Evaluation of fatty acid amides in the carrageenan-induced paw edema model

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Abstract

While it has long been recognized that Δ⁹-tetrahydrocannabinol (THC), the primary psychoactive constituent of cannabis, and other cannabinoid receptor agonists possess anti-inflammatory properties, their well-known CNS effects have dampened enthusiasm for therapeutic development. On the other hand, genetic deletion of fatty acid amide hydrolase (FAAH), the enzyme responsible for degradation of fatty acid amides, including endogenous cannabinoid N-arachidonoyl ethanolamine (anandamide; AEA), N-palmitoyl ethanolamine (PEA), N-oleoyl ethanolamine (OEA), and oleamide, also elicits anti-edema, but does not produce any apparent cannabinoid effects. The purpose of the present study was to investigate whether exogenous administration of FAAs would augment the anti-inflammatory phenotype of FAAH (−/−) mice in the carrageenan model. Thus, we evaluated the effects of the FAAs AEA, PEA, OEA, and oleamide in wild-type and FAAH (−/−) mice. For comparison, we evaluated the anti-edema effects of THC, dexamethasone (DEX), a synthetic glucocorticoid, diclofenac (DIC), a nonselective cyclooxygenase (COX) inhibitor, in both genotypes. A final study determined if tolerance to the anti-edema effects of PEA occurs after repeated dosing. PEA, THC, DEX, DIC elicited significant decreases in carrageenan-induced paw edema in wild-type mice. In contrast, OEA produced a less reliable anti-edema effect than these other drugs, and AEA and oleamide failed to produce any significant decreases in paw edema. Moreover, none of the agents evaluated augmented the anti-edema phenotype of FAAH (−/−) mice, suggesting that maximal anti-edema effects had already been established. PEA was the most effective FAA in preventing paw edema and its effects did not undergo tolerance. While the present findings do not support a role for AEA in preventing carrageenan-induced edema, PEA administration and FAAH blockade elicited anti-edema effects of an equivalent magnitude as produced by THC, DEX, and DIC in this assay.

Keywords: Endogenous cannabinoid; Fatty acid amide hydrolase (FAAH); Inflammation; THC; N-Arachidonoyl ethanolamine (anandamide); N-Palmitoyl ethanolamine (PEA); Edema

1. Introduction

While the primary active cannabinoid constituent of cannabis, Δ⁹-tetrahydrocannabinol (THC), has long been known to possess anti-edema properties (Sofia et al., 1974), the occurrence of psychotropic side effects greatly limits the therapeutic utility of this and other cannabinoid receptor agonists that act directly at the CB₁ receptor (Compton et al., 1996; Jarbe et al., 1981; Wiley et al., 1993). An alternative target to treat inflammation is to block fatty acid amide hydrolase (Cravatt et al., 1996), the enzyme that is predominantly responsible for the biodegradation of the endogenous cannabinoid N-arachidonoyl ethanolamine or anandamide (Devane et al., 1992), as well as other fatty acid amides (FAAs) including the analgesic/antiinflammatory compound N-palmitoyl ethanolamine (Lo Verme et al., 2005a), the appetite modulating ligand N-oleoyl ethanolamine (Lo Verme et al., 2005b), and the sleep-inducing agent oleamide (Basile et al., 1999; Cravatt et al., 1996).
support that FAAH has a key role in regulating the activities of exogenous and endogenous FAAs was revealed in FAAH (−/−) mice (Cavavatt et al., 2001). These mice are severely impaired in their ability to degrade FAAs (Clement et al., 2003), possess significantly elevated brain levels of endogenous AEA and other FAAs, and exhibit CB1 mediated phenotypic hypalgesia in the tail immersion, hot plate, and formalin pain models (Cavavatt et al., 2001, 2004; Lichtman et al., 2004). Importantly, FAAH (−/−) mice and transgenic mice that express FAAH in the nervous system, but not in the periphery, exhibit reduced edema following an intraplantar injection of carrageenan into the paw (Cavavatt et al., 2004; Lichtman et al., 2004) suggesting that this enzyme represents a viable target to treat edema and that specific FAAs may mediate this effect. The observation that the irreversible carbamate inhibitor of FAAH, URB597, produces anti-edema effects in the carrageenan model (Holt et al., 2005) further supports that this enzyme and/or specific FAAs may be a clinically relevant treatment for edema.

Genetic (Cavavatt and Lichtman, 2004; Lichtman et al., 2004) and pharmacological (Holt et al., 2005) inhibition of FAAH decreases carrageenan-induced paw edema. Thus, the purpose of the experiments in the present study was to identify specific FAAs that reduce carrageenan-induced edema and to determine whether these FAAs would augment the anti-edema phenotype of FAAH (−/−) mice. FAAH (Willoughby et al., 1997) rapidly degrades AEA in wild type animals, thereby presenting a formidable challenge in investigating AEA’s in vivo effects. Thus, another fundamental goal of these studies was to assess whether AEA augments the FAAH (−/−) mouse anti-inflammatory phenotype. To this end, we assessed the anti-edema effects of AEA, PEA, OEA, and oleamide in FAAH (−/−) and wild-type mice, as assessed in the carrageenan model of paw edema. These effects were compared to those produced by positive control compounds including THC, the synthetic glucocorticoid dexamethasone (DEX), and the non-selective cyclooxygenase (COX) inhibitor diclofenac (DIC) in both FAAH (−/−) and wild-type mice. A final study examined whether the anti-edema effects of PEA undergo tolerance in the carrageenan model by administering either PEA or vehicle twice daily for 5 days and determining the anti-edema effects in the carrageenan model of a challenge dose of either PEA (25 mg/kg) or vehicle.

2. Methods

2.1. Subjects

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) and male and female FAAH (−/−) mice backcrossed for 13 generations onto a C57BL/6 background served as subjects. Subjects weighed between 20 and 30 g, and were housed 4–5 per cage in a temperature-controlled (20–22 °C) facility. Mice were given unlimited access to food and water in their home cages and were maintained on a 12/12 h light/dark cycle. Food and water were available ad libitum. All animal protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee and were in concordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

2.2. Drugs

AEA, which was chemically synthesized as described previously (Cavavatt et al., 1996), N-palmitoyl ethanolamine (PEA; Tocris, Ellisville, MO), N-oleoyl ethanolamine (OEA; Tocris, Ellisville, MO), oleamide (Tocris, Ellisville, MO), and Δ9-tetrahydrocannabinol (THC; National Institute on Drug Abuse, Bethesda, MD) were dissolved in a vehicle that consisted of a mixture of ethanol, alkamuls-620 (Rhone-Poulenc, Princeton, NJ), and saline at a ratio of 1:1:18. Dexamethasone (DEX; Tocris, Ellisville, MO) and diclofenac (DIC; Tocris, Ellisville, MO) were dissolved in saline. Each drug was given through the i.p. route of administration in a volume of 10 μl/g body weight.

2.3. Induction of paw edema with carrageenan

These procedures have been previously described and used by our laboratory (Cavavatt et al., 2004; Lichtman et al., 2004). Edema was induced by giving an intraplantar injection of 0.3% carrageenan (Sigma, St Louis) in a 20 μl volume into the hind left paw using a 26 gauge needle. Paw thickness was measured with electronic digital calipers (Traceable Calipers, Friendswood, TX), prior to 1, 3, 5, and 24 h following carrageenan administration. Peak effects occurred by 5 h and carrageenan-induced paw edema returned to almost baseline levels by 24 h in both FAAH (−/−) and wild-type mice (see Fig. 1), thus only the 5 h data are reported.

2.4. Procedures

In the initial screening studies, we tested whether 50 mg/kg of PEA, OEA, AEA, or oleamide administered 30 min (i.p.) before carrageenan would reduce paw edema in wild-type mice. This dose of each compound was used in a previous study to characterize the behavioral effects of FAAs in FAAH (−/−) mice (Lichtman et al., 2002). Additionally, THC was tested initially at a dose of 50 mg/kg administered 30 min (i.p.) before carrageenan. Each drug that was found to reduce edema at the 50 mg/kg dose was then evaluated in dose–response studies. DEX and DIC were assessed for comparison. DIC was injected 30 min before carrageenan and DEX was injected 1 h before carrageenan. These doses and administration times were based on previous reports (Amann and Schuligoi, 2000; Buritova et al., 1996) and pilot studies in our laboratory in which these doses and time points maximally decreased edema in the carrageenan model. Next, the effects of AEA (50 mg/kg), PEA

![Fig. 1](https://example.com/image1.png)
(50 mg/kg), OEA (50 mg/kg), THC (50 mg/kg), DEX (10 mg/kg), and DIC (5 mg/kg) were tested in FAAH (−/−) mice to determine if they would augment the anti-edema phenotype. Each of these concentrations represented maximal doses and were derived from the appropriate scientific literature. In a final experiment we sought to determine whether repeated dosing of PEA would induce tolerance or augment the effects of PEA on edema. Subjects were given two daily i.p. injections of vehicle or PEA (50 mg/kg) for five consecutive days and the effects of a challenge dose of PEA (25 mg/kg) or vehicle administered 30 min before carrageenan were assessed in the carrageenan model on the following day. Experimentally naïve mice were used for all control groups and test conditions.

2.5. Data analysis

Data analyses included differences in paw edema from baseline measures at the 5 h time point when the effect of carrageenan on paw edema peaks. Analysis of variance (ANOVA) were used to analyze the effect of genotype and drugs. Dunnett’s test was used for post hoc analysis in the dose–response experiments to compare the effects of each drug dose to those of vehicle. The Tukey–Kramer test was used for post hoc analyses comparing the drug effects in the FAAH (−/−) and (+/+) genotypes and between challenge doses in the PEA repeated dosing study. Differences were considered significant at the p < 0.05 level.

3. Results

3.1. FAAs, DEX, DIC, and THC reduce the development of carrageenan-induced paw edema

In the first set of experiments we sought to determine if pretreatment with the FAAs PEA, OEA, AEA, and oleamide, as well as the reference compounds DEX, DIC, and THC would reduce carrageenan-induced paw edema in C57BL/6 mice. All drugs were administered 30 min before intraplantar injection of carrageenan, with the exception of DEX, which was administered 1 h before carrageenan. As expected both DEX (t(1.8) = 53, p < 0.001), and DIC (t(1.7) = 25, p < 0.001) significantly reduced carrageenan induced paw edema (see Fig. 2a). Systemic administration of THC (Fig. 2b) also elicited a significant decrease in carrageenan-induced paw edema, F(4,25) = 18.9, p < 0.0001 at the 12.5 (p < 0.05), 25 (p < 0.01), and 50 (p < 0.01) mg/kg doses. The effects of the FAAs are shown in Fig. 2c. Systemic treatment with PEA (F(4,31) = 45.41, p < 0.0001) at the 12.5 (p < 0.01), 25 (p < 0.01), and 50 (p < 0.01) mg/kg doses reduced paw edema in carrageenan-treated mice. OEA also reduced carrageenan-induced edema (F(3,20) = 6.36, p < 0.01), but this effect was only significant at the highest dose (50 mg/kg) tested. In contrast, systemic treatment with AEA (p < 0.10) and oleamide (p < 0.54) failed to elicit significant reductions in carrageenan-induced paw edema.

3.2. FAAs, DEX, DIC and THC fail to augment the anti-inflammatory phenotype in FAAH (−/−) mice

In the next set of experiments, we evaluated the ability of PEA (25 mg/kg), OEA (50 mg/kg), AEA (50 mg/kg), THC (50 mg/kg), DEX (10 mg/kg) and DIC (5 mg/kg), to augment the anti-edema phenotype previously reported in FAAH (−/−) mice (Cravatt et al., 2004; Lichtman et al., 2004). As expected, FAAH (−/−) mice consistently displayed a significant reduction in carrageenan-induced paw swelling, regardless of injection treatment (see Fig. 3). In wild type mice, PEA (Fig. 3a) F(3,16) = 43, p < 0.0001, THCC (Fig. 3b) F(3,20) = 50, p < 0.0001, DEX (Fig. 3c), F(3,26) = 15, p < 0.0001, and DIC (Fig. 3d), F(3,17) = 9.8, p < 0.001, again reduced carrageenan-induced paw edema. None of these compounds augmented the anti-edema phenotype of FAAH (−/−) mice. In contrast to the data presented in Fig. 1c, OEA failed to decrease paw edema in wild-type mice and did not further enhance the anti-edema phenotype of FAAH (−/−) (Fig. 3e), though a significant group effect was found due to the FAAH (−/−) phenotype, F(3,20) = 23, p < 0.0001. Similarly, AEA (Fig. 3f) failed to reduce edema in either wild-type or FAAH (−/−) mice, while the significant ANOVA was due to genotype differences, F(3,20) = 10.6, p < 0.01.

3.3. PEA-induced anti-edema does not undergo tolerance

To the best of our knowledge, there are presently no published reports examining whether the anti-edema effects of PEA undergo tolerance in the carrageenan model. Thus, in the final experiment, we investigated whether repeated administration of either PEA (50 mg/kg) or vehicle given twice daily for 5 days would result in diminished anti-edema effects upon a challenge dose of either PEA (25 mg/kg) or vehicle injected the following day 30 min before carrageenan. As shown in Fig. 4, a significant overall effect was found, F(3,18) = 11.9, p < 0.001. An acute challenge of PEA elicited an equivalent degree in magnitude of anti-edema effects regardless of whether mice received repeated PEA or vehicle injections.

4. Discussion

In the present study, PEA was the only FAA that reliably and efficaciously reduced the magnitude of paw edema when administered before the carrageenan. The positive controls DEX and DIC, as well as the cannabinoid agonist THC also reduced paw edema when administered before the carrageenan. In contrast, neither the endocannabinoid AEA nor the sleep-inducing lipid oleamide had any significant effects on carrageenan-induced paw edema. No agent tested augmented the anti-edema phenotype of FAAH (−/−) mice. However, the efficacies for DEX, DIC, PEA, and THC were similar in magnitude to the anti-edema FAAH (−/−) phenotype suggesting that ceiling effects may have prevented the occurrence of further reductions in paw edema. Strikingly, the anti-edema effects of PEA did not undergo tolerance following repeated administration of high doses.

PEA was the only FAA evaluated in the present study that reliably inhibited carrageenan-induced paw edema in wild-type mice. Indeed, the anti-inflammatory properties of PEA were first described over 50 years ago (Coburn et al., 1954) and are well established (Conti et al., 2002; Costa et al., 2002; De Petrocellis et al., 2001; Di Marzo et al., 2001b; Lo Verme et al., 2005a; Mazzari et al., 1996; Re et al., 2007).
PEA does not bind to CB₁ or CB₂ receptors (Di Marzo et al., 2001b; Lambert and Di Marzo, 1999; Lambert et al., 2002), however, SR144528 has been found to block the anti-edema effects of PEA that is administered before carrageenan (Conti et al., 2002; Lichtman et al., 2004; Malan et al., 2002). On the other hand these same investigators also found that SR144528 failed to antagonize the PEA-induced anti-edema effects on established carrageenan-induced edema (Costa et al., 2002; Farquhar-Smith and Rice, 2001; Ross et al., 2000). Additionally, it has been proposed that PEA can also act by inhibiting FAAH expression (Di Marzo et al., 2001b) which subsequently enhances AEA effects at CB or transient vanilloid 1 channel (TRPV1) receptors (De Petrocellis et al., 2001). PEA’s anti-inflammatory effect also may be produced by its local antagonism or down-regulation of mast cell degranulation (Jack, 1996; Mazzari et al., 1996; Re et al., 2007). Another possible mechanism by which PEA exerts its anti-edema effects is by activating peroxisome proliferator activated receptor-α (PPAR-α), as PEA lacks efficacy in models of edema in PPAR-α (−/−) mice (Lo Verme et al., 2005a). However, this suggestion requires further study as OEA which is 10-fold more potent than PEA at PPAR-α produced unreliable effects in the present study and has recently been reported to reduce inflammatory pain through a PPAR-α-independent mechanism of action (Suardiaz et al., in press). Nonetheless, though it should be noted that these results do not provide direct evidence that PEA mediates the FAAH (−/−) anti-edema phenotype, the results of the present study are consistent with the idea that elevated levels of PEA may contribute to the anti-edema phenotype of FAAH (−/−) mice and that PEA has anti-edema effects in the carrageenan model.

A novel observation found here was that repeated dosing of PEA failed to lead to tolerance to an acute injection of PEA in the carrageenan model. Similarly, PEA retained its anti-hyperalgesic effects to a mechanical noxious stimulus in the sciatic nerve ligation model of neuropathic pain even after subchronic dosing (i.e., 30 mg/kg once a day for 14 days (Lo Verme et al., 2005a). In contrast, other studies have reported tolerance to the behavioral effects of FAAAs with repeated dosing. Tolerance to AEA-induced hypothermia, analgesia, catalepsy, and suppression of spontaneous activity has been found in a number of studies (Costa et al., 2000; Fride, 1995; Pertwee et al., 1993; Welch, 1997). Tolerance to oleamide-induced hypothermia, analgesia, and suppression of open field activity was found

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**Fig. 2.** Effects of various agents on carrageenan-induced paw edema. All data are reflected as post-injection paw thickness – pre-injection paw thickness ± SE. Paw diameter was measured prior to and 5 h after carrageenan administration. Samples sizes were 5–10 mice per group. Baseline paw thickness values ranged between 1.89 and 2.25 mm. Separate groups of experimentally naïve mice were used for each set of experiments. (A) The positive control compounds dexamethasone (DEX) and diclofenac (DIC) significantly reduced carrageenan-induced paw edema. **p < 0.01 versus vehicle (VEH). (B) Effects of PEA, OEA, AEA, and oleamide (OLE) on carrageenan induced paw edema. All drugs were administered 30 min before carrageenan was injected into the paw. *p < 0.05 versus VEH, **p < 0.01 versus VEH. (C) THC dose-dependently reduced carrageenan-induced edema. *p < 0.05 versus VEH, **p < 0.01 versus VEH. (D) Both rimonabant and SR144528 completely blocked the anti-edema effects of THC. **p < 0.01 versus VEH + VEH.
after eight, but not three, days of repeated dosing (Fedorova et al., 2001). Thus, the observation that tolerance does not develop to the anti-edema effect of PEA, unlike other FAAs, is a critical finding with respect to the potential therapeutic utility of this compound.

A significant challenge to investigate AEA’s in vivo effects in wild-type animals is its rapid degradation by FAAH (Willoughby et al., 1997), thus a prominent goal of the present study was to determine if AEA further reduced the FAAH (−/−) mouse anti-inflammatory response in the carrageenan model to confirm the role of AEA in inflammation. In the present study systemic administration of AEA failed to decrease carrageenan-induced edema in wild-type and FAAH (−/−) mice. Given that no compound tested was able to further augment the anti-inflammatory phenotype of FAAH (−/−) mice, the role of AEA in inflammation cannot be ruled out. Nonetheless, it is not surprising that the effects of the endogenous cannabinoid AEA in carrageenan-induced edema are somewhat mixed because of its rapid degradation in vivo (Willoughby et al., 1997). In contrast to the results of the present study, intraplantar administration of AEA elicited anti-edema effects in the rat carrageenan model (Richardson et al., 1998). A report in which the carbamate FAAH inhibitor URB597 elicited an anti-edema effect in pentobarbital-anesthetized mice that was blocked by the CB2 receptor antagonist SR144528 (Clayton et al., 2001a; Di Marzo et al., 2001a) suggests that AEA acting at CB2 receptors may play a role in edema. Similarly, SR144528 elicited a partial attenuation of the FAAH (−/−) anti-edema phenotype in one study (Lichtman et al., 2004), but not in another study (Cravatt et al., 2004). AEA also has been reported to produce anti-inflammatory effects via its actions at TRPV1 receptors (De Petrocellis et al., 2001; Di Marzo et al., 2001a).

Only the highest dose of OEA tested was found to inhibit carrageenan-induced paw edema, though this effect was much less in magnitude than the effects elicited by PEA, DIC, DEX, and THC (see Fig. 1b). Moreover, OEA failed to elicit a significant decrease in paw edema in a subsequent experiment (see Fig. 2e). There are no previous reports of OEA inhibiting carrageenan-induced paw edema but topical treatment with a single dose of OEA was found to inhibit inflammation in the phorbol ester ear pinna edema model in wild type, but not in PPAR-α (−/−), mice (Lo Verme et al., 2005a). OEA was reported to bind to PPAR-α receptors and to induce satiety and lipolysis by activating this receptor (Fox et al., 2001; Guzman et al., 2004; Rodriguez de Fonseca et al., 2001). However, OEA has recently been reported to reduce inflammatory pain through a PPAR-α-independent mechanism of action, though inflammation data was not reported (Suardiez et al., in press). On the other hand, OEA has been found to reduce visceral pain and reduce food intake through its actions at TRPV1 receptors (Wang et al., 2005).
THC was first reported to have anti-edema properties in the carrageenan model over 30 years ago (Sofia et al., 1974). Since this time, other non-selective cannabinoid receptor agonists, including HU210, WIN55,212-2 and anandamide, as well as selective CB2 receptor agonists AM1241 and JWH-133 were found to elicit anti-edema effects in this model (Clayton et al., 2002; Elmes et al., 2005; Nuckley et al., 2003a,b; Quartilho et al., 2003; Richardson et al., 1998). Our findings in the present study confirm THC’s anti-edema actions. However, the 12.5, 25, and 50 mg/kg doses of THC that decreased edema have also been shown to reduce locomotor activity (Cravatt et al., 2001; Lichtman et al., 2002) and consequently this change in weight bearing activity may indirectly affect edema. Indeed a significant challenge in developing THC as an anti-inflammatory agent is that it produces profound CNS effects (Compton et al., 1996; Jarbe et al., 1981; Wiley et al., 1993).

The mechanisms underlying the FAAH (−/−) anti-edema phenotype and the anti-edema effects of PEA are not addressed in the present study. However, they may involve the inhibition of early proinflammatory mediators including cytokines, inducible cyclooxygenase-2 (COX-2), inducible and/or endothelial nitric oxide synthase, TNF-alpha, and neutrophil influx (Costa et al., 2002; Farquhar-Smith and Rice, 2001; Ross et al., 2000). PEA has been found to inhibit mast cell degranulation (Mazzari et al., 1996) as well as reduce nitric oxide production (Costa et al., 2002), neutrophil influx (Farquhar-Smith and Rice, 2003) and TNF-alpha levels (Berdyshev et al., 1998). PEA also inhibits COX activity (Costa et al., 2002). The actions of PEA may also be due to the actions of its metabolites, particularly in the repeated dosing study, or downstream targets of this FAAH. Recently, activation of the large conductance potassium channel (KCa 1.1, BK, slo) a downstream target of PPAR-α receptor agonists, which may be an important target for PEA, was found to reduce pain in the formalin model and to act synergistically with anandamide to reduce pain (Russo et al., 2007). The PPAR-α receptor agonist GW7647 and anandamide were found to produce synergistic antinociception in this same study. In addition, the combination of anandamide and the COX-2 inhibitor, rofecoxib, synergistically decrease pain in the formalin model and increase levels of AEA, OEA, and PEA in the formalin treated paw (Guindon et al., 2006). These findings suggest that the anti-edema effects of FAAH inhibition may be mediated by multiple mechanisms and that the results from these combination studies should be extended to investigate their effects on inflammation.

An additional consideration in regard to the role of PEA and other FAAs in the anti-edema phenotype, as well as the finding that no compound tested augmented the anti-edema phenotype of FAAH (−/−) mice is that compensatory mechanisms related to the absence of FAAH also could account for the observed phenotype. The identification of N-acylethanolamine-hydrolyzing acid amidase (also referred to as PEA-preferring acid amidase) which can metabolize PEA and other FAAs (Sun et al., 2005; Tsuboi et al., 2004, 2005; Ueda et al., 2001, 2005) suggests a role of this enzyme in FAAH (−/−) mice. It has been demonstrated that FAAH, and COX-2 to a lesser and perhaps by a downstream mechanism, play a significant role in the inactivation of anandamide in the rat small mesenteric artery (Ho and Randall, 2007). Certainly, determining the mechanisms of action for PEA, FAAH blockade, and cannabinoid receptor agonists will be an important aspect of this research to address in future studies. Additionally, we have found that repeated administration of PEA does not induce tolerance in the carrageenan model; however, a related question to be addressed in future studies is the ability of these compounds to treat chronic inflammatory disease states. Future studies should also address the clinically relevant question of whether these agents can reduce edema when administered after edema has been established. This is a particularly important question as anti-edema agents are most often administered after inflammation has already occurred.

In conclusion, DEX, DIC, PEA, THC, and, to a lesser extent, OEA administered before carrageenan, reduced paw edema. In contrast, neither AEA nor oleamide produced any anti-edema effects. The failure of all agents to produce additional anti-edema effects in FAAH (−/−) mice, further suggests that deletion of FAAH results in near maximal anti-edema effects. Importantly, repeated administration of PEA did not induce tolerance in the carrageenan model, which would provide a clear therapeutic advantage. These findings strengthen assertions that PEA as well as FAAH inhibitors have therapeutic potential as anti-inflammatory agents.

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**Fig. 4. Effects of vehicle (VEH) or PEA (25 mg/kg) challenge doses following repeated dosing of VEH or PEA (50 mg/kg twice a day for 5 days). Baseline paw thickness values ranged between 1.97 and 2.06 mm. Data are reflected as post-injection paw thickness – pre-injection paw thickness ± SE. Paw diameter was measured prior to and 5 h after carrageenan administration. Sample sizes were 5–6 mice per group. **p < 0.01 versus VEH + VEH, †p < 0.01 versus PEA + VEH.**
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