induce vasodilation (NO and cGMP), while the boost of NO production during nitric hypoxia activates ET synthesis. Such give-and-take between the regulatory systems is evidently required by the organism to provide for effective regulation involving negative feedback. The latter is known to underlie the maintenance of homeostasis in the living organism and is, as a rule, realized on the basis of multiparametric regulation.

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Effects of Inhibition of Ca^{2+} Mobilization and Ca^{2+} Chemocontrolled Entry by 15-Hydroxyeicosatetraenoic Acid on the Modulation of Cholinoreceptor Plasticity in *Helix lucorum*

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Arachidonic acid and its noncyclic derivatives have long been recognized as secondary messengers [6,10]. Together with other secondary messengers, eicosanoids are involved in the intracellular regulation of the plasticity of neurons [8,9] and their cholinoreceptors (CR) [1-4]. It cannot be ruled out that endogenous 15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-HETE) participates in the intracellular regulation of CR plasticity, since previously we have shown that exogenous 15-HETE

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is involved in CR desensitization of the RPa3 and LPa3 neurons of *Helix lucorum*, as manifested in a reversible extinction of the acetylcholine-induced inward current (ACh current) for repeated applications of the transmitter on the soma [2]. Two contralateral modulatory effects of 15-HETE were found depending on the duration of exposure. The short-latency effect of 15-HETE (from 10 to 60-80 min), which manifests itself in a lower degree of extinction, was related to the well-known relapse mechanism, namely inhibition of 5- and 12-lipoxygenases by 15-HETE, while the long-latency effect was related to the action of 15-HETE via the direct regulatory pathway that controls CR plasticity.

It is known that the leukotrienes formed by the 5-lipoxygenase pathway increase the intracellular Ca^{2+} concentration [7,11,12]. Previously, we have shown that 5-lipoxygenase eicosanoids induce a greater degree of ACh-current extinction [3]. One of the components of the mechanism of leukotriene C_4 action is Ca^{2+} mobilization via the inositol-1,4,5-trisphosphate-dependent (IP_3-d) pathway, which stimulates CR plasticity [1]. It seems reasonable to assume that the mechanism underlying the modulation of CR by 15-HETE via a direct pathway in its second phase includes potentiation of the increase in the intracellular Ca^{2+} concentration by 5-lipoxygenase eicosanoids. The present study provides experimental evidence confirming this hypothesis.

MATERIALS AND METHODS

Experiments were performed on identified RPa3 and LPa3 neurons of the garden snail *Helix lucorum taurica Kryn* in a preparation of isolated ganglia. The bathing Ringer's solution contained (in mM): NaCl 100, KCl 4, $CaCl_2$ 10, $MgCl_2$ 4, and Tris-HCl 10 (pH 7.5). Transmembrane currents were recorded using the double-electrode voltage-clamp technique. The microelectrodes were of

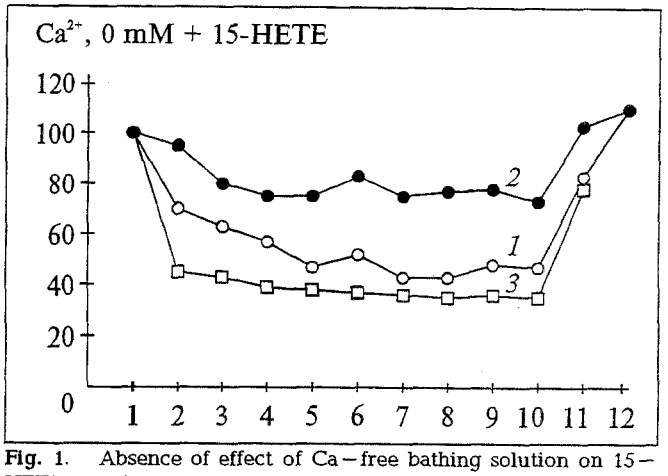

Fig. 1. Absence of effect of Ca-free bathing solution on 15-HETE modulation of CR of RPa3 neurons. *1*) frequency inhibition of ACh current in Ca-free solution; *2, 3*) after application of 15-HETE (7 μM) with a duration of exposure of 44 min (*2*) and 159 min (*3*). Abscissa: number of applications in the series; ordinate: maximum amplitude of ACh current in % of its response to the first stimulus in the series.

Pyrex and filled with 2.5 M KCl or 1 M potassium acetate; the electrode resistance was 9-50 (26.5±2.9; *M±m*) or 48-200 (114.3±13.7) MOhm, respectively. Acetylcholine was ionophoretically applied to the soma in the current amplitude range of 686-1042 nA, 1-6 sec (3.6±0.2 sec).

The series included 11-13 consecutive ionophoretic ACh applications using a current of constant direction, amplitude, and duration. To inhibit the ACh current, the first 10 stimuli were applied at 60-240-sec intervals (117.0±8.0 sec). For evaluation of the degree and rate of restoration of the inhibited reaction, subsequent stimuli were applied at 10-min intervals. The modulatory effects of 15-HETE (synthesized at the Moscow Institute of Fine Chemicals Technology) were assessed after altering the Ca^{2+} concentration in neurons with the use of: 1) modified Ca-free solution (the divalent ion concentration was compensated with Mg^{2+}); 2) tetracaine, an inhibitor of Ca^{2+}-dependent Ca^{2+} mobilization (Sigma, USA); 3) 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester (TMB-8, Sigma,

TABLE 1. Effect of Ca-Free Ringer's Solution, Tetracaine, and TMB-8 on Amplitude of ACh Current in Neurons

Experimental conditions	Number of Neurons			
	total	increase	decrease	no effect
Ca^{2+}, (0 mM) +15-HETE	9[1]/9[2]	2/5	6/3 (−24.1±4.5)[3]	1/1
Tetracaine +15-HETE	14/12	2/1	12*/10* (−16.4±10.4)/(−37.5±7.3)	0/1
TMB-8 +15-HETE	16/16	0/0	16**/15** (−32.8±3.8)/(−32.9±4.7)	0/1

Note. Here and in Table 2: [1] – 15-HETE exposure<70 min, [2] – >70 min, [3] – degree of inhibition of ACh current (in %) after pharmacological treatment. *: significance of differences $p<0.01$; **: $p<0.001$.

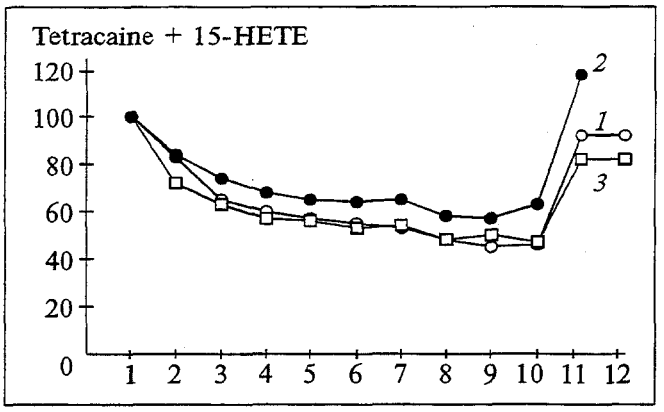

Fig. 2. Tetracaine–induced alterations in long–latency modulatory effect of 15–HETE on CR plasticity of LPa3 neurons. *1*) frequency inhibition of ACh current caused by tetracaine (50 μM); *2*, *3*) after extracellular application of 15–HETE (6 μM) with a duration of exposure of 28 min (*2*) and 87 min (*3*).

USA), an inhibitor of IP_3-dependent Ca^{2+} mobilization. Tetracaine and TMB-8 were dissolved in water, 15-HETE was dissolved in 10% ethanol.

The results were statistically analyzed with the nonparametric Wilcoxon and sign tests using DIASTA and STADIA software packages. Forty neurons (23 RPa3 and 17 LPa3) from 40 ganglia preparations were examined. The effect of 15-HETE was tested in Ca-free solution on 10 cells, after tetracaine, on 14 cells, and after TMB-8, on 16 cells. The membrane potential ranged from -37 to -75 mV (-55.7±1.3 mV).

RESULTS

Removal of Ca^{2+} from the extracellular solution weakened the effect of 15-HETE in the first phase of its action (exposure<70 min) and abolished it in the second phase (exposure>70 min) (Table 1). Tetracaine (50-90 μM, 57.9±3.0) and TMB-8 (10-60 μM) had no effect on the ability of 15-HETE (3-10 μM, 5.9±0.2) to inhibit the ACh current in either phase of action (Table 1). Presumably, 15-HETE-induced inhibition of the ACh current in the neurons of *Helix lucorum* is associated with the receptor-operated Ca^{2+} entry. Since the effect is more prominent in the second phase, it is likely to be an indirect one.

The absence of Ca^{2+} in the extracellular solution did not change the degree or direction of the modulatory effects of 15-HETE on CR plasticity for short- and long-latency effects of the eicosanoid (Table 2; Fig. 1.). Tetracaine and TMB-8 affected the long-latent modulatory effect of 15-HETE but did not change the short-latent effect (Table 2; Figs. 2 and 3). These results demonstrate that inhibition of intracellular Ca^{2+} mobilization via Ca^{2+}- and IP_3-dependent pathways affects the modulatory effect of 15-HETE action on CR plasticity in the second phase of 15-HETE action and dose not affect the first phase of modulation. The intracellular Ca^{2+} concentration may increase at the expense of extracellular Ca^{2+} entering the cytoplasm through ion channels which may be operated by CR [1,5]. 15-HETE inhibits the CR-operated inward Ca^{2+} current, however this pathway for altering the free Ca^{2+} concentration in the cytoplasm is not employed by eicosanoids for modulation of CR plasticity. It can therefore be concluded that one of the mechanisms of enhanced plasticity of

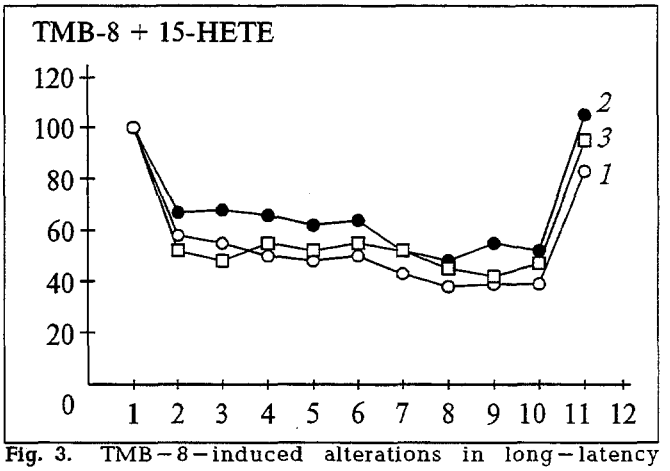

Fig. 3. TMB–8–induced alterations in long–latency modulatory effect of 15–HETE on CR plasticity of LPa3 neurons. *1*) frequency inhibition of ACh current caused by TMB–8 (5 μM) with a duration of exposure of 67 min (*2*) and 115 min (*3*).

TABLE 2. Effect of Ca–Free Ringer's Solution, Tetracaine, and TMB–8 on the Degree of Inhibition of ACh Current in Neurons

Experimental conditions	Number of Neurons			
	total	increase	decrease	no effect
$[Ca^{2+}]_o$, (0 mM) +15–HETE	9[1]/9[2]	4*/1	0/5* (33.2±5.8)[3]	5/3
Tetracaine +15–HETE	14/12	10*/5	3/3 (9.9±2.4)	1/4
TMB–8 +15–HETE	16/16	9*/5	2/4 (9.6±1.8)	5/7

Note. *: $p<0.05$.

CR in the second phase of 15-HETE action involves potentiation of Ca^{2+} mobilization from the intracellular pools by 5-lipoxygenase eicosanoids via Ca^{2+}-and IP_3-dependent pathways.

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PHARMACOLOGY

Comparison of the Effects of Piracetam and N-Acetylaspartic Acid on Memory and on the Content of Transmitter Amino Acids in the Rat Brain during Simulation of a Neurotic State

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Recently, glutamic and aspartic acids as excitation transmitters have attracted the attention not only of neurobiologists but also of clinicians specializing in neuropsychiatry. These amino acids and their receptors have been found to be involved in the pathogenesis of a broad spectrum of diseases of the central nervous system (CNS), primarily of neurodegenerative states [2,4,6,7,10].

Studies of the properties of excitatory amino acid (EAA) receptors have shown that blocking of the latter may produce a therapeutic effect during ischemia, hypoxia, hypoglycemia of the brain, sei-

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