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Comparison of the Effects of 5-Hydroxyeicosatetraenoic Acid and Hepoxilin on Cholinoreceptor Plasticity of *Helix lucorum* Neurons

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Natural polyunsaturated fatty acids (dihomo-γ-linolenic, arachidonic and thymnodonic) are components of cell membrane lipids. Enzymatic transformation of these compounds under the action of lipoxygenases (LO), cytochrome P_{450}, and cyclooxygenase results in the formation of low-molecular-weight oxygenized metabolites - eicosanoids which are universal regulators of cell metabolism [5,15]. Since eicosanoids freely penetrate through the cell membrane, they may act both as intracellular (secondary mediators) and as extracellular (retrograde messengers) signal molecules and participate in transsynaptic regulation of neuron activity as local neuromodulators [5,6,10-15].

Among lipoxygenase eicosanoids, three main groups formed under the influence of 5-, 12-, and 15-LO can be distinguished [15]. In studying the role of eicosanoids in the regulation of cholinoreceptor (ChR) plasticity of *Helix lucorum* neurons, we used polyacethylene analogs of dihomo-γ-linolenic, arachidonic, and thymnodonic acids, which are known to inhibit different lipoxygenases [2]. The results allowed us to suggest the main regulatory role of 5-lipoxygenase eicosanoids including those from the 15-lipoxygenase pathway. On the other hand, involvement of 12-lipoxygenase derivatives in this regulation seemed most unlikely. 15-Hydroxyeicosatetraenoic acid (15-HETE) regulates ChR plasticity [1]. In the present study we tested

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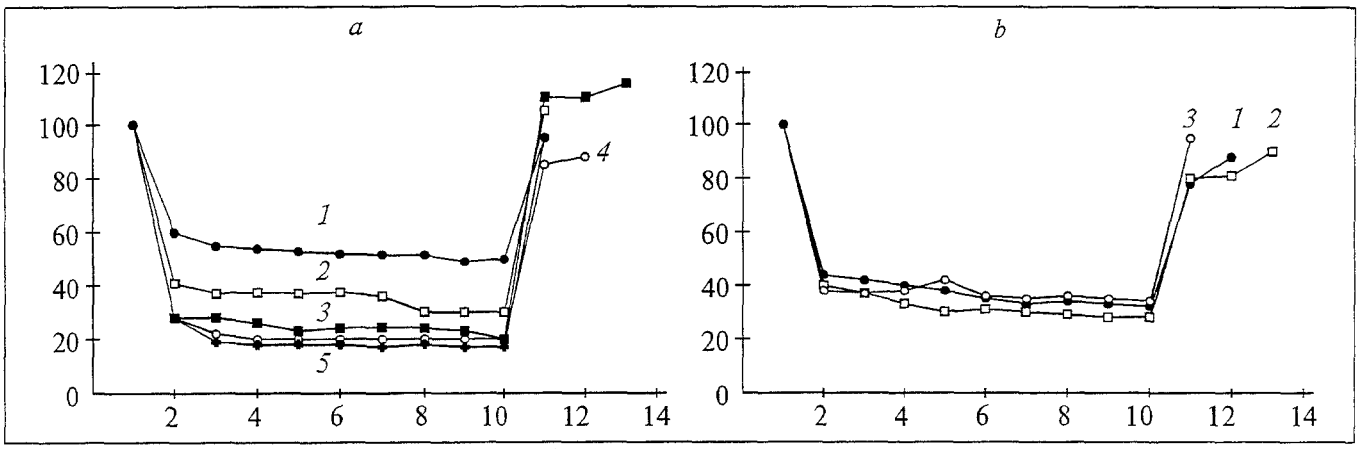

Fig. 1. Effect of 5-HETE (*a*) and HXA_3 (*b*) on ACh-current extinction curves of identified RPa3 neuron. *a, b*) ACh-current extinction curves of RPa3 neurons from different preparations. *a*: *1*) ACh-current extinction before pharmacological influence; *2–4*) against the background of 5-HETE action (34.2 μM) with a 50 min. (*2*), 140 min (*3*), and 190 min (*4*) exposure; *5*) after a 50 min washing of the preparation in Ringer solution; *b*: *1*) ACh-current extinction before pharmacological influence; *2*) against the background of HXA_3 action (80 μM, 30 min); *3*) after a 40 min washing of the preparation in Ringer solution. Abscissa: No. of ACh application in the series; ordinate: maximal amplitude of AC current (%) with respect to its value in response to the first stimulus in the series.

eicosanoids of two other lipoxygenase groups: 5-HETE, a derivative of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and hepoxilin A_3 (HXA_3), a epoxyhydroxymetabolite of 12-HPETE [9,10,15].

The accumulated data on neurobiological effects of monohydroxy- and monohydroperoxyeicosatetraenoic acids and hepoxilins are scant. Endogenous 5- and 12-HETE were found in synaptosomes and neuronal soma of the sea snail *Aplysia* [12,14]. It was shown that 5-HPETE, 5-HETE, and 12-HETE did not affect the membrane potential and slightly prolonged the action potential of *Aplysia* sensory neurons, whereas 12-HPETE effectively influenced the indicated parameters [12,14]. Hepoxilin biosynthesis was recorded in the rat brain [10] and the nervous system of *Aplysia* [13]. HXA_3 was shown to stimulate the transmembrane transfer of Ca^{+2} ions [7,8]. In addition, HXA_3 and its conjugates with 11-glutathione hyperpolarize neurons of the CA1 region of the rat hippocampus, increase the amplitude of orthodromic IPSP and postspike hyperpolarization, and reduce the action potential generation threshold [6,11]. HXA_3 also hyperpolarizes *Aplysia* neurons and activates K^+-channels of the S type increasing the probability of their opening [4,13]. On the basis of these data it was concluded that eicosanoids play a neuromodulatory role as intracellular secondary mediators and extracellular retrograde messengers.

MATERIALS AND METHODS

The experiments were performed on identified neurons RPa3 and LPa3 of *Helix lucorum taurica* Kryn in isolated ganglia at room temperature. The circumesophageal nerve ring was fixed in a flow-through chamber of 1 ml volume. After enzymatic treatment of the preparation in 2% type IA collagenase (Sigma, USA) or in 0.5% digestase (Seatec, Russia-Luxembourg) during 30 and 40 min, respectively, at room temperature the connective tissue membranes covering the ganglia were removed. Ringer solution of the following composition: 10 mM Tris-HCl, 100 mM NaCl, 4 mM KCl, 10 mM $CaCl_2$, and 4 mM $MgCl_2$, pH 7.5, was run through the chamber.

Transmembrane currents were recorded using the double-electrode voltage clamp technique. The intracellular microelectrodes were made of Pyrex and filled with 2.5 M KCl; microelectrode resistance constituted 7-22 MOhm, (12.9±1.8 MOhm; $M \pm m$). The double-barreled micropipette was terminated at the outer surface of the neuronal soma. The phoretic channel was filled with acetylcholine chloride (ACh, Serva, Germany) dissolved in dis-

TABLE 1. Effect of 5-HETE and HXA_3 on ACh-Current Amplitude ($M \pm m$)

Compound	Number of neurons investigated			
	total	increase	decrease	no effect
5-HETE, (22.8–57.0 μM)	5	0	4* −33.2±5.8	1
HXA_3, (40–100 μM)	6	0	6* −49.2±6.5	0

Note. Variations in the ACh-current amplitude (in %) after pharmacological influence are indicated in parentheses. One asterisk here and in Table 2 designates $p<0.05$. Two asterisks designate $p<0.01$.

tilled water (4 M, pH 7.5), and the balance channel was filled with Ringer solution. Cationic currents were run through the phoretic channel (735-933 nA, 1 sec). Micropipette resistance was 20-36 MOhm.

Every series included 11-13 successive ionophoretic ACh applications by a current of constant direction, strength and duration. First 10 stimuli were delivered at intervals of 120-180 sec (125.5±5.5 sec) to extinguish the ACh current. Subsequent stimuli were delivered at intervals of 10 min to assess the degree and rate of restoration of the extinguished reaction. The experiment consisted of several extinction series - control (before pharmacological influence), experimental (against the background of agent action), and restoration (after washing of the ganglia in running Ringer solution). In the stimulation series Ringer solution was not run through the chamber. In the experimental series stimuli were delivered 10-70 min after administration of 5(S)-hydroxy-(6E,8Z,11Z,14Z)-eicosatetraenoic acid (5-HETE, synthesized at the A. N. Belozerskii Research Institute of Physicochemical Biology, Moscow State University) and methyl ester of hepoxilin A_3 [(8R/S)-hydroxy-(11S,12S)-epoxy-5Z,9E,14Z-eicosatrienoic acid - (mixture of threo- and erythro-epimers)] (HXA_3, synthesized at the M. V. Lomonosov Institute of Fine Chemical Technology). 5-HETE was dissolved in ethanol and HXA_3 in dimethylsulfoxide (Sigma, USA). The final concentration of eicosanoids was adjusted to the desired values by adding aliquots of their solutions (5.7 μM 5-HETE and 10 mM HXA_3) with a microsyringe to the flow-through chamber. This procedure made it possible to achieve the calculated concentration of the substances (5.7-57 mM and 10-100 μM, respectively) and provided homogeneity of the surrounding solution. The maximal content of ethanol and dimethylsulfoxide in the flow-through chamber after the addition of eicosanoids did not exceed 1%.

Reliability of the pharmacological influence on ACh-current extinction was assessed using the Wilcoxson paired *t* test from the comparison of experimental and control values of the ACh current in the series in response to ACh applications of the same number (on separate neurons) and the nonparametric sign test (on the whole cell population analyzed). Reliability of the pharmacological influence on the ACh response was assessed according to the Wilcoxson (Mann-Whitney) *U* test and nonparametric sign test. The data were processed in an IBM XT personal computer using special software packages "DIASTA" and "STADIA". The results were obtained for 11 neurons (7 RPa3, 4 LPa3) from 11 ganglionic preparations. The membrane potential was in the range of (-54)-(-78) mV (-66.8±2.5 mV).

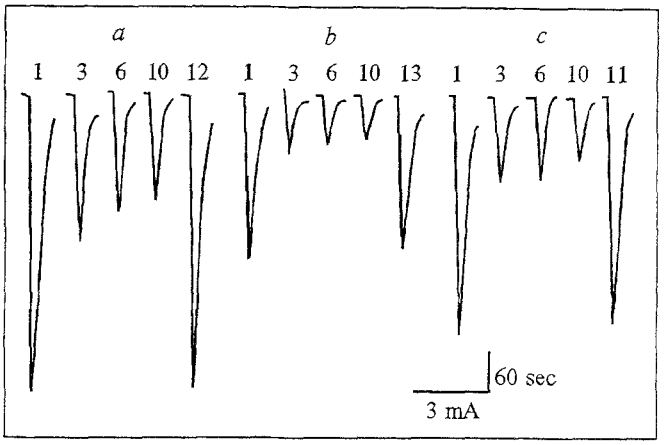

Fig. 2. Deepening of ACh-current extinction by 5-HETE recorded for neuron RPa3. The following recordings are presented: input current (deviation downward) in response to the 1st, 3rd, 6th, and 10th repeated ionophoretic ACh applications (816 nA, 1 sec) at 120 sec intervals and test applications of ACh from the same pipette 10 (*a*, *c*) and 20 (*b*) min after completion of rhythmic stimulation (11, 12 stimuli). *a*) before pharmacological influence; *b*) after extracellular application of 5-HETE (34.2 μM, 190 min), *c*) after a 50 min washing of the preparation in Ringer solution. Holding voltage 75 mV. Calibration: current 3 nA, time 60 sec.

RESULTS

It was shown that 5-HETE (22.8-57/32.3±5.2/μM; 10-70/42.0±7.3/min) lowered the ACh-current amplitude by 33.2±5.8% on average in 4 out of 5 cells ($p<0.05$) (Table 1, Fig. 2). In addition, 5-

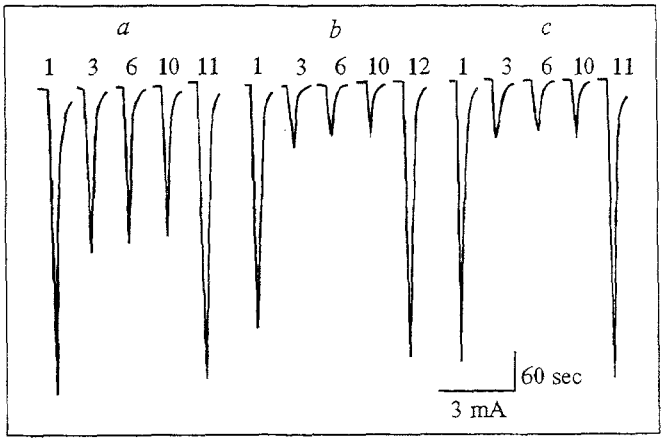

Fig. 3. Absence of HXA_3 effect on ACh-current extinction for LPa3 neuron. The following recordings are presented: input current (deviation downward) in response to the 1st, 3nd, 6th, and 10th repeated ionophoretic ACh-applications (782 nA, 1 sec) at 120 sec intervals and test applications of ACh from the same pipette 10 (*c*), 20 (*a*), and 30 (*b*) min after completion of rhythmic stimulation (11, 12, and 13 stimuli). *a*) before pharmacological influence; *b*) after extracellular HXA_3 application (80 μM, 30 min); *c*) after a 40 min washing of the preparation in Ringer solution. Other notation as for Fig. 2.

TABLE 2. Effect of 5-HETE and HXA_3 on ACh-Current Extinction ($M \pm m$)

Compound	Number of neurons investigated			
	total	weakening	deepening (enhancement)	no effect
5-HETE, (22.8-57.0 μM)	5	0	4* −17.12±1.1	1
HXA_3, (40-100 μM)	6	0	2	4

Note. Variations in ACh-current extinction (in %) after pharmacological influence are indicated in parentheses.

HETE deepened ACh-current extinction in 4 out of 5 cells ($p<0.05$) by 17.1±1.1% (Table 2; Fig. 1, *a* and Fig. 2, *b*). The effect of 5-HETE intensified with prolongation of exposure and was irreversible: washing free of 5-HETE did not restore the initial extinction curve (Fig. 1, *a*).

In the case of HXA_3, the compound (40-100/68.6±7.4/μM; 20-70/41.1±6.6/min) reduced the ACh-current amplitude in 6 out of 6 cells ($p<0.01$) by 49.2±6.5% (Table 1; Fig. 3). HXA_3 did not exert a coordinated effect on extinction of the ACh current (Table 2; Fig. 3, *b*), which correlated with a higher value of its effective dose in comparison with 5-HETE in the modulation of ACh-current amplitude.

The results obtained confirm the earlier assumption that eicosanoids from the 5-lipoxygenase pathway regulate cholinoreceptor plasticity of *Helix lucorum* neurons, while 12-lipoxygenase derivatives lack such a regulatory effect [2]. However, our results contradict those obtained on *Aplysia* neurons [12-14], where a higher efficiency of 12-lipoxygenase derivatives in comparison with 5-lipoxygenase derivatives was revealed. These differences may be explained by a probable regulatory specificity of 5- and 12-lipoxygenase derivatives in respect to functionally different membrane regions studied on *Helix* and *Aplysia* neurons.

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