Anandamide – the other side of the coin

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Since the discovery that anandamide (arachidonylethanolamide) is a cannabinoid receptor ligand[1], knowledge of the biochemical pathways responsible for the synthesis, uptake and inactivation of anandamide has grown rapidly. Despite several important discoveries, final proof that anandamide has a physiological role as an endogenous activator of cannabinoid receptors is still lacking. Our study, which describes the first endogenous ligand for the vanilloid receptor present on primary sensory nerves, addresses a new molecular target for anandamide in addition to cannabinoid receptors. Above all, our findings highlight a new potential mechanism whereby anandamide, related lipids, or both, modulate vascular tone[2]. In his comment on our paper, Szolcsányi raises the important question of whether anandamide is produced at high enough concentrations *in vivo* to cause activation of vanilloid receptors.

Anandamide is formed in many organs and cell types including neurones, macrophages and endothelial cells[3–5]. Measurements of anandamide in skin and different brain regions have yielded basal levels of 10–90 pmol g^{-1} tissue[6,7]. This could easily lead to local anandamide concentrations of 10–90 nM, particularly near the cell membrane where this highly lipophilic mediator is likely to accumulate. Considering that the anandamide precursor *N*-arachidonyl-phosphatidylethanolamine is present in concentrations up to 360 pmol g^{-1} tissue in the same brain regions[6] and is most abundant in the spinal cord[8], the concentration of anandamide could rise even further under conditions of enhanced stimulation.

How do these concentrations of anandamide compare with those required to cause activation of vanilloid and cannabinoid receptors? Anandamide produces a graded vanilloid-receptor-mediated vasorelaxation in mesenteric and hepatic arteries of rat and in basilar arteries of guinea-pig with threshold concentrations of ~30 nM, ~100 nM and ~300 nM and EC_{50} values close to 100 nM, 300 nM and 1000 nM, respectively[2]. Thus, anandamide is clearly active as a vanilloid receptor agonist in submicromolar concentrations in isolated arteries. At present, it is unknown whether such concentrations of anandamide are achieved locally *in vivo*, but it is not unlikely that activated neurones, macrophages or endothelial cells could produce the concentrations of anandamide required to elicit vasodilatation.

With only a few exceptions[9,10], the anandamide concentrations reported by us to activate vanilloid receptors are in fact similar to those known to interact with cannabinoid receptors[11]. For example, in a recent study using cells transfected with cannabinoid CB_1 and CB_2 receptors, anandamide inhibited forskolin-stimulated cAMP accumulation with EC_{50} values of 700 nM and 1100 nM, respectively[12]. In other accepted CB_1 receptor bioassay systems, such as the mouse vas deferens and longitudinal preparations of the guinea-pig ileum, anandamide inhibits electrically evoked contractions with EC_{50} values of 53–61 nM and 289 nM, respectively[13,14]. Anandamide was a substantially less potent inhibitor of electrically evoked contractions in the circular muscle of the guinea-pig ileum and mouse urinary bladder, and of noradrenaline release from rat atria – three other preparations in which the effects of anandamide have been attributed to activation of CB_1 receptors[15–17]. Other relevant proteins that are potentially involved in the turnover of anandamide, such as the anandamide transporter and fatty acid amide hydrolase, have K_m values in the micromolar range[18]. Thus, with the exception of the studies by Richardson *et al.*[9,10], who observed an inhibitory action of very low concentrations of anandamide (0.1–1 nM) on capsaicin-induced release of calcitonin gene-related peptide (CGRP) from superfused rat skin and lumbar spinal cord, many effects of anandamide that are attributed to activation of CB_1 receptors occur over the same concentration interval as those causing vasorelaxation.

Anandamide was less potent in patch-clamp experiments on cells that artificially express the vanilloid subtype 1 receptor (VR1) than in isolated arteries[2]. A similar drop in potency was seen with the well-defined vanilloid receptor agonist capsaicin. In contrast to the patch-clamp experiments, the physiological readout in isolated arteries (vasorelaxation) involves a whole sequence of events from activation of vanilloid receptors, Ca^{2+} influx and release of CGRP from sensory nerves to activation of CGRP receptors and stimulation of adenylate cyclase in smooth muscle cells. Thus, the differences in potency between these bioassay systems probably reflect differences in vanilloid receptor expression, signal amplification, or both.

No doubt, our finding that anandamide activates vanilloid receptors can explain most of the reports in the literature on the vasodilator action of anandamide in isolated arteries. There is now evidence that the vasodilator response to methanandamide, a stable analogue of anandamide, in the rat isolated perfused mesenteric vascular bed is also mediated by this mechanism (V. Ralevic and D. Kendall, pers. commun.). Previous observations, made by us and several other research groups, that micromolar concentrations of SR141716A, a selective CB_1 receptor antagonist, attenuate anandamide-induced vasodilator responses are not in conflict with this novel mechanism. There is now compelling evidence that such high concentrations of this antagonist have CB_1-receptor-independent effects[19,20] and might interfere with vanilloid-receptor-mediated vasorelaxation[2].

In anaesthetized rats, intravenous injection of a bolus dose of anandamide induces a complex haemodynamic response comprising an initial transient hypotension and bradycardia, followed by a vasopressor response and a final drop in blood pressure, the latter

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response being inhibited by SR141716A (Ref. 21). A similar multiphasic response to anandamide is observed in mice[22]. The lack of this response in CB_1 gene knockout mice provides strong evidence for the involvement of CB_1 receptors in the hypotensive response to anandamide and other cannabinoids[22]. However, we believe that this *in vivo* bioassay system might not be relevant for the physiological action of any potential endogenous vanilloid receptor ligand. The prototype vanilloid receptor agonist capsaicin, which induces a triphasic haemodynamic response similar to that evoked by anandamide, produces only a vasopressor response in pithed (spinalized) rats lacking nervous control of the cardiovascular system[23]. This lipophilic compound, which is known to induce vasodilator responses *in vitro* and following local application in, for example, skin and mucosal tissues[24–26], apparently fails to produce systemic hypotension when given as an intravenous bolus injection in pithed animals. The reason for this is unclear, but limitations in the access to its target on primary sensory nerves is one possible explanation that could also account for the lack of effect of anandamide in CB_1 gene knockout mice.

There is still much to be learned about the function and regulation of primary sensory neurones. Nociception is only one aspect of the function of these nerves, which are also involved in visceral reflexes, inflammation and regulation of vascular tone. It is possible that anandamide plays a physiological role in several of these situations via activation of either vanilloid or cannabinoid receptors. The fact that anandamide inhibits CGRP release in skin via CB_1 receptors[9] does not necessarily mean that this compound does the same in other tissues or that actions at other receptor targets should not be considered. The occurrence of either of these two regulatory mechanisms might simply depend on whether sensory nerve fibres express CB_1 or vanilloid receptors and on the local concentration of anandamide. In fact, current studies by Herkenham's group show that CB_1 receptors are expressed on a limited population (8–13%) of CGRP or substance-P-containing sensory neurones[27]. Furthermore, anandamide might synergize with other inflammatory mediators (e.g. bradykinin, prostaglandin E_2 and ATP) or physiological factors (pH and temperature) to modulate vanilloid receptor activity under normal or pathological conditions. As suggested by Szolcsányi, the potential existence of more potent lipids than anandamide that can regulate the function of vanilloid receptors *in vivo* should also be considered. Thus, much work still needs to be done to fully establish the molecular targets for anandamide in different tissues and to understand the physiological role of this endogenous lipid.

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Chemical name

SR141716A: *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide hydrochloride