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Paradoxical effects of the cannabinoid CB_2 receptor agonist GW405833 on rat osteoarthritic knee joint pain

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SUMMARY

Objective: The present study examined whether local administration of the cannabinoid-2 (CB_2) receptor agonist GW405833 could modulate joint nociception in control rat knee joints and in an animal model of osteoarthritis (OA).

Method: OA was induced in male Wistar rats by intra-articular injection of sodium monoiodo-acetate with a recovery period of 14 days. Immunohistochemistry was used to evaluate the expression of CB_2 and transient receptor potential vanilloid channel-1 (TRPV1) receptors in the dorsal root ganglion (DRG) and synovial membrane of sham- and sodium mono-iodoacetate (MIA)-treated animals. Electrophysiological recordings were made from knee joint primary afferents in response to rotation of the joint both before and following close intra-arterial injection of different doses of GW405833. The effect of intra-articular GW405833 on joint pain perception was determined by hindlimb incapacitance. An *in vitro* neuronal release assay was used to see if GW405833 caused release of an inflammatory neuropeptide (calcitonin gene-related peptide – CGRP).

Results: CB_2 and TRPV1 receptors were co-localized in DRG neurons and synoviocytes in both sham- and MIA-treated animals. Local application of the GW405833 significantly reduced joint afferent firing rate by up to 31% in control knees. In OA knee joints, however, GW405833 had a pronounced sensitising effect on joint mechanoreceptors. Co-administration of GW405833 with the CB_2 receptor antagonist AM630 or pre-administration of the TRPV1 ion channel antagonist SB366791 attenuated the sensitising effect of GW405833. In the pain studies, intra-articular injection of GW405833 into OA knees augmented hindlimb incapacitance, but had no effect on pain behaviour in saline-injected control joints. GW405833 evoked increased CGRP release *via* a TRPV1 channel-dependent mechanism.

Conclusion: These data indicate that GW405833 reduces the mechanosensitivity of afferent nerve fibres in control joints but causes nociceptive responses in OA joints. The observed pro-nociceptive effect of GW405833 appears to involve TRPV1 receptors.

Introduction

Osteoarthritis (OA) is the most common form of arthritis and is characterized by extensive remodelling of subchondral bone and permanent destruction of articular cartilage leading to joint pain. Currently, no disease modifying drugs are available; therefore, treatment of OA is primarily restricted to analgesics which often have limited efficacy and hazardous side-effects. An established animal model of OA pain involves the intra-articular injection of the glycolysis inhibitor sodium mono-iodoacetate (MIA) which disrupts cartilage metabolism, leading to OA like lesions[1–5], nerve sensitization and joint pain[6,7].

A family of agents which have shown great promise for the treatment of chronic pain are cannabinoids. Cannabinoid agonists suppress nociceptive transmission and inhibit pain related behaviour in different models of arthritis pain[7–10]. Two cannabinoid receptors, cannabinoid receptor 1 (CB_1 receptor) and cannabinoid receptor 2 (CB_2 receptor), have been cloned and characterized. The CB_1 receptor is present in the central and peripheral nervous system while the CB_2 receptor is predominantly associated with the immune system[11,12].

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The potential anti-inflammatory and analgesic effects of CB_2 specific cannabinoid agonists have been tested in various pain models[13–18]. Current cannabinoid pain therapies are frequently limited by central nervous system (CNS)-mediated side-effects; however, selective CB_2 receptor agonists have been shown to be devoid of such effects[14,18,19]. When applied systemically, the CB_2 receptor agonist GW405833 partially reversed the inflammation and hyperalgesia found in multiple acute inflammatory models[20,21].

In addition to being agonist ligands at cannabinoid receptors, several cannabinoids are also activators of the transient receptor potential vanilloid channel-1 (TRPV1)[22–25]. TRPV1 is expressed on nociceptive afferent neurones throughout the periphery and has been demonstrated to play a critical role in the induction of thermal hyperalgesia in inflammatory pain models[26–28]. Several studies have shown that the TRPV1 channel is involved in mediating the anti-nociceptive effect of CB_1 agonists[22,24,27–29]. Thus, certain cannabinoids may act as dual cannabinoid–vanilloid mediators, particularly under conditions of inflammatory hyperalgesia.

This study examined whether local application of the CB_2 receptor agonist GW405833 can reduce nociceptive activity of afferent nerve fibres and pain behaviour in control and MIA-treated rat knee joints. Since the pro-nociceptive neurotransmitter calcitonin gene-related peptide (CGRP) modulates joint mechanonociception centrally[30] and CGRP knockout arthritic mice do not develop secondary hyperalgesia[31], we looked at the release of CGRP from spinal cord homogenates in order to examine GW405833 activity at native TRPV1 receptors[32]. Activation of the receptor was measured in both the basal and stimulated/phosphorylated states to simulate the normal and diseased conditions respectively.

Methods

Animals

Experiments were performed on 217 male Wistar rats (250–450 g). The animal handling and surgical procedures outlined in this study all adhered to the Canadian Council guidelines for the care and use of experimental animals which also review animal ethics.

MIA model of OA

Fifty-three rats were deeply anaesthetised with 2% isoflurane in 100% O_2 (1 L/min). To induce OA, 50 μl of 3 mg sodium MIA in 0.9% saline was injected into the joint cavity through the patellar ligament. Animals were allowed to recover for 14 days which has consistently been shown to cause severe end-stage OA in this species[1,4,5]. In a further 42 animals, 50 μl of saline was injected into the knee joint and this cohort served as a sham control group.

Immunohistochemistry

CB_2 and TRPV1 expressions were evaluated in DRGs and synovial membrane from sham-injected ($n = 4$) and MIA-injected ($n = 4$) male Wistar rats. Animals were anaesthetised and perfused transcardially with saline followed by 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS), pH 7.4. DRG (L4–L6) and synovial membrane were dissected, post-fixed in the 4% PFA/PBS solution for 3 h at room temperature, and then transferred to a 30% sucrose/PBS solution and maintained overnight at 4°C. Tissue then was mounted in M1 embedding media (Thermo Shandon, Pittsburgh, PA, USA) and stored at −80°C. Sections 12–14 μm were cut from fixed tissue on a cryostat and mounted serially onto Superfrost Plus microscope slides (Fisher Scientific, Pittsburgh, PA, USA). After equilibration to room temperature, the sections were blocked for endogenous proteins for 1 h with 1× Dako Tris-buffered saline with Tween 20 wash buffer (Dako, Carpinteria, CA, USA) containing 1% BSA, 0.1% triton X100 and 10% normal serum of the host species of the second antibody. Sections were incubated overnight at 4°C with a cocktail of primary antibody of rabbit anti-CB_2 (gift from Dr. Ken Mackie, Indiana University, Bloomington, IN) and either guinea pig anti-TRPV1 for synovium (Neuromics, GP14100, Edina, MN, USA) or goat anti-TRPV1 for DRG (Santa Cruz, SC-12498, Santa Cruz, CA, USA), followed by several washes the next morning with 1× wash buffer. Negative staining was confirmed using a blocking peptide (CB_2) or omission of the primary antibody (TRPV1). The sections then were incubated for 1 h at room temperature with a cocktail of goat anti-guinea pig Alexa 594 and goat anti-rabbit Alexa 488 for synovium and a cocktail of donkey anti-goat Alexa 594 and donkey anti-rabbit 488 for the DRG. All secondary antibodies were from Molecular Probes (Invitrogen, Carlsbad, CA, USA). Following several washes with 1× wash buffer, the slides were cover-slipped with fluorescent mounting media and stored in the dark at 4°C for later analysis. Labeling was visualized on a Leica fluorescence microscope (Bannockburn, IL, USA) equipped with the appropriate filter blocks. Merged images were a composite of single-labeled files using a feature of Spot software (Diagnostic Instruments, Sterling Heights, MI, USA). For DRG, cell profiles were outlined and immunohistochemical staining (staining intensity) was recorded and analyzed using Image Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

Surgical procedures

The detailed methods for the surgical procedure have been described previously[7]. Rats were deeply anaesthetized with urethane (25% stock solution; 2 g/kg, i.p.). The left jugular vein was cannulated so that the muscle relaxant gallamine triethiodide (50 mg/kg) could be injected to eliminate neural interference arising from the hindlimb musculature. The right saphenous artery was cannulated below the knee joint to permit local close intra-arterial injection of drugs to the knee joint. Rotational torque was applied to the knee joint by placing the right hindpaw into a shoe-like holder which was connected to a force transducer and a torque meter.

Extracellular electrophysiological recording

The technique used for recording afferent activity from articular nerve fibres in the rat knee joint has been described previously[33,34]. The saphenous nerve was isolated in the inguinal region and cut centrally to prevent the generation of spinally-mediated reflexes. Neural strands were then placed over a platinum electrode to record single afferent fibre activity. The indifferent electrode was a silver wire electrode placed in the muscle tissue of the ipsilateral hindlimb. The receptive field of the fibres was identified by the elicitation of a response to gentle probing of the knee joint with a glass rod. The mechanical threshold of each recorded joint afferent was determined by a gradual increase of the torque applied to the joint until the fibre starts eliciting action potentials. The mechanosensitivity of articular afferents was measured in response to outward non-noxious rotation and noxious hyper-rotation of the knee joint. Since no differences were observed between the two movements in respect to the effect of the applied drugs, the data for both rotation movements were pooled for statistical analysis. The amount of force applied during the joint rotation ranged between 15 and 25 mNm for the non-noxious movement and between 35 and 45 mNm for the noxious movement. To establish a control baseline level of activity for each fibre, three movement cycles of the knee to discrete torque levels were performed at the beginning of the experiment and the mean afferent firing rate was taken as