### Title
Formation and action of 8-hydroxy-11,12-epoxy-5,9,14-icosatrienoic acid in Aplysia: a possible second messenger in neurons.




### Journal
Proceedings of the National Academy of Sciences of the United States of America, 86(5)

### ISSN
0027-8424



### Authors
Piomelli, D
Shapiro, E
Zipkin, R
et al.



### Publication Date
1989-03-01

### DOI
10.1073/pnas.86.5.1721

*Proc. Natl. Acad. Sci. USA*
Vol. 86, pp. 1721–1725, March 1989
Neurobiology

# Formation and action of 8-hydroxy-11,12-epoxy-5,9,14-icosatrienoic acid in *Aplysia*: A possible second messenger in neurons

(12-lipoxygenase/arachidonic acid/hepoxilins/presynaptic inhibition)

DANIELE PIOMELLI*, ELI SHAPIRO*, ROBERT ZIPKIN†, JAMES H. SCHWARTZ*, AND STEVEN J. FEINMARK‡§

*Howard Hughes Medical Institute and ‡Departments of Pharmacology and Medicine, Columbia University, New York, NY 10032; and †Biomol Research Laboratories, Plymouth Meeting, PA 19462

*Communicated by Bengt Samuelsson, December 5, 1988 (received for review September 2, 1988)*

ABSTRACT **In *Aplysia* neural tissue, the release and metabolism of arachidonic acid are stimulated by histamine or by activation of the identified L32 nerve cell circuit of the abdominal ganglion. Previously we found that histamine and intracellular stimulation of L32 cells, which are putatively histaminergic neurons, cause the production of 12-hydroxy-5,8,10,14-icosatetraenoic acid (12-HETE), a product of the 12-lipoxygenase pathway formed through 12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE). 12-HPETE, but not 12(*S*)-HETE, mimics the dual-action response of L14 ink motor neurons to histamine and stimulation of L32. 12-HPETE can also be further metabolized to 8-hydroxy-11,12-epoxy-5,9,14-icosatrienoic acid (8-HEpETE) which was identified by HPLC, enzymatic hydrolysis, and GC/MS. Production of 8-HEpETE is specific, as its positional isomer 10-hydroxy-11,12-epoxy-5,8,14-icosatrienoic acid is not formed after physiologic stimulation. 8-HEpETE can elicit the late component (hyperpolarization) of the dual-action response in L14 cells, suggesting that it may be a second messenger in *Aplysia*.**

12-Lipoxygenase converts arachidonic acid to 12-hydroperoxy-5,8,10,14-icosatetraenoic acid (12-HPETE), which may then be reduced to a more stable hydroxy acid, 12-hydroxy-5,8,10,14-icosatetraenoic acid (12-HETE). 12-HETE, which originally was discovered and characterized in human platelets (1, 2), has since been detected in nervous tissue from vertebrates (3, 4) and invertebrates. In the marine mollusk *Aplysia californica*, production of 12-HETE is evoked by applying histamine (5) or phenylalanylmethionylarginylphenylalaninamide (FMRFamide) (6), two modulatory neurotransmitters that produce presynaptic inhibition. Intracellular stimulation of L32 cells, identified neurons that produce presynaptic inhibition, also results in the production of 12-HETE, presumably through release of endogenous transmitter (5). Although 12-HETE is produced by intact ganglia, synthetic 12(*S*)-HETE is unable to induce electrophysiologic responses in any *Aplysia* neurons tested (6, 7). In contrast, application of its precursors, arachidonic acid and 12-HPETE, mimics the actions of FMRFamide on sensory neurons (6, 8). Further metabolism of 12-HPETE may be required to obtain these biologic activities. Belardetti *et al.* (8) reported that the hydroperoxy acid enhances the probability of $K_s^+$-channel opening in whole-cell patches of *Aplysia* sensory neurons but is not effective on cell-free patches, suggesting that a cytosolic component is needed to convert 12-HPETE into an active product.

Among other products, the metabolism of 12-HPETE has been reported to yield a pair of diastereomeric epoxy alcohols, (8*RS*)-8-hydroxy-11,12-epoxy-5,9,14-icosatrienoic acid (8-HEpETE) and (10*RS*)-10-hydroxy-11,12-epoxy-5,8,14-icosatrienoic acid (10-HEpETE), which are formed through an intramolecular rearrangement of the hydroperoxide (9). The epoxy alcohols then may be hydrolyzed either enzymatically (10) or nonenzymatically to isomeric trihydroxyicosatrienoic acids (TriHETEs), which originally were identified in platelets (11, 12). Both TriHETEs and epoxy alcohols have now been found in brain and several other tissues (10, 13, 14). It has been shown that 8-HEpETE (for which the name hepoxilin $A_3$ has been proposed) potentiates glucose-induced insulin secretion (15) and transport of calcium across the guinea pig yolk sac (16).

In this report we show that 8-HEpETE is synthesized by intact abdominal ganglia. 12-HPETE and 8-HEpETE [but not 12(*S*)-HETE] have marked electrophysiologic effects on follower cells of L32, suggesting that these metabolites may act as second messengers in *Aplysia* neurons. A preliminary account has been published (17).

## MATERIALS AND METHODS

**Preparation of Nervous Tissue for Biochemical Analysis.** Central ganglia from 10 *Aplysia* weighing 100–200 g each (Howard Hughes Medical Institute Mariculture Resource Facility, Woods Hole, MA, and Marinus, Sand City, CA) were removed, trimmed of connective tissue, and homogenized in a Polytron (Brinkmann) in 5 ml of chilled artificial seawater (5, 18). Homogenates were warmed to 15°C for 5 min before incubation for 10 min with [$^{14}$C]- or [$^3$H]arachidonic acid (50 $\mu$M; 2.5–25 $\mu$Ci; 1 $\mu$Ci = 37 kBq) or with 12-HPETE (50 $\mu$M; Biomol Research Labs).

**Extraction and Purification of Lipids.** Incubations were stopped with cold acetone (2 vol) and the resulting precipitates were removed by centrifugation. The supernatant was acidified (pH 3.6–4.0) with formic acid and extracted twice with ethyl acetate (2 vol). The combined organic layers were dried over sodium sulfate and then evaporated under reduced pressure. The lipid residue was dissolved in hexane, applied to a normal-phase high-performance liquid chromatography (HPLC) column (Silicar LC Si; 250 × 4.6 mm, Supelco), and eluted with hexane/2-propanol/acetic acid (98:2:0.1, vol/vol) at 1 ml/min. Fractions were collected for analysis by liquid scintillation counting, gas chromatography/mass spectrometry (GC/MS), or rechromatography by reversed-phase HPLC. Purified epoxy alcohols were applied to a Nucleosil $C_{18}$ column (250 × 4.6 mm; 5-$\mu$m particle size) and eluted with methanol/water/acetic acid (75:25:0.1, vol/vol) at 1 ml/min.

**Synthesis of Standard Epoxy Alcohols.** The methyl ester of (8*RS*)-8-hydroxy-11,12-*trans*-epoxy-5,14-*cis*-9-*trans*-icosa-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: 12-HPETE, 12-hydroperoxy-5,8,10,14-icosatetraenoic acid; 12-HETE, 12-hydroxy-5,8,10,14-icosatetraenoic acid; 8-HEpETE, 8-hydroxy-11,12-epoxy-5,9,14-icosatrienoic acid; 10-HEpETE, 10-hydroxy-11,12-epoxy-5,8,14-icosatrienoic acid; 12-KETE, 12-keto-5,8,10,14-icosatetraenoic acid; TriHETE, trihydroxyicosatrienoic acid; PFB, pentafluorobenzyl; TMS, trimethylsilyl; NICI, negative-ion chemical ionization.

§To whom reprint requests should be addressed.

trienoic acid was synthesized by the method of Corey and Wei-Guo (19). Its structure was confirmed by NMR and GC/MS and by GC/MS after catalytic hydrogenation, lithium aluminum hydride reduction, and methanolysis. Just before use, 8-HEpETE methyl ester was hydrolyzed by treatment for 3 hr with methanolic NaOH (methanol/50% NaOH, 9:1, vol/vol). The mixture was diluted with water, neutralized with solid $CO_2$, and applied to a preconditioned Sep-Pak $C_{18}$ column (Waters, Milford, MA). After washing with water, 8-HEpETE sodium salt was eluted with methanol. A vehicle was prepared by carrying out a blank hydrolysis through column purification. Standard 10-HEpETE as well as $^3$H-labeled epoxy alcohols were prepared with rat lung 12-lipoxygenase and purified by HPLC; rat lung epoxide hydrolase was prepared as reported (10).

**GC/MS.** Analyses were performed on a Hewlett–Packard 5987A fitted with an HP-1 (12 m) capillary column placed directly into the ion source; helium was used as the carrier gas at a constant head pressure of 55 kPa. The injector was kept at 250°C and the transfer lines were kept at 275°C. The source was operated at 200°C for the electron-impact mode and at 150°C for the negative-ion chemical ionization (NICI) mode.

For NICI analysis, purified samples were converted to the pentafluorobenzyl (PFB) esters prior to formation of trimethylsilyl (TMS) ethers (5). Derivatized samples were dissolved in hexane for the analyses carried out with methane as the ionizing gas (approximate source pressure, 106.6 Pa).

For electron-impact analysis (electron voltage, 70 eV), samples were converted to the corresponding methyl (Me) esters with diazomethane before formation of the TMS ethers as described above.

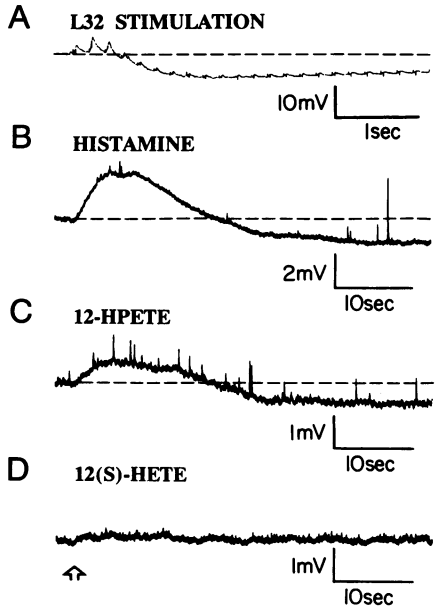


FIG. 1. Response of L14 to L32 and lipoxygenase metabolites. L14 was impaled for intracellular recording of membrane potential. (*A*) For L32 stimulation, the presynaptic cell, L32, was driven with a 5-sec train of depolarizing pulses of 200 msec at 5 Hz. Each pulse produced three spikes in L32 (not shown). The resting potential of L14 was −60 mV. L14 typically responds with a fast depolarization, followed by a slower hyperpolarizing response. (*B*) Histamine (100 pmol over 1 sec) was applied to L14 (resting potential, −70 mV). (*C*) 12-HPETE (100 pmol over 3 sec) was applied to L14 (resting potential, −70 mV). (*D*) 12(*S*)-HETE (250 pmol over 5 sec) was applied to L14 (resting potential, −64 mV). Fast upward deflections in histamine and 12-HPETE records are spontaneous excitatory postsynaptic potentials onto L14. Histamine and 12-HPETE records are from the same L14 cell. Arrow indicates beginning of application.

Table 1. Response of identified *Aplysia* neurons to histamine and 12-HPETE

| Neuron and stimulus | No. of experiments | No. of observations | Type of response | | | |
|---|---|---|---|---|---|---|
| | | | D | H | D/H | N |
| Cell L14 | | | | | | |
| Histamine | 26 | 54 | 6 | 1 | 44 | 3 |
| 12(*S*)-HETE | 7 | 20 | 1 | 3 | 0 | 16 |
| 12-HPETE | 15 | 34 | 1 | 8 | 19 | 6 |
| Cell L10 | | | | | | |
| Histamine | 14 | 34 | 0 | 32 | 0 | 2 |
| 12(*S*)-HETE | 5 | 12 | 0 | 1 | 0 | 11 |
| 12-HPETE | 9 | 18 | 2 | 4 | 0 | 12 |

Puffs of 25–250 pmol were applied to L10 or L14 cells at intervals of 5–10 min. Response amplitudes ranged from 1 to 10 mV and developed within 10–30 sec. Responses usually decayed within 1 min, although sometimes the membrane potential did not fully recover during the interval between puffs. The responses were classified as either depolarizing (D), hyperpolarizing (H), dual-action (D/H), or no response (N). Metabolites were tested up to four times in some ganglia, but in most instances each compound was tested only once or twice. Some of the results from these experiments are presented in this table; others are reported elsewhere (22).

**Intracellular Stimulation and Electrophysiology.** Labeling of isolated ganglia with [$^3$H]arachidonic acid and the identification and intracellular stimulation of neurons in the abdominal (L32 and its follower cells, L10 and L14) and cerebral (C2 cells) ganglia were performed as described (5). Abdominal ganglia were superfused with artificial seawater (1–2 ml/min) in a 5-ml chamber. Identified cells were impaled with standard glass microelectrodes (5–20 mΩ, filled with 2 M potassium citrate). Some cells were impaled with two electrodes, one for recording voltage, the other for passing current. Otherwise a single electrode connected to a bridge circuit was used. One to 10 μl of a test compound was puffed into the superfusion stream by air pressure (41.4–69.0 kPa) with a WPI (New Haven, CT) microejection unit over a

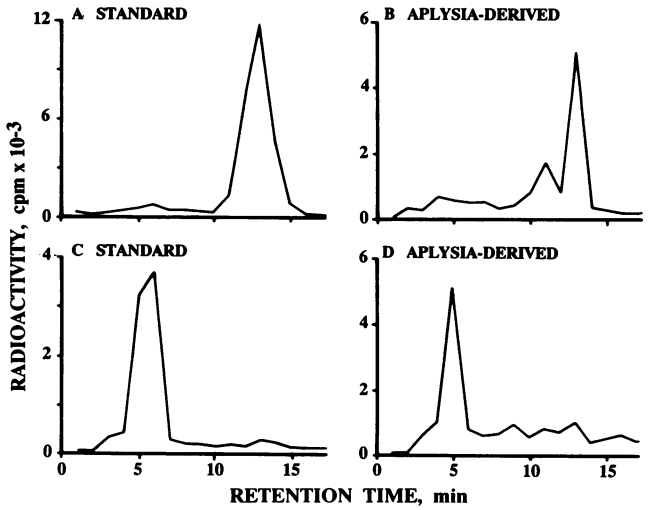


FIG. 2. Reversed-phase HPLC analysis of purified 8-HEpETE before and after hydrolysis. (*A*) [$^3$H]8-HEpETE, synthesized with rat lung 12-lipoxygenase, was applied to the HPLC. (*C*) Some of this material was incubated at 30°C for 30 min with epoxide hydrolase (2 ml of lung fraction B; ref. 10) before analysis. (*B*) [$^3$H]8-HEpETE was purified from a homogenate of the central ganglia of 10 *Aplysia* incubated with [$^3$H]arachidonic acid (50 μM, 25 μCi, for 10 min at 15°C as described in *Materials and Methods*) and half of the purified 8-HEpETE was applied to the reversed-phase HPLC column. (*D*) The other half was incubated with the epoxide hydrolase before analysis as described above. Fractions (1 min) of the HPLC effluent were assayed for radioactivity by liquid scintillation counting.

period of 1–10 sec. The puffing pipette (tip diameter, 10–20 μm), filled with the substance, was positioned about 0.5 mm above the cell body of a neuron. Solutions of 12-HPETE and 12(*S*)-HETE in ethanol were dried under nitrogen and resuspended in seawater by sonication. Histamine, 8-HEpETE sodium salt, and vehicle were dissolved in seawater.

## RESULTS

**Biological Activity of 12-HPETE on Identified *Aplysia* Neurons.** Stimulation of L32 neurons hyperpolarizes L10 cells and usually produces a dual-action (fast depolarization–slow hyperpolarization) in L14 cells (Fig. 1*A* and ref. 20). Histamine, suggested to be the neurotransmitter released by L32, evokes similar responses (Fig. 1*B* and ref. 21). In L14, 12-HPETE mimicked the response produced by stimulating L32 or application of histamine (Fig. 1*C*) but was only marginally active on L10 (Table 1). In contrast, 12(*S*)-HETE had no activity on either L10 or L14 (Fig. 1*D*, Table 1). The results of 29 experiments are summarized in Table 1.

**Identification of 8-HEpETE Produced in Neural Homogenates.** Homogenates of neural tissue were incubated with radioactive arachidonic acid for 30 min, and lipids were extracted and fractionated by normal-phase HPLC. Two previously unidentified labeled compounds were detected at retention times of 12–15 min and 30–32 min (data not shown; $n = 12$). These were absent from homogenates incubated in the presence of the lipoxygenase blocker nordihydroguaiaretic acid (30 μM; $n = 3$), but their synthesis was unaffected by aspirin (0.5 mM; $n = 3$), a cyclooxygenase blocker. Similar products were isolated from homogenates incubated with [$^3$H]12-HPETE (data not shown; $n = 2$). We suspected these two metabolites to be epoxy alcohols.

Preliminary identification of 8-HEpETE (retention time, 30–32 min) was confirmed by reversed-phase HPLC. The radioactive component, purified by normal-phase HPLC, was eluted from the reversed-phase column as a single peak (Fig. 2*B*) at the retention time of authentic 8-HEpETE (Fig. 2*A*). Treatment of authentic 8-HEpETE with rat lung epoxide hydrolase produced isomeric TriHETEs (Fig. 2*C*). We obtained identical results with the substance derived from *Aplysia* (Fig. 2*D*).

Identification of the two epoxy alcohol isomers was confirmed by GC/MS. The NICI spectrum of the PFB/TMS derivative of 8-HEpETE shows a single prominent ion at $m/z$ 407 (M − 181) that arises by loss of PFB. Analysis by selected ion monitoring of purified *Aplysia* material also shows a peak of $m/z$ 407 at the retention time of authentic 8-HEpETE (Fig. 3*A*). From the electron-impact mass spectrum of the Me/TMS derivative of synthetic 8-HEpETE (Fig. 3*B*), prominent or characteristic ions were chosen for mass fragmentography. Material derived from *Aplysia* was eluted at an equivalent chain length of 22.6, identical to that observed with synthetic 8-HEpETE under these conditions. Characteristic ions were detected at $m/z$ 422 ($M^+$), 407 ($M^+ - 15$), 311 ($M^+ - 111$; loss of the C13–20 tail), and 281 (α cleavage at C8 with loss of C1–7) (Fig. 3*C*). The less polar metabolite formed in the homogenates (retention time on normal-phase HPLC, 12–15 min) was eluted from the GC with an equivalent chain length of 22.1, identical to that observed for standard 10-HEpETE (11). Characteristic ions were detected at $m/z$ 407 ($M^+ - 15$), 391 ($M^+ - 31$), 311 ($M^+ - 111$), 282 (double

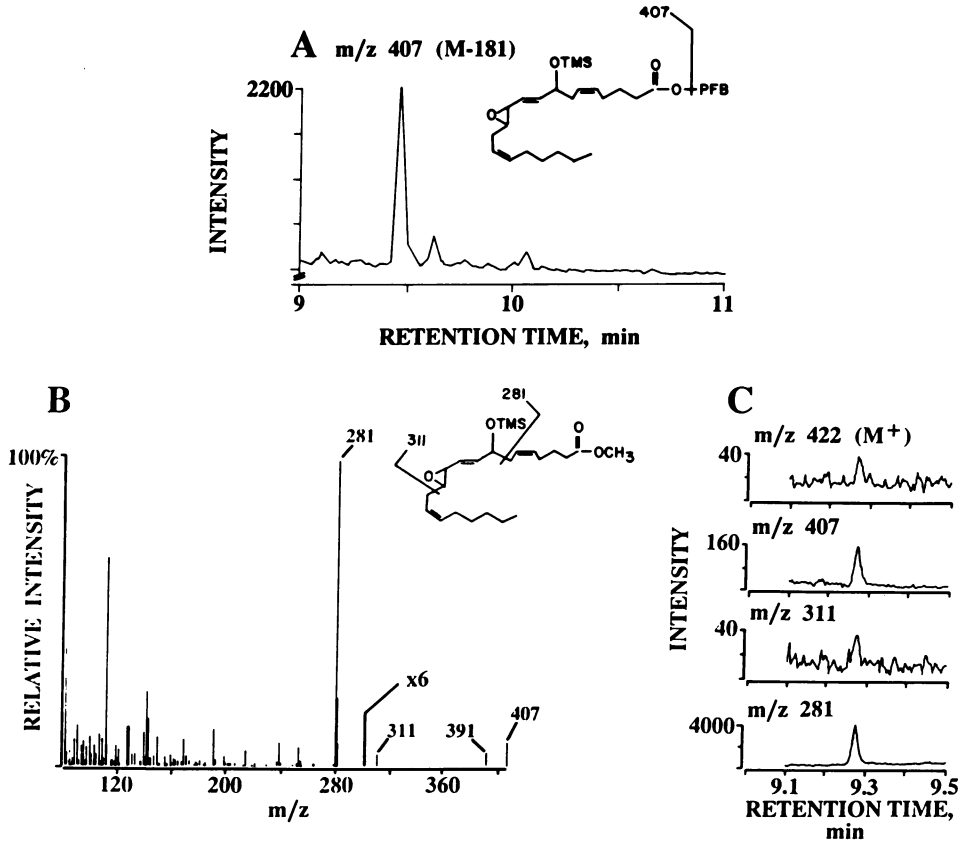


FIG. 3. GC/MS analysis of 8-HEpETE. (*A*) NICI GC/MS analysis of purified material from *Aplysia* homogenates (5% of the extract from 50 animals incubated with 50 μM arachidonic acid) after conversion to the PFB/TMS derivative. (*B*) Electron-impact mass spectrum of the Me/TMS derivative of synthetic 8-HEpETE (column conditions: 12-m HP-1 capillary, 60°C for 1 min and then increased to 280°C at 20°C/min). (*C*) Mass fragmentographic analysis of the Me/TMS derivative of purified 8-HEpETE derived from *Aplysia* homogenates (10 animals) incubated with 12-HPETE (50 μM in 6 ml).

ring cleavage of the epoxide), and 269 (base peak, $\alpha$ cleavage at C10).

**Intracellular Stimulation of L32 Cells.** L32 neurons in abdominal ganglia prelabeled with [$^3$H]arachidonic acid were stimulated intracellularly. [$^3$H]12-HETE and [$^3$H]8-HEpETE (along with other unidentified products) were found in the bathing medium (Fig. 4*A*). No [$^3$H]10-HEpETE was detected. [$^3$H]8-HEpETE was not observed without stimulation (Fig. 4*B*). After stimulation of L32, [$^3$H]8-HEpETE increased ≈16-fold, from 29 ± 8 cpm per ganglion to 487 ± 105 cpm per ganglion ($P < 0.05$; $n = 4$). In the same experiments, there was no significant increase of radioactivity in the 10-HEpETE fractions (Fig. 4*C*). In contrast to the results with L32, intracellular stimulation of C2, an identified histaminergic neuron in the cerebral ganglion, did not generate detectable amounts of epoxy alcohol (Fig. 4*C*; $n = 5$), even though we previously found that 12-HETE is formed under these conditions (5).

**Biologic Activity of 8-HEpETE on Identified *Aplysia* Neurons.** Application of 8-HEpETE to L14 neurons produced a slow hyperpolarization (Fig. 5*B*) in contrast to the dual-action response induced by stimulating L32 (Fig. 1*A*). The mean maximal response to 8-HEpETE at the cell's resting potential was −3.6 ± 0.9 mV (range 0 to −8 mV); application of the vehicle had little effect (0.6 ± 0.5 mV, mean ± SEM; range +1 to −2 mV; $n = 8$, $P < 0.02$). Hyperpolarization induced by 8-HEpETE was accompanied by increased membrane conductance (Fig. 5). 8-HEpETE appeared to be less active on L10, producing weak hyperpolarization in two of five cells tested (data not shown).

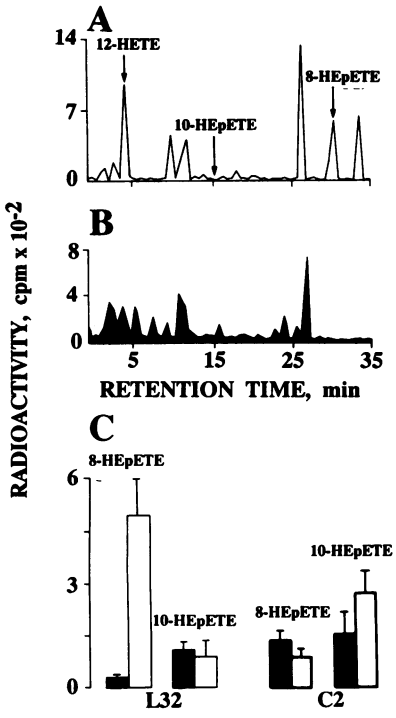


FIG. 4. Production of metabolites from prelabeled abdominal ganglia. Individual ganglia were labeled in artificial seawater (0.5 ml) containing 4 $\mu$Ci of [$^3$H]arachidonic acid for 18 hr at 15°C before L32 neurons were impaled and driven as described in *Materials and Methods* and ref. 5. Under these conditions, 95% of the label was incorporated. (*A*) L32 was stimulated to fire 40 spikes. The bath medium was analyzed for radioactive products by normal-phase HPLC (hexane/2-propanol/acetic acid, 98:2:0.1, vol/vol; 1 ml/min); 1-min fractions were collected. (*B*) Bath medium was collected as a control before impalement of L32. (*C*) $^3$H-labeled epoxy alcohols were quantified after L32 ($n = 4$) in the abdominal ganglion or C2 ($n = 5$) in the cerebral ganglion were stimulated intracellularly. Solid bars, controls; open bars, stimulated. Values are means ± SEM.

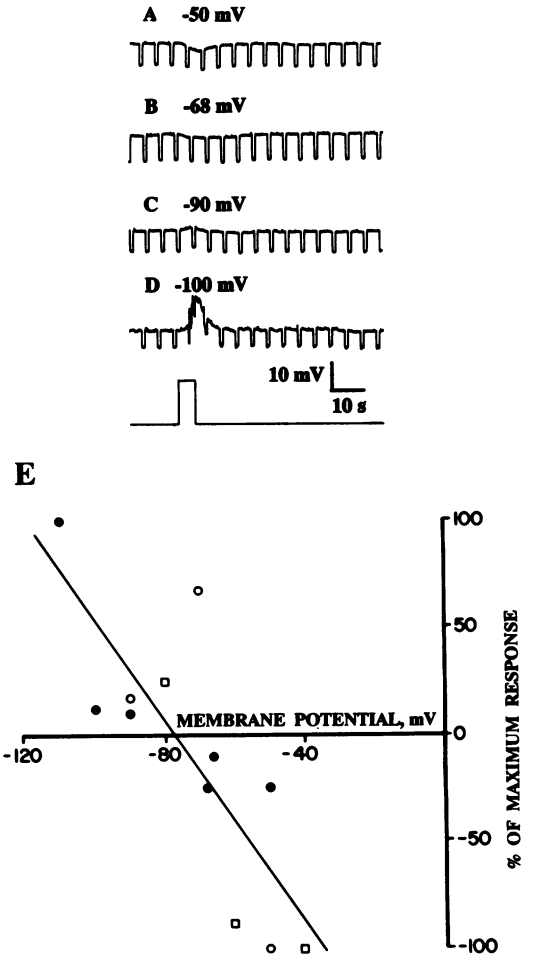


FIG. 5. Effect of membrane potential on the response of L14 to 8-HEpETE. L14 neurons were impaled with two electrodes, one to pass current and the other for recording membrane potential. (*A–D*) 8-HEpETE was applied to L14 (event marker in trace below *D*) while its membrane potential was held at −50 mV (*A*), −68 mV (resting potential) (*B*), −90 mV (*C*), or −100 mV (*D*). In all traces, conductance was measured with −4-nA current pulses of 1-sec duration at 0.2 Hz. 8-HEpETE was prepared as the sodium salt and dissolved in artificial seawater. The precise dose of 8-HEpETE was not known. Assuming no losses during hydrolysis, purification, and spontaneous decomposition in the puff pipette, the upper limit per puff would be 30 nmol. (*E*) The response of L14 to 8-HEpETE (percent of maximum within each experiment) was plotted against holding potential ($n = 3$). Linear regression analysis fit the data to a straight line ($r = 0.80$) with a calculated reversal potential of −77 mV.

In three other experiments the membrane potential of L14 was varied between −50 and −110 mV before application of 8-HEpETE. When the membrane potential was held above the normal resting potential (about −68 mV), 8-HEpETE produced a hyperpolarizing response (Fig. 5 *A*, *B*, and *E*). Below −77 mV, however, application of 8-HEpETE produced a depolarization (Fig. 5 *C–E*).

## DISCUSSION

This report shows that the 12-lipoxygenase metabolite 8-HEpETE is a biologically active product released by physiologic stimulation of identified neurons in intact abdominal ganglia of *Aplysia*. Identification of this metabolite was carried out by comparison to authentic 8-HEpETE on normal-phase and reversed-phase HPLC, as well as by enzy-

matic conversion of the unstable epoxide to triols. The material produced by *Aplysia* neural tissue had GC/MS characteristics similar to 8-HEpETE prepared by total synthesis. Results with synthetic 8-HEpETE differed in some respects from those previously published: the equivalent chain length for the Me/TMS derivative of 8-HEpETE in our system was 22.6 versus 23.1 (10). The spectrum of this derivative prepared by total synthesis also differs, primarily by lacking an ion at $m/z$ 243 ($\alpha$ cleavage at C8 with loss of C1–7). We find that this ion is resolved from the peak of 8-HEpETE and thus was not used during mass fragmentography. The differences in spectrum and GC retention possibly arise because of thermal instability of the epoxy alcohol derivative during capillary GC; earlier data were obtained with a packed GC column.

Epoxy alcohols arise by the rearrangement of 12-HPETE, which can be catalyzed by hemoglobin or hematin (23). This nonenzymatic process yields a mixture of two positional isomers, 8-HEpETE and 10-HEpETE. Neural homogenates from *Aplysia* are rich in cellular hemoglobin (24) and it is not surprising that this preparation, which is also rich in 12-lipoxygenase activity, can generate both isomers. Nevertheless, intracellular stimulation of L32 neurons in intact abdominal ganglia results in selective production of the more unstable isomer, 8-HEpETE. The absence of 10-HEpETE from intact ganglia contrasts with its generation in homogenates and suggests that, physiologically, the synthesis of 8-HEpETE is under enzymatic control.

The synthesis of 8-HEpETE is restricted to specific neural circuits. Although intracellular stimulation of C2 neurons in the cerebral ganglion leads to the release of monohydroxy acids, including 12-HETE (5), activation of this neuron does not result in detectable synthesis of 8-HEpETE. This observation further supports the idea that formation of epoxy alcohols in intact ganglia is enzymatic.

8-HEpETE is biologically active on some follower neurons of L32, producing slow hyperpolarization that is accompanied by increased membrane conductance. This response reverses at a membrane potential between −70 and −80 mV (Fig. 5*E*), suggesting that a $K^+$ channel mediates the effect. Ion substitution and patch-clamp studies are necessary to confirm this conclusion. Nevertheless, recent studies with 12-HPETE in sensory neurons are in agreement: Belardetti *et al.* (8) found that 12-HPETE enhances the probability of $K_s^+$-channel opening in whole-cell but not cell-free patches, suggesting that conversion of 12-HPETE into an active product, perhaps 8-HEpETE, is required. Preliminary trials on cell-free patches of sensory neurons indicate that synthetic 8-HEpETE induces $K_s^+$-channel opening (25).

In L14, Byrne (26) showed that the hyperpolarizing phase of the L32-induced dual-action postsynaptic potential is associated with increased conductance and reverses at about −80 mV, an observation that has been confirmed (E.S., unpublished data). The similarity in conductance change and reversal potential between the hyperpolarizing synaptic response and the response to 8-HEpETE is in accord with a common ionic mechanism.

The dual-action response in L14 induced by histamine or intracellular stimulation of L32 is mimicked by 12-HPETE. Application of 8-HEpETE induces only the hyperpolarizing phase, however, The fast depolarization might be produced by 12-HPETE itself or by another metabolite of 12-HPETE. We recently identified 12-keto-5,8,10,14-icosatetraenoic acid (12-KETE) as a candidate second messenger for this activity (22). 12-KETE is produced by *Aplysia* abdominal ganglia exposed to bath application of histamine and depolarizes L14.

8-HEpETE and 12-HPETE are less active when applied to L10, even though L10 responds to arachidonic acid with hyperpolarization and decreased transmitter release (7). It is possible that technical factors impede the penetration of active metabolites to some intracellular sites after extracellular application. Alternatively, as yet unidentified metabolites of arachidonate may be produced and act in L10.

Biochemical studies on intact *Aplysia* ganglia have shown the presence of the 12-lipoxygenase pathway. Although only small amounts of the arachidonate released after physiological stimulation are ultimately converted to 8-HEpETE, this metabolite comprises a substantial portion of the products. Based upon the extracellular application of 8-HEpETE, it is impossible to predict precisely how much must be generated *in situ* to produce a hyperpolarizing response in L14. Nevertheless, our results suggest that 8-HEpETE is important in evoking synaptic responses. Further, these studies indicate that epoxy alcohols may be synthesized enzymatically. Still further, our results would be another example of a second-messenger mechanism in which different metabolites formed from the same precursor produce dual, time-dependent conductance changes in the target cell (27). In L14, a compound, dual-action response may result from the parallel conversion of 12-HPETE into two metabolites, 12-KETE for depolarization and 8-HEpETE for hyperpolarization. In addition, experiments with L10 and other identified neurons (5) suggest that other metabolites of arachidonic acid remain to be discovered.

We thank Jillayn Lindahl for typing the manuscript, Arthur Monaghan for technical assistance in synthesizing 8-HEpETE, and Hensin Tsao for helping to prepare the rat lung enzymes. We are grateful to Francesco Belardetti for sharing his data before publication. S.J.F. is an Established Investigator of the American Heart Association.

1. Hamberg, M. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 3400–3404.
2. Nugteren, D. H. (1975) *Biochim. Biophys. Acta* **380,** 299–307.
3. Sautebin, L., Spagnuolo, C., Galli, C. & Galli, G. (1978) *Prostaglandins* **16,** 985–988.
4. Miyamoto, T., Lindgren, J. Å., Hökfelt, T. & Samuelsson, B. (1987) *FEBS Lett.* **216,** 123–127.
5. Piomelli, D., Shapiro, E., Feinmark, S. J. & Schwartz, J. H. (1987) *J. Neurosci.* **7,** 3675–3686.
6. Piomelli, D., Volterra, A., Dale, N., Siegelbaum, S. A., Kandel, E. R., Schwartz, J. H. & Belardetti, F. (1987) *Nature (London)* **328,** 38–43.
7. Shapiro, E., Piomelli, D., Feinmark, S. J., Vogel, S., Chin, G. & Schwartz, J. H. (1989) *Cold Spring Harbor Symp. Quant. Biol.* **53,** in press.
8. Belardetti, F., Rosolowsky, M. & Campbell, W. (1988) *Biophys. J.* **53,** 144 (abstr.).
9. Pace-Asciak, C. R. (1984) *J. Biol. Chem.* **259,** 8332–8337.
10. Pace-Asciak, C. R., Granström, E. & Samuelsson, B. (1983) *J. Biol. Chem.* **258,** 6835–6840.
11. Walker, I. C., Jones, R. C. & Wilson, N. H. (1979) *Prostaglandins* **18,** 173–178.
12. Bryant, R. W. & Bailey, J. M. (1979) *Prostaglandins* **17,** 9–18.
13. Pace-Asciak, C. R. (1988) *Biochem. Biophys. Res. Commun.* **151,** 493–498.
14. German, J. B. & Kinsella, J. E. (1986) *Biochim. Biophys. Acta* **877,** 290–298.
15. Pace-Asciak, C. R. & Martin, J. M. (1984) *Prostaglandins Leukotrienes Med.* **22,** 1–9.
16. Derewlany, L. O., Pace-Asciak, C. R. & Radde, I. C. (1984) *Can. J. Physiol. Pharmacol.* **62,** 1466–1469.
17. Piomelli, D., Feinmark, S. J. & Schwartz, J. H. (1987) *Soc. Neurosci. Abstr.* **13,** 598.
18. Eisenstadt, M., Goldman, J. E., Kandel, E. R., Koike, H., Koester, J. & Schwartz, J. H. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 3371–3375.
19. Corey, E. J. & Wei-Guo, S. (1984) *Tetrahedron Lett.* **25,** 5119–5122.
20. Kretz, R., Shapiro, E. & Kandel, E. R. (1986) *J. Neurophysiol.* **55,** 113–130.
21. Kretz, R., Shapiro, E., Bailey, C. H., Chen, M. & Kandel, E. R. (1986) *J. Neurophysiol.* **55,** 131–146.
22. Piomelli, D., Feinmark, S. J., Shapiro, E. & Schwartz, J. H. (1988) *J. Biol. Chem.* **263,** 16591–16596.
23. Pace-Asciak, C. R. (1984) *Biochim. Biophys. Acta* **793,** 485–488.
24. Wittenberg, B. A., Briehl, R. W. & Wittenberg, J. B. (1965) *Biochem. J.* **96,** 363–371.
25. Belardetti, F., Campbell, W., Chabert, P., Falck, J. R., Mioskowski, C. & Rosolowsky, M. (1988) *Soc. Neurosci. Abstr.* **14,** 1207.
26. Byrne, J. H. (1980) *J. Neurophysiol.* **43,** 896–911.
27. Higashida, H. & Brown, D. A. (1986) *Nature (London)* **323,** 333–335.