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The anti-inflammatory effect of tramadol in the temporomandibular joint of rats

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ABSTRACT

Tramadol is a centrally acting analgesic drug able to prevent nociceptor sensitization when administered into the temporomandibular joint (TMJ) of rats. The mechanism underlying the peripheral anti-inflammatory effect of tramadol remains unknown. This study demonstrated that intra-TMJ injection of tramadol (500 μg/TMJ) was able to inhibit the nociceptive response induced by 1.5% formalin or 1.5% capsaicin, suggesting that tramadol has an antinociceptive effect, acting directly on the primary nociceptive neurons activating the nitric oxide/cyclic guanosine monophosphate signaling pathway. Tramadol also inhibited the nociceptive response induced by carrageenan (100 μg/TMJ) or 5-hydroxytryptamine (225 μg/TMJ) along with inhibition of inflammatory cytokines levels, leukocytes migration and plasma extravasation. In conclusion, the results demonstrate that peripheral administration of tramadol has a potential antinociceptive and anti-inflammatory effect. The antinociceptive effect is mediated by activation of the intracellular nitric oxide/cyclic guanosine monophosphate pathway, at least in part, independently from the opioid system.

1. Introduction

Tramadol, 2-(dimethylaminomethyl)-1-(3-methoxyphenyl)cyclohexan-1-ol, is a classical analgesic drug that acts directly on the central nervous system (CNS) (Minami et al., 2015). The effect of tramadol on the CNS is well known to be important in pain modulation (Lewis and Han, 1997; Rawal et al., 2001; Sindrup et al., 1999; Mishra et al., 2008; Minami et al., 2015). Tramadol is a racemic mixture consisting of two isomers with different spectrums of pharmacological activity. In the CNS, tramadol activates both opioid and non-opioid (descending monoaminergic) systems, which are mainly involved in pain inhibition (Sindrup et al., 1999; Scott and Perry, 2000; Gillen et al., 2000; Altunkaya et al., 2003; Minami et al., 2015). The presence of tramadol in peripheral tissues results in interactions with additional cellular targets, such as sodium channels (Haeseler et al., 2006) associated with ionotropic glutamate receptor N-methyl-D-aspartate (NMDA) (Hara et al., 2005), transient receptor potential cation channel, subfamily V, member 1 (TRPV1) (Marincsák et al., 2008) and adenosine A1 receptors (Sawynok et al., 2013).

Local tramadol administration produces antinociception in the knee joint (Garlicki et al., 2006; Mert et al., 2007) and hind paw of rats (Souza et al., 2008). There is also evidence that locally administered tramadol produces analgesia in humans with few side effects (Pang et al., 1998; Demiraran et al., 2006; Ceccheti et al., 2014). In the temporomandibular joint (TMJ), it has been demonstrated that intra-articular injection of tramadol following arthrocentesis resulted in pain relief for up to 3 h after administration when compared to controls (Sipahi et al., 2015), suggesting an analgesic and a possible anti-inflammatory effect. Nevertheless, whether tramadol promotes analgesia in the peripheral nervous system remains to be established.

Temporomandibular disorders are among the commonest culprits of orofacial pain mediated by inflammatory mediators in substantial amounts, which, in many cases, lead to chronic orofacial pain (Cairns, 2010; Sessle, 2011). The management of symptomatic internal TMJ derangement using localized therapeutic approaches has always been challenging (Cairns, 2010; Sessle, 2011). Considering that tramadol has been used for localized therapeutic approaches in the TMJ, the purpose of this study was to evaluate the potential peripheral antinociceptive effect of tramadol on induced TMJ pain in rats as well as to elucidate the possible mechanisms involved therein.

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2. Methods

2.1. Animals

This study was performed on male Wistar rats (± 200 g), n=6 per group, 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. All animal experimental procedures and protocols were approved by the Committee on Animal Research of the University of Campinas (CEUA/UNICAMP no. 2983-1) and are in accordance with the guidelines by the National Council for Control of Animal Experimentation (CONCEA), ARRIVE (Kilkenny et al., 2010) and International Association for the Study of Pain (IASP) for the study of pain in conscious animals (Zimmermann, 1983). Each animal was used only once.

2.2. Drugs

Tramadol (O-Desmethyl-cis-tramadol-D6 hydrochloride solution), inhibitor of nitric oxide synthase (NOS) NG-Methyl-L-arginine acetate salt (L-NMMA), ATP-potassium sensitive channel blocker (glibenclamide), carrageenan, 1.5% capsaicin, 5-hydroxytryptamine (5-HT), PI3K inhibitor 5-(6Quinoxalinylmethylene)-2,4-thiazolidinedione (AS605240), AKT inhibitor IV trifluoroacetate salt and an aqueous solution of 37% formaldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). A nonselective antagonist of opioid receptor (5α)-4,5-Epoxy-3,14-dihydro-17-(2-propenyl) morphinan-6-one hydrochloride (naloxone hydrochloride); inhibitor of soluble cyclic GMP H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ); inhibitor of protein kinase G (9S,10R,12R)-2,3,9,10,11,12-Hexahydro-10-methoxy-2,9-dimethyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl] pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid, methyl ester (KT 5823) were obtained from Tocris Bioscience (Bristol, United Kingdom). Formalin solution was prepared from commercially available formalin further diluted in 0.9% NaCl to a final concentration of 1.5%. Tramadol, L-NMMA, glibenclamide, carrageenan, 5-HT, AS605240, AKT inhibitor, naloxone and KT5823 were dissolved in 0.9% NaCl solution. ODQ and glibenclamide were dissolved in saline and dimethyl sulfoxide (2%, Sigma, St. Louis, MO, USA) vehicle. Capsaicin solution was prepared from 10% capsaicin in ethanol, tween 80 and sterile saline in a 1:1:8 ratio by volume (Lam et al., 2005).

2.3. Experimental design

2.3.1. Effect of tramadol on formalin-induced TMJ nociception

To confirm that tramadol induces antinociception in the TMJ, groups of rats (n=6) were treated with co-administration of intra-TMJ tramadol (125, 250, 500 or 1000 μg/TMJ) plus 1.5% formalin (15 μl/TMJ). All animals received a total volume of 45 μl of the solutions into their TMJ. After injection of formalin into the TMJ, nociceptive behavior was evaluated over a period of 45 min (Roveroni et al., 2001; Clemente et al., 2004). At the end of this test, the animals were euthanized and their periarticular tissues removed to evaluate the release of cytokines, namely tumor necrosis factor alpha (TNF-α) and interleukin-1 beta (IL-1β).

2.3.2. Role of opioid receptors on tramadol-induced antinociception in the TMJ

To confirm that tramadol induces antinociception in the TMJ by activation of opioid receptors, a group of rats (n=6/ group) was pretreated (15 min) with an intra-TMJ injection of naloxone (10 μg/TMJ), a non-selective opioid receptor antagonist (Eisenberg et al., 1996). Subsequently, tramadol (500 μg/TMJ) was injected with 1.5% formalin (15 μl/TMJ). All animals received a total volume of 45 μl solution into their TMJ. After injecting formalin into the TMJ, the nociceptive behavior was assessed over a period of 45 min (Roveroni et al., 2001; Clemente et al., 2004).

2.3.3. Role of intracellular and PI3Kγ/AKT/nNOS/NO/K_{ATP} signaling pathway on tramadol-induced antinociception in the TMJ

Animals (n=6/ group) were pretreated (15 min) with an intra-TMJ injection of the PI3K inhibitor (AS605240, 30 μg/TMJ) (Cunha et al., 2012), or the selective inhibitor of protein kinase B (AKT inhibitor IV, 10 μg/TMJ) (Cunha et al., 2012), or the nonselective nitric oxide synthase inhibitor (NOS) (L-NMMA, 450 μg/TMJ) (Clemente-Napimoga et al., 2009), or the soluble cGMP enzyme inhibitor (ODQ, 8 μg/TMJ) (Clemente-Napimoga et al., 2009) or the protein kinase G inhibitor (KT5823, 1.5 μg/TMJ) (Cunha et al., 2012) or the ATP-sensitive potassium channel blocker (glibenclamide, 160 μg/TMJ) (Raffa et al., 1992). Tramadol (500 μg/TMJ) was then injected combined with 1.5% formalin (15 μl/TMJ). All animals received a total volume of 45 μl of solutions into their TMJ, after which the nociceptive behavior was evaluated over a period of 45 min (Roveroni et al., 2001; Clemente et al., 2004).

2.3.4. Effect of tramadol on capsaicin-induced TMJ nociception

In order to test whether tramadol induces antinociception in the TMJ via inhibition of primary C-fibers, groups of rats (n=6/group) were treated by co-administering an intra-TMJ injection of tramadol (500 μg/TMJ) plus the agonist of primary C-fibers capsaicin (1.5%, 15 μl/TMJ). All animals received a total volume of 45 μl the solution into their TMJ, after which, the nociceptive behavior was evaluated over a period of 30 min. The animals were euthanized upon conclusion of the experiment.

2.3.5. Effect of tramadol on carrageenan-induced TMJ inflammatory hypernociception

To confirm that tramadol exerts an anti-inflammatory effect on the TMJ, groups of rats (n=6/ group) were pretreated (15 min) with an intra-TMJ injection of tramadol (125, 250 or 500 μg/TMJ). Subsequently, carrageenan (100 μg/TMJ), a neutrophil recruiter, was also injected in the same TMJ. After 1 h, a low dose of 5-hydroxytryptamine (5-HT, 75 μg/TMJ) (Rodrigues et al., 2006) was injected into the TMJ ipsilaterally. All animals received a total volume of 45 μl of solutions into their TMJ. After TMJ injection of 5-HT, the nociceptive behavior was assessed over a 30-min period (Rodrigues et al., 2006). The animals were euthanized upon conclusion of the nociceptive test and their periarticular tissues removed for further analyses.

2.3.6. Effect of tramadol on 5-HT-induced TMJ inflammatory hypernociception

In order to check whether the anti-inflammatory effect of tramadol on the TMJ is mediated by inhibition of prostanoids and sympathetic amines release, groups of rats (n=6/ group) were pretreated (15 min) with an intra-TMJ injection of tramadol (125, 250 or 500 μg/TMJ). Subsequently, 5-HT (225 μg/TMJ) was injected into the same TMJ. All animals received a total volume of 45 μl of the respective solutions into their TMJ, after which, the nociceptive behavior was evaluated over a period of 30 min (Oliveira-Fusaro et al., 2012). The animals were euthanized upon completion of the experiment.

2.4. TMJ injections

Rats were briefly anesthetized by inhalation of isoflurane (1.5%, 30-s period) and a 30-gauge needle was introduced into the TMJ. A cannula consisting of a polyethylene tube was connected to the needle and also to a Hamilton syringe (50 μl). Each rat regained consciousness approximately 30 s after discontinuing the anesthetic (Clemente et al., 2004). All experiments were conducted in a double-blind fashion in which the person who injected the solutions was different from the one who performed the nociceptive behavior test. All animals received a total volume of 45 μl of the respective solutions into their TMJ.

2.5. Testing procedure for TMJ pain

The nociceptive assay was performed during the light cycle