Anandamide: an endogenous activator of the vanilloid receptor

Two recent articles[1,2] have discussed whether the cannabinoid anandamide is an endogenous agonist at the vanilloid receptor (VR1). Szolcsányi, although accepting the *in vitro* evidence of Zygmunt and colleagues[3], disputed this assertion[1] on the basis of the lack of effect of anandamide on blood pressure in cannabinoid CB_1 receptor knockout mice[1] and the low potency of anandamide at VR1, especially in electrophysiological studies[1,3]. However, Zygmunt's reply described good counter-arguments to these points[2], and more recent studies[4–6] provide further evidence to support his viewpoint.

We have recently reported that anandamide is a full receptor agonist at human VR1, with similar kinetic and electrophysiological properties to capsaicin[4]. Furthermore, the anandamide-induced response is inhibited by the vanilloid receptor antagonist capsazepine but not by cannabinoid receptor antagonists, and displays cross-desensitization with capsaicin[4]. This study also provided evidence that the potency reported for anandamide from the original electrophysiological studies[3] was an underestimate, probably caused by the biophysical properties of the ligand[4]. Indeed, the EC_{50} for anandamide at human VR1 (1 μM)[4] is identical to that reported in the guinea-pig basilar artery model (1 μM), and is similar to those (100–300 nM) reported in rat vasorelaxation models[2,3]. Moreover, these values compare well to those reported for inhibition of cAMP by anandamide (0.7–1 μM) in cells expressing cannabinoid receptors[2].

As mentioned by Zygmunt and colleagues[2], methanandamide also acts as a vasorelaxant in the isolated rat mesenteric bed via the same VR1-mediated mechanism[5]. However, although methanandamide is more potent than anandamide at the cannabinoid receptor[7], methanandamide is approximately tenfold less potent than anandamide in this organ bath model (V. Ralevic, pers. commun.). In keeping with this, we have shown that methanandamide is also tenfold less potent than anandamide at activating the human VR1 (Ref. 4).

Szolcsányi also raises the point that anandamide is analgesic at concentrations that do not activate VR1 (Ref. 1). This is probably correct, even allowing for the underestimation of the potency of anandamide at VR1 outlined above[3,4]. However, it not surprising that anandamide is analgesic because other cannabinoids are also analgesic[7] but have no affinity for VR1 (Refs 3, 4), which clearly indicates that the analgesia is mediated by cannabinoid receptors[7]. Yet this does not preclude anandamide from being an endogenous VR1 agonist as suggested by Szolcsányi[1], because no-one denies that anandamide possesses cannabinoid-, as well as VR1-, mediated actions.

Nevertheless, Szolcsányi made a valuable point when he raised the possibility that other ethanolamides and related compounds might also activate VR1 (Ref. 1). Indeed, we have reported that the putative endo-cannabinoid palmitoylethanolamide also activates VR1, albeit with a 10–30-fold lower potency than anandamide[4]. This is contrary to Zygmunt's earlier study[3], but probably reflects the relative sensitivity of the two assays used[4]. More recently, we have found that three other ethanolamides, oleoyl ethanolamide, dihomo-γ-linolenyl ethanolamide and docosatetraenyl ethanolamide, also activate VR1 with similar potencies to palmitoylethanolamide, whereas a fourth compound, linolenyl ethanolamide, is inactive (D. Smart and J.C. Jerman, unpublished observations). Interestingly, the potency of capsaicin is enhanced by moderately acidic conditions whereas the potency of anandamide is not[4], which suggests that ethanolamides might bind to a different site on the receptor.

In his article, Szolcsányi refers to the fact that the capsaicin analogue olvanil inhibits anandamide transport[1]. Indeed, this was one of the observations that led us to test anandamide as a VR1 agonist[4]. Recently, we have shown that the anandamide transport inhibitor AM404 is a full agonist at VR1, with a potency (EC_{50} = 110 nM) between that of capsaicin and anandamide and this response is blocked by capsazepine[6]. This, in conjunction with the actions of olvanil, implies that the anandamide transporter and VR1 might interact with a common pharmacophore, and thus *in vivo* studies using these agents must be interpreted with a degree of caution.

In conclusion, the data, taken collectively[2–6], support the view that anandamide is an endogenous ligand for VR1, although other structurally related, higher affinity ligands might also exist. Furthermore, because anandamide, and other ligands, clearly interact with what used to be considered two completely separate receptor systems, this exciting area of research will continue to challenge pharmacologists for years to come.

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Chemical name

AM404: (all z)-*N*-(4-hydroxyphenyl)-5,8,11,14-eicosatetraenamide