The ligand that came from within

Daniele Piomelli

Recent evidence suggests that the endogenous activators of neuronal vanilloid (capsaicin) receptors should be sought inside, not outside, the neuron. 12-Lipoxygenase metabolites of arachidonic acid, produced by pain-inducing mediators such as bradykinin, might trigger vanilloid receptor activation by interacting with an intracellular site on the receptor. Are these lipid-like second messengers the long-sought endogenous vanilloids?

The pungent principle of chili peppers, capsaicin, produces a sensation of intense pain by activating type 1 vanilloid (VR1) receptors found on the surface of peripheral pain-sensing neurons[1,2]. When these neurons are exposed to tissue-damaging stimuli, such as noxious heat or acidification, VR1 receptors become permeable to Na^+ and Ca^{2+} ions, causing the neurons to depolarize and fire action potentials. Sensory neuron firing transmits these pain signals towards the CNS, evoking at the same time a variety of local tissue responses, which include vascular dilatation and bronchial constriction[3]. VR1 receptors are also present in the brain, where they might be responsible for the decrease in body temperature and increase in motor activity elicited by centrally administered capsaicin[4]. The physiological significance of these central effects is not yet understood but the expression of VR1 receptors throughout the CNS implies that vanilloid signaling might possess functional roles well beyond the initial steps of pain perception[4].

What are the chemical signals that trigger VR1 receptor activation?

Although extracellular acidification has been unequivocally shown to regulate VR1 receptor gating, other factors are also likely to be involved. Consistent with this theory, H^+ ions do not stimulate VR1 receptor activity directly, but rather increase the sensitivity of the receptor to capsaicin[1]. Furthermore, brain VR1 receptors, unlike their peripheral counterparts, are normally protected from severe changes in pH. These considerations have prompted the search for endogenous capsaicin-like substances. Because of the hydrophobic properties of capsaicin, attention has focused primarily on lipid mediators such as anandamide, a brain-derived cannabinoid ligand[5]. However, anandamide has substantially higher affinity for cannabinoid CB_1 receptors than for VR1 receptors, and its *in vitro* and *in vivo* actions are strikingly different from those of capsaicin[6]. For example, anandamide-mediated activation of CB_1 receptors produces peripheral analgesia and cough suppression, two effects that are opposite to those observed with capsaicin[7,8]. Even the vascular hypotension induced by anandamide *in vivo*, which superficially resembles that induced by capsaicin, appears to be independent of VR1 receptor activation because it can be blocked by the CB_1 receptor antagonist SR141716A (Refs 9,10).

A recent study by Oh and collaborators[11] introduces important new elements to this discussion and takes us one step closer to the identification of the body's own capsaicin. Last year, Oh's laboratory reported that capsaicin interacts with a cytosolic component of the VR1 receptor and not, as it was generally assumed, with its extracellular domain[12]. A corollary of this unexpected observation is that endogenous capsaicin-like substances should not be sought outside the neuron, but instead should be sought inside the neuron. In agreement with this prediction, Oh and co-workers have now shown that a family of lipid intracellular second messengers, the lipoxygenase metabolites of arachidonic acid, might play a pivotal role in VR1 receptor activation[11].

Arachidonic acid lipoxygenation and cellular signaling

The metabolism of arachidonic acid begins with the mobilization of this fatty acid from membrane phospholipids and comprises an array of oxidative reactions that involves three main classes of oxygenase enzymes: cyclooxygenases (which initiate the conversion of arachidonic acid to prostanoids), cytochrome P450 enzymes (which produce epoxyeicosatrienoic acids) and a variety of different lipoxygenases. The first reaction catalyzed by all lipoxygenase enzymes is the addition of a hydroperoxide group to a select carbon atom in the arachidonic acid molecule to produce a hydroperoxy-eicosatetraenoic acid (HPETE). Thus, for example, the main product of mammalian 12-lipoxygenase is 12-HPETE, whereas that of 5-lipoxygenase is 5-HPETE. These short-lived intermediates have different fates in cells, and investigations of their metabolic routes have led to the discovery of important bioactive compounds, including key mediators of asthma and inflammation such as the leukotrienes (Fig. 1). But, in addition to this role as precursors for intercellular signaling molecules, lipoxygenase-derived HPETEs also appear to produce intracellular effects of their own. For example, biochemical and electrophysiological studies in the marine mollusk *Aplysia californica* have suggested that 12-HPETE acts as a second messenger that mediates presynaptic inhibition of neurotransmitter release[13,14]. One way by which 12-HPETE might exert these inhibitory effects is by increasing the opening probability of a class of K^+ channels known as K^+-S channels[13]. The regulation of K^+ channels by lipoxygenase metabolites of arachidonic acid has also been documented in mammalian neurons[15]. Moreover, experiments in the midbrain periaqueductal gray of the rat suggest that a

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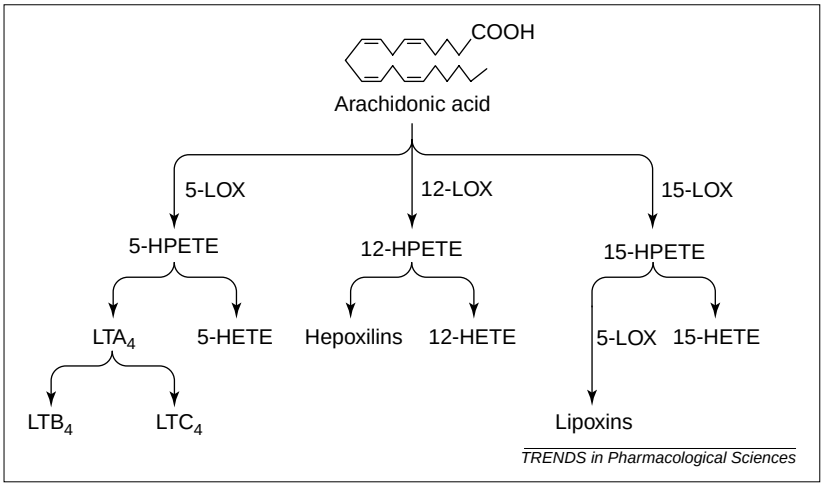

Fig. 1. Metabolism of arachidonic acid via the lipoxygenase (LOX) pathways to produce hydro(pero)xy-eicosatetraenoic acid [H(P)ETE] compounds and leukotrienes (LTs).

12-lipoxygenase product mediates the inhibition of GABA release produced by opioid peptides acting on presynaptic mu opioid receptors[16].

12-Lipoxygenase metabolites as capsaicin-like compounds

With this information at their disposal, Oh and collaborators studied the effects of various arachidonic acid derivatives on VR1 receptor activity in patch membranes of dorsal root ganglion neurons in primary culture. They found that 12-HPETE strongly activates single-channel currents, a phenomenon that was attributed to the opening of VR1 channels on the basis of three main observations. First, the currents elicited by 12-HPETE and capsaicin showed identical current–voltage relationships. Second, both of these currents were carried by Na^+ and K^+ ions, as determined by their equilibrium potentials. Third, both currents were blocked by the competitive VR1 antagonist capsazepine. The identification of 12-HPETE-sensitive channels as VR1 receptors was further confirmed by using a heterologous VR1 expression system. In a subsequent series of experiments, Oh and co-workers examined the potency and selectivity of the effects of 12-HPETE on VR1 channel activity. Among over 25 lipid-like molecules – including fatty acids, prostanoids and various lipoxygenase metabolites – 12-HPETE was shown to be the most potent and effective in activating VR1 receptors. Thus, 12-HPETE was five- and tenfold more effective than anandamide and arachidonic acid, respectively.

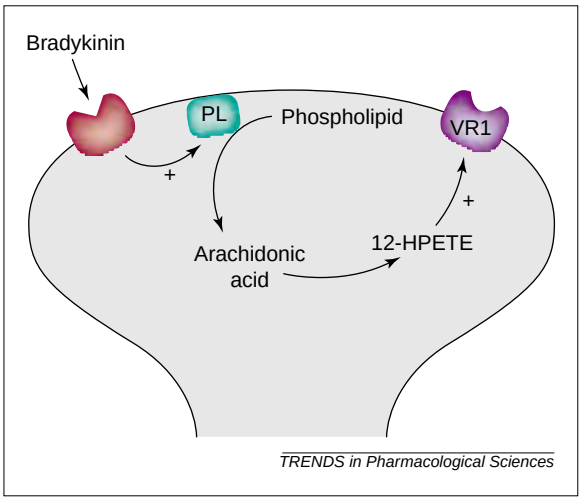

Fig. 2. Hypothetical mechanism by which 12-lipoxygenase metabolites of arachidonic acid might mediate vanilloid VR1 receptor activation. Membrane receptors for pain-inducing substances such as bradykinin might be coupled to the activation of phospholipases (PLs), which mobilize arachidonic acid from phospholipids. 12-Lipoxygenase converts free arachidonic acid to 12-hydroperoxyeicosatetraenoic acid (12-HPETE), which interacts with an intracellular component of the VR1 receptor channel causing an increase in its opening probability.

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On the basis of these observations, Oh and collaborators propose a model of vanilloid receptor activation that is both novel and thought provoking. According to this hypothesis (Fig. 2), pain-inducing substances such as bradykinin activate phospholipase-linked receptors in sensory neurons, mobilizing arachidonic acid from phospholipids and generating 12-HPETE. This lipid second messenger interacts in turn with a cytosolic domain of the VR1 receptor channel, increasing its opening probability and causing the sensory neuron to become depolarized.

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Testing the model

There are several ways by which this hypothesis can be tested further. Although it is known that bradykinin evokes arachidonic acid mobilization in many cells and tissues[17], it would be important to examine whether this bioactive peptide also causes formation of 12-HPETE in sensory neurons and whether an analogous effect is elicited by other pain-inducing substances. Another key test is to determine whether blockade of 12-lipoxygenase activity prevents hyperalgesia, an exaggerated response to painful stimuli that has been linked to VR1 receptor stimulation[18]. Initial experiments with the nonspecific lipoxygenase inhibitor nordihydroguaiaretic acid suggest that this might be the case[19], but studies with more selective agents (or, possibly, with mutant mice that lack the gene encoding 12-lipoxygenase) are necessary to confirm this possibility. Finally, a more complete characterization of the pharmacological properties of 12-HPETE is needed to show that these properties overlap significantly with those of capsaicin. These investigations should not only help determine whether 12-lipoxygenase products are indeed endogenous vanilloid compounds, but might also open new vistas on the molecular mechanisms of pain initiation and its pharmacological control.

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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

Ceramide: a new second messenger of cannabinoid action

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Cannabinoids, the active components of *Cannabis sativa* (marijuana), and their endogenous counterparts exert their effects by binding to specific $G_{i/o}$-protein-coupled receptors that modulate adenylyl cyclase, ion channels and extracellular signal-regulated kinases. Recent research has shown that the CB_1 cannabinoid receptor is coupled to the generation of the lipid second messenger ceramide via two different pathways: sphingomyelin hydrolysis, and ceramide synthesis *de novo*. Ceramide in turn mediates cannabinoid-induced apoptosis, as shown by *in vitro* and *in vivo* studies. These findings provide a new perspective on how cannabinoids act, and raise exciting physiological and therapeutic questions.

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Marijuana, a well-known preparation from *Cannabis sativa* L., has been used for many centuries both medicinally and recreationally. However, the structure of its active components – the cannabinoids – was not determined until the early 1960s. Because of their high hydrophobicity, cannabinoids were thought to exert their actions by inserting into biomembranes. However, in the late 1980s, binding sites for cannabinoids were discovered in the brain[1]. Nowadays it is widely accepted that marijuana-derived cannabinoids act via specific receptors that are normally bound by a family of endogenous ligands: the endocannabinoids[2–5]. Two cannabinoid receptors have been characterized and cloned so far: CB_1 (Ref. 6) and CB_2 (Ref. 7). The central action and many of the peripheral effects of cannabinoids rely on activation of CB_1 receptors, which are particularly abundant in the nervous system but also present in various extra-neural sites. By contrast, CB_2 receptor expression is almost exclusively restricted to the immune system.

Extensive molecular and pharmacological studies have demonstrated that cannabinoid receptors are $G_{i/o}$-protein-coupled receptors that signal inhibition of adenylyl cyclase and activation of the extracellular signal-regulated kinase (ERK) cascade. Furthermore, the CB_1 receptor modulates ion channels, inducing, for example, inhibition of N- and P/Q-type voltage-sensitive Ca^{2+} channels and activation of G-protein-activated inwardly rectifying K^+ channels[8] (Fig. 1). In addition to these well-established G-protein-coupled events, recent observations have shown that CB_1 receptor activation triggers the generation of ceramide. This ubiquitous lipid second messenger plays an important role in the control of cell fate at different sites, including the CNS (Refs 9–11). Thus, exposure of neural cells to physical, chemical, bacterial or viral stimuli can increase the intracellular concentration of ceramide and therefore evoke changes in the decision between cell survival and cell death[9,10]. In addition, changes in ceramide metabolism exert important regulatory effects on neuronal growth and development[11]. Moreover, intracellular ceramide accumulation, which might in turn induce apoptotic cell death, has been shown to occur in neurodegenerative disorders such as Alzheimer's and Parkinson's disease, epilepsy and ischaemia (stroke)[9–11]. This new aspect of

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