g. The endpoint was characterized by the removal of the paw in a clear flinch response after the paw withdrawal. The animals were tested before and after treatments. A different investigator performed each test, prepared the solution, and administered the intraplantar and intranganglionic injections. The results
are expressed by the withdrawal threshold (in gravity) that was calculated by subtracting the average of the last three measurements after the treatments from the average of three measurements before treatments.

**Temporomandibular Joint Injections.** Animals were anesthetized briefly by inhalation of halothane to allow temporomandibular joint (TMJ) injection, which was performed with a 30-gauge needle introduced into the left TMJ at the moment of injection. A cannula consisting of a polyethylene tube was connected to the needle and also to a Hamilton syringe (50 μl). Injection volumes were 15 μl in all cases. Each animal regained consciousness approximately 30 s after discontinuing the anesthetic.

**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 mirrored wood chamber with a glass at the front side) for a 15-min habituation period to minimize stress. Each animal immediately recovered from anesthesia after TMJ injection and was returned to the test chamber for counting nociceptive responses during the following 45-min observation period. 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. All animals that received saline injections.

**Formalin Paw Test.** Rats were placed in an open Plexiglas observation chamber for 30 min to become acclimated to their surroundings and then removed for formalin administration. Rats were gently restrained while the dorsum of the hind paw was s.c. injected with 50 μl of 1% formalin (1:100 dilution of stock formalin solution, 37% formaldehyde in 0.9% saline) using a 30-gauge needle. After injection, the rat was returned to the observation chamber for a 60-min observation period. A mirror was placed behind the chamber to enable unhindered observation of the formalin-injected paw. The recording time was divided into 12 blocks of 5 min, and a pain score was determined for each block by measuring the number of lifts or flinches of the affected limb during the observation time. Such behavior could vary from a simple lift of the paw (not associated with locomotion) to a vigorous shaking of the limb, or it could be a rippling of the back muscle associated with limb movement. Lifts or flinches were discrete and easily quantifiable.

**Cytokine Measurements.** Two hours after carrageenan intraplantar injection, animals were terminally anesthetized, and skin tissues of the plantar region were removed from the injected and control paws (saline). The samples were triturated and homogenized in 500 μl of the appropriate buffer containing protease inhibitors followed by a centrifugation of 10 min/2000 g. The supernatants were used to determined the levels of TNF-α (Cunha et al., 2005) by enzyme-linked immunosorbent assay, as described previously. The results are expressed as picograms of each cytokine per paw. As a control, the concentrations of these cytokines were determined in animals that received saline injections.

**Experimental Protocols**

**Effect of 15d-PGJ₂ on Carrageenan, Formalin, and TNF-α-Induced Mechanical Hypernociception.** Rats were pretreated with 15d-PGJ₂ (30, 100, and 300 ng/50 μl/paw i.pl.), and after 30 min, they received an i.pl. injection of carrageenan (100 μg/50 μl/paw i.pl.), formalin (1%/50 μl/paw i.pl.), or TNF-α (1 pg/50 μl/paw i.pl.). Mechanical hypernociception was evaluated after the carrageenan and TNF-α challenge by the electronic von Frey test.

**Effect of 15d-PGJ₂ on Formalin-Induced TMJ Nociception.** Rats were pretreated with 15d-PGJ₂ (100 ng/15 μl/TMJ), and after 30 min, they received an intra-articular injection of 1.5% formalin. Behavioral nociception response was evaluated for 45 min.

**Effect of 15d-PGJ₂ on PGE₂-Induced Mechanical Hypernociception.** Rats were pretreated with 15d-PGJ₂ (30, 100, and 300 ng/50 μl/paw i.pl.), and after 30 min, they received an i.pl. injection of PGE₂ (100 ng/50 μl/paw i.pl.). Mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test.

**Effect of PPAR-γ Receptor Antagonist on 15d-PGJ₂-Induced Antinociception.** Rats were pretreated (30 min) with the PPAR-γ receptor antagonist GW9662 (0.3, 1, and 3 ng/50 μl/paw i.pl.) followed by 15d-PGJ₂ (100 ng/50 μl/paw i.pl.) administration. After 30 min, PGE₂ (100 ng/50 μl/paw i.pl.) was injected. The mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test. All animals received a final volume of 150 μl of solutions.

**Effect of 15d-PGJ₂ Administered Directly into the DRG.** Rats were pretreated (30 min) with intraganglionic (100 ng/10 μl DRG) or intraplantar (100 ng/50 μl/paw) injection of 15d-PGJ₂, followed by PGE₂ (100 ng/50 μl/paw i.pl.) injection. Mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test.

**Effect of the Nonselective Opioid Receptor Antagonist Naloxone on 15d-PGJ₂-Induced Antinociception.** Rats were pretreated (30 min) with naloxone (1 μg/50 μl/paw i.pl.) followed by 15d-PGJ₂ (100 ng/50 μl/paw i.pl.) administration. After 30 min, PGE₂ (100 ng/50 μl/paw i.pl.) was injected. Mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test. All animals received a final volume of 150 μl of solutions.

**The Role of Macrophage for the Antinociceptive Effect of 15d-PGJ₂.** Rats were pretreated with 1% thioglycollate (100 μl/paw i.pl.). After 3 days, mechanical hypernociception was evaluated before animals received the following treatments: pretreatment (30 min) with naloxone (1 μg/50 μl/paw i.pl.) followed by 15d-PGJ₂ (30 ng/50 μl/paw i.pl.) administration. After 30 min, PGE₂ (100 ng/50 μl/paw i.pl.) was injected. Mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test. All animals received a final volume of 150 μl of solutions.

**Quantification of Macrophage Tissue Accumulation by N-Acetylglucosaminidase Activity Measurement.** Plantar skin tissue of thioglycollate- or saline-injected rats were homogenated in 1.0 ml of cooled (4°C) 0.9% saline containing 0.1% N-acetyl-β-glucosaminidase (Sigma), dissolved in citrate/phosphate buffer (0.1 M citric acid, 0.1 M Na₃HPO₄, pH 4.5) in a final concentration of 2.24 mM to 100 μl of supernatant from tissue sample processing, dissolved in citrate/phosphate buffer at appropriate dilutions. The reaction was terminated by the addition of 100 μl of 0.2 M glycine buffer, pH 10.6, and was quantified at 405 nm in a spectrophotometer (EMax; Molecular Devices, Sunnyvale, CA). The macrophage content was calculated from a standard curve based on NAG activity expressed as absorbance increase at 405 nm from 3% thioglycollate peritoneal-induced macrophages assayed in parallel. The results were expressed in relative number of macrophages per milligram of wet tissue (Belo et al., 2004).

**Role of NOcGMP/PKG/γₐTrp Channel Pathway on 15d-PGJ₂-Induced Antinociception.** Rats were divided in groups of five animals, and each group received different pretreatments with the following drugs: nonselective inhibitor of nitric-oxide synthase.
(NOS) L-NMMA (50 μg/50 μl/paw i.pl.; 30 min), inhibitor of soluble guanylate cyclase enzyme ODQ (8 μg/50 μl/paw i.pl.; 30 min), inhibitor of protein kinase GKT5823 (1.5 μg/50 μl/paw i.pl.; 10 min), or the ATP-potassium sensitive channel blocker glibenclamide (160 μg/50 μl/paw i.pl.; 30 min). Afterward, all groups received 15d-PGJ₂ (100 ng/50 μl/paw i.pl.). After 30 min, PGE₂ (100 ng/50 μl/paw i.pl.) was injected. Mechanical hypernociception was evaluated 3 h after the PGE₂ challenge by the electronic von Frey test. All animals received a final volume of 150 μl of solutions. The selected doses of all other antagonists used were obtained from previous studies (Sachs et al., 2004).

Statistical Analysis. Two independent experiments were performed, and the results were presented as the mean ± S.E.M. of the results (n = 5 per experiment). The differences between the groups were compared using one-way analysis of variance (ANOVA) to obtain the degree of significance, followed by the Bonferroni multiple comparison test to compare the groups and doses (behavioral experiments). The established level of significance was P < 0.05.

Results

15d-PGJ₂ Inhibits Carrageenan-Induced Mechanical Hypernociception and Formalin-Induced Nociception in the TMJ Joint. Intraplantar administration of 15d-PGJ₂ (30, 100, and 300 ng/paw) inhibited the mechanical hypernociception induced by intraplantar administration of carrageenan (100 μg/paw; Fig. 1A). This effect was significant 1, 3, and 5 h after carrageenan administration (Fig. 1B). The administration of 15d-PGJ₂ (100 ng/paw) in the contralateral paw did not inhibit the mechanical hypernociception induced by the carrageenan, indicating that it was acting locally (Fig. 1A). In contrast with these results, it was observed that 15d-PGJ₂ was not able to inhibit carrageenan-induced thermal hypernociception (data not shown). We also observed that the administration of 15d-PGJ₂ did not inhibit the formalin-induced nociception in the rat paw on both phases I and II pain behavior (data not shown). However, it inhibited formalin-induced nociception in the TMJ of rats (Fig. 1C).

15d-PGJ₂ Did Not Inhibit Carrageenan-Induced TNF-α Release but Inhibited TNF-α-Induced Mechanical Hypernociception. We have previously demonstrated that the carrageenan-induced hypernociception depends on TNF-α production (Cunha et al., 1992). In this study, 15d-PGJ₂ did not alter the release of TNF-α induced by carrageenan (Fig. 1D), but TNF-α hypernociception was inhibited by 15d-PGJ₂ (Fig. 1E). Therefore, the antinociceptive effect of 15d-PGJ₂ does not depend on the inhibition of the cytokine release but rather on the inhibition of cytokine hypernociceptive action.

15d-PGJ₂ Inhibits PGE₂-Induced Mechanical Hypernociception. Further addressing the 15d-PGJ₂ mechanism of action, we investigated its effect upon the mechanical hypernociception induced by a directly acting hypernociceptive mediator, PGE₂. This approach is related to the demonstration that, after carrageenan stimulus, there is the release of a cascade of cytokines, which include TNF-α. Cytokines then are responsible for inducing PGE₂ production that directly sensitizes the nociceptors (Verri et al., 2006). Thus, considering that 15d-PGJ₂ inhibits the TNF-α-induced hypernociception (Fig. 1E), but not carrageenan-induced production of TNF-α (Fig. 1D), it is conceivable that if 15d-PGJ₂ inhibits PGE₂-induced mechanical hypernociception, its mechanism of action may be different from conventional NSAIDs and inhibitors of cytokine production, thus opening the possibility of direct blockade of ongoing hypernociception by 15d-PGJ₂. In fact, it was detected that local treatment with 15d-PGJ₂ inhibited PGE₂-induced mechanical hypernociception.

![Fig. 1. Peripheral antinociceptive effect of 15d-PGJ₂. A, 15d-PGJ₂ was injected in the rats’ hind paws at doses 30, 100, and 300 ng (100 μl/i.pl.) 30 min before the intraplantar administration of carrageenan (Cg, 100 μg/50 μl/i.pl.), and hypernociception was evaluated 3 h after stimulus injection. B, time dependence of 15d-PGJ₂ (100 ng/paw) in the Cg (100 μg)-induced hypernociception. C, effect of 15d-PGJ₂ (100 ng/paw) in the formalin-induced nociception in the TMJ joint. D, effect of 15d-PGJ₂ (100 ng/paw) on Cg-induced TNF-α production in the paw skin. E, effect of 15d-PGJ₂ (100 ng/paw) on TNF-α (1 pg/paw)-induced hypernociception. The results are expressed as the mean ± S.D. of five animals per group. * statistical significance compared to saline; **, statistical significance compared to vehicle plus group Cg (A and B), formalin (C), and TNF-α (E). P < 0.05, one-way ANOVA followed by the Bonferroni’s test.](image-url)
ception (100 ng/paw; Fig. 2A). These results suggest that 15d-PGJ2 presents a peripheral opioid-like mechanism, because, as mentioned before, this type of peripheral antinociceptive drug is able to inhibit directly acting sensitizing mediator-induced hypernociception. Furthermore, intraplantar administration of 15d-PGJ2 (100 ng/paw) did not alter the nociceptive threshold of the animals, disproving a possible hypalgesic effect and suggesting an effect restricted to the inflammatory process. It is noteworthy to mention that the doses of carrageenan and PGE2, which were used, induced hypernociception only in the ipsilateral paw (Vivancos et al., 2004).

The Antinociceptive Action of 15d-PGJ2 Depends on PPAR-γ Activation. In an attempt to investigate whether the antinociceptive action of 15d-PGJ2 depends on PPAR-γ activation, we tested the effect of the selective PPAR-γ antagonist GW9662 upon 15d-PGJ2-induced antinociception. Local pretreatment of rats with GW9662 (0.3, 1, and 3 ng/paw), 15 min before 15d-PGJ2 (100 ng/paw) injection, reversed in a dose-dependent manner (P < 0.05) the antinociceptive activity of 15d-PGJ2 on PGE2-induced mechanical hypernociception (Fig. 2B).

The Antinociceptive Effect of 15d-PGJ2 Depends on Peripheral Resident Cells. To verify whether 15d-PGJ2 was exerting its antinociceptive effect by acting in the resident cells or directly in the peripheral sensitive neuron, animals were pretreated with 15d-PGJ2 by two different routes, intraplantar or intraganglionar. When intraplantarly administered, 15d-PGJ2 (100 ng/paw) inhibited the PGE2-induced hypernociception (100 ng/paw) as observed with morphine (6 μg/paw), which presents peripheral antinociceptive effects by acting directly on sensitive neurons. It is noteworthy that, when administered alone, 15d-PGJ2, did not show any alteration on the nociceptive threshold (right column; intraplantar administration). However, the intraganglionar injection of 15d-PGJ2 (100 ng/DRG) did not produce antinociceptive an effect upon PGE2-induced hypernociception (Fig. 3), whereas, as expected, intraganglionar administration with morphine

Fig. 2. 15d-PGJ2 inhibits PGE2-induced hypernociception: role of PPAR-γ. A, rats were treated with 15d-PGJ2 (30, 100, or 300 ng/paw) before i.pl. stimulus with PGE2 (100 ng/paw). B, animals were pretreated (30 min) with GW9662 (0.3, 1, or 3 ng/50 μl/i.pl.) or vehicle (saline), followed by 15d-PGJ2 administration (100 ng/50 μl/i.pl.). After 15 min, PGE2 was administered intraplantarly (100 ng/50 μl/i.pl.). Mechanical hypernociceptive threshold was evaluated 3 h after administration of PGE2 by electronic von Frey test. The results are expressed as the mean ± S.D. of 10 animals per group. *, statistical significance compared to saline group; **, statistical significance compared to PGE2 control group; and #, statistical significance compared to vehicle plus 15d-PGJ2 group. P < 0.05, one-way ANOVA followed by the Bonferroni’s test.

Fig. 3. 15d-PGJ2 does not inhibit PGE2-induced hypernociception when administered into the DRG. Mechanical hypernociception induced by i.pl. injection of PGE2 (100 ng/100 μl/i.pl.) was inhibited by intraplantar injection of 15d-PGJ2 (100 ng/10 μl/i.pl.) but not by intraganglionar injection of 15d-PGJ2 (100 ng/10 μl/intraganglionar) 15 min previously. Morphine (6 μg/i.pl. or intraganglionar) was used as a control. Direct injection of 15d-PGJ2 by intraplantar (black bars) or intraganglionar (open bars) administration did not show any threshold alteration (right column). Mechanical hypernociceptive threshold was evaluated 3 h after administration of PGE2 by electronic von Frey test. The results are expressed as the mean ± S.D. of 10 animals per group. *, statistical significance compared to vehicle group. P < 0.05, one-way ANOVA followed by the Bonferroni’s test.

Activation of Peripheral Opioid Receptors Mediates 15d-PGJ2-Antinociceptive Effects. Figure 4A shows that locally administered naloxone (1 μg/paw) abolished the antinociceptive effect of 15d-PGJ2 (100 ng/paw). This result suggests that the antinociceptive action of 15d-PGJ2 depends on local opioid receptor activation. Considering that one of the characteristics of opioid agonists is its capacity to reverse already established hypernociception induced by PGE2 (Ferreira, 1979), we sought to verify the effect of post-treatment with 15d-PGJ2 on mechanical hypernociception induced by

Fig. 4. 15d-PGJ2 induces antinociception by stimulating the release of endogenous opioids. A, 15d-PGJ2 (100 ng/50 μl/i.pl.) or saline (50 μl/i.pl.) was administered 30 min before PGE2 (100 ng/50 μl/i.pl.) injection. Naloxone (NLX; 1 μg/50 μl/i.pl.) abolished the antinociceptive effect of 15d-PGJ2. B, the antinociceptive effect of post-treatment with 15d-PGJ2 (1 h after PGE2 injection) on PGE2-evoked hypernociception was evaluated. Mechanical hypernociceptive threshold was evaluated 3 h after administration of PGE2 by electronic von Frey test. The results are expressed as the mean ± S.D. of 10 animals per group. *, statistical significance compared to saline group; **, statistical significance compared to vehicle plus PGE2 group; #, statistical significance compared to vehicle plus 15d-PGJ2 group. P < 0.05, one-way ANOVA followed by the Bonferroni’s test.
PGE2. Similar to pretreatment (30 min before PGE2 injection), local post-treatment with 15d-PGJ2 (100 ng/paw), 1 h after PGE2 injection, significantly reduced \(P < 0.05\) PGE2-induced mechanical hypernociception (Fig. 4B).

**Macrophages Seem to Be Involved in the Antinociceptive Effect of 15d-PGJ2:** Participation of Opioid Receptors. In an attempt to investigate whether the source of opioid release induced by 15d-PGJ2 could be the result of resident macrophages, we tested the antinociceptive effect of a subliminal dose of 15d-PGJ2 in a group of animals presenting an increased accumulation of macrophages in their paws induced by a previous administration of thioglycollate. Animals that received an intraplantar administration of thioglycollate did not show significant change in their nociceptive threshold after 3 days (data not shown). Although the intensity of hypernociception induced by PGE2 was similar in naive rats and rats that received thioglycollate injection, this latter group of animals (100 ng/paw) presented a marked antinociception induced by the subliminal dose of 15d-PGJ2 (30 ng/paw). This effect was also reversed by the local pretreatment with naloxone (Fig. 5A). To confirm that thioglycollate administration increased the number of macrophages in the rat paw, it was observed that NAG activity in the injected paw increased significantly (Fig. 5B). The increase in paw tissue macrophage population by thioglycollate injection was also confirmed by histopathological sections (data not shown).

**The NO/cGMP/PKG/KATP Pathway Mediates the Antinociceptive Effect of 15d-PGJ2.** The pretreatment with an inhibitor of the enzyme NOS l-NMMA (50 µg/paw), the specific inhibitor of the soluble guanylate cyclase ODQ (8 µg/paw), the PKG inhibitor KT5823 (1.5 µg/paw), or the K\(_{ATP}\) channel blocker glibenclamide (160 µg/paw) 30 min before 15d-PGJ2 injection abolished the antinociceptive effect of 15d-PGJ2 upon PGE2-induced hypernociception (Fig. 6, A–D, respectively).

**Discussion**

Several investigations showed that 15d-PGJ2 presents anti-inflammatory effects (Jiang et al., 1998; Ricote et al., 1998). For instance, its systemic administration ameliorates experimental inflammatory diseases, including ischemia-reperfusion injury and arthritis (Kawahito et al., 2000; Chatterjee et al., 2004). In the present study, we demonstrated for the first time an antinociceptive action of 15d-PGJ2 upon mechanical hypernociception. The local treatment of rats’ hind paw with 15d-PGJ2 inhibited carrageenan- and PGE2-induced mechanical hypernociception. It also inhibited formalin-induced TMJ nociception. Furthermore, our results showed that such effects might depend on PPAR-\(\gamma\)

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**Fig. 5.** Role of macrophages in the 15d-PGJ2 antinociceptive effect: participation of opioid receptors. A, control groups received an i.pl. injection of PGE2 (100 ng/50 µl; first bar) or pretreatment with a subliminal dose of 15d-PGJ2 (30 ng/50 µl.i.pl.) and, after 15 min, PGE2 (100 ng/50 µl.i.pl.; second bar). Rats were pretreated with 1% thioglycollate (100 µl.i.pl.), and after 3 days, it was administered in the following treatments: PGE2 (100 ng/50 µl.i.pl.; third bar); pretreatment with 15d-PGJ2 (30 ng/50 µl.i.pl.) and, after 15 min, PGE2 (100 ng/50 µl.i.pl.; fourth bar); and pretreatment (30 min) with naloxone (1 µg/50 µl.i.pl.) followed by injection of PGE2 (100 ng/50 µl.i.pl.; fifth bar) or pretreatment (30 min) with naloxone (1 µg/50 µl.i.pl.) followed by 15d-PGJ2 (30 ng/50 µl.i.pl.) administration. After 15 min, PGE2 (100 ng/50 µl.i.pl.) was injected (sixth bar). Mechanical hypernociceptive threshold was evaluated 3 h after administration of PGE2 by electronic von Frey test. The results are expressed as the mean ± S.D. of 10 animals per group. *, statistical significance compared to saline group; **, statistical significance compared to groups treated with 15d-PGJ2 plus PGE2 group. P < 0.05, one-way ANOVA followed by the Bonferroni’s test.

**Fig. 6.** Inhibition of 15d-PGJ2 antinociception by antagonists of the NO/cGMP/PKG/K\(_{ATP}\) pathway. Rats were pretreated with the antagonists of the NO pathway 15 min before 15d-PGJ2 and 30 min before PGE2-induced hypernociception. A, pretreatment with l-NMMA (50 ng/50 µl.i.pl.). B, pretreatment with ODQ (8 µg/50 µl.i.pl.). C, pretreatment with KT5823 (1.5 µg/50 µl.i.pl.). D, pretreatment with glibenclamide (Gbl; 160 µg/50 µl.i.pl.). The intensity of hypernociception was evaluated 3 h after administration of PGE2 by electronic von Frey test. Data are expressed as the mean ± S.D. of 10 animals per group. *, statistical significance compared to saline group; **, statistical significance compared to PGE2 control group; #, statistical significance compared to vehicle plus 15d-PGJ2 group. P < 0.05, one-way ANOVA followed by the Bonferroni’s test.
activation, peripheral opioid receptor activation, and resident macrophages.

Previous studies have demonstrated that inflammatory hypernociception induced by carrageenan results in release of a cascade of mediators initiated by the production of the hypernociceptive cytokines TNF-α, interleukin-1β, and chemokines. These cytokines stimulate the release of the directly acting hypernociceptive mediators represented mainly by prostaglandins, which directly act on nociceptive neurons (Cunha et al., 2005; Verri et al., 2006). Regarding the anti-inflammatory mechanism of 15d-PGJ2, it has been demonstrated that it inhibited the expression of several inflammatory molecules, such as COX-2 and TNF-α (Jiang et al., 1998; Ricote et al., 1998). However, in our experimental model, 15d-PGJ2 was not able to inhibit the production of TNF-α but inhibited TNF-α and PGE2-induced mechanical hypernociception. In agreement, the hypernociceptive effect of TNF-α is dependent on prostaglandin production (Cunha et al., 1992). It is important to mention that analogues that act by inhibition of COX (NSAID) or cytokine production (thalidomide) are not able to affect PGE2-induced hypernociception (Ribeiro et al., 2000). On the other hand, there are peripheral antinociceptive drugs, such as peripheral opioids, that inhibit the ongoing hypernociception induced by PGE2 (Ferreira, 1972; Sachs et al., 2004). Therefore, we are suggesting that 15d-PGJ2 presents a peripheral opioid-like effect.

The fact that 15d-PGJ2 did not inhibit carrageenan-induced thermal hypernociception suggests that different mechanisms are involved in the genesis of thermal and mechanical hypernociception. In fact, the thermal test detects mainly the superficial nociceptive fiber activation, whereas our mechanical test detects s.c. nociceptor activation. Recently, we demonstrated that different mechanisms are involved in the activation of these different nociceptive fibers (Vivancos et al., 2003). Further supporting the antinociceptive action of 15d-PGJ2, although it did not affect formalin induced-nociception in cutaneous tissues, our results demonstrated an inhibition of formalin-induced nociception in TMJ. It is well demonstrated that this test has an inflammatory component (Clemente et al., 2004). It is clear that inflammatory conditions can result in hyperalgesia produced by peripheral sensitization of nociceptors and by central sensitization of the nociceptive neurons. The difference between the s.c. and TMJ nociceptors may result from the fact that both tissues are predominantly innervated by different subsets of primary nociceptive neurons. Because deep inputs may be more effective in inducing central neuronal excitation than cutaneous inputs, greater sensory disturbances may occur in pain conditions involving deep tissues than in those involving cutaneous tissues (Imbe et al., 2001). Otherwise, it has been demonstrated that the TMJ tissues are more sensitive to sympathomimetic amines and PGE2 than cutaneous tissues (Rodrigues et al., 2006). Thus, we hypothesized that TMJ is more sensitive to prostaglandins in comparison to paw tissue, which could explain our results.

Studies show that the activity of 15d-PGJ2 depends either on PPAR-γ activation and/or on mechanisms independent of this nuclear receptor (Straus et al., 2000). The blockade of ongoing hypernociception by 15d-PGJ2 was dependent on PPAR-γ activation. In line with our results, the antinociceptive role of PPAR-γ has already been demonstrated in different models of inflammatory pain. Oliveira et al. (2007) observed that systemic administration of pioglitazone, a thiazolidinedione that selectively activates PPAR-γ, reduces the second phase of formalin test (inflammatory phase). However, pioglitazone is ineffective upon the first phase of the formalin test, which is considered to be a noninflammatory phase. Likewise, paw administration of 15d-PGJ2 did not alter the peripheral nociceptive threshold of naive animals, confirming that the antinociceptive effect of PPAR-γ activation is restricted to the ongoing inflammatory process. On the other hand, injection of the PPAR-γ agonist in the mouse paw did not reduce the second phase of formalin-induced nociception (LoVerme et al., 2006). Corroborating this effect, we observed that local administration of 15d-PGJ2 did not inhibit formalin-induced nociception in the rat paw (data not shown). Another contradiction between our study and the study conducted by Oliveira et al. (2007) is the fact that, in this latter study, the PPAR-γ agonist did not inhibit carrageenan-induced mechanical hypernociception. These contradictions could be a result of differences in PPAR-γ agonists that could have different pharmacological profiles, as well the routes of administration of the PPAR-γ agonist used, or doses of carrageenan. Moreover, Oliveira et al. (2007) used a variation of the von Frey method in which only one filament was used and the frequency of the paw withdrawals was analyzed. In the present study, an electronic device detected the pressure necessary to induce a withdrawal response, which is more sensible than the von Frey filaments that detect differences between doses (Vivancos et al., 2004). Thus, all of these differences may explain the discrepant results.

The antinociceptive effect of 15d-PGJ2 seems to be limited to the periphery. Actually, previous evidence indicated that peripheral endogenous antinociceptive mechanisms may counteract inflammatory pain through the release of endogenous opioid peptides or anti-inflammatory cytokines (Stein et al., 2003; Verri et al., 2006). These findings, together with the fact that 15d-PGJ2 post-treatment, similar to peripheral opioids, directly blocked established hypernociception induced by PGE2, raised the hypothesis that 15d-PGJ2 is promoting peripheral analgesia by the stimulation of endogenous opioid release. Accordingly, naloxone prevented the antinociceptive action of 15d-PGJ2. It is noteworthy that these results do not exclude the possibility that 15d-PGJ2 is directly activating opioid receptors present in primary sensitive neurons. Nevertheless, we observed that, unlike morphine (Ferrari et al., 2007), 15d-PGJ2 was not able to cause antinociception when administered intraganglionarly, supporting the suggestion that 15d-PGJ2 is not acting directly on sensitive neurons and pointing to an action through endogenous opioid release by paw resident cells. The involvement of peripheral endogenous opioid release in the antinociceptive effect of other drugs has already been demonstrated (Mousa et al., 1996; Ibrahim et al., 2005). For instance, CB2 cannabinoid receptor activation produces peripheral antinociception by stimulating β-endorphin release from resident keratinocytes, which act at local neuronal μ-opioid receptors (Ibrahim et al., 2005).

In an attempt to determine the cell source of peripheral opioid release stimulated by 15d-PGJ2, it was observed that increasing the number of peripheral macrophages by previous administration of thioglycollate in the rat paw enhances the antinociceptive effect of 15d-PGJ2. This result suggests
that the naloxone-sensitive antinociceptive effect of 15d-PGJ\(_2\) probably depends on paw skin macrophages. Indeed, there is evidence that opioid-containing macrophages are involved in the endogenous control of inflammatory pain (Brack et al., 2004). Furthermore, PPAR-\(\gamma\) is markedly expressed in activated macrophages, which also suggests the possibility of PPAR-\(\gamma\) activation by 15d-PGJ\(_2\) resulting in the release of opioids by resident macrophages (Ricote et al., 1998). However, the supernatants of macrophage cultures stimulated with 15d-PGJ\(_2\) were not able to inhibit PGE\(_2\)-induced hypernociception (data not shown). A possible explanation is that the amount released in vitro was not enough to produce antinociception in vivo. Alternatively, the production of endogenous opioids depends on macrophages but also on other resident cells present in the paw of the animals. Keratinocytes are a possible alternative, which are known sources of endogenous opioids as mentioned previously (Ibrahim et al., 2005).

Similar to peripheral opioids, the antinociceptive mechanism of 15d-PGJ\(_2\) involves activation of the \(\alpha\)-arginine/NO/cGMP/PKG pathway. This conclusion is supported by the observation that the peripheral antinociceptive activity of 15d-PGJ\(_2\) was prevented by inhibitors of NOS, cGMP, and PKG. The analgesic activity of the \(\alpha\)-arginine/NO/cGMP/PKG pathway is opened by opening \(\mathcal{K}_{\text{ATP}}\) channels (Sachs et al., 2004). This concept is based on the observation that the peripheral antinociceptive activities of opioids, NO donors and cGMP, are inhibited by \(\mathcal{K}_{\text{ATP}}\) channel blockers (Sachs et al., 2004). Further investigating peripheral opioids in the antinociceptive effect of 15d-PGJ\(_2\), it was observed that glibenclamide prevented the peripheral antinociceptive effect of 15d-PGJ\(_2\). Although the activation of \(\alpha\)-arginine/NO/cGMP/PKG/\(\mathcal{K}_{\text{ATP}}\) channel pathway seems to be involved in peripheral analgesic activity of opioids, there is overwhelming evidence that this analgesic effect of opioids also depends on inhibition of cAMP formation and of Ca\(^{2+}\) channels present in the peripheral nociceptive neurons (Levine and Taiwo, 1989; Stein et al., 2003). Thus, although the activation of \(\alpha\)-arginine/NO/cGMP/PKG/\(\mathcal{K}_{\text{ATP}}\) channel pathway is pharmacologically relevant for 15d-PGJ\(_2\) activity, it did not disprove the involvement of inhibition of cAMP formation and of Ca\(^{2+}\) translocation as well in the 15d-PGJ\(_2\) analgesic effect.

Besides the opioids system, there is also evidence of interaction between PPAR-\(\gamma\) and cannabinoids (Burstein, 2005). Indeed, it has been demonstrated that cannabinoid receptor agonists present effect via activation of the PPAR-\(\gamma\) receptor. Although we did not investigate the participation of endogenous cannabinoids in the antinociceptive action of 15d-PGJ\(_2\), there is evidence that PPAR-\(\alpha\) synergizes with cannabinoids to produce analgesia (Russo et al., 2007). Therefore, further studies are necessary to elucidate the contribution of endocannabinoids to the antinociceptive effect of 15d-PGJ\(_2\).

In summary, we demonstrated that 15d-PGJ\(_2\) presents an aperipheral antinociceptive effect, which depends on activation of PPAR-\(\gamma\) and peripheral opioid receptors. It seems that 15d-PGJ\(_2\) promotes an endogenous opioid release, which may be related to macrophages. In conclusion, we are adding further data supporting the potential therapeutic use of 15d-PGJ\(_2\) in the control of inflammatory pain. Furthermore, our results may contribute to a better comprehension of experimental data in which 15d-PGJ\(_2\) is used as a pharmacological tool and may provide new insights for the development of novel analgesics.

Acknowledgments
We thank Ieda Regina dos Santos Schivo and Sérgio Roberto Rosa for technical assistance.

References


