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Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide

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Abstract

The endogenous fatty acid ethanolamide, palmitylethanolamide, alleviated, in a dose-dependent manner, pain behaviors elicited in mice by injections of formalin (5%, intraplantar), acetic acid (0.6%, 0.5 ml per animal, intraperitoneal, i.p.), kaolin (2.5 mg per animal, i.p.), and magnesium sulfate (120 mg per kg, i.p.). The antinociceptive effects of palmitylethanolamide were prevented by the cannabinoid CB_2 receptor antagonist SR144528 [*N*-([1*s*]-endo-1.3.3-trimethylbicyclo[2.3.1]heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide], not by the cannabinoid CB_1 receptor antagonist SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide · HCl]. By contrast, palmitylethanolamide had no effect on capsaicin-evoked pain behavior or thermal nociception. The endogenous cannabinoid, anandamide (arachidonylethanolamide), alleviated nociception in all tests (formalin, acetic acid, kaolin, magnesium sulfate, capsaicin and hot plate). These effects were prevented by the cannabinoid CB_1 receptor antagonist SR141716A, not the cannabinoid CB_2 receptor antagonist SR141716A. Additional fatty acid ethanolamides (oleylethanolamide, myristylethanolamide, palmitoleylethanolamide, palmitelaidylethanolamide) had little or no effect on formalin-evoked pain behavior, and were not investigated in other pain models. These results support the hypothesis that endogenous palmitylethanolamide participates in the intrinsic control of pain initiation. They also suggest that the putative receptor site activated by palmitylethanolamide may provide a novel target for peripherally acting analgesic drugs.

Keywords: Cannabinoid; Anandamide; Palmitylethanolamide; Pain; Inflammation

1. Introduction

Cannabinoid drugs exert potent analgesic effects in animals by interacting with cannabinoid CB_1 receptor-type receptors located in various pain-processing areas of the central nervous system (see Walker et al., 1999 for a review). In addition to these centrally mediated effects, cannabinoids may also act at peripheral sites to reduce pain and hyperalgesia evoked by irritant chemicals such as formalin, turpentine and carrageenan (Calignano et al., 1998; Jaggar et al., 1998; Ko and Woods, 1999; Richardson et al., 1998). Pharmacological, genetic and anatomical evidence indicates that cannabinoid CB_1 receptors expressed in nociceptive neurons (Hohmann and Herkenham, 1999; Ahluwalia et al., 2000) are involved in the peripheral actions of cannabinoid drugs (Calignano et al., 1998; Richardson et al., 1998; Zimmer et al., 1999). In addition, cannabinoids inhibit the release of calcitonin gene-related peptide (CGRP) in isolated skin preparations (Richardson et al., 1998), suggesting that one mechanism by which these drugs may modulate pain is the inhibition of neuropeptide release from peripheral sensory terminals.

An important question raised by these findings is whether endogenous cannabinoid (endocannabinoid) compounds, generated spontaneously or as a result of tissue injury, participate in the control of peripheral pain signaling. The endocannabinoids, which include anandamide (arachidonylethanolamide) and 2-arachidonylglycerol, are a class of lipid compounds that are produced in the brain and other tissues through stimulus-dependent cleavage of membrane lipid precursors (see Piomelli et al., 2000 for a review). In the case of anandamide, this precursor is represented, in all likelihood, by a minor *N*-acylated species of phosphatidylethanolamine, termed *N*-arachidonyl phosphatidylethanolamine (Di Marzo et al., 1994; Cadas et al., 1996, 1997). Depolarization or activation of cell surface

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receptors may trigger the enzymatic hydrolysis of *N*-arachidonyl phosphatidylethanolamine by an unknown phospholipase D activity and result in the extracellular release of anandamide (Giuffrida et al., 1999). Some peripheral tissues, such as the skin, may contain relatively high levels of anandamide, suggesting that under appropriate circumstances, this compound can attain tissue concentrations that are sufficient to activate local cannabinoid CB_1 receptors (Calignano et al., 1998). In agreement with this possibility, intraplantar application of the selective cannabinoid CB_1 receptor antagonist SR141716A in mice (Rinaldi-Carmona et al., 1994) was found to enhance pain behaviors elicited by formalin injection in the same paw (Calignano et al., 1998). This response appears, however, to be species-specific or environment-dependent, as no effect of SR141716A on formalin-evoked pain could be demonstrated in rats (Beaulieu et al., 2000).

The anandamide precursor, *N*-arachidonyl phosphatidylethanolamine, belongs to a family of *N*-acylated phosphatidylethanolamines that differ in the fatty acid moiety linked to the primary amino group of phosphatidylethanolamine. Thus, catalytic hydrolysis of *N*-acyl phosphatidylethanolamines by phospholipase D activity gives rise to various saturated and monounsaturated fatty acid ethanolamides, including palmitylethanolamide and oleylethanolamide (Cadas et al., 1997). Palmitylethanolamide, which was isolated from tissues more than three decades ago (Bachur et al., 1965), was shown to have marked anti-inflammatory (Benvenuti et al., 1968; Facci et al., 1995; Mazzari et al., 1996) and antinociceptive effects when administered as a drug (Calignano et al., 1998; Jaggar et al., 1998). Although palmitylethanolamide does not bind to either cannabinoid CB_1 or CB_2 receptors (Devane et al., 1992; Griffin et al., 2000), its antinociceptive actions may be prevented by the selective cannabinoid CB_2 receptor antagonist SR144528. These results have led to suggestions that palmitylethanolamide-evoked antinociception may be mediated by an uncharacterized receptor with cannabinoid CB_2 receptor-like pharmacology (Calignano et al., 1998, 2000). The cellular localization of this putative receptor and its possible structural relationship with the cloned cannabinoid CB_2 receptor subtype, which is primarily expressed in immune cells (Munro et al., 1993), remain unknown. As is the case with anandamide, endogenous palmitylethanolamide may serve important pain-modulating functions in peripheral tissues. Indeed, biochemical analyses have revealed that nonstimulated skin contains concentrations of palmitylethanolamide in the high nanomolar range (Calignano et al., 1998). Furthermore, administration of the cannabinoid CB_2 receptor antagonist SR144528, was found to enhance formalin-induced pain behaviors in mice, suggesting that locally generated palmitylethanolamide may participate in modulating peripheral nociception (Calignano et al., 1998, but see Beaulieu et al., 2000 for discordant results in rats). The biological effects of oleylethanolamide, if any, are still undetermined. Biochemical studies in vitro have shown, however, that oleylethanolamide can inhibit anandamide inactivation by interfering with both transport and hydrolysis of this lipid molecule (Désarnaud et al., 1995; Di Tomaso et al., 1996; Piomelli et al., 1999).

In the present study, we extended our previous investigations on the antinociceptive properties of palmitylethanolamide in mice. Our results show that palmitylethanolamide alleviates pain behaviors in several animal models, and that these antinociceptive effects are blocked by the cannabinoid CB_2 receptor antagonist SR144528.

2. Materials and methods

2.1. Animals

Male Swiss mice weighing 20–25 g (Charles River, Italy) were used. For writhing tests (acetic acid, kaolin, magnesium sulfate) the animals were food-deprived overnight. Ad libitum-fed mice were used for all other tests.

2.2. Chemicals

All fatty acid ethanolamides were synthesized and purified following standard procedures (Giuffrida et al., 2000). SR144528 [*N*-([1*s*]-endo-1.3.3-trimethylbicyclo[2.3.1]heptan-2-yl)-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide] was a gift from Sanofi; SR141716A [*N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide ·HCl] was provided by RBI as part of the Chemical Synthesis Program of the NIMH; all other chemicals were from Sigma. Stock solutions of drugs were stored in dimethyl sulfoxide (DMSO). Fresh drug suspensions were prepared in warm (37°C) physiological saline (0.9% NaCl in distilled water) to a final DMSO concentration of 10%.

2.3. Formalin and capsaicin-evoked hind-paw licking

Mice received intraplantar injections of formalin (5% vol/vol, 10 μl) or capsaicin (0.4–1.6 μg per animal, 10 μl) in saline, containing 10% DMSO. The duration of paw licking was monitored by an observer blind to the experimental treatment for periods of 0–15 min (early phase) and 15–30 min (late phase) immediately after formalin administration, and for a period of 15 min after capsaicin administration. Initial tests determined that the presence of 10% DMSO in the injection vehicle did not significantly affect formalin responses (data not shown).

2.4. Acetic acid-, kaolin- and magnesium sulfate-evoked writhing

Mice received intraperitoneal (i.p.) injections of acetic acid (0.6%, 0.5 ml per animal), kaolin (2.5 mg per animal,

suspended in 0.2 ml of saline) or magnesium sulfate (120 mg per kg, dissolved in 10 ml of saline). Writhing episodes were monitored for a period of 30 min after administration of each irritant by an observer blind to experimental treatment.

2.5. Hot plate test

Mice who received i.p. injections of vehicle (10% DMSO in saline), anandamide or palmitylethanolamide, were placed on a hot plate (55.5°C), and latencies for the occurrence of nocifensive behavior (jumping, licking) were measured by trained observers blind to the experimental conditions. Test cutoff time was 60 s.

2.6. Statistical analysis

Statistical significance was determined by two-way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

3.1. Effects of palmitylethanolamide on formalin-evoked nociception

We have previously reported that intraplantar administration of palmitylethanolamide results in a marked inhibition of formalin-evoked pain behavior in mice, and that this effect is reversed by the cannabinoid CB_2 receptor antagonist SR144528 (Calignano et al., 1998). A similar response was observed in the present experiments, when palmitylethanolamide was administered into the paw, together with formalin (Fig. 1(A); data not shown).

To investigate the structural specificity of this response, we examined the antinociceptive activities of various fatty acid ethanolamide analogs of palmitylethanolamide. Oleylethanolamide (shorthand fatty acid designation, 18:1Δ^9 *cis*) produced a weak inhibition of the first phase of formalin-evoked pain (Fig. 1(B)). The antinociceptive effect of oleylethanolamide (50 μg per animal) was reduced by pretreatment with either the cannabinoid CB_2 receptor antagonist SR144528 (0.2 mg per kg, intravenous, i.v., 30 min before oleylethanolamide) or the cannabinoid CB_1 receptor antagonist SR141716A (0.2 mg per kg, i.v., 30 min before oleylethanolamide) (formalin, 149 ± 7 s; formalin plus oleylethanolamide, 75 ± 5 s; formalin plus oleylethanolamide and SR144528, 126 ± 21 s; formalin plus oleylethanolamide and SR141716A, 161 ± 4 s; $n = 6$). Oleylethanolamide exerted no significant antinociceptive effect on the second phase of the formalin response (Fig. 1(C)). Myristylethanolamide (14:0, 50 μg per animal) was inactive in either phase (Fig. 1(C)). Palmitoleylethanolamide (16:1Δ^9 *cis*, 50 μg per animal) and palmitelaidylethanolamide (16:1Δ^9 *trans*, 50 μg per animal) reduced the early phase of the formalin response in a manner that was not statistically significant and had no effect on the late phase (Fig. 1(C)).

3.2. Effects of palmitylethanolamide on acetic acid-evoked nociception

Administration of an irritating dose of acetic acid (0.6%) produced a robust writhing response, which peaked 5–20 min after injection of the acid. The response was attenuated in a dose-dependent manner by systemic administration of palmitylethanolamide (1–20 mg per kg, i.p., 30 min before acetic acid) (Fig. 2(A)). The antinociceptive effects

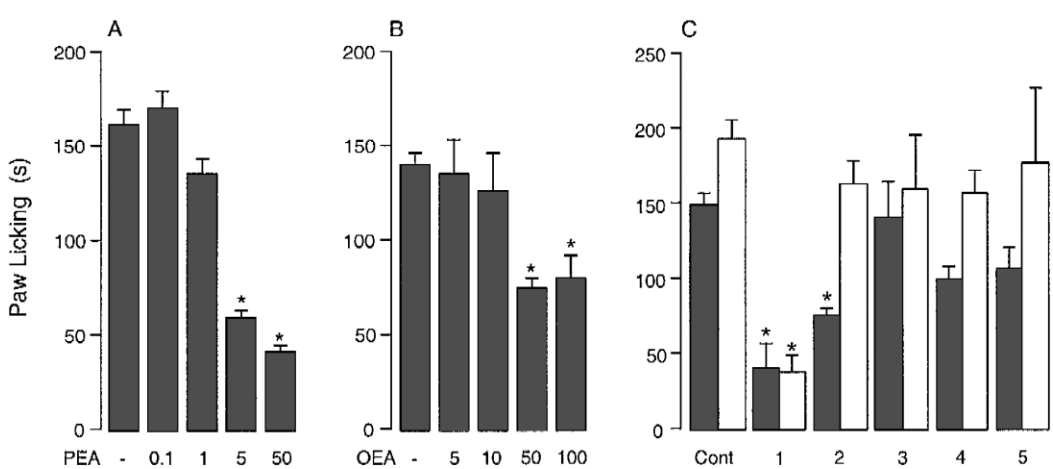

Fig. 1. Dose-dependent effects of palmitylethanolamide (PEA) (A) and oleylethanolamide (OEA) (B) on the early phase of nociception in the formalin test. Fatty acid ethanolamides (0.1–50 μg per animal) were administered by intraplantar injection together with formalin (5%, 10 μl per animal). (C) Effects of palmitylethanolamide and other fatty acid ethanolamides on the early (closed bars) and late (open bars) phases of the formalin test. Cont, formalin alone; 1, palmitylethanolamide; 2, oleylethanolamide; 3, myristylethanolamide; 4, palmitoleylethanolamide; 5, palmitelaidylethanolamide. Fatty acid ethanolamides (50 μg per animal) were administered by intraplantar injection together with formalin (5%). *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 4$–14 for each condition).

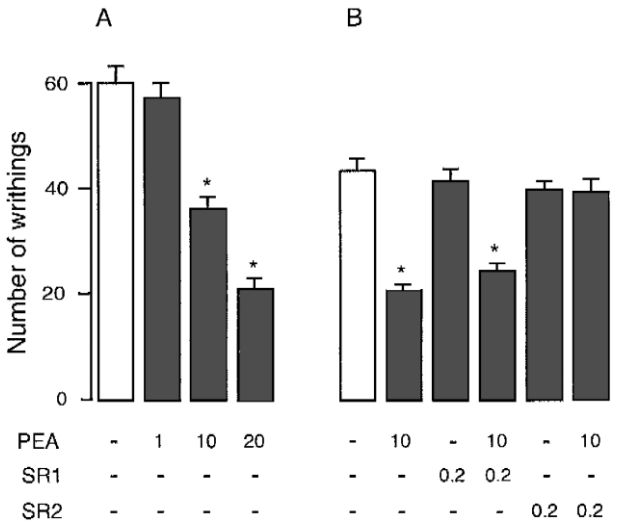

Fig. 2. Dose-dependent effects of palmitylethanolamide (PEA) on acetic acid-evoked writhing (A), and inhibition of these effects by the cannabinoid CB_2 receptor antagonist SR144528 (B). Palmitylethanolamide was administered by i.p. injection at doses of 1–20 mg per kg, 30 min before acetic acid. The cannabinoid CB_1 receptor antagonist SR141716A (SR1) or the cannabinoid CB_2 receptor antagonist SR144528 (SR2) were administered i.v. at 0.2 mg per kg, 30 min before palmitylethanolamide. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

of palmitylethanolamide were reversed by pretreatment with the cannabinoid CB_2 receptor antagonist SR144528 (0.2 mg per kg, i.v., 30 min before palmitylethanolamide), not with the cannabinoid CB_1 receptor antagonist SR141716A (0.2 mg per kg, i.v., 30 min before palmitylethanolamide) (Fig. 2(B)).

Anandamide administration resulted in a dose-dependent inhibition of acetic acid-induced pain behavior (Fig. 3(A)), which was blocked by the cannabinoid CB_1 receptor antagonist SR141716A (0.2 mg per kg, i.v., 30 min before palmitylethanolamide), but not by the cannabinoid CB_2 receptor antagonist SR144528 (0.2 mg per kg, i.v., 30 min before palmitylethanolamide) (Fig. 3(B)). Alone, SR144528 and SR141716A had no significant effect on acetic acid-evoked writhing at doses that inhibited the effects of palmitylethanolamide and anandamide, respectively (Figs. 2(B) and 3(B)).

3.3. Effects of palmitylethanolamide on kaolin-evoked nociception

Acetic acid-evoked writhing responses are accompanied by a profound, often lethal peritoneal inflammation. To determine whether palmitylethanolamide and anandamide inhibit visceral pain in experimental models that exhibit a less marked inflammatory component, we tested the effects of these lipid compounds on kaolin-evoked and magnesium sulfate-evoked writhing.

The administration of a kaolin suspension (2.5 mg per animal, i.p.) caused an average of 6.6 ± 1.1 writhing episodes ($n = 6$), which mostly occurred during the first 5–10 min after injection. The nociceptive response to kaolin was potently inhibited by systemic injections of palmitylethanolamide (0.1–10 mg per kg, i.p.) (Fig. 4(A)) or anandamide (1–10 mg per kg, i.p.) (Fig. 4(B)) (both administered 30 min before kaolin). The antinociceptive actions of palmitylethanolamide were blocked by the cannabinoid CB_2 receptor antagonist SR144528 (0.2 mg per kg, i.v., 30 min before palmitylethanolamide) (Fig. 4(A)), whereas those of anandamide were blocked by the cannabinoid CB_1 receptor antagonist SR141716A (0.2 mg per kg, i.v., 30 min before anandamide) (Fig. 4(B)).

3.4. Effects of palmitylethanolamide on magnesium sulfate-evoked nociception

Like kaolin, magnesium sulfate produces a reversible nocifensive response when injected intraperitoneally in rodents. The administration of magnesium sulfate (120 mg per kg, i.p.) caused an average of 9.1 ± 0.3 writhing episodes in mice ($n = 6$). Palmitylethanolamide (1–10 mg per kg, administered 30 min before magnesium sulfate) inhibited this response in a dose-dependent manner (Fig. 5(A)). In a second group of mice, in which magnesium sulfate produced an average of 6.8 ± 0.6 writhing episodes ($n = 6$), the inhibitory effects of palmitylethanolamide were blocked by the cannabinoid CB_2 receptor antagonist SR144528 (0.2 mg per kg, i.v., 30 min before palmitylethanolamide), not by the cannabinoid CB_1 receptor

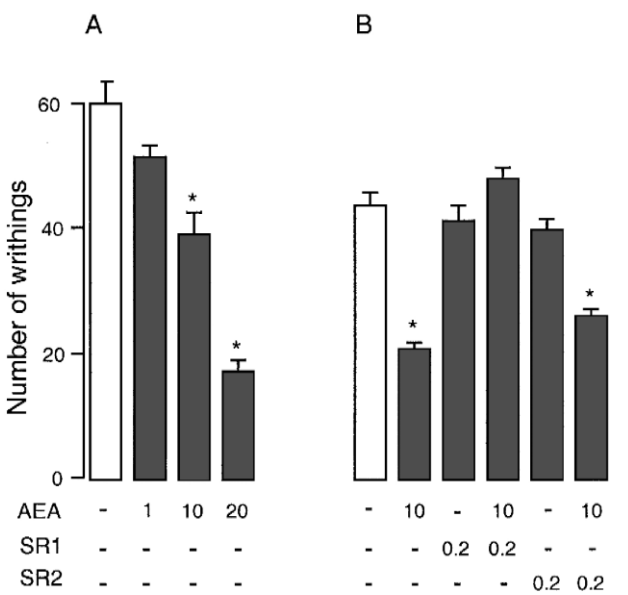

Fig. 3. Dose-dependent effects of anandamide (AEA) on acetic acid-evoked writhing (A), and inhibition of these effects by the cannabinoid CB_1 receptor antagonist SR141716A (B). Anandamide was administered by i.p. injection at doses of 1–20 mg per kg, 30 min before acetic acid. The cannabinoid CB_1 receptor antagonist SR141716A (SR1) and the cannabinoid CB_2 receptor antagonist SR144528 (SR2) were administered i.v. at 0.2 mg per kg, 30 min before anandamide. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

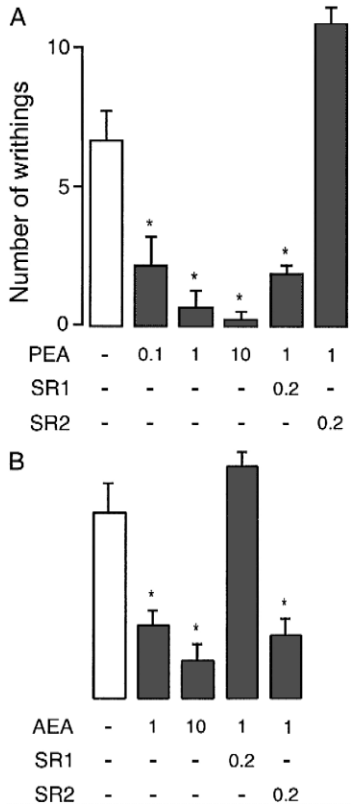

Fig. 4. Dose-dependent effects of palmitylethanolamide (PEA) (A) or anandamide (AEA) (B) on kaolin-evoked writhing, and inhibition of these effects by the cannabinoid CB_2 receptor antagonist SR144528. Palmitylethanolamide and anandamide were administered by i.p. injection at doses of 0.1–10 mg per kg, 30 min before kaolin. The cannabinoid CB_1 receptor antagonist SR141716A (SR1) and the cannabinoid CB_2 receptor antagonist SR144528 (SR2) were administered i.v. at 0.2 mg per kg, 30 min before palmitylethanolamide or anandamide. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

antagonist SR141716A (0.2 mg per kg, i.v., 30 min before palmitylethanolamide) (Fig. 5(B)).

3.5. Anandamide and palmitylethanolamide synergistically inhibit nociception

We have previously shown that anandamide and palmitylethanolamide may act synergistically to reduce formalin-evoked pain behavior (Calignano et al., 1998). To determine if a similar synergistic interaction also occurs in other pain models, we tested the effects of systemic co-administration of anandamide and palmitylethanolamide on kaolin-induced writhing. When injected separately, very low systemic doses of anandamide (0.1 mg per kg, i.p.) or palmitylethanolamide (0.01 mg per kg, i.p.) did not significantly reduce the number of writhing episodes elicited by kaolin (2.5 mg per animal, i.p.) (Fig. 6). However, concomitant administration of the two compounds produced a marked antinociceptive effect, which was abrogated by treatment with either SR141716A or SR144528 (each at 0.2 mg per kg, i.v., 30 min before anandamide plus palmitylethanolamide) (Fig. 6).

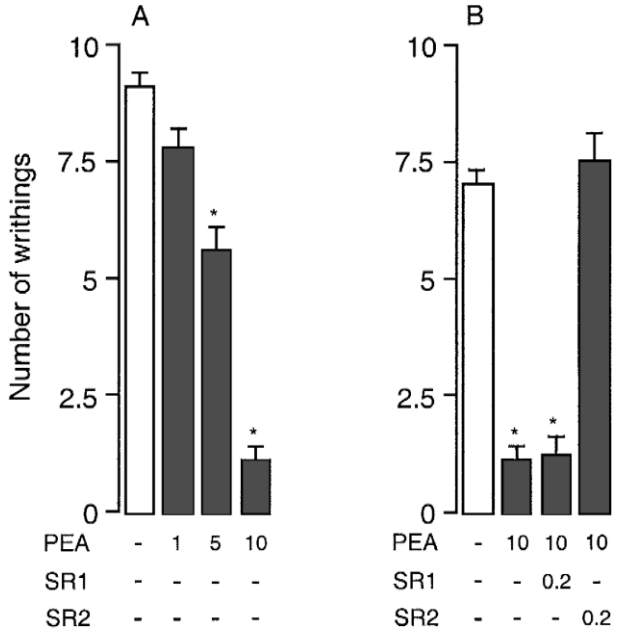

Fig. 5. Dose-dependent effects of palmitylethanolamide (PEA) on magnesium sulfate-evoked writhing (A), and inhibition of these effects by the cannabinoid CB_2 receptor antagonist SR144528 (B). Palmitylethanolamide was administered by i.p. injection at doses of 1–10 mg per kg, 30 min before magnesium sulfate. The cannabinoid CB_1 receptor antagonist SR141716A (SR1) and the cannabinoid CB_2 receptor antagonist SR144528 (SR2) were administered i.v. at 0.2 mg per kg, 30 min before palmitylethanolamide. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

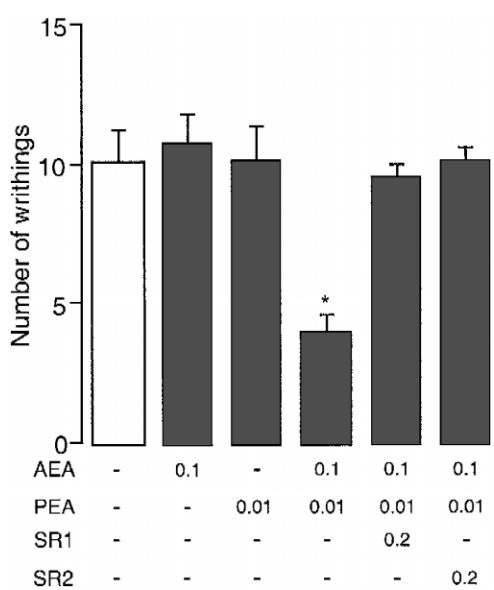

Fig. 6. Anandamide (AEA) and palmitylethanolamide (PEA) synergistically inhibit kaolin-evoked writhing. Anandamide (0.1 mg per kg, i.p.) and palmitylethanolamide (0.01 mg per kg, i.p.) were administered either separately or together, 30 min before kaolin. The cannabinoid CB_1 receptor antagonist SR141716A (SR1) and the cannabinoid CB_2 receptor antagonist SR144528 (SR2) were administered separately (0.2 mg per kg, i.v.) 30 min before anandamide plus palmitylethanolamide. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

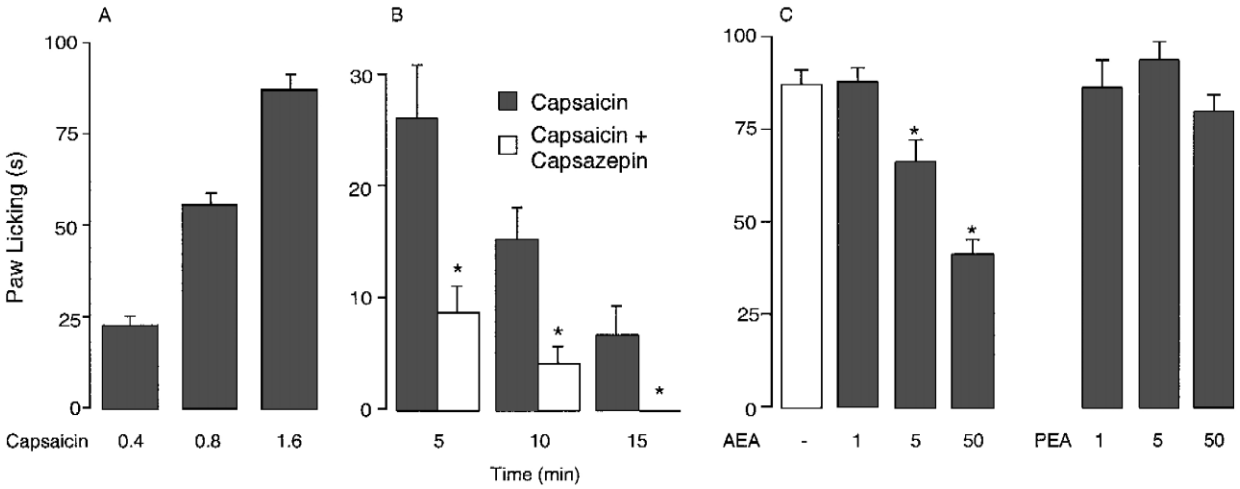

Fig. 7. Dose-dependent nociceptive effects of capsaicin (A), and inhibition of these effects by the vanilloid receptors antagonist capsazepin (B). Capsaicin (0.4–1.6 μg per animal) and capsazepin (12 μg per animal) were administered by intraplantar injection; nocifensive behavior (paw licking) was assessed for a period of 15 min (A) or for three subsequent 5-min periods (B) after capsaicin injection. In (B), the dose of capsaicin was 1.6 μg per animal. (C) Dose-dependent effects of anandamide (AEA) and palmitylethanolamide (PEA) on capsaicin-evoked nociception. Capsaicin (1.6 μg per animal), anandamide or palmitylethanolamide (1–50 μg per animal) were administered by intraplantar injection. *, $P < 0.05$. ANOVA followed by Dunnett's test ($n = 6$ for each condition).

3.6. Effects of palmitylethanolamide on capsaicin-induced nociception

To further investigate the mechanism of action of palmitylethanolamide, we examined the effects of this compound on capsaicin-induced pain. The nocifensive behavior that follows peripheral injection of capsaicin is thought to be triggered by activation of VR1-type vanilloid receptors located on primary sensory afferents (Caterina et al., 1997; Szallasi and Blumberg, 1999; Davis et al., 2000). In agreement with this possibility, intraplantar capsaicin produced a nociceptive response in mice, which was dose-dependent (Fig. 7(A)), short-lasting (Fig. 7(B)) and attenuated by the vanilloid antagonist capsazepine (12 μg per animal, intraplantar) (Fig. 7(B)). The effect of capsaicin was dose-dependently inhibited by the local co-administration of anandamide (Fig. 7(C)), which, per se, did not elicit any significant nocifensive behavior (data not shown). By contrast, capsaicin-induced pain behavior was not affected by local injections of palmitylethanolamide (Fig. 7(C)), even at doses that produced a complete inhibition of the early phase of the formalin response (50 μg per animal) (Fig. 1(A)).

Table 1
Time-course of the effects of vehicle (10% DMSO in saline, i.p.), anandamide (AEA, 20 mg per kg, i.p.) and palmitylethanolamide (PEA, 20 mg per kg, i.p.) on thermal nociception, as assessed in the hot plate test (55.5°C)
ANOVA followed by Dunnett's test ($n = 6$ for each condition).

Time (min)	Hot plate latencies (s)		
	Vehicle	AEA	PEA
5	22±4	19±4	21±4
10	20±4	25±6	28±6
15	18±2	33±2[a]	21±5
20	16±4	36±3[a]	23±3
25	22±8	22±4	21±7
30	24±6	26±3	19±4

[a] $P < 0.05$.

3.7. Effects of palmitylethanolamide on thermal nociception

The fact that palmitylethanolamide does not affect capsaicin-induced pain suggests that this compound may not directly interfere with primary afferent-mediated transmission of pain signals to the central nervous system. To further investigate this possibility, we compared the antinociceptive effects of palmitylethanolamide and anandamide in the hot plate test. In mice that received anandamide (20 mg per kg, i.p.), the latency to jump 15 and 20 min after administration was significantly higher than in mice that received vehicle alone (10% DMSO in saline solution) (Table 1). By contrast, in mice that received palmitylethanolamide (20 mg per kg, i.p.), hot plate latency values were identical to those of control, vehicle-injected animals (Table 1).

4. Discussion

The fatty acid amide, palmitylethanolamide, was previously found to inhibit formalin-evoked pain behavior in rodents (Calignano et al., 1998; Jaggar et al., 1998). In the present study, we have further characterized the antinociceptive activity of this endogenous lipid molecule in several models of phasic and tonic pain.

Our initial structure–activity relationship studies suggest that the ability of palmitylethanolamide to reduce formalin-evoked nociception may have distinct structural

requirements, insofar as small variations in chemical structure were found to produce substantial losses of biological activity. For example, myristylethanolamide (shorthand fatty acid designation, 14:0) and palmitoleylethanolamide (16:1Δ^9 *cis*) displayed no significant antinociceptive activity, despite their close structural resemblance with palmitylethanolamide (16:0). These findings, which need to be extended by testing a wider range of structural analogs, are consistent with the possibility that the effects of palmitylethanolamide are mediated by activation of a selective receptor site. This hypothesis is further supported by the ability of the cannabinoid CB_2 receptor antagonist, SR144528, to prevent palmitylethanolamide-evoked antinociception (Calignano et al., 1998; present study). The relationship of the putative receptor activated by palmitylethanolamide with the cloned cannabinoid CB_2 receptor subtype, if any, currently remains undetermined. There is general agreement that palmitylethanolamide does not productively interact with the cloned cannabinoid CB_2 receptor (Devane et al., 1992; Griffin et al., 2000), a negative finding that we have reproduced in our lab (S. Kathuria and D. Piomelli, unpublished observations). However, activation of cannabinoid CB_2 receptors by the selective agonist, HU308, was recently shown to inhibit pain in the formalin model (Hanus et al., 1999).

Oleylethanolamide was found to exert a weak antinociceptive effect in the formalin test, which was reduced by systemic administration of either cannabinoid CB_1 or CB_2 receptor antagonists. These results suggest that oleylethanolamide may reduce nociception through a dual mechanism. Oleylethanolamide may weakly interact with a receptor site sensitive to the cannabinoid CB_2 receptor antagonist SR144528. Moreover, by inhibiting anandamide inactivation (Désarnaud et al., 1995; Di Tomaso et al., 1996; Piomelli et al., 1999), oleylethanolamide may cause anandamide to accumulate in the injected paw and activate local cannabinoid CB_1 receptors. The possibility that oleylethanolamide directly binds to and activates cannabinoid CB_1 receptors is unlikely, because oleylethanolamide displays no affinity for these receptors in vitro (S. Kathuria and D. Piomelli, unpublished observations). In previous experiments, using an identical protocol, we failed to observe the antinociceptive effects of oleylethanolamide (50 μg per animal, intraplantar) in mice (Calignano et al., 1998). We do not understand the reasons for this discrepancy, but possible explanations include the weak antinociceptive activity of oleylethanolamide or small changes in rearing conditions, which may strongly affect behavioral responses in mice.

Local and systemic administration of palmitylethanolamide alleviated pain behaviors elicited by a diverse group of chemical irritants, which included formalin, acetic acid, kaolin and magnesium sulfate. In all cases, treatment with the cannabinoid CB_2 receptor antagonist SR144528 (but not of the cannabinoid CB_1 receptor antagonist SR141716A) prevented the effects of palmitylethanolamide, indicating that a common mechanism may be involved in these responses. Such broad antinociceptive properties strengthen the hypothesis that palmitylethanolamide may play a key role in the intrinsic control of pain initiation. Moreover, these properties support the notion that the putative receptor activated by palmitylethanolamide may provide a useful target for analgesic drug development (Calignano et al., 1998, 2000; Piomelli et al., 2000). Further support to this proposal comes from the fact that palmitylethanolamide may exert both analgesic (Calignano et al., 1998; Jaggar et al., 1998; present study) and anti-inflammatory effects (Benvenuti et al., 1968; Facci et al., 1995; Mazzari et al., 1996). Thus, drugs aimed at the putative palmitylethanolamide receptor might offer the advantage of combining these two complementary therapeutic properties. Additional experiments are needed, however, to unequivocally establish whether the analgesic and anti-inflammatory actions of palmitylethanolamide are mediated by the same putative receptor blocked by SR144528.

Despite its ability to attenuate a variety of phasic pain responses, such as those elicited by formalin and magnesium sulfate, palmitylethanolamide had no effect on capsaicin-induced nocifensive behavior. Capsaicin, the active principle in chili pepper, is thought to produce pain by selectively activating VR1-type vanilloid receptors on peripheral sensory fibers (Caterina et al., 1997; Szallasi and Blumberg, 1999; Davis et al., 2000). Accordingly, the fact that palmitylethanolamide did not inhibit capsaicin-induced pain at doses that completely prevented other nociceptive responses suggests that palmitylethanolamide does not directly interfere with nociceptor-mediated pain transmission. In keeping with this possibility, palmitylethanolamide had no effect on thermal nociception (Table 1), which is also thought to require phasic nociceptor activation (Besson and Chaouch, 1987). By contrast, and in agreement with the presence of cannabinoid CB_1 receptors in sensory neurons (Hohmann and Herkenham, 1999), we report here that anandamide inhibits capsaicin nociception. Moreover, in previous studies, we have shown that anandamide prevents thermal nociception and that this effect is also blocked by the cannabinoid CB_1 antagonist SR141716A (Beltramo et al., 1997). A plausible explanation for our findings is that anandamide and palmitylethanolamide exert their peripheral antinociceptive effects by interacting with different molecular and cellular targets. According to this hypothesis, anandamide may activate cannabinoid CB_1 receptors located on capsaicin-sensitive primary afferents, resulting in the decreased responsiveness of these afferents to noxious stimuli. Palmitylethanolamide, on the other hand, may stimulate an uncharacterized receptor, blocked by SR144528 and located on non-neuronal peripheral cells or capsaicin-insensitive neurons. The existence of distinct molecular and cellular targets for anandamide and palmitylethanolamide might also account for the synergistic effects displayed by these compounds on formalin-evoked or kaolin-evoked nocicep-

tion. Whether or not these speculations turn out to be correct, the analgesic properties of palmitylethanolamide and anandamide underscore the important functions served by these lipid messengers in pain signaling, and the potential interest of their receptor systems as targets for analgesic drugs.

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