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Introduction

N-Palmitoyl-ethanolamine (PEA), a member of the fatty-acid ethanolamide family (1), which is released together with anandamide from a common phospholipid precursor, exerts a similar effect by activating peripheral CB2-cannabinoid receptors (2). Evidence suggests that PEA is synthesized endogenously during inflammation and tissue damage to induce a number of beneficial effects, including the relief of inflammation and the control of neurogenic and neuropathic pain. PEA treatment in animals has demonstrated the efficacy of and great promise for its use in the treatment of neurogenic, inflammatory (3 – 6), and postoperative pain (7). These effects of PEA may occur through several possible mechanisms (8).

ATP-sensitive K+ channels (KATP) have been implicated in several studies as the final step of the peripheral antinociceptive mechanism of various analgesic drugs (9 – 11). The molecular mechanism of this process begins with the activation of nitric oxide (NO) synthase, which produces the second messenger NO. NO then increases the intracellular level of cGMP, which can activate KATP (12, 13). Based on experimental evidence that the peripheral antinociceptive effect of NO donors (14) and exogenous cGMP analogs (15) is mediated by KATP activation, it has been proposed that a link exists between the L-arginine/NO/cGMP pathway and KATP in the antinociceptive model (14, 15).

The L-arginine/NO/cGMP pathway (16, 17) has recently been implicated in the peripheral antinociceptive effect of PEA (18). Although the anti-inflammatory and antinociceptive effects of PEA were first characterized nearly 50 years ago, the identity of the final mechanism that mediates these actions has not been elucidated. Therefore, the aim of this present study was to verify the participation of KATP in the peripheral antinociception effect induced by local injection of PEA.

Abstract. Although the antinociceptive effects of N-palmitoyl-ethanolamine (PEA) were first characterized nearly 50 years ago, the identity of the mechanism that mediates these actions has not been elucidated. The present study investigated the contribution of K+ channels on peripheral antinociception induced by the CB2 agonist PEA. Nociceptive thresholds to mechanical paw stimulation of Wistar rats treated with intraplantar prostaglandin E2 to induce hyperalgesia were measured, and other agents were also given by local injection. PEA (5, 10, and 20 μg/paw) elicited a local peripheral antinociceptive effect. This effect was antagonized by glibenclamide, a selective blocker of ATP-sensitive K+ channels (20, 40, and 80 μg/paw). In addition, neither the voltage-dependent K+ channel–specific blocker tetraethylammonium (30 μg/paw) nor the small and large conductance blockers of Ca2+-activated K+ channels, dequalinium (50 μg/paw) and paxilline (20 μg/paw), respectively, were able to block the local antinociceptive effect of PEA. These results indicate that the activation of ATP-sensitive K+ channels could be the mechanism that induces peripheral antinociception by PEA and that voltage-dependent K+ channels and small and large conductance Ca2+-activated K+ channels do not appear to be involved in this mechanism.

Keywords: N-palmitoyl-ethanolamine (PEA), K+ channel, cannabinoid agonist, CB2 agonist, peripheral antinociception

Full Paper

N-Palmitoyl-ethanolamine (PEA) Induces Peripheral Antinociceptive Effect by ATP-Sensitive K+-Channel Activation

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Abstract. Although the antinociceptive effects of N-palmitoyl-ethanolamine (PEA) were first characterized nearly 50 years ago, the identity of the mechanism that mediates these actions has not been elucidated. The present study investigated the contribution of K+ channels on peripheral antinociception induced by the CB2 agonist PEA. Nociceptive thresholds to mechanical paw stimulation of Wistar rats treated with intraplantar prostaglandin E2 to induce hyperalgesia were measured, and other agents were also given by local injection. PEA (5, 10, and 20 μg/paw) elicited a local peripheral antinociceptive effect. This effect was antagonized by glibenclamide, a selective blocker of ATP-sensitive K+ channels (20, 40, and 80 μg/paw). In addition, neither the voltage-dependent K+ channel–specific blocker tetraethylammonium (30 μg/paw) nor the small and large conductance blockers of Ca2+-activated K+ channels, dequalinium (50 μg/paw) and paxilline (20 μg/paw), respectively, were able to block the local antinociceptive effect of PEA. These results indicate that the activation of ATP-sensitive K+ channels could be the mechanism that induces peripheral antinociception by PEA and that voltage-dependent K+ channels and small and large conductance Ca2+-activated K+ channels do not appear to be involved in this mechanism.

Keywords: N-palmitoyl-ethanolamine (PEA), K+ channel, cannabinoid agonist, CB2 agonist, peripheral antinociception
Materials and Methods

Animals
All experiments were performed on 160 – 200 g male Wistar rats (from CEBIO-UFMG, Belo Horizonte, Brazil). The rats were housed in a temperature-controlled room (23°C ± 1°C) on an automatic 12 h light/dark cycle (06:00 – 18:00 h). All tests were conducted during the light phase (08:00 – 15:00 h). Food and water were freely available until the onset of the experiments.

All animal procedures and protocols were approved by the Ethics Committee on Animal Experimentation (CETEA) of the Federal University of Minas Gerais (UFMG).

Measurement of hyperalgesia
Hyperalgesia was induced by subcutaneous injection of prostaglandin E2 (PGE2, 2 μg) into the plantar surface of the hind paw. Hyperalgesia was measured according to the paw pressure test described by Green and Young (19) and Randall and Sellito (20). An analgesimeter (Ugo-Basile, Comerio, Italy) was used with a cone-shaped paw-presser with a rounded tip, which applies a linearly increasing force to the hind paw. The weight in grams (g) 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 the three consecutive trials recorded before and 3 h after PGE2 injection. The hyperalgesia was calculated as the difference between these two averages (Δ of nociceptive threshold) and expressed in grams (g).

Drugs administration
In this study, we used the analgesic drug N-palmitoyl-ethanolamine (PEA), specific KATP blocker glibenclamide (21), nonselective voltage-dependent K+-channel blocker tetraethylammonium (22), selective blocker of small conductance Ca2+-activated K+ channels dequalinium chloride (23), and selective blocker of large conductance Ca2+-activated K+ channels paxilline (24).

PEA (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 6% DMSO in saline; tetraethylammonium chloride ([56-34-8] (C2H5)4N(Cl), Sigma-Aldrich), dequalinium chloride [1,1-(1,10-decanediyl)bis(4-amino-2-methylquinolinium)] dichloride; Calbiochem, San Diego, CA, USA] and paxilline chloride ((2R,4bS, 6As,12bS,12cR,14aS)-5,6,6a,7,1,12,12b,12c,13,14,14a-decahydro-4b-hydroxy-2-(1-hydroxy-1-methylethyl)-12b,12c-dimethyl-2H-pyranolo[2″,3″:5′,6′]benz[1′,2′:2,3′]indenol-3(4bh)-one, Sigma-Aldrich) were dissolved in isotonic saline. Glibenclamide {glyburide/5-chloro-N[2-[4-[[cylohexylamino]carbonyl]sulfonyl]phenyl][ethyl]-2methoxybenzamide, Sigma-Aldrich} was dissolved in 1% Tween in isotonic saline. PGE2 (Sigma-Aldrich) was dissolved in 2% ethanol in saline. All drugs were administered using an injected volume of 50 μl/paw, with the exception of PGE2 (100 μl/paw).

Experimental protocol
PEA was administered in the right hind paw 2 h and 55 min after local injection of PGE2. In the protocol used to determine whether PEA was acting outside the injection paw, PGE2 was injected into both hind paws, while PEA was administered 2 h and 55 min later into the right paw, after which the nociceptive threshold was measured in both hind paws.

Tetraethylammonium was administered 30 min prior to PEA and the other K+-channel blockers, glibenclamide, dequalinium, and paxilline, were administered 5 min prior to PEA.

The protocols concerning dose and time of administration of each drug used in this study were obtained through literature data and pilot experiments.

Statistical analysis
The data were statistically analyzed 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

Peripheral antinociceptive effect of PEA
Figure 1 shows the dose-dependent antinociceptive effect induced by PEA (05, 10, and 20 μg) into the right hind paw in PGE2-induced hyperalgesia (2 μg/paw) model. In addition, PEA in a dose of 20 μg injected alone did not alter the nociceptive threshold. Although the dose of 20 μg/paw was able to reverse the hyperalgesia induced by PGE2 almost completely, this dose did not produce any antinociceptive effect in the left paw, indicating that, at this dose, it presented only a peripheral site of action.

Involvement of the K+ channels in peripheral PEA-induced antinociception
The KATP specific blocker glibenclamide (20, 40, and 80 μg/paw) dose-dependently blocked the PEA (20 μg/paw) induced peripheral antinociception (Fig. 2). Glibenclamide alone did not induce hyperalgesia or any overt behavioral effect (same figure).

Another experiment verified that the voltage-dependent K+-specific-channel blocker tetraethylammonium (30
μg), the selective blocker of small conductance Ca²⁺-activated K⁺ channels dequalinium (50 μg/paw), and the blocker of large conductance Ca²⁺-activated K⁺ channels paxilline (20 μg/paw) injected into the rat paw did not significantly reduce the peripheral PEA-induced antinociception (20 μg/paw) in the PGE₂-induced hyperalgesia model (Fig. 3).

Discussion

PEA demonstrated an antinociceptive effect (3 – 6, 25) and its action has been confirmed in primary afferent neurons (26) and in the dorsal root ganglion (27). Intraplantar injection of PEA has been shown to reduce nocifensive behaviors elicited by the intraplantar injection of formalin, complete Freund’s adjuvant, and carrageenan in models of inflammatory pain (25). The present study demonstrated that a local dose of PEA (20 μg/paw) was...
able to elicit a peripheral antinociceptive effect in rat paw PGE$_2$-induced hyperalgesia observed in the paw pressure test. A single injection of PGE$_2$ in the rat paw is able to sensitize nociceptors to chemical and mechanical stimuli (28) with the advantage of excluding the possibility that the peripheral effects of the analgesic drugs result from blockage of the release or the action of mediators produced during the inflammatory process.

In order to exclude the possibility that the antinociception effects of PEA at a dose of 20 μg/paw did not arise at sites outside the paw, PEA was administered into the right paw under the same tissue conditions, and the nociceptive threshold was measured in both hind paws. PEA did not produce antinociception in the left paw, indicating that a dose of 20 μg/paw caused only local peripheral action.

Recently, it was proposed that PEA could activate NO/cGMP in sensory neurons through neuronal NOS, producing peripheral antinociception (18). It has been observed that this pathway can activate KATP (29, 30).

Sulphonylureas, like glibenclamide, alter single-channel kinetics in a manner similar to ATP; they reduce the open time and burst durations and increase the frequency and duration of the interburst intervals (31, 32), thereby inducing a block in the antinociceptive effect of analgesic drugs. In the current work, the specific KATP blocker glibenclamide (21) was used to show that KATP is involved in the peripheral antinociception induced by PEA.

Felder et al. (33) demonstrated that in contrast to CB$_1$ cannabinoid–receptor agonists, CB$_2$ cannabinoid–receptor agonists had no effect on the K$^+$ ion currents in transfected Chinese hamster ovary cells. In their study, these results are explained by the expression of three different Gai protein in the cells used. Different β-subunits determine how a G-protein interacts with transmembrane receptors (34). The lack of the appropriate βγ subunit expression in these cells is unlikely to cause the failure of the CB$_2$ cannabinoid receptor to couple to K$_v$ (33). These results contradict this present work; however, this could be explained by the fact that the first work was performed only in vitro, whereas the current study was performed in vivo.

To verify whether CB$_2$ cannabinoid receptor could activate other K$^+$ channels, specific blockers of voltage-dependent K$^+$ channels and small and large conductance Ca$^{2+}$-activated K$^+$ channels were used. However, none of these blockers were able to antagonize the peripheral antinociceptive effect of PEA.

This result indicates that similarly to the CB$_1$ cannabinoid agonist anandamide (35), activation of KATP is the peripheral antinociceptive mechanism of the CB$_2$ cannabinoid receptor agonist PEA.

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**References**


