Lipid Turnover in The Central Nervous System

Arachidonic acid metabolism: potential for diverse signalling within the same neuron

James H. Schwartz
Howard Hughes Medical Institute, Center for Neurobiology & Behavior, Columbia University, 722 West 168 Street, New York, NY 10032, U.S.A.

The receptor-mediated generation of a second messenger most often is thought of as designed to produce a single physiological effect. Even though a given second messenger typically influences the functioning of several enzymic reactions and ion channels within a nerve cell, it is usual to regard these actions as parallel or syntelic (that is, working toward the same physiological goal). Receptor-mediated release of arachidonate from membrane phospholipids results in the production of many metabolites through at least three major pathways: lipoxygenases, (predominantly the 12- and 5-enzymes), cyclo-oxygenase, and $P_{450}$ epoxygenases. In tissues other than the nervous system, numerous metabolites with known biological activities are produced by each of these pathways (for example [1, 2]).

Multiplicity of possible bioactive metabolites suggests the potential for diverse, combinatorial signalling. As second messengers, metabolites of arachidonic acid are exceptional: they can function both within a single nerve cell and, because most of the metabolites produced from arachidonate can pass through membranes readily, in neighbouring neurons as well. Transcellular signalling, which has been reported in other tissues [3-5], might influence neighbouring neurons, both presynaptically (as retrograde messengers) or locally (as interneuronal signalling).

We have focused on the effects of arachidonate itself and on intermediates produced in the 12-lipoxygenase pathway, because these substances have been found to be active in identified neurons of the marine mollusc, *Aplysia californica* (Fig. 1). In *Aplysia* nervous tissue the arachidonic acid released is converted rapidly by 12-lipoxygenase to 12(S)-hydroperoxyeicosatetraenoic acid (12-HPETE) and other metabolites [6-8] (Fig. 1); it also appears to be capable of stimulating some isoforms of protein kinase C (PKC) (T. C. Sacktor & J. H. Schwartz, unpublished work).

We have found two examples of apparently opposing physiological effects produced by the

**Fig. 1**

12-lipoxygenase pathways in *Aplysia* nervous tissue

Arachidonic acid is metabolized to 12-HPETE, which is further converted to several other metabolites. Only two have been shown to be active when applied to *Aplysia* neurons: 12-KETE and 8-HEpETE. The other metabolites shown, although formed in *Aplysia* tissue, are not biologically active (from [37]).

Abbreviations used: 12-HPETE, 12(S)-hydroperoxyeicosatetraenoic acid; 12-KETE, 12-ketoicosatetraenoic acid; 8-HEpETE, 8-hydroxy-11,12-epoxy-5,9,14-eicosatrienoic acid; PKC, protein kinase C; FMRFamide, Phe-Met-Arg-Phe-amide.
receptor-mediated release of arachidonic acid, each in the same identified neurons of the abdominal ganglion. While the behavioural functions of these two examples of second-messenger dialectics are not fully understood, we suggest that they may best be explained by differences in the kinetics of producing different metabolites in cell L14, on the one hand, and subcellular compartmentation in mechanosensory neurons, on the other.

**Effects of 12-lipoxygenase metabolites on cell L14**

The modulatory transmitter, histamine, has two distinct neurophysiological effects on L14: a rapid depolarization followed by a low hyperpolarization [8, 10]. These actions, which are produced by modulation of two distinct ion channels, are both mimicked by application of the 12-lipoxygenase product, 12-HPETE (Fig. 2).

12-HPETE, which can be shown by chiral chromatography to be the 12(S)-isomer (M. Aloe, M. Klein, J. H. Schwartz & S. J. Feinmark, unpublished work), is further converted to several metabolites in *Aplysia* nervous tissue, only two of which, 12-ketoeicosatetraenoic acid (12-KETE) and 8-hydroxy-11,12-epoxy-5,9,14-eicosatrienoic acid (8-HEpETE), have been found to be active.

**Fig. 2**

**Dual-action response of L14**

The identified *Aplysia* neuron, L14, was impaled for intracellular recording. Histamine (100 pmol during a period of 1 s) was applied (arrow) to a cell with a resting potential of −70 mV. Note the rapid depolarization followed by a slow hyperpolarization. Similar responses were seen when 12-HPETE (100 pmol during 3 s) was applied to the same cell. Application of 12-HETE (250 pmol during 5 s) was without effect (from [8]).

Pharmacological experiments reveal that the fast depolarizing response can be elicited by 12-KETE [7]. This metabolite produces its effect by opening a mixed ion channel. The slow hyperpolarization is produced by the epoxy alcohol, 8-HEpETE, acting on a K+ channel similar to the special K+ S-channel to be described below in sensory neurons [8].

We presume that the dual action produced by the transmitter is caused by the effects of the metabolites formed from 12-HPETE through two branches of the 12-lipoxygenase pathway. The sequential neurophysiological response to histamine suggests that the depolarization occurs first either because 12-KETE is formed more rapidly than the epoxy alcohol or because 12-KETE opens the mixed ion channel faster than 8-HEpETE shuts the K+ channel. Either explanation implicates differences in molecular kinetics or compartmentation in time. Presumably the behavioural effects of L14’s response is to sharpen the cell’s output, although there is no behavioural data to substantiate this idea as yet.

**Effect of arachidonate and 12-lipoxygenase metabolites on mechanosensory neurons**

Defensive withdrawal reflexes in *Aplysia* are mediated by identified sensory neurons that synapse on motor cells [12–14]. Sensory-to-motor neuron synapses can be strengthened by administering strong, noxious stimuli to the intact animal or by exposing isolated central ganglia to serotonin, a facilitatory neurotransmitter. The neurophysiological basis of sensitization, which is a simple form of learning, is thought to be the increased release of transmitter by the sensory neurons, a form of synaptic plasticity called presynaptic facilitation. Earlier work has focused on cyclic AMP-dependent protein phosphorylation of a special K+ ion channel, the K+S-channel, as the cause of the facilitation underlying short-term sensitization [12, 15–17]. More recently, activation of PKC has also been shown to increase the release of transmitter at sensory-to-motor synapses [18–21] (Fig. 3a).

A variety of neurophysiological experiments suggest that PKC produces presynaptic facilitation by mobilizing synaptic vesicles from a reserve pool in the terminals of sensory neurons, presumably by a mechanism similar to that proposed by Llinas et al. [22], and, unlike the cyclic AMP-dependent protein kinase, not through the phosphorylation of an ion channel. The two multifunctional protein kinases act independently: even though both are
**Fig. 3**

**Possible opposing actions of arachidonic acid and 12-lipoxygenase metabolites on *Aplysia* pleural sensory neurons**

(a) Translocation of PKC activity to sensory cell membranes. PKC is translocated and activated after training intact animals (Training) or during application of serotonin (20 μM-5-HT) for 5 min to isolated pleural-pedal ganglia. The activity remains associated with the membrane for periods up to 1 day after delivering the sensitizing stimuli. There is evidence indicating that this persistence of kinase activity is due to the continuous release of arachidonic acid. Translocation also occurs after application of 0.2 μM-phorbol dibutyrate (PDBu) to the ganglia for 45 min. Treatments with the inactive 4 a-phorbol (4a) for 45 min or with the permeating analogue, 8-(4-chlorophenylthio-cyclic AMP, (cpt-cAMP, 1 mM) for 45 min were ineffective (from [21]).

(b) Action of lipoxygenase metabolites on cultured *Aplysia* pleural sensory neurons. Application of 12-HPETE (1.5 μM, top trace) inhibits firing for about 2 min. During this period, the smaller spikes are below threshold. 5-HPETE (1.5 μM, bottom trace) does not affect the resting membrane potential or the simulated action potentials (brief upward spikes) (from [25]).

Activated by serotonin, there is no evidence for cross-talk between the two second messenger systems (Fig. 3a) [21].

All isoforms of PKC have been found to require a lipid activator that can be mimicked pharmacologically by phorbol esters [23]. For most isoforms diacylglycerol produced by phospholipase C is thought to be the endogenous activator; for some isoforms, however, arachidonate is also an effective activator [24]. We have some unpublished evidence to indicate that an isoform of PKC important to presynaptic facilitation in *Aplysia* sensory cells can use arachidonate as its activator. Paradoxically, however, sensory-to-motor synapses are also powerfully inhibited by metabolites of arachidonic acid [8, 25] (Fig. 3b). This opposing physiological action (presynaptic inhibition versus presynaptic facilitation) is brought about by the modulatory, neuropeptide, Phe-Met-Arg-Phe-amide (FMRFamide), which activates phospholipase A2 through a pertussis-toxin-sensitive G-protein-coupled receptor mechanism that results in the release of arachidonate [8, 26, 27].

The inhibition is produced by a metabolite of arachidonic acid which opens the same K⁺ channel that is closed by cyclic AMP-dependent protein phosphorylation [8, 25]. Despite the similarities in conductance change and reversal potential between the hyperpolarizing effects in cell L14 and sensory neurons, it is not yet certain whether the metabolite that produces the hyperpolarization in L14, 8-HEpETE, is the active metabolite in sensory neurons. Nevertheless, there is strong evidence that the effective second messenger is formed from 12-HPETE (which is much more effective than arachidonate when applied to whole cells), but which is much less effective in patch-clamped pieces of sensory neuron membrane [28, 29]. This indicates that arachidonate itself is not the second messenger, nor is it the peroxy acid, which must be further converted to an active substance with the aid of cytoplasmic factors not present in the ripped-off pieces of membrane.

In the abdominal ganglion, release of both FMRFamide and serotonin is known to occur in response to sensitizing stimuli [30, 31]. As in L14, it is not understood why inhibition accompanies facilitation in sensory neurons. Unlike the situation in L14, however, the transmitters eliciting opposing actions are known to differ, and it can reasonably be assumed that their receptors are separated on the sensory neuron's surface. Activation of PKC through a phospholipase A₂ could occur in a restricted region of membrane at some distance...
from the phospholipase A₂ activated by FMRFamide, especially since we have evidence that the activation produced by serotonin is not through a G-protein, but rather through a novel activating protein, lipotonin [32]. Lipotonin is similar to the phospholipase A₂-activating proteins (PLAP) previously reported in non-neural tissues of vertebrates [33–35].

**Conclusion**

Second messenger cascades are notoriously complex. For the most part, this complexity results from cross-talk between biochemical pathways that are now fairly well studied [36]. Despite their complexity, these pathways usually operate in the service of the same physiological output. Arachidonic acid metabolism, because it occurs along pathways with many branches, provides the opportunity for another degree of complexity. A variety of metabolites can be formed – even produced by the action of the same transmitter substance – that cause physiologically opposing effects in the same neuron.

9. Reference deleted
11. Reference deleted

Received 6 December 1990