Essential fatty acids and their metabolites in signal transduction

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Introduction

Linoleic acid (9,12-18:2) is the precursor of arachidonic acid and its C₃₀ metabolites, collectively known as eicosanoids, that are known to play important regulatory roles in diverse physiological systems [1–4]. Their significance in the cardiovascular, reproductive and immune systems has been explored in some detail, and binding sites with specificity for particular cyclooxygenase or lipooxygenase products have been defined. Because some effects of eicosanoids are mediated through interactions with G-protein-coupled receptors, it has been proposed that eicosanoids exert all their actions in this manner [2]. Many exceptions to this generalization suggest, however, that eicosanoids are members of a unique category of autacoids (local hormones) that act not only at extracellular receptors to activate various second message systems, but also directly on cellular targets. As eicosanoid production is itself usually a secondary response to an extracellular receptor-mediated event, such a scheme suggests a mechanism for interactive cellular signaling, whereby an eicosanoid-mediated intracellular response to the actions of hormones, growth factors or neurotransmitters can also be signalled to the local environment.

Eicosanoids are generally classified into two subsets, the cyclo-oxygenase and lipooxygenase products. Attention has been focused on those relatively stable compounds that are most easily assayed. Beginning with the elucidation of the structures of leukotrienes, an increasing number of eicosanoids have been catalogued, including several novel compounds. The enzymic machinery involved in the synthesis of such a range of molecules is complex and unlikely to exist fortuitously. However, our knowledge of eicosanoid receptors is limited, and chemical instability or lack of availability of radiolabelled ligands is likely to frustrate the characterization of many eicosanoid-binding sites for some time. Functional assays have nonetheless demonstrated that specific binding sites for particular eicosanoids are present on a variety of enzymes and channels, as well as on G-protein-coupled receptors [3].

Eicosanoid actions mediated by G-protein-coupled receptors

Eicosanoid receptors have been shown to couple via G-proteins to adenylate cyclase, phospholipase C and phospholipase A₂, as well as to K⁺ channels [3, 4]. As with other hormones or transmitters, the intracellular response to a particular eicosanoid may thus be cell-specific, depending on the complement of G-proteins expressed by a particular cell type. G-protein-mediated alterations in second message levels (e.g. cyclic AMP) provide an explanation for many physiological effects of eicosanoids, such as prostaglandin E₂-induced water resorption in the kidneys [2]. However, as well as acting ‘classical’ second message pathways, it is noteworthy that further eicosanoid metabolism can be induced by eicosanoid receptor activation. For example, the activation of leukotriene D₄ receptors on a human monocyte line, U937, leads via protein kinase C and gene transcription de novo to a further release and metabolism of arachidonic acid [5]. Second message responses involving elevated free intracellular Ca²⁺ levels are also likely to induce a concomitant activation of phospholipase A₂ with subsequent eicosanoid release. These events complicate the interpretation of eicosanoid involvement in intracellular signalling mechanisms.

Actions of eicosanoids on ion channels

Many examples of eicosanoid modulation of K⁺ channels now exist. The most thoroughly analysed example of direct ion channel modulation by eicosanoids comes from studies of Aplysia sensory neurons carried out at Columbia University [6–8]. The synaptic coupling between sensory and motor neurons that control the gill withdrawal reflex in Aplysia is influenced by interneurons that act presynaptically. 5-Hydroxytryptamine is known to inactivate (via cyclic AMP) a K⁺ channel (the S-channel) leading to increased transmitter release, whilst the inhibitory neurotransmitter FMRFamide activates the same channel through the release of arachidonic acid and the production of 12-lipoxygenase products. A careful patch-clamp analysis has demonstrated that 12-HPETE acts at a specific binding site associated with the extracellular face of the ion channel [7], whereas an oxi...
dized derivative (hepoxalin A3?) generated in the presence of hematin, interacts with an intracellular site [8], to increase the probability of channel opening. These binding sites must be directly associated with the channel or associated molecules, because mechanisms involving phosphorylation, second messengers or G-proteins have been ruled out.

Interestingly, a variety of eicosanoids have been shown to activate K⁺ channels in smooth muscle and cardiac muscle, as well as sensory neurons of *Aplysia*. Convincing evidence that muscarinic activation of heart K⁺ channels requires phospholipase A₂ activation and lipoxygenation activity has been presented [9] and leukotrienes A₄ and C₂ have been shown to exert such activity [10]. K⁺-channel activity in smooth muscle cells has also been shown to be regulated by arachidonic acid, although here the effect seems to be indirectly mediated through effects on membrane fluidity [11].

**Direct actions of eicosanoids on kinases**

Protein phosphorylation plays a pivotal intracellular regulatory role [12]. The discovery that, within the central nervous system, a kinase C isozyme exists that is activated by arachidonic acid [13] indicated that eicosanoids may directly modulate intracellular phosphorylation. The biochemical consequences of eicosanoid-stimulated kinase activity have been clearly demonstrated by Linden & Routtenberg [14] in studies of Na⁺ and Ca²⁺ channels in N1E-115 cells. Voltage-dependent Na⁺ currents were reversibly attenuated with a range of cis-fatty acids, the actions of which were inhibited by kinase C inhibitors. Interestingly, phorbol esters did not mimic this effect, although both phospholipase A₂ and cis-fatty acids attenuated Ca²⁺ currents in the same cells. Such observations suggest that eicosanoids may either dictate the substrate specificity of a kinase C or activate a specific kinase that is unaffected by phorbol esters.

Some of the eicosanoid actions on Ca²⁺-calmodulin-dependent kinase II (CaM kinase II) activity provide direct evidence for a regulatory role that may have significance in synaptic function [15]. The concentrations producing 50% inhibition for a number of hydroperoxy and hydroxy acids that inhibit CaM kinase II are in the nanomolar range, and evidence for eicosanoid inhibition in intact synaptosomes has been obtained with hydroxyacids that are known to be produced within the nervous system. CaM kinase II is found at high concentrations in synaptosomes and, together with kinase C, may cause enhanced transmitter release. Both enzymes have been shown to play a role in the establishment of long-term potentiation, an alteration in synaptic efficacy that may be involved in learning and memory [16], lending added significance to the modulatory effects of eicosanoids on these two enzymes.

**Eicosanoid effects on membrane fluidity**

Micromolar concentrations of arachidonic acid have been shown to influence ion channel activity [11], glial glutamate uptake [17] and gap junction conductances [18]. These effects are not altered by kinase inhibitors, and are also evoked by other cis-fatty acids that are not metabolizable to eicosanoid products. Such actions are attributed to alterations in the structure of the lipid bilayer causing an increase in membrane fluidity. Although the concentrations of eicosanoids that induce such effects are high, it is possible, particularly in damaged tissue, that transient local micromolar concentrations of eicosanoids may exist.

**Summary**

The above examples argue that the diversity of eicosanoid structures is reflected in distinct actions, that the membrane-permeable nature of these molecules provides a system for interactive cellular signalling that may be particularly important in the nervous system, and that convincing evidence exists for direct actions of eicosanoids on both ion channels and kinases, as well as G-protein-coupled receptors.


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