Brazilian Journal of Medical and Biological Research (2012) 45: 1240-1243
ISSN 0100-879X Short Communication

Participation of cannabinoid receptors in peripheral nociception induced by some NSAIDs

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Abstract

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used extensively to control inflammatory pain. Several peripheral antinociceptive mechanisms have been described, such as opioid system and NO/cGMP/KATP pathway activation. There is evidence that the cannabinoid system can also contribute to the *in vivo* pharmacological effects of ibuprofen and indomethacin. However, there is no evidence of the involvement of the endocannabinoid system in the peripheral antinociception induced by NSAIDs. Thus, the aim of this study was to investigate the participation of the endocannabinoid system in the peripheral antinociceptive effect of NSAIDs. All experiments were performed on male Wistar rats (160-200 g; N = 4 per group). Hyperalgesia was induced by a subcutaneous intraplantar (*ipl*) injection of prostaglandin E_2 (PGE_2, 2 µg/paw) in the rat's hindpaw and measured by the paw pressure test 3 h after injection. The weight in grams required to elicit a nociceptive response, paw flexion, was determined as the nociceptive threshold. The hyperalgesia was calculated as the difference between the measurements made before and after PGE_2, which induced hyperalgesia (mean = 83.3 ± 4.505 g). AM-251 (80 µg/paw) and AM-630 (100 µg/paw) were used as CB_1 and CB_2 cannabinoid receptor antagonists, respectively. *Ipl* injection of 40 µg dipyrone (mean = 5.825 ± 2.842 g), 20 µg diclofenac (mean = 4.825 ± 3.850 g) and 40 µg indomethacin (mean = 6.650 ± 3.611 g) elicited a local peripheral antinociceptive effect. This effect was not antagonized by *ipl* CB_1 cannabinoid antagonist to dipyrone (mean = 5.00 ± 0.9815 g), diclofenac (mean = 2.50 ± 0.8337 g) and indomethacin (mean = 6.650 ± 4.069 g) or CB_2 cannabinoid antagonist to dipyrone (mean = 1.050 ± 6.436 g), diclofenac (mean = 6.675 ± 1.368 g) and indomethacin (mean = 2.85 ± 5.01 g). Thus, cannabinoid receptors do not seem to be involved in the peripheral antinociceptive mechanism of the NSAIDs dipyrone, diclofenac and indomethacin.

Key words: Nonsteroidal anti-inflammatory drugs (NSAIDs); Peripheral antinociception; Cannabinoid system

Introduction

Cannabinoids can be broadly defined as compounds with actions on cannabinoid receptors together with chemically related compounds. They include compounds derived from the plant *Cannabis sativa* with Δ^9-THC as prototype, the related group of synthetic drugs and finally the endogenous eicosanoids, with anandamide as the compound most extensively studied (1). At the peripheral level, cannabinoid receptors are known to be involved in primary afferent neuron modulation, inhibiting membrane excitation and Ca^{2+} conductance and also increasing potassium conductance, inducing a similar antinociceptive effect. The antinociceptive effect of the endocannabinoid system has been implicated in pain models (2).

Nonsteroidal anti-inflammatory drugs (NSAIDs) like dipyrone, diclofenac and indomethacin are widely prescribed for their antinociceptive and analgesic activity (3). The search for different mechanisms of NSAID-induced antinociception has greatly increased after investigators observed that inhibition of prostaglandin synthesis in the inflamed tissue is not the only pathway for this response. Previous studies have demonstrated that the opioid system and the NO/cGMP/KATP pathway could be involved in the antinociceptive mechanism of NSAIDs (4,5). There is evidence indicating that the cannabinoid system can contribute to the *in vivo* pharmacological effects of ibuprofen and indomethacin (6). Gühring et al. (7) have suggested that indomethacin may allow an increased synthesis of endocannabinoids from arachidonic acid by blocking cyclooxygenase (COX). The same investigators have shown that spinal pretreatment with AM-251 blocks the antinociception caused by indomethacin. However, there is no evidence of involvement of the endocannabinoid system in the peripheral antinociception induced by NSAIDs.

Thus, the objective of the present study was to investigate the participation of the CB_1 and CB_2 cannabinoid receptors in the peripheral antinociceptive effect of the NSAIDs dipyrone, diclofenac and indomethacin.

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Received March 10, 2012. Accepted August 3, 2012. Available online September 21, 2012. Published December 17, 2012.

Material and Methods

Animals

All experiments were performed on male Wistar rats (160-200 g) from CEBIO-UFMG (Universidade Federal de Minas Gerais) housed in a temperature-controlled room (23 ± 1ºC) on an automatic 12-h light/dark cycle (6:00-18:00 h). Food and water were freely available until the beginning of the experiments. Animals were used only once and sacrificed after the experiments.

All animal procedures and protocols were approved by the Ethics Committee for Animal Experimentation (CETEA) of the UFMG.

Measurement of hyperalgesia

Hyperalgesia was induced by a subcutaneous injection of prostaglandin E_2 (PGE_2; 2 µg) into the plantar surface of the hind paw and measured using the paw pressure test described by Randall and Selitto (8). An analgesimeter was used (Ugo-Basile, Italy) with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the hind paw. The weight in grams required to elicit the nociceptive response of paw flexion was determined as the nociceptive threshold. A cutoff value of 300 g was used to reduce the possibility of damage to the paws. The nociceptive threshold was measured in the right paw and determined as the average of three consecutive trials recorded before and 3 h after PGE_2 injection. The hyperalgesia was calculated as the difference between these two averages (Δ of nociceptive threshold) and reported in grams.

Drug administration

All drugs were administered by injecting a volume of 50 µL/paw, with the exception of PGE_2 (100 µL/paw). Diclofenac (Purifarma, Brazil) and dipyrone (Sigma, USA) were dissolved in isotonic saline, while indomethacin (Sigma) was dissolved in Tris-base buffer. The CB_1 and CB_2 cannabinoid receptor antagonists, AM-251 (Tocris, USA) and AM-630 (Tocris) were dissolved in 10% DMSO in saline. PGE_2 (Cayman, USA) was dissolved in 2% ethanol in saline.

Experimental protocol

NSAIDs were injected into the right hind paw 2:55 h after local injection of PGE_2. AM-251 and AM-630 were administered 10 min prior to the NSAIDs. The nociceptive threshold was assessed 3 h after local administration of PGE_2.

Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA) and the *post hoc* Bonferroni test for multiple comparisons. Probabilities of less than 5% ($P < 0.05$) were considered to be statistically significant.

Results

Dipyrone (40 µg), diclofenac (20 µg) and indomethacin (40 µg) injected into the right hind paw produced an antinociceptive response against the hyperalgesia induced by local injection of PGE_2 (2 µg/paw; Figure 1A and B). The

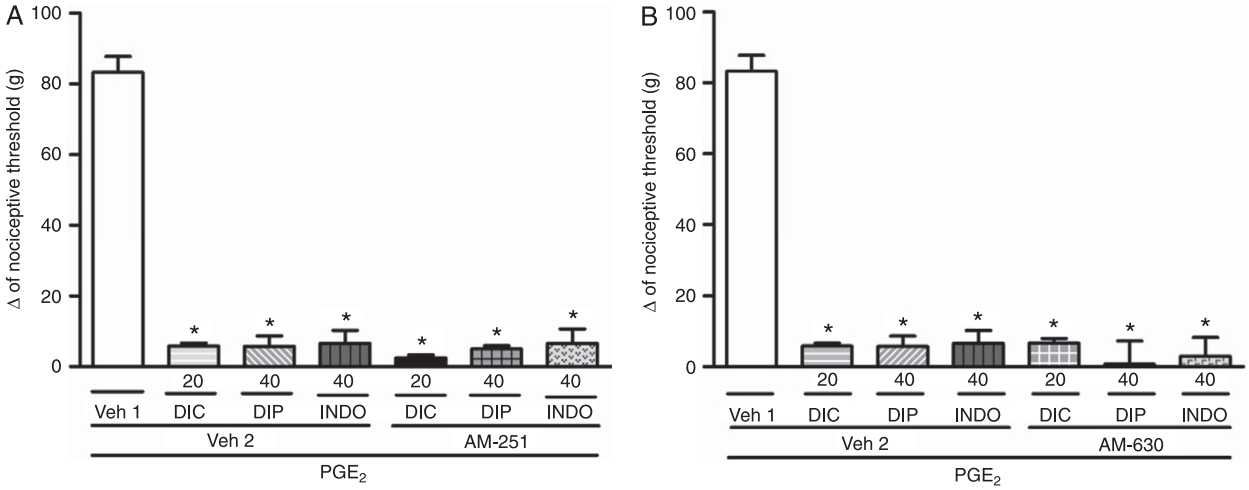

Figure 1. *A*, Effect induced by intraplantar administration of AM-251 (80 µg/paw) on nonsteroidal antinociceptive drug (NSAID)-induced peripheral antinociception in the hyperalgesic paw [prostaglandin E_2 (PGE_2) 2 µg]. AM-251 was injected 15 min prior to the NSAIDs diclofenac (DIC, 20 µg/paw), dipyrone (DIP, 40 µg/paw) and indomethacin (INDO, 40 µg/paw), which were administered 2:55 h after local administration of PGE_2. Data are reported as means ± SEM for N = 4 animals. *$P < 0.001$ compared to PGE_2 (ANOVA followed by the Bonferroni post-test). Veh 1 = saline; Veh 2 = 10% DMSO. *B*, Effect of intraplantar administration of AM-630 (100 µg/paw) on NSAID-induced peripheral antinociception in the hyperalgesic paw (PGE_2, 2 µg). AM-630 was injected 15 min prior to the NSAIDs DIC (20 µg/paw), DIP (40 µg/paw) and INDO (40 µg/paw), which were administered 2:55 h after local administration of PGE_2. Data are reported as means ± SEM for N = 4 animals. *$P < 0.001$ compared to PGE_2 (ANOVA followed by the Bonferroni post-test).

antinociceptive effects were not antagonized by the CB_1 cannabinoid receptor antagonist AM-251 (80 µg/paw; Figure 1A) or by the CB_2 cannabinoid receptor antagonist, AM-630 (100 µg/paw; Figure 1B). AM-251 and AM-630 had no effect when each was administered alone (data not shown).

Discussion

Since the discovery of the G-protein-coupled cannabinoid receptor (9,10) and the identification of anandamide, an endogenous cannabinoid ligand (11), there has been increased interest in the actions of cannabinoids.

To understand the physiological role of endocannabinoids, in-depth knowledge of their actions, biosynthesis and the factors that control it is required. Arachidonoyl ethanolamide (anandamide) is an endogenous ligand for CB_1 receptors that was shown to be a metabolite of arachidonic acid. Although it is not clear through which pathway arachidonic acid contributes to the synthesis of endocannabinoids, it was shown that arachidonic acid mobilization increases anandamide synthesis (12).

Endocannabinoids are metabolized by fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL) (12) and by other enzymes such as COX-2 (13). Thus, the inhibition of spinal COX-2 produces analgesia by inhibiting not only pronociceptive prostaglandins, but also endocannabinoid breakdown (14). A possible interaction between the analgesic activity of COX inhibitors and endocannabinoids was proposed in the behavioral study by Gühring et al. (7). This study showed that pretreatment of the spine with AM-251 blocked the indomethacin-induced antinociception of the formalin response or of zymosan-induced hyperalgesia. Moreover, these investigators added further evidence that indomethacin acts at the spinal level at least at three sites. First, it blocks the COXs, an action that results in higher levels of arachidonic acid used for endocannabinoid synthesis. Second, it lowers nitric oxide (NO) production, reducing the breakdown of endocannabinoids. Third, indomethacin inhibits FAAH, contributing to the sparing of endocannabinoid levels (7). On the basis of these interactions between COX inhibitors and the endocannabinoid system the present study assessed the participation of the endocannabinoid system in the peripheral antinociception induced by NSAIDs.

A recent study using the model of knee inflammation in rats demonstrated that administration of just COX-2 into the spine, but not COX-1 and non-selective inhibitors, reversed the spinal hyperexcitability. This result might be explained by the prevention of 2-arachidonoyl glycerol (2-AG) breakdown observed only when a selective COX-2 inhibitor was used and this response was prevented or partially reversed by AM-251. Thus, this interaction may depend on the sites of action of different COX inhibitors (14).

Our study showed that dipyrone, diclofenac and indomethacin induced antinociceptive effects. These effects were considered to be peripheral since NSAIDs administered into the right paw did not produce an antinociceptive effect in the left paw (data not shown). Moreover, NSAIDs did not induce antinociception when injected into non-hyperalgesic paws (data not shown). In addition to the classical inhibition of COX (15,16), some studies have indicated that opioids and the NO/cGMP/KATP pathway could be involved in the antinociceptive mechanism of NSAIDs (5,17). Additionally, the literature indicates that the suppression of sodium currents in sensory neurons by diclofenac and flufenamic acid would contribute to their analgesic activity (18). However, this does not seem to be the case in the present study because the doses used were specifically reversed by NO synthase and opioid receptor antagonists (Romero TRL, Duarte IDG, unpublished data).

It has been reported that AM-251 and AM-630, at the same doses and time of administration as used in the present study, were able to significantly decrease the antinociceptive effect of the CB_1 and CB_2 cannabinoid receptor agonists arachidonyl-2-chloroethylamide and N-palmitoyl-ethanolamine, respectively (data not shown), and the µ-opioid receptor agonist morphine (19). However, the present study demonstrated that AM-251 and AM-630 were not able to decrease the peripheral antinociceptive effect of dipyrone, diclofenac and indomethacin injected into the hind paw of rats.

The present results provide evidence that CB_1 and CB_2 cannabinoid receptors do not seem to be involved in the peripheral antinociceptive mechanism of the NSAIDs dipyrone, diclofenac and indomethacin.

Acknowledgments

Research supported by CNPq grants and fellowships.

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