



## N-(4-Methoxy-2-nitrophenyl)hexadecanamide, a palmitoylethanolamide analogue, reduces formalin-induced nociception

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### ABSTRACT

**Aims:** To investigate the local antinociceptive effect as well as the possible mechanisms of action of a novel analogue of palmitoylethanolamide (PEA) N-(4-methoxy-2-nitrophenyl)hexadecanamide (HD) in the rat formalin test.

**Main methods:** The formalin test was used to assess the antinociceptive activity of HD in vivo. The hydrolysis of anandamide catalyzed by fatty acid amide hydrolase (FAAH) was used to determine the action of HD on FAAH activity in vitro.

**Key findings:** Local peripheral ipsilateral, but not contralateral, administration of HD (10–100 µg/paw) produced a dose-dependent antinociceptive effect in rats. The CB<sub>1</sub> and CB<sub>2</sub> receptor antagonists AM281 (0.3–30 µg/paw) and SR144528 (0.3–30 µg/paw), respectively, reduced the antinociceptive effect of HD (100 µg/paw). In addition, methiothepin (0.03–0.3 µg/paw) and naloxone (5–50 µg/paw) significantly reduced HD-induced antinociception (100 µg/paw). In vitro, HD reduced only to a minor extent the hydrolysis of anandamide catalyzed by FAAH.

**Significance:** HD local administration produces antinociception that probably results from an indirect activation of peripheral CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors. Data suggest that 5-HT<sub>1</sub> and opioid receptors also participate in the antinociceptive effect of this compound. HD may have potential as analgesic drug.

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### Introduction

There is considerable evidence showing that cannabinoid receptor agonists have analgesic activity in rodent models of inflammatory (Smith et al., 1998; Farquhar-Smith et al., 2002; Kehl et al., 2003) and neuropathic (Bridges et al., 2001; Scott et al., 2004) pain. These effects are mediated via both CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors (Fox et al., 2001; Scott et al., 2004). However, there is a need to improve the therapeutic index of existing cannabinoids, particularly with respect to short-term motor and psychotropic side effects and the longer-term risk of psychosis associated with Cannabis use, which are mediated by CB<sub>1</sub> cannabinoid receptors expressed in brain. One approach is to increase the tonus of the endogenous cannabinoid (endocannabinoid) system indirectly, for example by inhibiting the metabolism of the endocannabinoid ligands anandamide (AEA, structure shown in Fig. 1) and 2-arachidonoylglycerol (2-AG). Compounds with this mechanism, for example, inhibitors of the AEA hydrolytic enzyme fatty acid amide

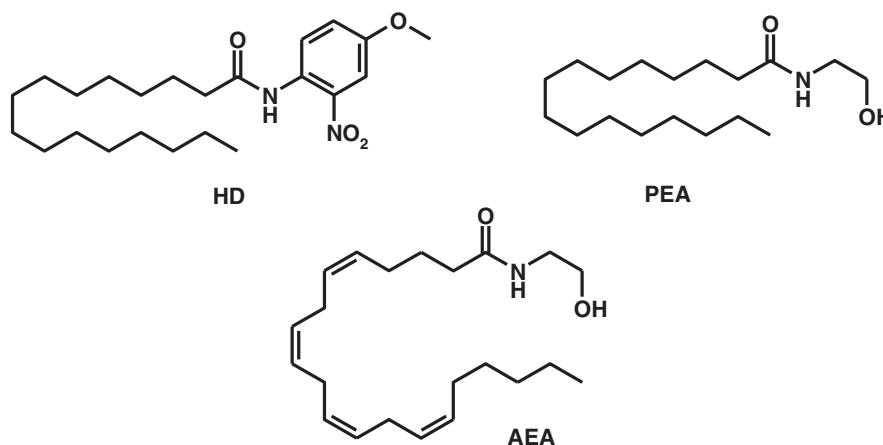
hydrolase (FAAH), are effective in animal models of pain (see e.g. Lichtman et al., 2004; Chang et al., 2006; Karbarz et al., 2009). Another approach is to administer the drugs locally in the site of injury.

Palmitoylethanolamide (N-(2-hydroxyethyl)hexadecanamide; PEA, structure shown in Fig. 1) is an endogenous compound structurally related to AEA that produces potentially useful effects upon pain and inflammation in animal models (Jaggar et al., 1998; Calignano et al., 1998, 2001; Conti et al., 2002; Quartilho et al., 2003; Johaneck and Simone, 2004; LoVerme et al., 2006; Gutiérrez et al., 2007) and possibly in man (Calabrò et al., 2010; Conigliaro et al., 2011). Although PEA has no direct effects upon CB receptors, its antinociceptive effects involve the endocannabinoid system, possibly as a result of its action as a competing substrate at FAAH (Lambert et al., 2002). The ability of PEA to activate the peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) pathway has also been proposed to be involved in its anti-nociceptive and anti-inflammatory effects (LoVerme et al., 2005, 2006).

The lack of direct effect of PEA upon CB cannabinoid receptors together with its antinociceptive properties makes it an attractive molecule for the design of novel analogues. In this regard, PEA, which acts in vitro reduce AEA metabolism (Vandevoorde et al., 2003), has been reported to produce analgesia in animal models of neuropathic pain

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**Fig. 1.** Chemical structures of *N*-(4-methoxy-2-nitrophenyl)hexadecanamide (HD) and palmitoylethanolamide (PEA). The structure of the endocannabinoid homologue anandamide (AEA) is shown for comparative purposes.

(Wallace et al., 2007). In preliminary studies we have found that systemic administration of *N*-(4-methoxy-2-nitrophenyl)hexadecanamide (HD, structure shown in Fig. 1), a novel analogue of PEA, inhibits acetic acid-induced and thermal nociception in mice (Déciga-Campos et al., 2007). Here, we report the analgesic activity of HD *in vivo* after local administration in the formalin test of persistent pain. In addition, the mechanisms of action of HD on *in vivo* and *in vitro* were investigated.

## Materials and methods

### Chemical synthesis of HD

A mixture of 4-methoxy-2-nitroaniline (5 g, 0.0297 mol), triethylamine (3.3 g, 0.0327 mol, 1.1 eq) and methylene chloride (50 ml) was stirred for 30 min. After this time, palmitoyl chloride (8.9 g, 0.0327 mol, 1.1 eq), was added dropwise to the solution. The mixture was stirred 5 h under nitrogen atmosphere at room temperature. Thin layer chromatography was used to monitor the reaction. After cooling, the mixture was neutralized with saturated NaHCO<sub>3</sub> solution and the precipitate formed was filtered by suction. The crude product HD was purified by recrystallization from ethanol yielding 9.96 g (83%) of yellow crystals with a melting point of 77.5–78.9 °C. Fig. 1 shows the chemical structure HD. The compound was fully characterized by <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance spectroscopy and also by mass spectrometry. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>), δ 0.80 (t, 3H, CH<sub>3</sub>), 1.22–1.62 (m, 28H, CH<sub>2</sub>), 2.26 (t, 2H, CH<sub>2</sub>), 3.83 (s, 3H, CH<sub>3</sub>O), 7.20 (dd, 1H, H-5), 7.41 (d, 1H, H-3), 7.77 (dd, 1H, H-6), 13.21 (s, 1H, NH); <sup>13</sup>C NMR (75.5 MHz, CDCl<sub>3</sub>), δ 14.3 (CH<sub>3</sub>), 28.1 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 29.5 (10 CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 37.3 (CH<sub>2</sub>-CO), 56.1 (CH<sub>3</sub>-O), 108.7 (C-3), 123.6 (C-6), 124.1 (C-5), 127.9 (C-1), 136.9 (C-2), 155.1 (C-4), 171.1 (C=O) PPM and MS: *m/z* (% relative intensity) 406 (M<sup>+</sup>, 98), 168 (100); High resolution mass spectrometry (HRMS): Calculated for C<sub>23</sub>H<sub>38</sub>N<sub>2</sub>O<sub>4</sub>: 406.2832. Found: 406.314.

### Pharmacological evaluation

#### Animals

Female Wistar and Sprague–Dawley (180–200 g) rats were used in this study for behavioral and *in vitro* experiments, respectively. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmerman, 1983) and their care were conducted in conformity with Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). Protocols were approved by the ethics committee of Cinvestav (Mexico, D.F.) and Umeå University (Sweden). Efforts were made to minimize animal suffering and to reduce the number of animals used. Rats were used once only. They

were given free access to standard chow and tap water. Animals were housed in a climate controlled room with a 12 h light/dark cycle. At the end of the experiment, animals were killed in a CO<sub>2</sub> chamber.

#### Formalin test

The procedure used has been described previously (Rocha-González et al., 2005). Rats were placed in an open Plexiglass observation chambers for 30 min to allow them to accommodate their surroundings, then they were removed for formalin administration. Fifty microliters of dilute formalin (1%) was injected subcutaneously into the dorsal surface of the right hind paw with a 30-gauge needle. The animal was then returned to the chamber for observation and nociceptive behavior was observed immediately after formalin injection. A mirror was placed behind the chamber to enable unhindered observation. Nociceptive behavior was quantified as the numbers of flinches of the injected paw during 1-min periods every 5 min up to 60 min after injection. Flinching was readily identified and was characterized as a rapid and brief withdrawal or flexing the injected paw. Formalin-induced flinching behavior is biphasic; the initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min).

#### Assessment of the antinociceptive activity of HD in the rat formalin test

Rats received 20% DMSO (50 μl, the effect of 20% DMSO was not different from that of saline on formalin-induced nociception) or increasing doses of HD (10–100 μg/paw) 20 min before formalin injection into the dorsal surface of the right hindpaw. Then, flinching behavior was assessed for 1 h. Rats in all groups were tested for possible side effects such as reduction of righting, stepping, and corneal/pinna reflexes before and after drug treatment as previously reported (Malmberg and Yaksh, 1992).

#### Analysis of the possible mechanism of action of HD on formalin-induced nociception in rat

Rats received a s.c. injection (50 μl) of vehicle (sterile 0.9% saline or 20% DMSO) or increasing doses of the selective CB<sub>1</sub> and CB<sub>2</sub> receptor antagonists AM281 (0.3 and 30 μg/paw) and SR144528 (0.3 and 30 μg/paw), respectively, as well as the 5-HT receptor antagonist methiothepin (0.03 and 0.3 μg/paw) or opioid receptor antagonist naloxone (5 and 50 μg/paw) 35 min before formalin injection and 15 min before HD administration (100 μg/paw) into the dorsal surface of the right hind paw. Then, flinching behavior was assessed for 1 h.

## Biochemical evaluation

### FAAH inhibition assay

FAAH activity was assayed in homogenates of female Sprague-Dawley rat brain (minus cerebellum) as described previously (Boldrup et al., 2004). Briefly, test compound or ethanol carrier (10 ml) were incubated at 37 °C for 10 min with diluted homogenates (165 ml, in 10 mM Tris-HCl-1 mM EDTA, pH 7.6) and 25 ml of a mixture of 16 mM non-radioactive AEA (2 mM final assay concentration) containing trace amounts of [<sup>3</sup>H]-AEA with the label in the ethanolamine side chain (30–60 Ci/mmol) and 1% w/v fatty acid free bovine serum albumin. Reactions were terminated by putting the samples on an ice-bath followed by the addition of 80 µl charcoal mixed with 320 µl of 0.5 M HCl. After vigorous vortexing followed by centrifugation, the radioactivity (corresponding to the [<sup>3</sup>H]-ethanolamine produced as a result of the FAAH-catalyzed breakdown of [<sup>3</sup>H]-AEA) found in aliquots (200 µl) of the aqueous phase was determined by liquid scintillation counting with quench correction. Blank values used buffer in place of homogenates. Results are expressed as % of concomitantly assayed vehicle controls.

### Drugs

1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide (AM281), methiothepin mesylate and naloxone hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO, USA). Non-radioactive AEA and 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-N-[(1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]hept-2-yl]-1H-pyrazole-3-carboxamide (SR144528) were purchased at Cayman Chemical (Ann Arbor, Michigan, USA). [<sup>3</sup>H]-Anandamide was obtained from American Radiolabeled Chemicals, Inc (St. Louis, MO, USA). All other substances were of analytical grade. AM281, SR144528 and HD were dissolved in 20% DMSO in saline solution. AEA was dissolved in ethanol. Methiothepin and naloxone were dissolved in saline.

### Data and statistical analysis

All in vivo experimental results are given as the mean ± S.E.M. of the data obtained in 6 animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curve (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule.

One- or two-way analysis of variance (ANOVA) followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when  $P < 0.05$ .

## Results

### Antinociceptive effect of HD

Subcutaneous injection of 1% formalin into the hindpaw produced a biphasic flinching behavior. The first phase started immediately after formalin injection and declined gradually in about 10 min. The second phase initiated 15 min after formalin injection and it reached a maximum effect between 30 and 40 min declining gradually at about 60 min (Fig. 2). Fig. 2 depicts the time course of the antinociceptive effect of HD in the formalin test at the greatest dose tested. Local peripheral ipsilateral, but not contralateral, administration of HD (10–100 µg/paw) significantly reduced ( $P < 0.05$ ) the number of flinches in a dose-dependent manner during the second phase of the formalin test (Figs. 2 and 3). In contrast, HD was unable to significantly reduce the AUC for phase 1 of the test (Figs. 2 and 3). Since no effect was observed during phase 1, data regarding this phase in subsequent experiments have not been presented here. No side effects were observed in any of the studied groups of animals.

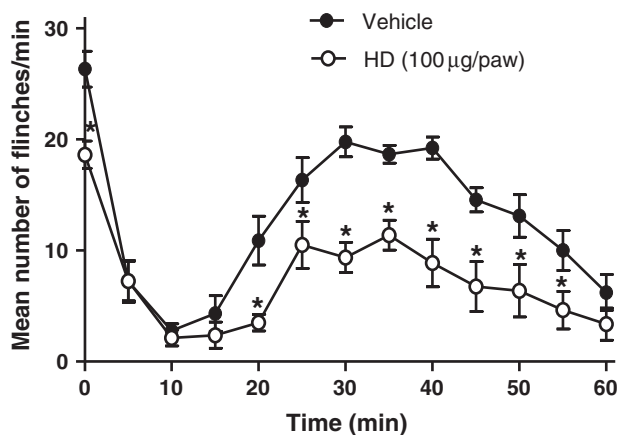


Fig. 2. Time course of the flinching behavior observed after local peripheral administration of vehicle and HD (100 µg/paw) in rats submitted to the formalin test. Each value represents the mean of at least 6 animals ± S.E.M. \* Significantly different with respect to the vehicle ( $P < 0.05$ ), determined by two-way ANOVA followed by Tukey's test.

### Effects of AM251 and SR144528 on HD-induced peripheral antinociception

Local peripheral administration of the selective CB<sub>1</sub> cannabinoid receptor antagonist AM281 (30 µg/paw) and the CB<sub>2</sub> cannabinoid receptor antagonist/PPARα receptor antagonist SR144528 (30 µg/paw), respectively, were unable to prevent formalin-induced nociception when given on their own. In marked contrast, both drugs dose-dependently (0.3–30 µg/paw) prevented HD-induced antinociception (100 µg/paw) during phase 2 of the formalin test (Fig. 4).

### Effect of methiothepin and naloxone on HD-induced peripheral antinociception

The non-selective 5-HT receptor antagonist methiothepin (0.03–0.3 µg/paw) as well as the non-selective opioid receptor antagonist naloxone (5–50 µg/paw) prevented in a dose-dependent manner HD (100 µg/paw)-induced antinociception (Fig. 5). When given alone, the highest dose of both antagonists did not affect formalin-induced nociception.

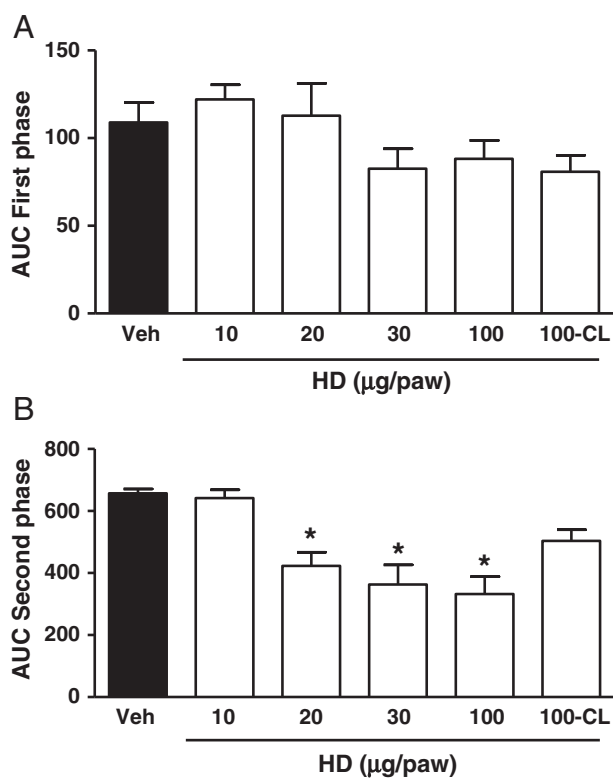
### Effect of HD upon FAAH activity in vitro

The ability of HD to inhibit FAAH was investigated using the endocannabinoid anandamide (2 µM) as substrate. A significant albeit modest inhibition was seen at the two greatest concentrations tested (20 and 50 µM) (Fig. 6).

## Discussion

### Antinociceptive effect of HD

In this study we have found that local peripheral ipsilateral, but not contralateral, administration of HD significantly reduces formalin-induced nociception in rats. Our data extend previous observations showing that systemic administration of HD inhibits acetic acid-induced and thermal nociception in mice (Déciga-Campos et al., 2007), and demonstrate that the local effect of HD on the second phase of the formalin test can be blocked by AM281, SR144528, naloxone and methiothepin. Taken together, data suggest that HD inhibits nociception in inflammatory and nociceptive pain models after local and systemic administration in rats or mice. Thus, HD retains the useful analgesic properties of PEA, which reduces nociceptive behavior induced by formalin (Jaggar et al., 1998; Calignano et al., 1998, 2001; LoVerme et al., 2006), acetic acid (Calignano et al., 2001) as well as capsaicin (Quartilho et al., 2003), carragenan (Conti et al., 2002; Gutiérrez et al., 2007) and mild heat injury



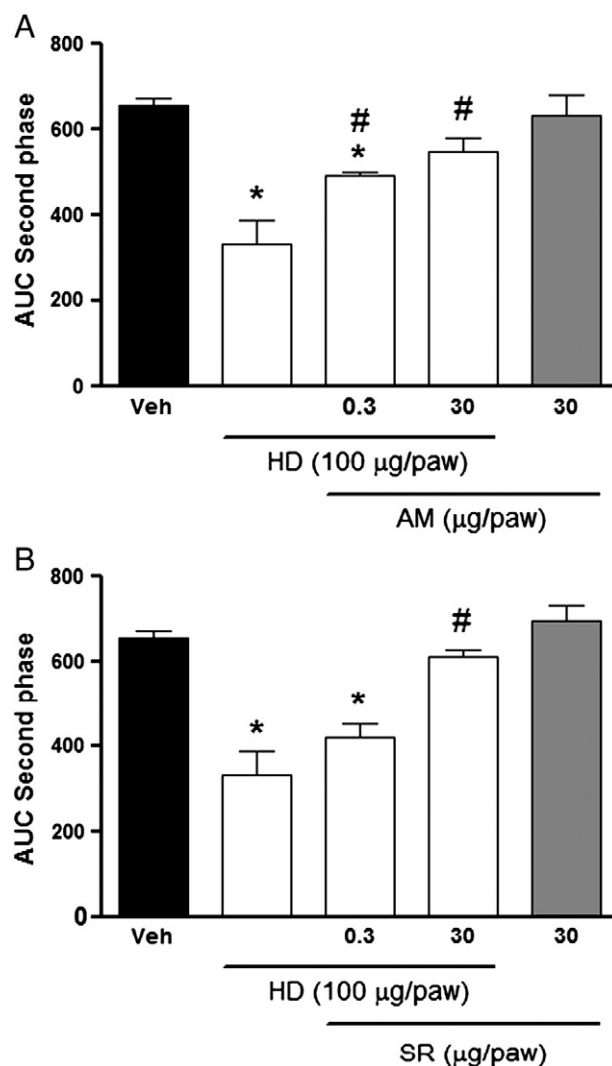
**Fig. 3.** Antinociceptive effect of the local peripheral administration of HD during the first (A) and second phase (B) of the formalin test in rats. Data are expressed as the area under the number of flinches against time curve (AUC). Bars are the means  $\pm$  S.E.M. for at least six animals. \* Significantly different ( $P < 0.05$ ) from the vehicle group (Veh), as determined by one-way ANOVA followed by Tukey's test. CL: Contralateral.

(Johanek and Simone, 2004). In addition, it has shown potential utility in chronic pelvic pain (Calabrò et al., 2010) and carpal tunnel syndrome (Conigliaro et al., 2011) in man.

The finding that the CB<sub>1</sub> cannabinoid receptor antagonist AM251 and the CB<sub>2</sub> cannabinoid antagonist/PPAR $\alpha$  receptor antagonist SR144528 block the effects of HD in the formalin test clearly implicate an activation of the endocannabinoid system in the mechanism of action of HD. However, PEA and its analogues do not interact directly with CB receptors (Vandevoorde et al., 2003), and so an indirect mechanism is presumably operative here. Some possibilities are considered below.

#### Role of FAAH inhibition in the antinociceptive effects of HD

Intraplantar administration of the selective FAAH inhibitor URB597 to sham-operated rats results in increased levels of the endocannabinoids oleoyl ethanolamide (OEA), AEA and 2-AG in the hind paws (Jhaveri et al., 2006). Given that both AEA (via CB<sub>1</sub> cannabinoid receptors) and 2-AG (via CB<sub>2</sub> cannabinoid receptors) are efficacious in the formalin test when given locally to the hind paws of rats (Guindon et al., 2006, 2007), and that FAAH inhibitors reduce the behavioral and biochemical consequences of formalin in this test (Lichtman et al., 2004; Sit et al., 2007; Clapper et al., 2010; Thors et al., 2010), inhibition of FAAH could in theory be involved in the action of HD. The compound, however, was a rather weak inhibitor of FAAH in vitro, producing inhibition of AEA hydrolysis only at concentrations of 20  $\mu$ M and higher. As a caveat, it should be pointed out that solubility issues in the FAAH assay are problematical with PEA analogues (Vandevoorde et al., 2003), so that the compound may be more potent as an inhibitor of FAAH than is seen here. However, HD is less potent than PEA (IC<sub>50</sub> value 5  $\mu$ M) when tested in the same incubation conditions (Jonsson et al., 2006). In addition, a recent study found that the FAAH inhibitor ST4070 increased brain levels of the endocannabinoids PEA and AEA, but not 2-AG (Caprioli et al., 2012).

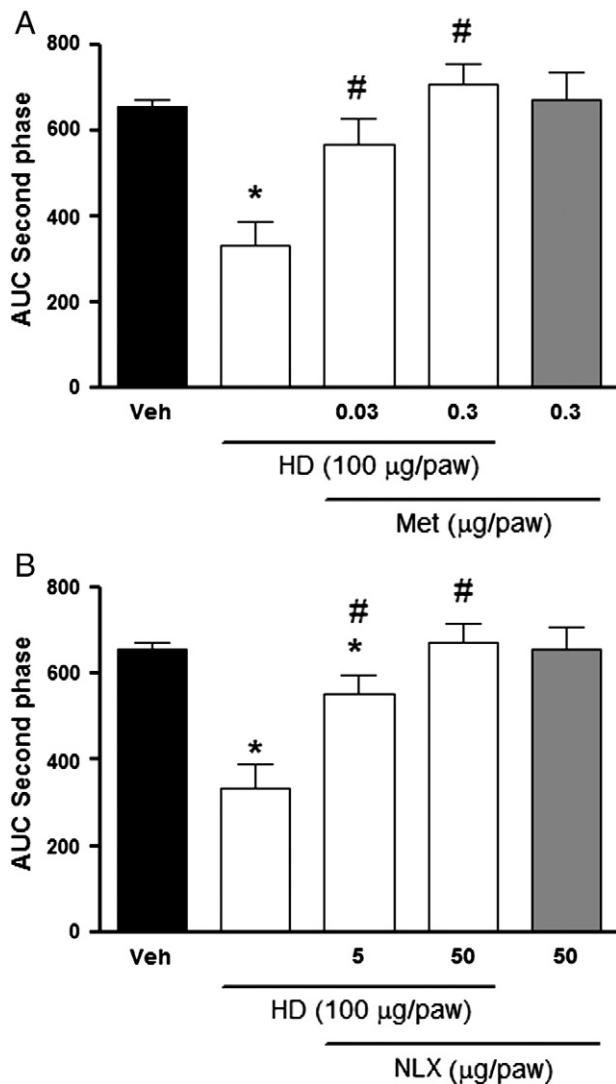


**Fig. 4.** Effect of the local peripheral administration of AM281 (AM, 0.3, 30  $\mu$ g/paw) and SR144528 (SR, 0.3, 30  $\mu$ g/paw) on HD-induced antinociception in rats submitted to the formalin test. Bars are the means  $\pm$  S.E.M. of 6 animals. \* $P < 0.05$ , significantly different from the control group (Veh) and # significantly different from the HD (100  $\mu$ g/paw) group, as determined by one-way ANOVA followed by Tukey's test.

We thus conclude that although it is possible that FAAH inhibition may be involved in the mechanism of action of HD, other mechanisms are probably of greater importance. An alternative, however, may be that HD can inhibit other metabolic pathways of PEA and/or AEA and that this may contribute to the effects seen here. The best candidate in this respect is N-acyl ethanolamine-hydrolyzing acid amidase (Sun et al., 2005). Relatively little work has been undertaken on the pharmacology of this enzyme, but a recent report has shown that a selective inhibitor of N-acyl ethanolamine-hydrolyzing acid amidase reduces carrageenan-induced leukocyte infiltration in vivo (Solorzano et al., 2009). To our knowledge, inhibitors of this enzyme have not been tested locally in rats submitted to the formalin test.

#### "Entourage", CB<sub>2</sub> cannabinoid receptor-like and PPAR $\alpha$ -mediated effects of HD

In view of the lack of direct effect of PEA upon CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors, the SR144528-sensitive effect of PEA in the formalin test (Calignano et al., 1998) has been attributed to effects on an unidentified CB<sub>2</sub>-like receptor and to an "entourage" effect whereby the PEA increases the efficacy of endocannabinoids (review, see Lambert et al., 2002). Some studies have reported the ability of PEA to negatively

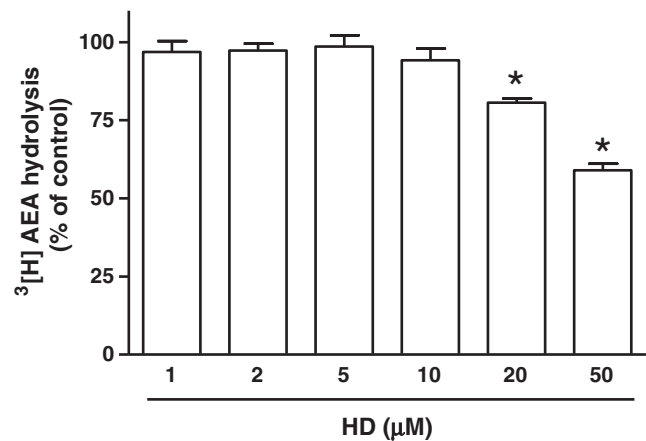


**Fig. 5.** Effect of the local peripheral administration of methiothepin (Met; 0.03, 0.3 µg/paw) and naloxone (NLX; 5, 50 µg/paw) on HD-induced antinociception in rats submitted to the formalin test. Bars are the means  $\pm$  S.E.M. of 6 animals. \* $P < 0.05$ , significantly different from the control group (Veh) and # significantly different from the HD (100 µg/paw) group, as determined by one-way ANOVA followed by Tukey's test.

modulate mast cells activation through CB<sub>2</sub> cannabinoid receptors (Facci et al., 1995; Mazzari et al., 1996; Cerrato et al., 2010), although other authors have not seen such effects (Jonsson et al., 2001). Another candidate mechanism to produce antinociception is PPAR- $\alpha$ , which is present in peripheral sensory neurons (LoVerme et al., 2006). It is involved in the analgesic effects of PEA, and which acts synergistically with CB<sub>1</sub> cannabinoid receptors to reduce pain (LoVerme et al., 2006; Russo et al., 2007; Sagar et al., 2008). Wallace et al. (2007) reported that the beneficial effect of the PEA analogue palmitoylallylamide was partially blocked by a PPAR $\alpha$  antagonist in the partial sciatic nerve injury model of neuropathic pain, and a PPAR $\alpha$  component of the antinociceptive effects of the FAAH inhibitor URB597 has also been seen in a model of inflammatory pain (Sagar et al., 2008). The possible participation of PPAR- $\alpha$  in the antinociceptive effect of HD in the rat formalin test is currently under investigation in our laboratory.

#### Involvement of opioid receptors

Local peripheral administration of the non-selective opioid receptor antagonist naloxone prevented antinociception induced by local HD. These results suggest that HD produces its peripheral effect following



**Fig. 6.** Inhibition of FAAH by HD in vitro. Bars are the mean  $\pm$  S.E.M. of 3 rates of hydrolysis of the FAAH substrate anandamide (AEA, 2 µM) expressed as % of vehicle controls. \* $P < 0.05$  versus control.

activation of opioid receptors at the peripheral terminal of the sensory neuron. We hypothesize that this effect is downstream of the mobilization of the endocannabinoid system by HD, given that systemic, spinal or supraspinal administration of naloxone is able to block the antinociceptive effect of anandamide and  $\Delta^9$ -tetrahydrocannabinol (Reche et al., 1996; Haller et al., 2008), of the CB<sub>2</sub> cannabinoid receptor-selective agonist AM1421 (Ibrahim et al., 2005; Curto-Reyes et al., 2010) and of the FAAH inhibitors URB59, OL135 and JNJ-161010 (Jhaveri et al., 2006; Chang et al., 2006; Karbarz et al., 2009) in experimental pain models.

#### Involvement of serotonin receptors

HD-induced antinociception was prevented by the non-selective 5-HT receptor antagonist methiothepin. Since methiothepin is a high-affinity 5-HT<sub>1/2/6/7</sub> receptor antagonist (Hoyer et al., 1994), data suggest that these receptors could be involved in HD-induced local antinociception in the formalin test, again presumably downstream of the effects on the endocannabinoid system. If this downstream effect is local, the most likely candidate peripheral receptor would be the 5-HT<sub>1</sub> receptor, whose activation is linked to local antinociception (Doak and Sawynok, 1997; Granados-Soto et al., 2010), but not the 5-HT<sub>2/6/7</sub> receptors as their local activation is associated with pronociception (Rocha-González et al., 2005; Castañeda-Corral et al., 2009; Nakajima et al., 2009). There may be a difference as to which 5-HT receptor subtypes are recruited following local and systemic activation of the endocannabinoid system, since systemic cannabinoids interact with descending serotonergic pathways via CB<sub>1</sub>-mediated mechanisms to produce antinociception involving spinal 5-HT<sub>7</sub> and 5-HT<sub>2A</sub> receptors (Seyrek et al., 2010), and since activation of spinal 5-HT<sub>2</sub> (Sasaki et al., 2001), 5-HT<sub>3</sub> (Sasaki et al., 2001) and 5-HT<sub>7</sub> (Dogrul et al., 2009; Yanarates et al., 2010) receptors produce antinociception. Further experiments are needed to determine which 5-HT systems are involved in the actions of HD.

#### Conclusion

Our results indicate that HD produces peripheral antinociception in the formalin test. The antinociceptive effects of HD are prevented by AM281 and SR144528 as well as by methiothepin and naloxone. The effect of HD on FAAH in vitro could contribute at best in part to the local effect of HD. Our data are consistent with the hypothesis that HD may have potential as an analgesic in inflammatory pain.

#### Conflict of interest statement

We do not have conflict of interest.

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