Metabolites of Arachidonic Acid in the Nervous System of Aplysia: Possible Mediators of Synaptic Modulation

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Release of arachidonic acid from membrane phospholipids is receptor-mediated and might generate second messengers in neurons. We tested this idea using the simple nervous system of the marine mollusk, Aplysia californica. Aplysia neural components metabolize arachidonic acid through lipoxygenase and cyclo-oxygenase pathways. We identified 2 major lipoxygenase products, 12- and 5-hydroxyeicosatetraenoic acids (12-HETE and 5-HETE), and 2 cyclo-oxygenase products, PGE$_2$ and PGF$_2$. These metabolites of arachidonic acid are formed in synaptosomes, as well as in identified nerve cell bodies, indicating that both lipoxygenase and cyclo-oxygenase pathways are active within neurons. Application of the modulatory neurotransmitter histamine to cerebral ganglia that had been labeled with $^3$H-arachidonic acid induced the formation of $^3$H-12-HETE. This response was inhibited by the histamine antagonist cimetidine. Furthermore, release of radioactive 5-HETE and 12-HETE was observed after intracellular stimulation of the histaminergic cell C2 in cerebral ganglia labeled with $^3$H-arachidonic acid. Cimetidine also inhibited this response. Application of serotonin or stimulation of the giant serotonergic cell (GCN) in the cerebral ganglion did not cause detectable amounts of the labeled eicosanoids to be released. We found that intracellular stimulation of putative histaminergic neurons in the L32 cluster of the abdominal ganglion, which produces presynaptic inhibition in L10 neurons, also elicited the release of $^3$H-12-HETE and $^3$H-PGE$_2$. Thus, for the first time we provide evidence that synaptic stimulation promotes turnover of arachidonic acid in neurons. We suggest that metabolites of arachidonic acid are likely to participate in some postsynaptic responses to histamine and may be second messengers for presynaptic inhibition.

Although biochemical actions of neurotransmitters were recognized soon after Sutherland discovered cAMP (Sutherland and Rall, 1960), for many years the predominant mechanism of synaptic transmission was thought to be direct: the binding of neurotransmitter to ionophoric receptors, causing rapid changes in membrane conductance. Because of recent work on transmembrane transduction (see Stryer and Bourne, 1986), it seems likely that, more commonly, neurotransmitters act indirectly through the generation of intracellular second messengers. Attention has been focused primarily on the receptor-stimulated activation of adenylate cyclase and phospholipase C. Release of arachidonic acid from membrane phospholipids also might be mediated by receptors in neurons and could represent the transducing event for as-yet unidentified second messenger cascades (Wolfe, 1982). In non-neuronal mammalian cells, free arachidonate is released through receptor-mediated reactions triggered by hormones, autacoids, and growth factors; once released from the membrane, the fatty acid is rapidly metabolized to a family of bioactive products (the eicosanoids), many of which have been implicated in either intracellular or intercellular signaling (Needleman et al., 1986).

Formation of cyclo-oxygenase products, the prostaglandins PGD$_2$, PGE$_2$, and PGF$_2$, has been shown in neural tissue (Wolfe, 1982). In addition, Lindgren et al. (1984) and Adesuyi et al. (1985) have demonstrated lipoxygenase activities in mammalian brain, as indicated by the formation of 12- and 5-hydroxyeicosatetraenoic acids (HETE) and the peptide-containing leukotrienes. Despite these biochemical advances, direct evidence that eicosanoids play a role in neuronal function has been difficult to obtain because of the heterogeneity of nervous tissue and the small size of vertebrate nerve cells. These obstacles are minimized in Aplysia and other invertebrates that offer the experimental advantage of relative simplicity and large, identifiable neurons with known properties.

In this study, we examined the arachidonic acid cascade in the nervous system of Aplysia. We first found that arachidonic acid is a significant constituent of neural phospholipids. Then we showed that arachidonic acid is metabolized through both lipoxygenase and the cyclo-oxygenase pathways. In order to provide evidence that metabolism occurs within neurons, we next examined the formation of eicosanoids in isolated neuronal cell bodies and in synaptosomes. Experiments with histamine, or intracellular stimulation of the histaminergic neuron C2 or of putatively histaminergic cells in the L32 cluster, indicate that physiologically appropriate stimuli can evoke the metabolism of arachidonic acid, presumably through activation of postsynaptic receptors that are linked to a phospholipase.

Some of these results have been published previously as an abstract (Piomelli et al., 1986).

Materials and Methods

Aplysia californica, weighing 70–200 gm (Howard Hughes Medical Institute Marine Culture Resource Facility, Woods Hole Oceanographic Institution, Woods Hole, MA and Marinus, Sand City, CA), were kept in
and L14 by hyperpolarizing these cells by injecting current. After the stimulation, GCN was stimulated alone, using 3 intracellular current pulses to produce 20 spikes each at 1 min intervals. In another experiment, the L32 cells, which are putatively histaminergic and are clustered in the left abdominal hemisegment, were stimulated with 3 DC pulses of 2 sec duration, each producing 40 spikes and a suprathreshold slow excitatory synaptic potential onto GCN (top trace). (This response is not blocked by cimetidine.) After the stimulation, the bath was assayed for 3H-arachidonic acid metabolites. HPLC chromatograms from this experiment are shown in Figure 8. In other experiments with the histamine antagonist cimetidine, ganglia were incubated for 10 min at 10–20 °C before the cell was stimulated. After washing the cimetidine out, we waited 10 min before stimulating again.

Intracellular stimulation of identified neurons

Cerebral or abdominal ganglia were pinned to silicone plastic (Sylgard, Dow Chemical Corp., Midland, MI) in a chamber containing 0.3–0.4 ml of a supplemented artificial seawater (Eisenstadt et al., 1973). Cerebral ganglia were pinned dorsal side up to allow access to the identified histaminergic cell C2 and to the serotoninergic giant cerebral neuron (GCN). Abdominal ganglia were pinned ventral side up to allow access to L32 and their follower cells. The 2 bilaterally symmetrical C2 cells and the ipsilateral GCNs were exposed by partial removal of the overlying connective tissue sheath. Abdominal ganglia were desheathed to expose the cell body of L10 and the neurons in the L32 cluster. Ganglia were then incubated overnight in the seawater containing 3H-arachidonic acid (25 μCi/ml, 135 Ci/mmol) (Amersham, Arlington Heights, IL). After incubation, the ganglia were washed extensively with seawater to remove any unincorporated 3H-arachidonic acid. C2 or L32 cells were impaled with standard intracellular glass-capillary microelectrodes (10–20 MΩ, filled with 2 M potassium citrate) connected to bridge circuits for recording intracellularly and passing current. C2 and GCN were identified by their characteristic size, position, and spontaneous activity, and by the presence of a slow, excitatory synaptic potential produced by C2 onto GCN (Fig. 1A). Neurons in the L32 cluster were identified by their size, location, spontaneous activity, and their ability to produce a slow, dual-action PSP in L14 cells (n = 6); in some instances, L32 neurons were identified by their ability to produce a slow, inhibitory synaptic potential in L10 (n = 5) (Fig. 1B) (Byrne, 1980a, b; Kretz et al., 1986a). In each experiment, follower cells were impaled first, and a 0.2 ml sample of the bath was taken before C2 or L32 was impaled. C2 or L32 was stimulated with intracellular current pulses to produce spikes and release their transmitter. In most experiments, the stimulation protocol consisted of delivering 3 depolarizing DC pulses lasting 2.5–5 sec at 30 sec intervals, each of which elicited 25–50 spikes (Fig. 1). To control for the effects of depolarization and spike activity in the GCN follower cell, as well as for any nonspecific effects of intracellular stimulation, we stimulated GCN alone, using 3 intracellular current pulses to produce 20 spikes each at 1 min intervals. In another experiment, we controlled for the postsynaptic inhibitory responses of L10 and L14 by hyperpolarizing these cells by injecting current. After the stimulation, 0.2 ml of the bath was collected for assaying metabolites of arachidonic acid. After experiments with the histamine antagonist cimetidine, ganglia were incubated for 10 min at 10–20 °C before the cell was stimulated. After washing the cimetidine out, we waited 10 min before stimulating again.

Preparation of nervous tissue for biochemical analysis

Isolated ganglia. Intact ganglia were labeled for 2 hr or overnight with 32P (carrier-free, 25 μCi/ml) and with 3H-ethionine, 3H-choline, 3H-inositol, or 14C-serine (each at 2 μCi/ml), both from Amersham.

Neural components. Connective tissue sheaths were removed by dissection from central ganglia, and the neural components (cell bodies and neuropil) were placed in the supplemented seawater. In some experiments, the isolated neural components were incubated for 2 hr or overnight in seawater (0.2 ml) containing 2.5–25 μCi/ml 3H-arachidonic acid (Amersham; sp act, 85–135 Ci/mmol).

Isolated cell body of R2. Pinned abdominal ganglia from specimens weighing 50–70 gm were incubated for 50 min in seawater containing trypsin (Sigma, St. Louis, MO; type IIIIS, 4 mg/ml) to facilitate removal of the sheath for exposing underlying neurons (Conn et al., 1986). The trypsin-containing bathing solution was then removed and the ganglia washed with seawater containing soybean trypsin inhibitor (Sigma), followed by seawater. The isolated cell body of the cholinergic neuron R2 was incubated for 2 hr in seawater (50 μl) containing 3H-arachidonic acid (25 μCi/ml) and washed gently by successively diluting the bath. The cell was mechanically stimulated by agitating the 50 μl bath by pipetting 1 or 2 times during a period of 1 min. Phospholipases are sensitive to mechanical stimulation (Van den Bosch, 1980); because of the small amount of material, it was necessary to stimulate the metabolism of arachidonic acid to ensure detection of the labeled eicosanoids. Metabolites were analyzed after an equal volume of methanol was added.
10,000 x g. The pellet resulting from this centrifugation (about 0.5 mg protein), which is enriched in pinched-off synaptic terminals, as judged by biochemical and morphological criteria, was divided into 3 parts, and each was gently resuspended in 0.1 ml seawater containing H-arachidonic acid (5 μCi/ml). After 2 hr, the synaptosomes were sedimented by low-speed centrifugation and resuspended in 0.1 ml seawater; then arachidonic acid metabolism was mechanistically stimulated for 1 min by stirring with a pipette.

**Analytical methods**

Values are given as means ± SEM.

**Extraction of lipid.** Neural components were homogenized at 0°C in 0.1 M of CaCl2-free seawater containing 2 μM EGTA. The homogenate was extracted twice with hexane/isopropanol (3:2, vol/vol) for 30 min at room temperature. Subsequently, the organic phases were dried under nitrogen, and reconstituted in 0.1 ml of hexane prior to further analysis.

**Extraction of eicosanoids.** Samples of bath solutions were acidified to pH 3.5 with formic acid and extracted twice with 5 volumes of chloroform. The organic phases were combined, dried, and reconstituted in methanol (0.1 ml). Alternatively, neural tissue homogenates were shaken with cold acetone, centrifuged, and the supernatant was extracted with 2 volumes of hexane, acidified to pH 3.5, and extracted again with 5 volumes of diethyl ether. The ether phase was dried under N2 and reconstituted in hexane/isopropanol/acetic acid (98:2:0.1, 0.25 ml) for purification by high-performance liquid chromatography (HPLC).

**Thin-layer chromatography (TLC).** TLC was performed on silica gel G plates (Analtech, Newark, DE) that had been activated for 30 min at 80°C. The major lipid classes were separated in hexane/ethyl ether/acetate/methanol (85:20:2:4; solvent system A). Phospholipids were separated in chloroform/methanol/20% aqueous dimethylamine (60:36:10; system B). The migration of lipid standards was determined by staining with 5% phosphomolybdic acid in ethanol.

**Gas-chromatography-mass spectrometry (GC/MS).** Total lipid extracts were subjected to transmethylation with 12% boron trifluoride in methanol for 1 hr at 70°C. The reaction mixture was extracted twice with an equal volume of hexane, and the organic layer dried under N2 and reconstituted in 10 μl hexane for analysis. GC/MS of the fatty acid methyl esters was carried out on a Hewlett-Packard 5890 GC (Hewlett-Packard) equipped with a glass column packed with 10% Silar 10 CP. Helium was the carrier gas, with a temperature program from 10°C to 220°C at a rate of 10°C/min. Quantitative GC analysis was performed on a Hewlett-Packard 5890 GC on a SP 2340 fused silica-capped column (30 m x 0.32 mm I.D.; Supelco, Bellefonte, PA). Samples were injected in the split mode (ratio, 100:1), and helium was the carrier (1 ml/min). The injector and flame ionization detector were kept at 250°C. In other experiments, we performed these determinations on individual phospholipid classes after they had been separated by TLC.

**GC/MS of the hydroxy acid derivatives was carried out on a Hewlett-Packard 5987A for analysis.** The hydroxy acids were converted to the corresponding methyl ester trimethylsilyl ether (Me-TMS) derivatives by reaction with bis(trimethylsilyl) trifluoroacetamide (BSTFA)/acetontitrile (1:1; 50 μl) for 15 min at 70°C. The TMS derivatives were separated on a column (10 μm i.d.) of capillary silicone (50 m x 0.32 mm I.D.; Supelco, Bellefonte, PA). The injector was kept at 250°C and the source at 150°C. Oven temperature was kept for 1 min at 60°C and then raised to 320°C at a rate of 30°C/min.

**HPLC.** HETE were purified from unextracted bath samples on a Novapak C18 column eluted isocratically with a mobile phase of acetonitrile/water/acetic acid (26:74:0.1) at a flow rate of 2.5 ml/min. Unextracted bath samples were also analyzed for prostaglandins by reversed-phase HPLC on a Novapak C18 column, eluted isocratically with a mobile phase of acetonitrile/water/acetic acid (30:70:0.1) at a flow rate of 2.5 ml/min (Elgin et al., 1982). Retention times of the prostaglandins were obtained with tritium-labeled PGE1, PGD2, 6-keto-PGF1α, thromboxane B2, and PUF2α (New England Nuclear). For spectral and GC/MS analysis, 12-HETE was purified from ether extracts by normal-phase HPLC on a SiliaC18 column, eluted with hexane/isopropanol/acetic acid (98:2:0.1, 1 ml/min). This system separates all monoxygenated metabolites (12-5-, 8-, 9-, 11-, 15-HETE). Ultraviolet absorbance was monitored continuously, using a photodiode array detector (Hewlett-Packard).

**Radioimmunoassay.** In some experiments, intact, isolated ganglia were incubated for 30 min with unlabeled arachidonic acid (15 μM) in seawater (0.2 ml) to ensure production of adequate amounts of the metabolites for assay. The bath was fractionated by reversed-phase HPLC to purify either the hydroxy acids or the prostaglandins. HPLC fractions were evaporated to dryness and reconstituted in Dulbecco’s phosphate-buffered saline (Gibco, Grand Island, NY) immediately before the assay. For assaying 12-HETE, the fractions were dried, reconstituted in methanol, and subjected to further purification by reversed-phase HPLC (system I). Kits from Sun-Lab (Boston, MA) were used to assay 5-HETE, 12-HETE, PGE2, 6-keto-PGF1α, and PGF2α.

**Fate of exogenous J3H-12-HETE.** Isolated neural components were incubated for 10 min in seawater (0.25 ml) containing 0.25 μCi/ml ['3H]-12-HETE (New England Nuclear; sp act, 225 Ci/mmol). Samples of the bath, diluted with methanol, were analyzed by reversed-phase HPLC (solvent system I). Phospholipids were extracted from tissue and separated into classes by TLC using solvent system B. In other experiments, the extracts were dried and the residue saponified for 1 hr in methanolic KOH (methanol/50% KOH, 3/0.2 vol/vol) at 70°C. After cooling, water was added and the pH adjusted to 3.5. Fatty acids were extracted twice with 2 volumes of chloroform. The combined organic phases were dried, reconstituted in methanol, and subjected to reversed-phase HPLC. Neuronal extracts were also analyzed by reversed-phase HPLC and high-performance liquid chromatography (HPLC). We obtained phosphatidylcholine, sphingomyelin, and phospholambinic acid from Sigma; arachidonic acid from Nu-Check-Prep (Ellysian, MN); phospholipid standards from Avanti Polar Lipids (Birmingham, AL); 15-, 13-, 12-, 5-, 8-, 9-, 11-, and 9-HETE, eicosatetraynoic acid (ETYA), nortrihydroxouacetic acid, 5,6-methanoleukotriene A4, methyl ester, and 5,6-dehydroarachidonic acid from Biopol.
Acid PE PC PS + PI

Arachidonic acid is a constituent of Aplysia neural lipids and is formed by Aplysia neural components. Addition of labeled arachidonic acid was converted to labeled metabolites, such as 12-HETE and 5-HETE, stable end products of arachidonic acid metabolism through the 12- and 5-lipoxygenase pathways. Separation of these products by HPLC system 1 is shown in Figure 2A. In 12 similar experiments, 0.5 μCi of 3H-arachidonic acid were added to 0.1 ml of artificial seawater containing on average about 8 mg wet weight of tissue; 4925 ± 108 cpm were recovered after reversed-phase HPLC purification at the retention time of 12-HETE, and 3713 ± 76 cpm at the retention time of 5-HETE. Background radioactivity in the fractions around these peaks was 98 ± 8 cpm.

The identity of 12-HETE was confirmed using several methods. In 2 experiments in which the radioactive material eluting with 12-HETE on reversed-phase HPLC was subjected to methylation and analyzed by normal-phase HPLC, the labeled product comigrated with 12-HETE methyl ester (Fig. 2B). Also, radioimmunoassay after isolation by reversed-phase HPLC revealed a single immunoreactive component at the retention time expected for 12-HETE (Fig. 2A, shaded area) (n = 2). Further structural analysis of this material was performed by UV spectrometry and GC/MS. For this purpose, the ganglia from the nervous system of 100 Aplysia were homogenized and incubated for 30 min with arachidonic acid (50 μM); 12-HETE was purified from an ether extract by normal-phase HPLC. Ultraviolet absorbance was monitored continuously at 235 nm (λ_{max}), and full spectra were taken using a photodiode array detector. The HPLC chromatogram (Fig. 3A) shows a major peak of absorbance at 4.6 min, the retention time of authentic 12-HETE.

Lipid extracts from neural components labeled overnight with 3H-arachidonic acid were fractionated by TLC using solvent system A (total lipids) or B (phospholipid classes) (n = 4). Total radioactivity on the TLC plate was, on average, 70,000 cpm (A) and 40,000 cpm (B). A similar distribution was obtained in 4 other determinations after labeling for 2 hr with 3H-arachidonic acid.

**Research Laboratories (Philadelphia, PA), cimetidine from Smith, Kline & French (Philadelphia, PA); HPLC and TLC solvents from Burdick & Jackson (Muskegon, MI); and boron trifluoride from Kodak (Rochester, NY).**

**Results**

**Arachidonic acid is a constituent of Aplysia neural lipids**

Arachidonic acid accounted for about 10% of the total fatty acids of Aplysia neural lipids, as determined by quantitative GC analysis (Table 1). Comparable proportions of arachidonic acid were measured in each isolated phospholipid class (Table 2). In addition, 3H-arachidonic acid was readily incorporated into the lipids of isolated neural components (Table 3). Neural components were used for these experiments because, with intact ganglia, most of the 3H-arachidonic acid was preferentially incorporated into the connective tissue sheath and did not reach the neurons. The presence of polyunsaturated fatty acids has been demonstrated in other mollusks (Roots and Johnston, 1965), was not investigated.

**Arachidonic acid metabolites are produced in Aplysia nervous tissue**

To explore the physiological relevance of the eicosanoids in the nervous system of Aplysia, it was first essential to characterize the major metabolic pathways of arachidonic acid and to show that they are present in neurons. We found that both lipoxygenase and cyclooxygenase products of arachidonic acid could be formed by Aplysia neural components.

**Identification of lipoxygenase products.** 3H-Arachidonic acid added to neural components was converted to labeled metabolites with the chromatographic properties of 12-HETE and 5-HETE, stable end products of arachidonic acid metabolism formed through the 12- and 5-lipoxygenase pathways. Separation of these products by HPLC system 1 is shown in Figure 2A. In 12 similar experiments, 0.5 μCi of 3H-arachidonic acid were added to 0.1 ml of artificial seawater containing on average about 8 mg wet weight of tissue; 4925 ± 108 cpm were recovered after reversed-phase HPLC purification at the retention time of 12-HETE, and 3713 ± 76 cpm at the retention time of 5-HETE. Background radioactivity in the fractions around these peaks was 98 ± 8 cpm.

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**Table 2. Fatty acid composition of Aplysia neural phospholipids**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>PE</th>
<th>PC</th>
<th>PS + PI</th>
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<tr>
<td>14:0</td>
<td>N.D.</td>
<td>3.1 ± 0.5</td>
<td>N.D.</td>
</tr>
<tr>
<td>14:1</td>
<td>5.4 ± 1.4</td>
<td>1.4 ± 1.2</td>
<td>4.3 ± 1.5</td>
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<tr>
<td>16:0</td>
<td>2.8 ± 0.9</td>
<td>2.2 ± 1.0</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>16:1</td>
<td>1.8 ± 1.4</td>
<td>2.1 ± 1.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>18:0</td>
<td>4.3 ± 0.7</td>
<td>5.4 ± 2.6</td>
<td>19.0 ± 4.8</td>
</tr>
<tr>
<td>18:1</td>
<td>2.0 ± 1.3</td>
<td>8.6 ± 3.3</td>
<td>3.2 ± 1.7</td>
</tr>
<tr>
<td>18:2</td>
<td>2.7 ± 0.9</td>
<td>4.1 ± 1.7</td>
<td>2.3 ± 1.1</td>
</tr>
<tr>
<td>20:1</td>
<td>2.2 ± 0.1</td>
<td>2.8 ± 1.3</td>
<td>3.3 ± 1.2</td>
</tr>
<tr>
<td>20:4</td>
<td>7.5 ± 2.5</td>
<td>8.3 ± 3.8</td>
<td>6.9 ± 4.9</td>
</tr>
<tr>
<td>20:5</td>
<td>2.3 ± 0.8</td>
<td>4.7 ± 1.0</td>
<td>3.6 ± 1.1</td>
</tr>
<tr>
<td>22:2</td>
<td>4.3 ± 1.2</td>
<td>1.8 ± 0.9</td>
<td>4.9 ± 1.3</td>
</tr>
<tr>
<td>22:4</td>
<td>24.7 ± 6.8</td>
<td>11.8 ± 4.0</td>
<td>5.9 ± 2.4</td>
</tr>
<tr>
<td>22:6</td>
<td>4.5 ± 1.1</td>
<td>4.9 ± 2.1</td>
<td>5.9 ± 2.1</td>
</tr>
<tr>
<td>24:0</td>
<td>1.2 ± 0.6</td>
<td>0.8 ± 0.2</td>
<td>N.D.</td>
</tr>
<tr>
<td>Others</td>
<td>31.0 ± 6.4</td>
<td>37.6 ± 8.3</td>
<td>35.3 ± 1.2</td>
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</table>

**Table 3. Incorporation of 3H-arachidonic acid into Aplysia neural lipids**

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Radioactivity (cpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE</td>
<td>15.8 ± 1.9</td>
</tr>
<tr>
<td>PC</td>
<td>36.3 ± 3.2</td>
</tr>
<tr>
<td>PS + PI</td>
<td>40.0 ± 4.1</td>
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</table>

**Lipid extracts from neural components labeled overnight with 3H-arachidonic acid were fractionated by TLC using solvent system A (total lipids) or B (phospholipid classes) (n = 4). Total radioactivity on the TLC plate was, on average, 70,000 cpm (A) and 40,000 cpm (B). A similar distribution was obtained in 4 other determinations after labeling for 2 hr with 3H-arachidonic acid.**
12-HETE had been injected. (Thus the total amount of 12-HETE recovered from the nervous systems of 100 animals in this experiment would be 1 µg.) Analysis by EI mass spectrometry further confirmed the identification (Fig. 4B). Three characteristic ion fragments [m/z 391 (M-15), 295 (M-111), and 73] were chosen from the spectrum of synthetic 12-HETE-Me-TMS. These fragments we identified to coelute from the GC at an equivalent chain length value identical to that of 12-HETE-Me-TMS.

Evidence that the second labeled metabolite is 5-HETE was provided only by normal-phase HPLC and by radioimmunoassay. The 2-step chromatographic procedure used to characterize 12-HETE was not practical for 5-HETE. In 3 control experiments, after the second reversed-phase HPLC step less than 10% of standard 3H-5-HETE was recovered. (In contrast, the recovery of 3H-12-HETE under the same conditions was 40-60%.) Therefore, the labeled metabolites were extracted directly from bath samples and analyzed by normal-phase HPLC either as free fatty acids or after methylation with diazomethane. In both analyses, major radioactive components were found to elute with standards of 5-HETE or 5-HETE-Me (not shown).

Similarly, radioimmunoassay was performed on the fractions collected from the reversed-phase HPLC separation (using system I; see Materials and Methods). Although the neural components released 100 pg/ganglion of immunoreactive 5-HETE with the same retention time of the 5-HETE standard (19.5 min), this accounted for only 13% of total immunoreactive 5-HETE recovered. Most of the fractions yielded background values in the range of 11-26 pg/ganglion of immunoreactive material. In addition, 3 components, eluting at 7.5, 10.5, and 24 min, contained 50, 50, and 58 pg/ganglion of immunoreactivity. Detection of these additional components may be due to cross-reactivity of the antibody with other arachidonic acid metabolites, or with metabolites of different polyunsaturated fatty acids that are present in Aplysia (Tables 1 and 2). Because of the poor recovery, the identification of 5-HETE must be tentative.

The formation of 12-HETE and 5-HETE that we have shown suggests that 12- and 5-lipoxygenases are present in Aplysia neural components. The presence of these enzymes is further indicated by the following experiments. Neither metabolite was formed by neural components that had been boiled for 5 min. Agents that block mammalian lipoxygenases inhibit the production of these metabolites in Aplysia. Nordihydroguaiaretic acid (NDGA) and ETYA inhibited the formation of 3H-12-HETE and 3H-5-HETE by neural components incubated with 3H-arachidonic acid (0.5 µCi/0.1 ml for 10 min) (Table 4). Half-maximal inhibition of 3H-12-HETE formation was obtained with 15 µM ETYA, and of 3H-5-HETE with 0.5 µM ETYA. NDGA blocked the generation of both hydroxy acids with equal effectiveness (IC50, 3 µM). Two specific inhibitors of mammalian 5-lipoxygenase, 5,6-methanoleukotriene A4, methyl ester (MLTA4) and 5,6-dehydroarachidonic acid (DHA), were also tested. Aplysia 5-lipoxygenase activity was inhibited by DHA with an IC50 of 10 µM. Inhibition of 3H-5-HETE release by 50% was obtained with 5 µM MLTA4. The tentative biochemical identification of 5-HETE is strengthened by these pharmacological results with mammalian 5-lipoxygenase blockers.

**Fate of 3H-12-HETE and 3H-5-HETE.** We found that the primary metabolic fate of the hydroxy acids in Aplysia nervous tissue is esterification to glycerophospholipids and neutral lipids, as in mammalian retina (Birkle and Bazan, 1984). When 3H-12-HETE was incubated for 10 min with Aplysia ganglia, 24.5% of the label was incorporated into lipid (n = 4); the radioactivity was found to be equally distributed between phospholipid and neutral lipids: phosphatidyl-ethanolamine, 11.6 ± 3.2%; -choline, 28.8 ± 4.1%; -serine and -inositol, 9.3 ± 2.9%. To determine whether the label represented esterified 12-HETE or a metabolite, the lipid extract was saponified and the hydroxy acids examined by reversed-phase HPLC. The distribution of radioactivity on the chromatogram was identical to that of control experiments in which authentic 3H-12-HETE had been subjected to hydrolysis, extraction, and purification by HPLC (data not shown). In 4 similar experiments on the fate of 3H-5-HETE, Figure 2. Identification of 12-HETE formed by Aplysia neural components. A. Reversed-phase HPLC and immunochromatography. Bath samples from neural components, incubated with 3H-arachidonic acid (0.5 µCi in 0.1 ml for 30 min), were subjected to reversed-phase HPLC (solvent system I); each 30 sec fraction was counted by liquid scintillation; the solid line (cpm) was drawn by connecting each point. The 2 major components that are separated from the solvent front eluted at the retention times of 12-HETE and 5-HETE. The material at the solvent front represents unidentified polar metabolites of arachidonic acid. The amounts of radioactivity on the chromatogram correspond to the production of the neural components from the cerebral, paired pleuropedal and abdominal ganglion of one specimen. In another experiment, bath samples from neural components incubated with arachidonic acid (15 µM) were fractionated by HPLC and the material eluting at the retention time of 12-HETE was chromatographed again on the same HPLC system. After the second HPLC step, the eluted fractions were dried, reconstituted in phosphate buffer, and analyzed by radioimmunoassay, using commercial antibody against 12-HETE. The immunoreactive material, indicated by the shaded area, had the same retention time as did the 12-HETE standard. Recovery of standard 3H-12-HETE after 2 chromatographic steps was typically 40-60% (n = 3). B. Normal-phase HPLC. The radioactive material eluting at 12-HETE on reversed-phase HPLC was collected, dried, subjected to methylation, and chromatographed by normal-phase HPLC. The major radioactive component was eluted at the retention time of 12-HETE methyl ester. Recovery was only 10-20%.
which was also incorporated into tissue lipids, we found 30% in phospholipids (phosphatidyl-ethanolamine, 11.6 ± 3.2%; -choline, 11.0 ± 2.1%; -serine and -inositol, 4.8 ± 1.9%); and the rest in neutral lipids. No polar metabolites were found in bath samples of ganglia incubated with 3H-12-HETE or 3H-5-HETE analyzed by reversed-phase HPLC (n = 2; not shown), indicating that in Aplysia, as in mammalian tissues (Stenson and Parker, 1979; Birkle and Bazan, 1984), these hydroxy acids are not rapidly metabolized.

Identification of cyclo-oxygenase products. Levine and Kobayashi (1983) previously found immunoreactive PGE₂ and PGF₆ in unextracted aqueous homogenates of Aplysia ganglia. They did not purify the products before immunological analysis, however. Christ and Van Dorp (1972) reported the production of prostaglandins in non-neural tissues of other invertebrates. We investigated the formation of prostaglandins using neural components labeled overnight with ³H-arachidonic acid (Materials and Methods). A major radioactive substance with HPLC characteristics identical to those of PGE₂ was released into the bath from neural components during a 30 min incubation (Fig. 5). Minor labeled components were also released, with retention times of PGF₆ and of 6-keto-PGF₁α, the stable hydrolysis product of prostacyclin. Six similar experiments identifying these prostanooids were performed. The radioactive material eluting with the solvent front (Fig. 5) has not yet been identified. [It is likely to include, however, more polar metabolites of arachidonic acid such as dihydroxy, epoxy-hydroxy, and trihydroxy derivatives that have been described in other systems (Bryant and Bailey, 1979; Pace-Asciak et al., 1983; German and Kinless, 1986). These metabolites are currently being studied in our laboratory (Piomelli et al., 1987a).]

The identities of PGE₂ and PGF₆ were confirmed by radioimmunoassay after purification by reversed-phase HPLC. The bath contained a single component of PGE₂-like immunoreactivity, which eluted from the reversed-phase HPLC at the retention time expected for this substance (Fig. 5). Similarly, immuno-reactive PGF₆ (0.72 pmol) was detected at the retention characteristic of this compound (n = 2; data not shown). Some material that reacted with the PGF₆ antibody was also found in fractions with the retention time of PGE₂. This immuno-reactivity may be due to cross-reactivity of the antibody used with PGE₂ (0.3% according to information provided by Sera-gen). Alternatively, it may result from cross-reactivity with cyclo-oxygenase products derived from polyunsaturated fatty acids other than arachidonic acid (Levine and Kobayashi, 1983). No immunoreactive 6-keto-PGF₁α was detected in HPLC fractions obtained from similar experiments (n = 2; lower limit of detection, 75 pg/0.1 ml).

The formation of PGE₂ and PGF₆ suggests that cyclo-oxygenase activity is present in Aplysia nervous tissue. This is fur-

### Table 4. Effects of various lipoxygenase inhibitors on formation of ³H-12-HETE and ³H-5-HETE by Aplysia neural tissue

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µM)</th>
<th>³H-12-HETE (cpm/ganglion)</th>
<th>³H-5-HETE (cpm/ganglion)</th>
<th>³H-12-HETE (% of control)</th>
<th>³H-5-HETE (% of control)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>4295 ± 1091</td>
<td>3632 ± 736</td>
<td>100</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>ETYA</td>
<td>30</td>
<td>71 ± 17</td>
<td>72 ± 17</td>
<td>1.6</td>
<td>1.9</td>
<td>4</td>
</tr>
<tr>
<td>NDGA</td>
<td>30</td>
<td>70 ± 15</td>
<td>70 ± 8</td>
<td>1.6</td>
<td>1.9</td>
<td>3</td>
</tr>
<tr>
<td>DHA</td>
<td>30</td>
<td>972 ± 205</td>
<td>41 ± 7</td>
<td>22.6</td>
<td>11.1</td>
<td>3</td>
</tr>
<tr>
<td>MLTA₆</td>
<td>15</td>
<td>1835 ± 15</td>
<td>68 ± 3</td>
<td>42.7</td>
<td>18.8</td>
<td>5</td>
</tr>
</tbody>
</table>

Neural tissue from Aplysia ganglia was incubated for 10 min in artificial seawater containing ³H-arachidonic acid (0.5 µCi/0.1 ml) with or without a lipoxygenase blocker in n experiments. Labeled hydroxy acids were purified from bath samples by reversed-phase HPLC (solvent system I), ETYA, eicosatetraynoic acid; NDGA, nordihydroguaiaretic acid; DHA, 5,6-dehydroarachidonic acid; MLTA₆, 5,6-methanoleukotriene A, methyl ester.

* Means ± SEM.
ther indicated since the cyclo-oxygenase blocker indomethacin inhibited the formation of these products with an IC₅₀ of 0.5 μM.

**Arachidonic acid metabolism in single nerve cell bodies and terminals**

We have shown that neural components of *Aplysia* nervous tissue metabolize arachidonic acid. In order to provide evidence that lipoxygenase and cyclooxygenase activities actually occur in nerve cells, we have studied the metabolism of arachidonic acid in synaptosomes and isolated cell bodies.

**Synaptosomes.** Gentle mechanical stimulation of synaptosomes labeled by incubation with ^3H-arachidonic acid resulted in the release of radioactive eicosanoids into the bath. No me-

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**Figure 4.** Identification of 12-HETE formed by *Aplysia* neural tissue: analysis by gas-chromatography/mass spectrometry. The lipid extract from the homogenate of nervous tissue incubated with arachidonic acid (see legend to Fig. 3) was fractionated by normal-phase HPLC. Material with the retention time of 12-HETE was collected and derivatized as described (see Materials and Methods). A, Analysis by negative-ion chemical ionization. We monitored a diagnostic ion at m/z 391 produced by loss of PFB. The peak observed had the expected retention time for authentic 12-HETE-PFB-TMS. B, Analysis by electron-impact ionization. Characteristic ions for 12-HETE-Me-TMS (m/z 391, 295, and 73) were observed at the retention time expected for this compound. Total ion current (TIC) is displayed in the lower panel.

---

**Figure 5.** Identification of PGE₂. Neural components from the ganglia of one animal were labeled overnight with ^3H-arachidonic acid (2.5 μCi/ml). After washing out unincorporated label, we incubated the tissue for 30 min. Bath samples were analyzed by reversed-phase HPLC (solvent system II); 30 sec fractions were collected for counting. Arrows indicate the position of PGE₂, PGF₂α, and 6-keto-PGF₁α standards. Bath samples from an incubation of neural components with arachidonic acid (15 μM) were fractionated by HPLC (system II); each fraction was dried and reconstituted in phosphate buffer. PGE₂ (dotted line) was identified by radioimmunooassay.

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**Figure 6.** Arachidonic acid metabolism in *Aplysia* synaptosomes. Synaptosomes were labeled for 2 hr with ^3H-arachidonic acid (25 μCi/ml) and stimulated to release eicosanoids by gentle agitation. After 1 min, samples of the bath were subjected to reversed-phase HPLC; 30 sec fractions were collected for counting. ^3H-arachidonic acid was incorporated into the major phospholipid classes in the synaptosomes. Of the total label present after separation of the lipids on TLC in system B, 7.3% was incorporated in phosphatidyl-choline, 4.8% in phosphatidyl-ethanolamine, and 22% in phosphatidyl-serine and -inositol (average of 2 determinations). A, Formation of hydroxy acids (HPLC system I). The retention times of 12-HETE and 5-HETE are indicated. B, Formation of prostaglandins (HPLC solvent system II). ^3H-PGF₂α and ^3H-PGE₂ were detected. Two labeled components, designated I and II, had the retention times of standard ^3H-6-keto-PGF₁α and PGD₂.
Stimulation of 3H-HETE formation by histamine

Histamine (McCaman and Weinreich, 1985; Kretz et al., 1986b) and serotonin (Kupfermann, 1979) are the modulatory transmitters most thoroughly characterized in Aplysia. In vertebrates, both substances stimulate the release of arachidonic acid from lung (Blackwell et al., 1978) and vascular smooth muscle and endothelial cells (Raenziger et al., 1980; Coughlin et al., 1984). We therefore approached the possibility that metabolites of arachidonic acid are produced from endogenous lipids as a consequence of receptor activation by testing whether a 1 min application of histamine or serotonin releases eicosanoids from ganglia previously labeled with 3H-arachidonic acid. Histamine caused the production of 3H-HETE (1710 ± 358 cpm; n = 4) after reversed-phase HPLC. In 2 experiments, 3H-PGE₂ accounted for 3.8 and 4.0% and 3H-PGF₂α for 3.9 and 5.7% of an average of 10,000 total counts per minute. In preliminary experiments, we found that the hydroxy acids are also formed by isolated cell bodies of other identified neurons (pleural sensory cells, bag cells, and L7).

Intracellular stimulation of specific neurons causes release of lipoxygenase and cyclooxygenase products

We next tested whether endogenous transmitter released by stimulating individual identified histaminergic or serotoninergic neurons might also activate arachidonic acid metabolism.

C2 neurons: C2 is a multiaction neuron in the cerebral ganglion of Aplysia and is a well-characterized histaminergic neuron (Weinreich et al., 1975; McCaman and McKenna, 1978; McCaman and Weinreich, 1982, 1985; Schwartz et al., 1986). Both histamine (Hintzen and Riehl, 1985) and stimulation of C2 have been shown to mediate presynaptic inhibition at specific synapses in the cerebral ganglion (Chiel et al., 1983). We stimulated C2 in ganglia labeled with 3H-arachidonic acid and analyzed the bath for 3H-hydroxy acids. Stimulation of C2 at physiological rates evoked the release of radioactive hydroxy acids. The major product was 3H-5-HETE (1274 ± 358 cpm/ganglion; n = 4), and of material eluting as 3H-5-HETE (116 ± 20 cpm), indicating that formation of the hydroxy acids is promoted by activation of specific receptors.

Depolarization produced by increasing the concentration of extracellular K⁺ to 100 mM (substituted for Na⁺; n = 3) only weakly stimulated release of 3H-12-HETE (151 ± 42 cpm) and of material eluting as 3H-5-HETE (116 ± 20 cpm), indicating that formation of the hydroxy acids is promoted by activation of specific receptors.
Figure 9. Formation of 12-HETE and PGE₂ produced by intracellular stimulation of L32 neurons. Labeled abdominal ganglia were stimulated as described in the legend to Figure 1B. HPLC chromatograms of bath samples taken before (C, D) and after (A, B) stimulation. Stimulation of L32 evoked production of labeled 12-HETE and PGE₂. Samples were analyzed by reversed-phase HPLC for 12-HETE, as described for Figure 2, and for PGE₂, as described for Figure 3.

The minimal stimulation protocol tested (20 spikes/s) was sufficient to cause production of the labeled hydroxy acids. Control samples were taken both before stimulation and after each bath wash. No counts above background were detected at the retention times of the hydroxy acids in samples from the same ganglia that had been used to examine the action of C2 (n = 3).

To rule out the possibility that cell depolarization contributes to eicosanoid release, we also stimulated intracellularly the serotonergic cell GCN; no detectable metabolites were found. [In Aplysia, no synaptic connections of GCN are known to occur within the cerebral ganglion (see Granzow and Fraser Rowell, 1981); postsynaptic targets are therefore absent in these experiments. Thus we cannot exclude the possibility that stimulation of GCN might result in the release of eicosanoids from its follower cells.] In 2 experiments, C2 was again stimulated in the same ganglion, and ³H-hydroxy acids were produced, indicating that the failure with GCN did not result from depleting the labeled precursor. Cimetidine (10⁻⁴ M) blocked the release of HETE produced by stimulating C2 (n = 3). In 2 experiments, the effect of cimetidine was found to be reversible after the drug was washed out. These experiments suggest that the histamine released by stimulating C2 neurons promotes the formation of lipoxygenase products through cimetidine-sensitive receptors.

L32 neurons The L32 cells, a group of putative histaminergic interneurons in the abdominal ganglion, make slow inhibitory connections on L10 and most left-upper-quadrant neurons, and a dual-action fast-excitatory/slow-inhibitory connection on L14. Presynaptic inhibition of L10 is produced by the L32-L10 IPSP (Byrne, 1980a; Kretz et al., 1986a). Intracellular stimulation of L32 neurons in ganglia labeled with ³H-arachidonic acid induced the release of ³H-12-HETE (Fig. 9). Stimulation of L32, unlike that of C2, did not promote formation of material eluting as 5-HETE. In 5 separate experiments, the ³H-12-HETE fraction contained 450 ± 85 cpm/ganglion after stimulation of L32. No counts above background were detected in the fractions containing 12-HETE and 5-HETE without stimulation.

We also examined the production of prostaglandins. In 2 experiments, intracellular stimulation resulted in the appearance of 377 and 619 cpm of ³H-PGE₂, with only background values in unstimulated controls.

Discussion

Metabolism of arachidonic acid in Aplysia neurons
Arachidonic acid is present in substantial amounts in nervous tissue of Aplysia, mainly esterified in glycerophospholipids, and, when released, can be metabolized through both lipoxygenase and cyclooxygenase pathways (Figs. 2-6). Using HPLC, radioimmunoassay, and GC/MS, we identified the 12-lipoxygenase product, 12-HETE. A second arachidonic acid metabolite was identified tentatively as 5-HETE on the basis of its chromatographic behavior and immunoreactivity, and because its formation was blocked by 5-lipoxygenase inhibitors. We found that both of these hydroxy acids can be incorporated into cell lipids as in mammalian tissue (Stenson and Parker, 1979; Birkle and Bazan, 1984). Best characterized in platelets, neutrophils, and other blood cells (Needleman et al., 1986), the distribution of 12-lipoxygenase is widespread; 12-lipoxygenase is present in mammalian brain (Adesuyi et al., 1985) and neuroblastoma cells (Birkle and Ellis, 1983). 5-Lipoxygenase, which is characteristic of inflammatory cells, is also found in the mammalian central nervous system (Lindgren et al., 1984; Adesuyi et al., 1985; Shimizu et al., 1987). The prostaglandins PGE₂ and PGF₂α were prominent products of the cyclooxygenase pathway, but other prostanoids are also likely to be formed (Figs. 6, 7). These cyclooxygenase products are present in virtually all vertebrate tissues, including the brain (Wolfe, 1982).

Even though some glial cells remain associated with the cell...
membrane of isolated nerve cell bodies (Giller and Schwartz, 1971), our experiments with isolated R2 somata suggest that it is likely that both lipoxygenase and cyclo-oxygenase pathways function within neurons: non-neural elements (connective tissue, muscle, blood vessels, and blood cells) are absent. The presence of multiple pathways of arachidonic acid metabolism in neurons, each with several identified intermediates and possibly many as-yet unidentified ones, may make the task of assigning function difficult, but it offers a rich variety of candidates for participation in neuronal action.

In Aplysia, as in mammals, hydroxy acids and prostaglandins can rapidly pass through cell membranes to appear in the bath. We do not know whether the stable metabolites that were recovered extracellularly present a distorted picture of the products actually formed. Answering this question requires analysis of metabolites within the neuron, which we have not attempted to do.

**Receptor mediation**

Birkle and Bazan (1984) have shown that membrane depolarization causes arachidonate turnover and formation of 12-HETE in rat brain. Depolarizing Aplysia neuronal membranes in the cerebral ganglion or in synaptosomes by increasing the concentration of extracellular K+ ion only marginally stimulated the formation of hydroxy acids. While depolarization at some sites may be effective, experiments in which histamine was applied to neural components suggest that the stimulation of arachidonic acid metabolism observed is receptor-mediated, and the promotion of the metabolism by firing C2, an identified histaminergic neuron, provides strong support for this idea. Not all histamine receptors appear to stimulate turnover of arachidonic acid, however. Cimetidine in Aplysia selectively blocks hyperpolarizing responses to histamine, but not responses that are depolarizing (like the one produced on GCN; Gruol and Weinreich, 1979). We found that cimetidine blocks the turnover evoked by stimulating C2. This suggests that release of arachidonic acid is mediated by histamine receptors responsible for some forms of inhibition, and not by receptors with excitatory effects. Not all modulatory transmitters stimulate the formation of hydroxy acids from arachidonic acid: application of serotonin was without effect.

Intracellular stimulation of L32 neurons, a cluster of identified cells that mediate presynaptic inhibition in the abdominal ganglion, also promotes the formation of 12-HETE and PGE2. Although there is some evidence that L32 cells are also histaminergic, this has not been established definitively (Kretz et al., 1986a, b).

We suggest that receptors for histamine are coupled to an effector phospholipase that hydrolyzes arachidonate from the membrane phospholipids. This enzyme has not yet been identified in Aplysia neurons. Receptor-mediated stimulation of eicosanoid production in mammalian systems invariably depends on the release of free arachidonate. This rate-limiting reaction can be triggered by receptor-linked activation of phospholipase A2, phospholipase C followed by diacylglycerol lipase, or both (Irvine, 1982).

**Specificity of responses to various treatments**

Exposure of Aplysia neural components to histamine and stimulation of C2 or L32 neurons each resulted in considerably different patterns of eicosanoid production. Histamine, when applied to neural components from the cerebral ganglion, elicited formation of 12-HETE; with C2 stimulation, a product that we have tentatively identified as 5-HETE was the most abundant metabolite. We have not yet determined the reason for these differences. Nevertheless, there are many possible biological causes for these results that may be interesting and important. The endowment of enzymes in the 2 lipoxygenase pathways may vary in different neurons, or the enzymes might be differentially distributed among the different parts of all neurons (for example, cell body and nerve terminals). These explanations make use of differences in either cell type or accessibility, but other factors might also account for the puzzling differences observed. Interestingly, in mammals, 5-lipoxygenase activity is dependent on Ca2+, but 12-lipoxygenase is not (Rouzer and Samuelsson, 1985). Thus the same nerve cell might metabolize arachidonic acid differently depending on the concentration of intracellular Ca2+, which, in turn, would depend on its previous history with regard to synaptic inputs regulating Ca2+ channels and mobilization of Ca2+ from intracellular stores. The differences observed between the products formed in response to stimulating C2 and L32 could likewise be explained by differences in cell type or dependence on activity. In addition, it is important to caution that our cell-stimulation experiments were performed under conditions in which activation of a single neuron may have driven polysynaptic networks of unmonitored nerve cells. These networks would, of course, be different for the 2 identified neurons stimulated.

**Possible physiological function**

Application of histamine and stimulation of the histaminergic neuron C2 or neurons in the L32 cluster promote the formation of lipoxygenase products of arachidonic acid. These treatments share the common feature of causing presynaptic inhibition. Involvement of lipoxygenase products in presynaptic inhibition is also indicated by pharmacological experiments carried out on the identified Aplysia interneuron, L10 (Shapiro et al., 1987) and on Aplysia sensory cells (Piomelli et al., 1987a). In both cells, the responses to inhibitory transmitters are mimicked upon application of arachidonic acid and suppressed by 4-bromophenacyl bromide, a phospholipase inhibitor. The neuropeptide FMRFamide produces inhibition in Aplysia (Ocorr and Byrne, 1985; Brenzina et al., 1987) and mediates presynaptic inhibition in sensory cells (Belardetti et al., 1987). NDGA, a lipoxygenase inhibitor, suppresses the membrane hyperpolarization elicited by FMRFamide, whereas indomethacin, a cyclooxygenase blocker, is without effect. The response to FMRFamide can also be mimicked by the lipoxygenase metabolite 12-HPFETE, whereas its derivative, 12-HETE, is inactive. These observations suggest that a lipoxygenase metabolite of arachidonic acid, the hydroperoxy acid, or an unidentified metabolite mediates the inhibitory response to FMRFamide in Aplysia sensory neurons. Similarly, 12-HPETE was found to inhibit the release of neuropeptide from the juxtaglomerular cells in mammalian kidney at concentrations 100 times lower than that of 12-HETE (Antonipillai et al., 1987). In other cells, 12 HPETE and 5-HPETE have biochemical effects that are not shared by the corresponding HETE; for example, they stimulate formation of cGMP (Hidaka and Asano, 1977; Snider et al., 1984), which is also observed in Aplysia ganglia (Piomelli and Schwartz, unpublished observations). The hydroperoxy acids also inhibit prostacyclin synthase (Moncada and Vane, 1979) and stimulate leukotriene production (Maelouf et al., 1982), whereas the hydroxy acids do not.
The case with which both lipoxigenase and cyclooxygenase products pass through the neuronal membrane raises the question as to whether these compounds might mediate intracellular as well as intracellular signaling. Some hypotheses explaining synaptic plasticity underlying learning and memory make use of second-messenger mechanisms in the presynaptic terminal (see Kandel and Schwartz, 1982). Others would implicate changes in the postsynaptic neuron (see Teyle and DiScenna, 1987). These 2 kinds of mechanisms need not be mutually exclusive. Hedqvist (1977) showed that PGE₂ released from adrenergic neurons in the sympathetic nervous system has an autocrine function. It is therefore possible that some metabolites of arachidonic acid act as intracellular second messengers (in the presynaptic element), while others pass through the terminal membrane and cross the cleft to act as first messengers on the postsynaptic cell.

References


