

Interactions between synthetic vanilloids and the endogenous cannabinoid system

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Abstract The chemical similarity between some synthetic agonists of vanilloid receptors, such as olvanil (*N*-vanillyl-*cis*-9-octadecenoamide), and the ‘endocannabinoid’ anandamide (arachidonoyl-ethanolamide, AEA), suggests possible interactions between the cannabinoid and vanilloid signalling systems. Here we report that olvanil is a stable and potent inhibitor of AEA facilitated transport into rat basophilic leukemia (RBL-2H3) cells. Olvanil blocked both the uptake and the hydrolysis of [¹⁴C]AEA by intact RBL-2H3 cells (IC₅₀ = 9 μM), while capsaicin and pseudocapsaicin (*N*-vanillyl-nonanamide) were much less active. Olvanil was more potent than previously reported inhibitors of AEA facilitated transport, i.e. phloretin (IC₅₀ = 80 μM), AM404 (12.9% inhibition at 10 μM) or oleoylethanolamide (27.5% inhibition at 10 μM). Olvanil was a poor inhibitor of [¹⁴C]AEA hydrolysis by RBL-2H3 and N18TG2 cell membranes, suggesting that the inhibitory effect on [¹⁴C]AEA breakdown observed in intact cells was due to inhibition of [¹⁴C]AEA uptake. Olvanil was stable to enzymatic hydrolysis, and (i) displaced the binding of high affinity cannabinoid receptor ligands to membrane preparations from N18TG2 cells and guinea pig forebrain (*K*_i = 1.64–7.08 μM), but not from cells expressing the CB2 cannabinoid receptor subtype; (ii) inhibited forskolin-induced cAMP formation in intact N18TG2 cells (IC₅₀ = 1.60 μM), this effect being reversed by the selective CB1 antagonist SR141716A. Pseudocapsaicin, but not capsaicin, also selectively bound to CB1 receptor-containing membranes. These data suggest that some of the analgesic actions of olvanil may be due to its interactions with the endogenous cannabinoid system, and may lead to the design of a novel class of cannabimimetics with potential therapeutic applications as analgesics.

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Key words: Vanilloid receptor; Cannabinoid receptor; Endocannabinoid; Anandamide; Capsaicin; Inflammation; Antinociception; Mast cell

1. Introduction

Evidence exists for the presence, in sensory neurons and some peripheral tissues, of distinct types of vanilloid receptors that are activated by the natural component of chili peppers, capsaicin (*N*-(3-methoxy-4-hydroxy-benzyl)-8-methyl-6-*trans*-nonenamide or *N*-vanillyl-8-methyl-6-*trans*-nonenamide, Fig. 1), and antagonized by capsazepine (for a review [1]). One

of these receptors was recently cloned and shown to mediate capsaicin effects in dorsal root ganglia [2]. The presence of at least one additional vanilloid receptor is suggested by studies carried out with resiniferatoxin, a phorbol derivative, the pattern of whose pharmacological actions differs from that of capsaicin [3]. A synthetic vanilloid derivative and anti-inflammatory/analgesic compound, *N*-(3-methoxy-4-hydroxy-benzyl)-*cis*-9-octadecenoamide (olvanil, [4,5], Fig. 1), was also suggested to bind to a special subtype of vanilloid receptors based on its non-pungency, lower toxicity and slower activation kinetics of Ca²⁺ inward currents as compared to capsaicin [5,6]. It is not known whether olvanil binds to the recently cloned VR1 vanilloid receptor [2].

The pharmacology and biochemistry of cannabinoid receptors are better known than those of vanilloid receptors (for a recent review see [7]). Two different receptor subtypes, named CB1 and CB2, have been identified and cloned from mammalian tissues [7], and endogenous ligands for these proteins have been found. The best known ‘endocannabinoid’ is anandamide (arachidonoyl-ethanolamide, AEA, [8]), whose human and rat tissue distribution, biosynthetic and catabolic pathways, and pharmacological actions have been thoroughly studied (recently reviewed in [9]). It has been proposed that the action of AEA is terminated by facilitated transport into cells followed by enzymatic hydrolysis of the amide bond. Evidence for a membrane ‘carrier’ protein selective for AEA has been provided for rat central neurons [10,11] and rat cerebellar granule cells [12] – which selectively express CB1 receptors [7] – and rat basophilic leukemia (RBL-2H3) cells [13] – which contain CB2 receptors [7,16]. AEA facilitated transport is inhibited by alkylating agents [13], phloretin and oleoylethanolamide [12], and some aromatic derivatives of AEA such as *N*-benzyl-arachidonamide [12] and *N*-(4-hydroxyphenyl)-arachidonamide (AM404) [11]. Once inside cells, AEA is hydrolyzed to arachidonate and ethanolamine via the action of ‘fatty acid amide hydrolase’ (FAAH), an enzyme which recognizes as substrates several long chain fatty acid amides and esters [14,15], including a potent anti-inflammatory congener of AEA, palmitoylethanolamide (PEA, [13,16]).

Starting from the chemical similarity between long chain capsaicin analogues and AEA or, particularly, aromatic inhibitors of AEA facilitated transport [11,12], we have examined whether capsaicin-like compounds could interact with the best characterized proteins of the ‘endogenous cannabinoid system’, i.e. the AEA ‘carrier’, FAAH and the two cannabinoid receptor subtypes. We report that olvanil is a potent inhibitor of AEA facilitated transport into RBL-2H3 cells, and both olvanil and another capsaicin congener, *N*-vanillyl-nonan-

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amide (pseudocapsaicin), but not capsaicin, weakly bind to CB1, but not CB2, cannabinoid receptors.

2. Materials and methods

Mouse neuroblastoma N18TG2, mouse J774 macrophages and RBL-2H3 cells were cultured as described previously [9,13]. [¹⁴C]AEA and [¹⁴C]PEA (5 mCi/mmol) were synthesized as described previously [8] starting from [¹⁴C]ethanolamine and the corresponding fatty acyl chlorides. Anandamide and oleylethanolamide were synthesized likewise from ethanolamine and arachidonoyl- or oleoyl-chlorides. [³H]SR 141716A (55 Ci/mmol) was purchased from Amersham, whereas [³H]CP 55940 (164 Ci/mmol) and [³H]WIN 55212-2 (43 Ci/mmol) were purchased from NEN. Phloretin, capsaicin and pseudocapsaicin were purchased from Sigma, forskolin from Fluka, and AM404 from Cayman Chemical. Olvanil was synthesized in the Naples laboratory by reacting 30 mg of 3-methoxy-4-hydroxy-benzylamine with an excess of oleoylchloride at 4°C for 30 min. The mixture was then brought to dryness under a flow of N₂ and purified by normal phase high pressure liquid chromatography (NP-HPLC) carried out with a semi-preparative column (Spherisorb S5W) eluted with a 40-min linear gradient from 90% to 80% of *n*-hexane in 2-propanol (flow rate 2 ml/min). Under these conditions olvanil was eluted after 27 min. Electron-impact mass spectrometric analysis, carried out on an HP-5989B quadrupole mass analyzer equipped with an electron impact source operating at 70 eV and 250°C, confirmed the chemical structure of the compound (*m/z* = 417, molecular ion; *m/z* = 294, loss of a 3-methoxy-4-hydroxy-phenyl group; *m/z* = 137 and 281, cleavage of the CH₂-NH bond). Experiments with intact cells were carried out as described previously [13] in six-well dishes (about 1.0 × 10⁶ cells/well). Cells were incubated at 37°C with 0.5 ml of serum-free media containing 20000 cpm (4 μM) of either [¹⁴C]AEA or [¹⁴C]PEA for 30 min with the substances shown in Figs. 1 and 2. After having washed the cells three times with 3 ml of 0.2% bovine serum albumin (BSA)-containing medium, [¹⁴C]AEA or [¹⁴C]PEA and [¹⁴C]ethanolamine in media and cells were quantified as described [13]. Some experiments were also performed in the presence of 50 μM *p*-hydroxymercuribenzoate. [¹⁴C]AEA and [¹⁴C]PEA hydrolysis by cell-free fractions was studied by using 10000 × *g* pellets from RBL-2H3 and N18TG2 cell homogenates prepared as described [13]. Aliquots of 40–80 μg total proteins were incubated in 0.5 ml of 50 mM Tris-HCl, pH 9, with 40000 cpm of either [¹⁴C]AEA or [¹⁴C]PEA for 30 min at 37°C. [¹⁴C]Ethanolamine produced from the enzymatic hydrolysis was determined as described elsewhere [13]. Experiments on the hydrolysis of olvanil (0.2 mg) by N18TG2 or RBL-2H3 cell membrane preparations were carried out with 30–300 μg total proteins suspended in 0.5 ml Tris-HCl, 50 mM, pH 9.0, and incubated for 30 min at 37°C. Aliquots of the membrane preparations were incubated in parallel with [¹⁴C]AEA to confirm the presence of FAAH activity. After the incubation, lipids were extracted and loaded on analytical thin layer chromatography plates in comparison with synthetic standards of olvanil and oleic acid. Plates were developed with chloroform/methanol/NH₃ 85:15:1 by vol. and the formation of oleic acid (*R_f* = 0.25) monitored by exposing the plate to iodine vapors. All the experiments on the effects of olvanil, capsaicin and pseudocapsaicin on AEA and PEA inactivation were performed in the Naples laboratory. Binding assays were carried out with guinea-pig forebrain (GPF) membranes or with membranes from CHO cells stably transfected with CB2 receptors (CHO-CB2 cells) [18] (in the Aberdeen laboratory), or with membranes from N18TG2 and RBL-2H3 cells (in the Naples laboratory). Forebrains from adult male Dunkin Hartley guinea-pigs were suspended in 50 mM Tris buffer (pH 7.4) and 0.32 M sucrose and homogenized with an Ultra-Turrax homogenizer. The homogenates were diluted with 50 mM Tris buffer and centrifuged at 100 000 × *g* for 1 h thus yielding the membranes for the binding assays. CHO-CB2, N18TG2 and RBL-2H3 cell membranes were prepared as described in [18,19] and [16], respectively. Binding assays were carried out by using the filtration procedures described previously [16,18,19] with slight modifications consisting in the presence of phenylmethylsulfonyl fluoride (PMSF, Sigma, 0.1 mM) in the respective binding buffers, and the use of different amounts of total membrane proteins and radiolabelled ligands ([³H]CP 55940 for GPF and CHO-CB2 membranes, [³H]SR 141716A for N18TG2 membranes, and [³H]WIN 55212-2 for RBL-2H3 membranes). Specific

binding was calculated by using either 1 μM unlabelled CP 55940, or 10 μM unlabelled SR 141716A (kindly donated by Sanofi Recherche, Montpellier) or HU-210 (kindly donated by Prof. R. Mechoulam, The Hebrew University of Jerusalem). The concentration of unlabelled ligand that produced a 50% displacement of the labelled ligands from specific binding sites was calculated using GraphPad Prism (GraphPad Software, San Diego, CA, USA). *K_i* values were calculated by means of the Cheng-Prusoff equation from the *K_d* values for each radiolabelled ligand in each of the membrane systems used. Cyclic AMP (cAMP) assays were performed in the Naples laboratory on intact confluent N18TG2 cells plated in six-well dishes and stimulated for 10 min at 37°C with 1 μM forskolin in 400 μl of serum-free Dulbecco's modified Eagle's Medium containing 20 mM HEPES, 0.1 mg/ml BSA, 0.1 mM 1-methyl-3-isobutylxanthine (Sigma) and either vehicle (ethanol) or anandamide, olvanil and olvanil plus SR 141716A at the concentrations shown in Fig. 3. After the incubation cells were extracted, and cAMP levels determined by means of a cAMP assay kit (Amersham), as advised by the manufacturer.

3. Results and discussion

In a previous study [13] we showed that, in RBL-2H3 cells, distinct uptake mechanisms exist for AEA and its anti-inflammatory congener PEA [16]. The diffusion of both acylethanolamides into intact RBL-2H3 cells was maximal after 20–30-min incubations, saturable, inhibited by *N*-ethylmaleimide and PMSF, and greatly reduced at 0–4°C. However, the two compounds did not compete for each other's uptake. RBL-2H3 cells were also found to express an 'AEA amidohydrolyase' activity [13] which was later identified as FAAH [17]. In agreement with the presence of distinct transporter proteins for the two acylethanolamides in RBL-2H3 cells, here we found that olvanil inhibits the facilitated diffusion of [¹⁴C]AEA, but not [¹⁴C]PEA, thereby significantly increasing the amount of extracellular AEA potentially available for cannabinoid receptor activation (Fig. 2A and data not shown). The inhibitory effect of olvanil (estimated

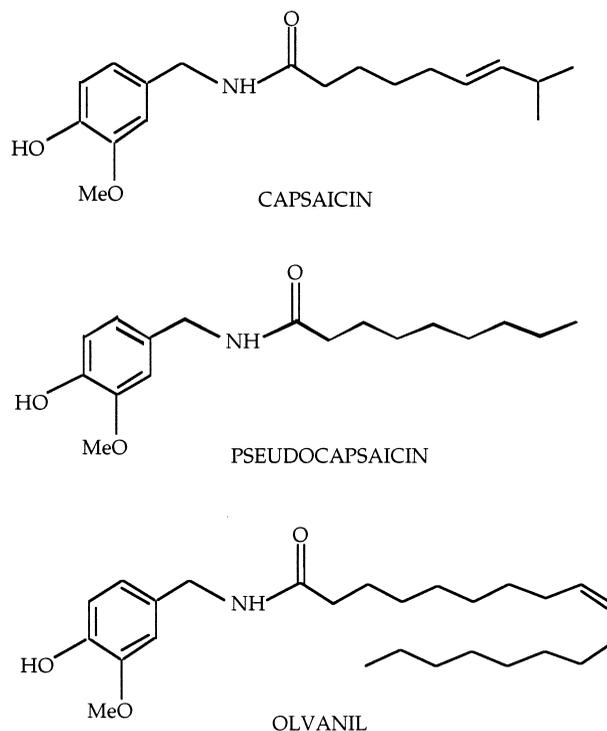
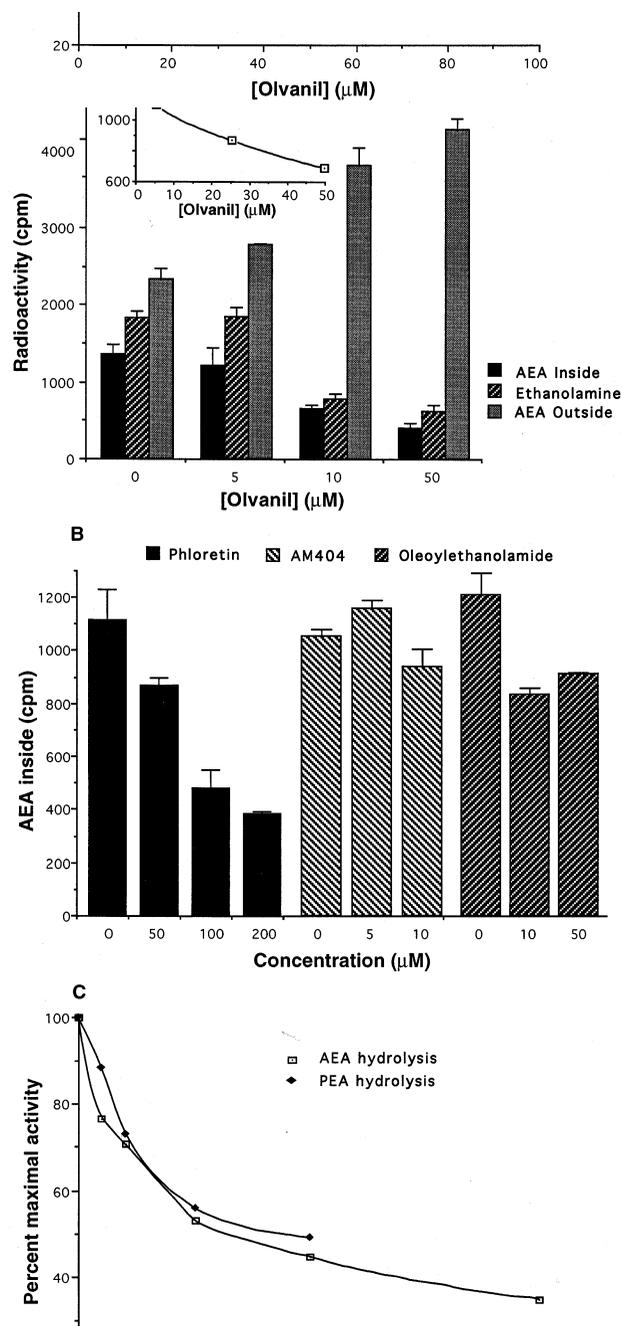


Fig. 1. Chemical structures of capsaicin, pseudocapsaicin and olvanil.

Fig. 2. Effect of olvanil and other inhibitors of AEA facilitated transport on [14 C]AEA inactivation by RBL-2H3 cells. A: Dose-dependent inhibition by olvanil of [14 C]AEA facilitated diffusion into and hydrolysis by intact cells. Data are expressed as radioactivity associated with AEA in the incubation media or inside cells, or with ethanolamine produced by AEA hydrolysis, after a 30-min incubation at 37°C, and are means \pm S.D. of three separate experiments carried out in duplicate. No significant effect of olvanil on [14 C]PEA facilitated diffusion and hydrolysis in intact cells was observed. The effect of olvanil in the presence of 50 μ M *p*-hydroxymercuribenzoate is shown in the inset. B: Dose-dependent effect of phloretin, AM404 and oleylethanolamide on [14 C]AEA facilitated diffusion into intact cells. Data are expressed as radioactivity associated with AEA inside cells after a 30-min incubation at 37°C, and are means \pm S.D. of three separate experiments carried out in duplicate. C: Dose-dependent inhibition by olvanil of [14 C]AEA and [14 C]PEA hydrolysis by RBL-2H3 membrane preparations (10000 \times g pellets). Data are expressed as the radioactivity associated with ethanolamine produced by AEA or PEA hydrolysis after a 30-min incubation at 37°C, and are means of three separate experiments carried out in duplicate. S.D. bars are not shown for the sake of clarity and were never higher than 5%.



$IC_{50} = 9 \pm 2 \mu$ M, $n = 3$) on [14 C]AEA diffused into cells after 30-min incubations at 37°C was more potent than that observed here with previously described [11,12] blockers of AEA facilitated diffusion such as phloretin (estimated $IC_{50} = 80 \mu$ M), oleylethanolamide (27.5% inhibition at 10 μ M) or AM404 (12.9% inhibition at 10 μ M) (Fig. 2B). The data obtained with the former two compounds are in good agreement with those previously reported for the inhibition of AEA diffusion into rat cerebellar granule cells [12], also in view of the fact that the concentration of radiolabelled AEA used in the previous study (0.3 nM) was much lower than that used here (4 μ M). The high concentration of the radiolabelled ligand used in the present study may indeed explain why AM404 was much less effective against [14 C]AEA uptake by RBL-2H3 cells than previously described for cortical astrocytes and neurons ($IC_{50} = 1\text{--}5 \mu$ M, [11]). We thought that this could have been due also to the longer incubation time used here as compared to the previous study [11], and, therefore, performed experiments looking at the effect of inhibitors on [14 C]AEA diffused into cells after 15-min incubations. This resulted in a slightly increased potency for oleylethanolamide (32.1% inhibition at 10 μ M) but not AM404 (14.5% inhibition at 10 μ M) (data not shown). With 30-min incubations, olvanil also inhibited [14 C]AEA hydrolysis by intact RBL-2H3 cells with an estimated IC_{50} of 9 μ M (Fig. 2A). Since the vanilloid compound inhibited [14 C]AEA hydrolysis by RBL-2H3 membrane preparations at higher concentrations ($IC_{50} = 32 \pm 1 \mu$ M, $n = 3$, Fig. 2C), it is possible that the inhibitory effect observed in intact cells was due to inhibition of AEA facilitated diffusion, which precedes enzymatic hydrolysis (as shown in [10], the $t_{1/2}$ values for AEA uptake and hydrolysis by rat cortical neurons are 2.5 and 6 min, respectively; moreover, the sub-cellular distribution of FAAH, described in [29], seems to rule out that anandamide is hydrolyzed by intact cells prior to its internalization). Olvanil also inhibited [14 C]AEA hydrolysis by N18TG2 membrane preparations – where FAAH expression has been shown [17] – but again only at high concentrations ($IC_{50} = 48 \pm 2 \mu$ M). However, in order to isolate the effect of olvanil on the AEA carrier from its weak effect on FAAH, we studied the uptake of [14 C]AEA by RBL-2H3 cells in the presence of *p*-hydroxymercuribenzoate at a concentration (50 μ M) found to counteract [14 C]AEA hydrolysis without

greatly affecting [14 C]AEA uptake (respectively, 42.2 ± 5.3 and $115.5 \pm 1.1\%$ of control, see also [13]). We found that, under these conditions, olvanil still inhibited [14 C]AEA accumulation ($IC_{50} = 35 \pm 2 \mu$ M, $n = 3$, Fig. 2A, inset) with little effect on residual [14 C]AEA hydrolysis ($41.8 \pm 2.3\%$ inhibition at 100 μ M), thus showing that the effect of the vanilloid on AEA uptake is not due to inhibition of AEA hydrolysis. Three further sets of data are in agreement with this conclusion: (i) olvanil also inhibited [14 C]AEA accumulation in J774 macrophages – where negligible FAAH activity is expressed [13,17] – with an estimated $IC_{50} = 27 \pm 9 \mu$ M ($n = 3$); (ii) oleamide, a competitive inhibitor of [14 C]AEA hydrolysis and a

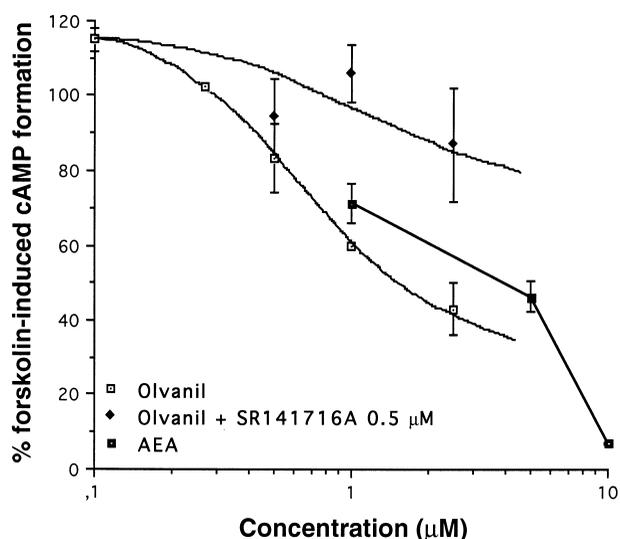


Fig. 3. Dose-dependent inhibition by olvanil and anandamide of forskolin-induced cAMP formation in N18TG2 cells. The effect of 0.5 μM SR 141716A on olvanil inhibitory action is also shown. Data are expressed as percent of cAMP levels in cells stimulated with 1 μM forskolin (66.6 ± 14.1 pmols/well), and are means \pm S.E.M. of three separate experiments. cAMP levels in unstimulated cells were 7.7 ± 3.3 pmols/well.

FAAH substrate [9,14,15,17], did not affect [^{14}C]AEA uptake by RBL-2H3 cells (data not shown); and (iii) olvanil did not counteract [^{14}C]PEA diffusion into RBL-2H3 cells even though it inhibited [^{14}C]PEA hydrolysis by membrane preparations as weakly as it did with [^{14}C]AEA hydrolysis ($\text{IC}_{50} = 49$ μM , Fig. 2C). We also found that olvanil is stable to FAAH-catalyzed hydrolysis since, under conditions leading to efficient [^{14}C]AEA hydrolysis, no oleic acid formation was observed from incubation of the vanilloid (0.4 mg/ml) with either RBL-2H3 or N18TG2 cell membranes (data not shown).

We wanted to assess whether capsaicin and its analogue pseudocapsaicin also inhibited AEA facilitated transport. The two compounds were found to reduce the uptake and the hydrolysis of [^{14}C]AEA by intact RBL-2H3 cells only at high μM concentrations, capsaicin being the most effective one on the uptake (estimated $\text{IC}_{50} = 46$ μM , with $\text{IC}_{50} > 100$ μM for the hydrolysis; the IC_{50} values for pseudocapsaicin were 83 and 60 μM for hydrolysis and uptake, respectively). The two compounds were not effective against [^{14}C]PEA in-

activation in intact cells ($\text{IC}_{50} > 100$ μM) and only slightly affected [^{14}C]AEA hydrolysis by membrane preparations ($\text{IC}_{50} = 83$ μM and 39 μM , respectively).

We next carried out a series of cannabinoid receptor binding experiments with olvanil, capsaicin and pseudocapsaicin. We used membrane preparations from guinea pig forebrain (GPF) and mouse neuroblastoma N18TG2 cells, which selectively express the CB1 receptor subtype [18,19], as well as membranes from CHO cells transfected with CB2 receptor cDNA (CHO-CB2 cells). The high affinity ligand [^3H]CP 55940, which binds to both cannabinoid receptor subtypes, was used for GPF and CHO-CB2 cell membranes, whereas the selective CB1 antagonist [^3H]SR 141716A was used for N18TG2 cell membranes [19]. We found that olvanil could displace both radiolabelled ligands from CB1 receptor-containing membranes, although only at μM concentrations, but not [^3H]CP 55940 from CHO-CB2 cell membranes (Table 1). Furthermore, olvanil was almost inactive in displacing [^3H]WIN 55212-2 from RBL-2H3 cell membranes, which also selectively express CB2 receptors [16]. The K_i values obtained for olvanil for the displacement of [^3H]CP 55940 from GPF membranes varied between 4.54 and 11.98 μM , with an average value of 7.08 μM . AEA in the same assay exhibited a K_i value of 0.48 μM . The K_i for olvanil displacement of [^3H]SR 141716A from N18TG2 cell membranes was significantly lower (1.64 ± 0.36 μM , $n = 3$) and similar to that found for AEA (1.91 ± 0.31 μM , $n = 3$). One reason for these discrepancies may be the use of different experimental procedures, membrane preparations and radioligands in these experiments (see Section 2). Indeed, fluctuations in the K_i values reported for anandamide (from low-middle to high nM concentrations) when using different procedures have been pointed out [7], and differences of more than 50-fold exist between some of the reported K_i values of CP 55940 for the displacement of [^3H]CP 55940 from CB1 receptors (see Table 2 in [7]). Another possibility, i.e. that olvanil is more potent in displacing [^3H]SR 141716A than [^3H]CP 55940 because olvanil and SR 141716A behave as inverse agonists, is ruled out by our data. In fact, although SR 141716A under certain conditions may be an inverse agonist ([7,9] and references cited therein), we found olvanil to behave as a cannabinoid receptor agonist. We measured the effect on forskolin-induced cAMP formation – an intracellular event whose inhibition is coupled to CB1 receptor activation [20] – in intact N18TG2 cells, which have been often utilized to study the effect of cannabimimetic compounds on adenylate cyclase [20]. As shown in Fig. 3, olvanil behaved as an agonist in this assay, and exhibited an

Table 1

K_i values (μM) for olvanil, pseudocapsaicin and anandamide binding to membrane preparations from various sources using different cannabinoid receptor ligands

	CB1		CB2	
	GPF+ [^3H]CP 55940	N18TG2+ [^3H]SR 141716A	CHO-CB2+ [^3H]CP 55940	RBL-2H3+ [^3H]WIN 55212-2
Olvanil	7.08 ± 1.41	1.64 ± 0.36	> 20	> 15
Pseudocapsaicin	6.16 ± 0.13	1.20 ± 0.28	> 20	N.D.
Anandamide	0.48 ± 0.04	1.91 ± 0.31	0.371 ± 0.102 [18]	0.03 [16]

Concentrations in the range 0.1–10 μM were tested. Data are means \pm S.E.M. of at least three separate experiments. For olvanil displacement of [^3H]CP 55940 from CHO-CB2 cell membranes and of [^3H]WIN 55212-2 from RBL-2H3 cell membranes, K_i values higher than 20 and 15 μM can be predicted from the observation that the compound exhibited, respectively, 0 and $6.7 \pm 2.3\%$ displacement at 1 μM and $19.5 \pm 1.5\%$ and $32.0 \pm 2.1\%$ displacement at 10 μM . The same applies to pseudocapsaicin ($15.8 \pm 2.8\%$ displacement at 10 μM for CHO-CB2 cell membranes). The K_i values for anandamide in these two cell membrane preparations, calculated by using a methodology similar to the ones described here, have been already reported [16,18]. N.D. = not determined.

IC₅₀ value of 1.60 μM similar to the K_i value for the displacement of [³H]SR 141716A observed in membranes from the same cells. Olvanil was more potent than AEA (IC₅₀ = 3.20 μM), probably due to its major stability to enzymatic degradation (see above). Furthermore, olvanil inhibition of forskolin-induced cAMP formation was reversed by 0.5 μM SR 141716A, thus proving that this effect was mediated by CB1 receptors. Clearly, further studies are still needed in order to characterize the CB1-binding properties of olvanil more fully. These studies may also indicate which of the structural features of vanilloids need to be modified in order to improve their weak CB1-binding activity. Interestingly, we found that also pseudocapsaicin displaced cannabinoid ligands from CB1, but not CB2, receptor-containing membrane preparations (K_i = 6.16 μM for GPF membranes and 1.20 μM for N18TG2 cell membranes, Table 1), whereas capsaicin caused no displacement at 10 μM (data not shown). This finding is in agreement with the widely accepted concept that a linear, saturated aliphatic chain of at least five carbon atoms (Fig. 1) must be present in the molecule to allow the interaction with CB1 receptors [30].

In summary, we have shown that the long chain analogue of capsaicin, olvanil, can efficiently interact with at least one of the four proteins of the 'endogenous cannabinoid system' studied here. Olvanil (i) inhibits AEA facilitated transport into RBL-2H3 cells; (ii) is a weak agonist at CB1, but not CB2, receptors; and (iii) very weakly inhibits AEA hydrolysis by FAAH-containing membrane preparations. Except for a weak inhibition of AEA uptake, capsaicin does not exhibit similar properties, whereas pseudocapsaicin is inactive on the AEA transporter but weakly binds to CB1 receptors. Both AEA and olvanil were shown previously to exert potent vasodilatory, analgesic and anti-inflammatory actions [4–6,21–24], although probably with different mechanisms of action. While such effects of AEA seem to be mediated by cannabinoid receptors located on sensory/sympathetic fibers, smooth muscle, endothelial cells and leukocytes [7,21–23], olvanil actions are probably due to interaction with – and subsequent desensitization of – selective polymodal sensory nociceptors, the vanilloid receptors [5]. While AEA has been recently shown to inhibit the release of calcitonin gene-related peptide (CGRP) from efferent sensory neurons and inflamed tissues [22,23], the opposite effect has been described for olvanil [6]. However, given the likely involvement of the 'endogenous cannabinoid system' in the control of thermal hyperalgesia [22] and the reported activation of VR1 vanilloid receptors by noxious heat [2], it is possible that common pathways underlie the analgesic actions of both compounds, at least during thermal nociception. On the basis of the data reported here, and of the spinal mechanism of action proposed for olvanil-induced antinociception [25], it may be hypothesized that part of the analgesic actions observed with olvanil in vivo [4,5] is due to potentiation of the 'tonal' down-regulation of thermal nociception effected by spinal endocannabinoids [22,23]. This potentiation may be effected through inhibition of AEA facilitated transport into peripheral fibers and/or blood cells and subsequent enhancement of endogenous AEA levels. Also activation of CB1 receptors may occur when using the high (>200 mg/kg body weight) concentrations of olvanil often reported in the literature [26–28]. Conversely, since AEA is not capable of inducing a typical receptor-mediated capsaicin response, i.e. CGRP release

from rat or mouse lumbar spinal cord [23], it is unlikely that the endocannabinoid interacts with vanilloid receptors. In conclusion, apart from suggesting the necessity of a general re-evaluation of the pharmacology and biochemistry of long chain capsaicin analogues (reviewed in [28]), the data described in this paper support a case for interactions between some synthetic vanilloids and the cannabinoid system and prompt further studies in this direction. Furthermore, the finding of the weak cannabimimetic actions of olvanil and pseudocapsaicin may open the way to the design of a novel class of analgesics with a multiple mechanisms of action.

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