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European Journal of Pharmacology 364 (1999) 75–78

ejp

Short communication

Anandamide transport inhibition by the vanilloid agonist olvanil

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Received 28 September 1998; revised 30 October 1998; accepted 6 November 1998

Abstract

The structural similarities between the anandamide transport inhibitor *N*-(4-hydroxyphenyl)-arachidonylamide (AM404) and the synthetic vanilloid agonist olvanil [(*N*-vanillyl)-9-oleamide], prompted us to investigate the possibility that olvanil may interfere with anandamide transport. The intracellular accumulation of [^{3}H]anandamide by human astrocytoma cells was prevented by olvanil with a K_i value of 14.1 ± 7.1 μM. By contrast, capsaicin [(8-methyl-*N*-vanillyl)-6-noneamide], a plant-derived vanilloid agonist, and capsazepine (*N*-[2-(4-chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2*H*-2-benzazepine-2-carbothioamide), a vanilloid antagonist, had no such effect (K_i > 100 μM). These results indicate that, although less potent than AM404 (K_i 2.1 ± 0.2 μM), olvanil may reduce anandamide clearance at concentrations similar to those needed for vanilloid receptor activation.

Keywords: Cannabinoid; Anandamide; Transport; Olvanil; Capsaicin; Analgesia

1. Introduction

Activation of vanilloid receptors by capsaicin, the pungent principle present in hot peppers of the genus *Capsicum*, produces a biphasic sensory response in which an initial nociceptive phase, characterized by a burning/pungent sensation, is followed by a longer-lasting analgesic and anti-inflammatory phase (Szallasi and Blumberg, 1996). To identify vanilloid agonists deprived of the initial nociceptive effects of capsaicin, but still capable to exert analgesic and anti-inflammatory actions, a variety of capsaicin congeners have been synthesized and tested. Among them, olvanil [(*N*-vanillyl)-9-oleamide] proved to be particularly promising because its lack of pungency is associated with a favorable pharmacological profile which includes analgesia, vasorelaxation and anti-inflammation (Brand et al., 1987; Szallasi and Blumberg, 1996). These effects do not completely overlap, however, with those produced by capsaicin (Brand et al., 1987). For example, although olvanil is approximately equipotent with capsaicin in eliciting antinociception in vivo, it activates nociceptors only at concentrations (≥ 500 μM) which are unlikely to be achieved following systemic administration (Dray et al., 1990). This raises the possibility that some of the olvanil effects may be mediated by an as yet unidentified mechanism. The structural similarities between olvanil and the anandamide transport inhibitor AM404 (Fig. 1) prompted us to investigate the hypothesis that olvanil may interfere with anandamide inactivation which is thought to occur by high-affinity transport in to cells followed by enzymatic degradation (Beltramo et al., 1997).

2. Materials and methods

The CCF-STTG1 human astrocytoma cell line possesses an anandamide transport system kinetically and pharmacologically indistinguishable from that previously described in rat brain astrocytes (M. Beltramo and D. Piomelli, unpublished observations) and was used as a model in this study. Confluent human astrocytoma cells (American Type Culture Collection, Rockville, MD), grown in 24-well plates, were incubated for 4 min in a Tris–Krebs' buffer (NaCl 136 mM, KCl 5 mM, $MgCl_2$ 1.2 mM, $CaCl_2$ 2.5 mM, glucose 10 mM, Trizma base 20 mM; pH 7.4) containing 30 nM anandamide (200,000 dpm/ml, 221 Ci/mmol, New England Nuclear, Wilmington, DE). After incubation, cells were rinsed with Tris–Krebs' buffer containing 0.1% (w/v) bovine serum albumin and subjected to sonification for 1–2 min in Tris–

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00821-8

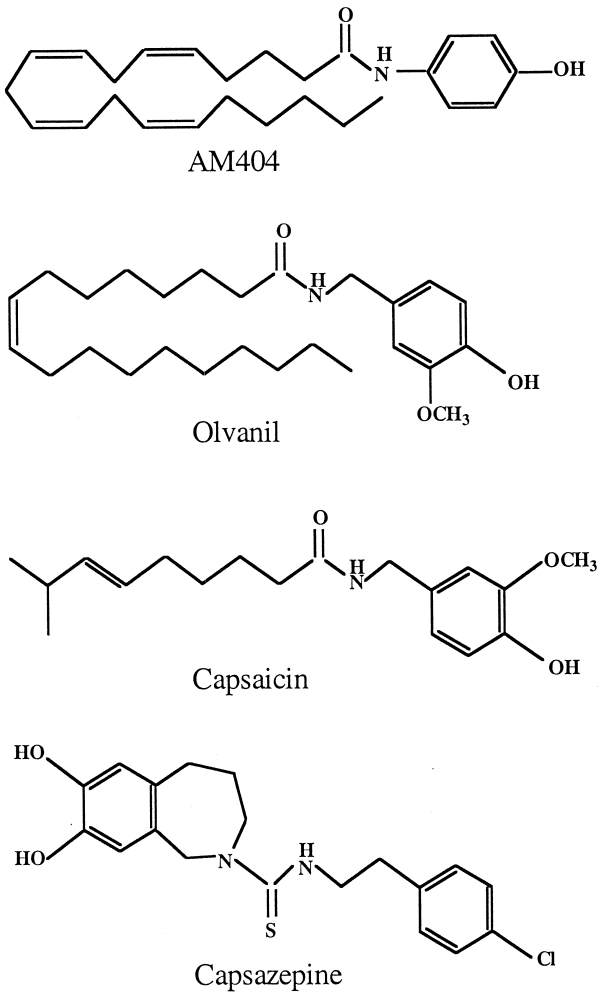

Fig. 1. Chemical structures of the anandamide transport inhibitor AM404, of the two vanilloid receptor agonists olvanil and capsaicin, and of the vanilloid receptor antagonist capsazepine.

Krebs containing 1% (v/v) Triton ×100 (Sigma). The samples were collected in glass vials and radioactive material was quantitated by liquid scintillation counting. For drug inhibition assays, test compounds were added to the incubation media at concentrations ranging from 0.1 to 100 μM from stock solution in dimethyl sulfoxide (DMSO). A preincubation step of 10 min in the presence of the same concentration of test compound was also performed. Control incubation were carried out in the presence of vehicle alone. IC_{50} values obtained by non-linear least square fitting of the data were converted to K_i values with the Cheng–Prusoff equation (Cheng and Prusoff, 1973) using a K_m value of 0.6 μM, which was determined in preliminary experiments.

Anandamide amidohydrolase activity was measured as previously described (Desarnaud et al., 1995). Briefly, rat brains were homogenized in ice-cold Tris buffer (20 mM) containing 0.32 M glucose and 1 mM EGTA. The homogenates were centrifuged, and supernatants collected and stored in liquid nitrogen until use. Amidohydrolase activity was measured for 10 min at 37°C in Tris buffer (50 mM pH 7.5) containing brain homogenate (0.2 mg of protein) and 3.15 nM [^{3}H]anandamide (labelled on the ethanolamide moiety, 15 Ci/mmol ARC, St. Louis, MO) brought to 100 nM with non radioactive anandamide (prepared as described by Devane et al., 1992). Olvanil (3–100 μM) was added to the incubation solution and 10 min preincubations with the same concentration of olvanil were performed. Reactions were stopped by adding ice-cold methanol. The samples were centrifuged and the supernatants applied to glass mini-column packed with Porapak type Q (100 mg/ml, 1 ml, Waters, Milford, MA). The eluant containing [^{3}H]ethanolamine was collected for liquid scintillation counting.

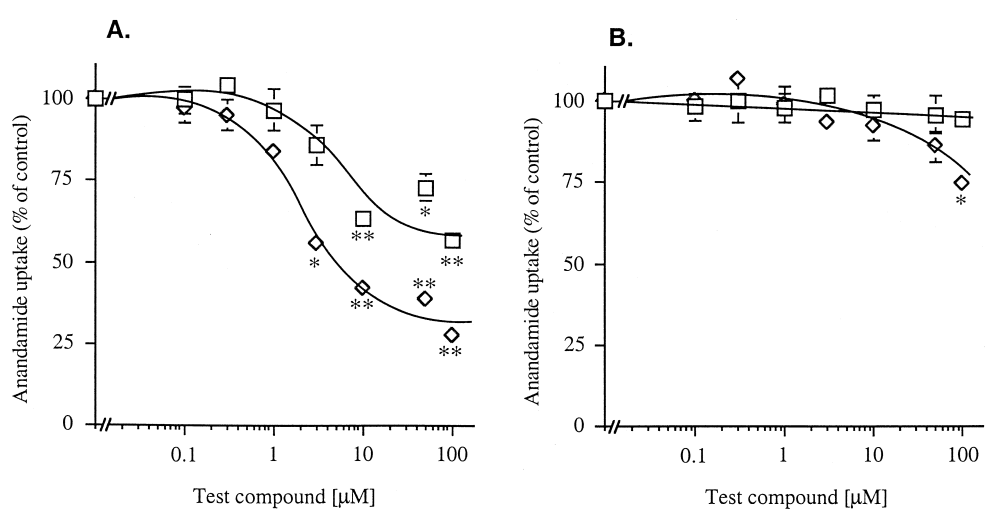

Fig. 2. Effects of AM404 (diamonds) and olvanil (squares) (A), and capsazepine (diamonds) and capsaicin (squares) (B), on [^{3}H]anandamide uptake in human astrocytoma cells. Each point represents the mean ± S.E.M. of at least six independent determinations. Nonspecific association (25% of total [^{3}H]anandamide accumulation) was not subtracted. Statistical differences were determined by Kruskal–Wallis non-parametric test. * $P < 0.01$, ** $P < 0.001$ compared to uptake in the absence of test compounds.

3. Results

Nonspecific association of [^{3}H]anandamide to astrocytoma cells, measured at 37°C in the presence of 100 μM unlabelled anandamide, was 25% of total [^{3}H]anandamide accumulation. This value was used for the calculation of both K_i values and maximal efficacies of the compounds tested.

As illustrated in Fig. 2A, olvanil inhibited anandamide transport with a K_i value of 14.1 ± 7.1 μM and a maximal efficacy of 57% (mean ± S.E.M., $n = 12$). By contrast, capsaicin, a plant-derived vanilloid agonist, and capsazepine, a vanilloid agonist, had little or no effect at any of the concentrations examined, displaying K_i values greater than 100 μM (Fig. 2B). Thus, even though less potent and efficacious than AM404, which in parallel assay displayed a K_i value of 2.1 ± 0.2 μM and a maximal efficacy of 96% (Fig. 2A), olvanil was able to produce a significant blockade of anandamide transport.

To characterize further the pharmacological properties of olvanil, we investigated its effect on anandamide amidohydrolase, the enzyme activity that catalyzes the hydrolysis of anandamide to arachidonic acid and ethanolamine (Desarnaud et al., 1995; Hillard et al., 1995; Ueda et al., 1995; Cravatt et al., 1996). Olvanil produced a partial inhibition of anandamide degradation (30.8 ± 3.0% of control, $n = 5$) only at the highest concentration tested (100 μM).

4. Discussion

The pharmacological profile of the vanilloid agonist olvanil can only partly be attributed to its ability to bind to, activate and desensitize vanilloid receptors (Dickenson et al., 1990; Dray et al., 1990; Hughes et al., 1992; Liu et al., 1997). In particular, the marked vasorelaxant and analgesic properties of olvanil do not closely agree with the limited potency of this compound on vanilloid receptors and with the effects of standard vanilloid agonists such as capsaicin.

The endogenous cannabinoid substance anandamide (Devane et al., 1992) produces vasorelaxation and analgesia by activating both peripheral and central CB1 type cannabinoid receptors (Fride and Mechoulam, 1993; Richardson et al., 1997; Wagner et al., 1997; Calignano et al., 1998; Randall and Kendall, 1998). These effects can be enhanced by the co-administration of the anandamide transport blocker AM404, which likely acts by protecting anandamide from biological inactivation (Beltramo et al., 1997; Calignano et al., 1997).

The structural similarity between AM404 and olvanil suggests that the latter may also be able to inhibit anandamide inactivation, and that this effect might account for some of its pharmacological properties. In support of this possibility, we found that olvanil inhibits anandamide transport in human astrocytoma cells with a potency comparable to that required for the displacement of resiniferatoxin binding to the vanilloid receptor (8.8 ± 3.1 μM; Winter et al., 1993). By contrast, both the vanilloid agonist capsaicin and the antagonist capsazepine are ineffective. Furthermore, olvanil has a weak inhibitory effect on anandamide amidohydrolase activity, which mediates the intracellular breakdown of anandamide to arachidonic acid and ethanolamide (Desarnaud et al., 1995; Hillard et al., 1995; Ueda et al., 1995; Cravatt et al., 1996).

In conclusion, our results indicate that olvanil inhibits anandamide transport in vitro, suggesting that this effect may contribute to its pharmacological properties in vivo. Experiments aimed at testing this hypothesis are currently underway. During the preparation of this manuscript, results similar to ours were reported in a preliminary form (Bisogno et al., 1998).

Acknowledgements

This study was supported by Neurosciences Research Foundation, which receives major support from Novartis. We thank Prof. Alexandros Makriyannis and Dr. Sonyuan Lin for the generous gift of AM404 and Ms. Suzanne Glasnapp for assistance.

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