PHYSIOLOGY

SHORT- AND LONG-LATENCY EFFECTS OF 15-HYDROXYEICOSATETRAENOIC ACID ON EXTINCTION OF THE ACETYLCHOLINE-INDUCED INWARD CURRENT IN *Helix lucorum* NEURONS

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Enzymic oxidation of arachidonic acid by lipoxygenases gives a large of products (eicosanoids), including polyunsaturated hydroperoxy- and hydroxy (mono- and di-) fatty acids, leukotrienes, and other compounds [4]. Lipoxygenase oxidation can take several different courses depending on the type and positional specificity of the enzymes [4]. Oxidation of arachidonic acid by the action of 15-lipoxygenase (15-LO) leads to the formation of 15(S)-hydroperoxy-5Z, 8Z, 11Z, 13E-eicosatetraenoic acid, which is further reduced to 15(S)-hydroxy-5Z, 8Z, 11Z, 13E-eicosatetraenoic acid (15-HETE). Just as for other eicosanoids, it probably has a signal function in the cell. Considering that activation of muscarinic acetylcholine receptors induces oxidation of arachidonic acid [3], involvement of 15-HETE in the regulation of acetylcholine receptors and their plasticity cannot be ruled out.

The aim of this investigation was to study the role of 15-HETE in short-term plasticity of acetylcholine receptors of *Helix lucorum* neurons.

EXPERIMENTAL METHOD

Experiments were carried out on identified neurons RPa3 and LPa3 of *Helix lucorum taurica* Kryn in a preparation of isolated ganglia at room temperature. The circumesophageal nerve ring was fixed in a continuous flow chamber with a volume of 1 ml. After treatment of the preparation with 2% collagenase solution (type IA, from "Sigma," USA) in Ringer's solution for 30 min at room temperature, the connective-tissue membranes covering the ganglia were removed. Ringer's solution of the following composition (in mM) flowed through the chamber containing the preparation: NaCl 100, KCl 4, $CaCl_2$ 10, $MgCl_2$ 4, Tris-HCl 10, pH 7.5. Transmembrane currents were recorded, using a method of voltage clamping on the membrane by two electrodes. The intracellular microelectrodes were drawn from "Pyrex" glass and filled with KCl (2.5 M), the resistance of the microelectrodes being 10-38 MΩ (21.4 ± 2.5 MΩ, M ± m). A double-barreled micropipet was applied to the outer surface of the soma. The iontophoretic channel was filled with acetylcholine (ACh) chloride ("Serva," West Germany) in distilled water (4 M, pH 7.4), the balancing channel with 2 M NaCl. Cationic currents (580-740 nA, 3-8 sec, 3.5 ± 0.4°C) were passed through the iontophoretic channel. The resistance of the pipets was 20-30 MΩ. The series included 11-13 successive iontophoretic applications of ACh by a current of constant direction, strength, and duration. The first 10 stimuli were applied at intervals of 60-90 sec (67.5 ± 3.1) to extinguish the ACh-current. Subsequent stimuli were applied at intervals of 10 min to assess the degree and rate of recovery of the extinguished reaction. The experiment consisted of several series of extinction — control (before the action of drugs), experimental, and recovery. During the series

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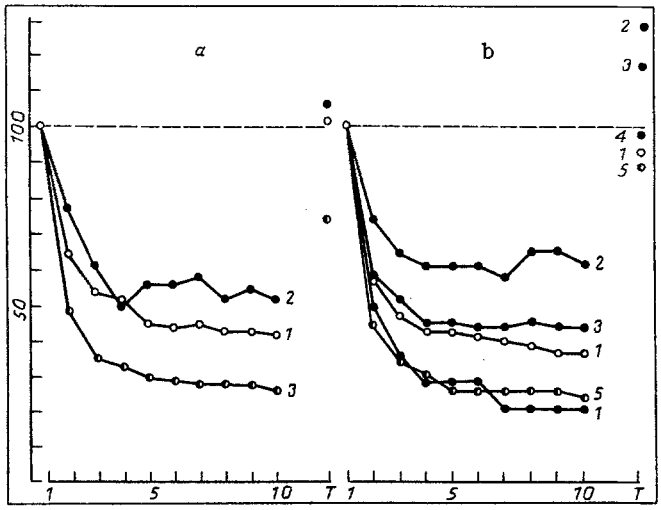

Fig. 1. Effect of 15-HETE on extinction curves of ACh-current of identified neurons. a, b) Extinction curves of ACh-current of LPa3 neurons from different preparations. a: 1) Extinction of ACh-current before pharmacological action; 2) against background of action of 15-HETE (4 μM, 40 min); 3) after rinsing out compound with Ringer's solution. b: 1) Extinction of ACh-current before action of 15-HETE; 2-4) against background of action of 15-HETE (8 μM) with exposure of 25 min (2), 65 min (3), 95 min (4); 5) after rinsing out compound. Abscissa, serial number of ACh application in series, T) test of spontaneous recovery of extinguished response (application of ACh to soma from the same iontophoretic pipet 10 min after end of series of repetitive stimulations); ordinate, maximal amplitude of ACh current (in % relative to its value in response to first stimulus in series).

the flow of Ringer's solution was stopped. The experimental series were applied 10-120 min after injection of 15-HETE (synthesized in the M. V. Lomonosov Moscow Institute of Fine Chemical Technology). The necessary final 15-HETE concentrations were created by adding aliquots of its 0.8 mM aqueous-ethanol solution (ethanol content 10%) from a microsyringe into the continuous-flow chamber, maintaining the calculated concentration of the test substance (0.8-16 μM) and the homogeneity of the solution. The maximal concentration of ethanol in the continuous-flow chamber after addition of the 15-HETE solution was 0.2%. The significance of the effect of the compound on extinction was estimated by a nonparametric test, namely Wilcoxon's paired T test (on single neurons) and by the signs test (on the whole population of cells investigated), and its effect on the magnitude of the ACh-response was determined by the Wilcoxon—Mann-Whitney U test. Results were obtained on 12 neurons (five RPa3, seven LPa3). The cell membrane potential was between —55 and —80 mV (—64.1 ± 2.5 mV).

EXPERIMENTAL RESULTS

15-HETE in a concentration of 4-16 μM reduced the amplitude of the ACh-current in 11 of the 12 cells ($p < 0.001$) on average by 48.5 ± 6.2%, but did not change it in one neuron. Exposure to 15-HETE for between 10 and 60-80 min reduced the depth of extinction in seven cells ($p < 0.05$) on average by 12.0 ± 0.7% (Figs. 1 and 2), in one cell extinction was strengthened, and in four it was unchanged. Rinsing out the 15-HETE led, not to recovery of the extinction curve, but to deepening of extinction compared with the control in seven of 10 neurons ($p < 0.05$) (Fig. 1a) on average by 13.1 ± 0.6%, in two neurons recovery of extinction was complete, and in one it was partial. This change in extinction after rinsing out the compound can be explained on assuming that 15-HETE has two opposite effects: short- and long-latency. Additional experiments confirmed the validity of this hypothesis. Several additional series of extinction were carried out on three neurons using a constant concentration of 15-HETE, but with increasing exposure. The same effect was observed on

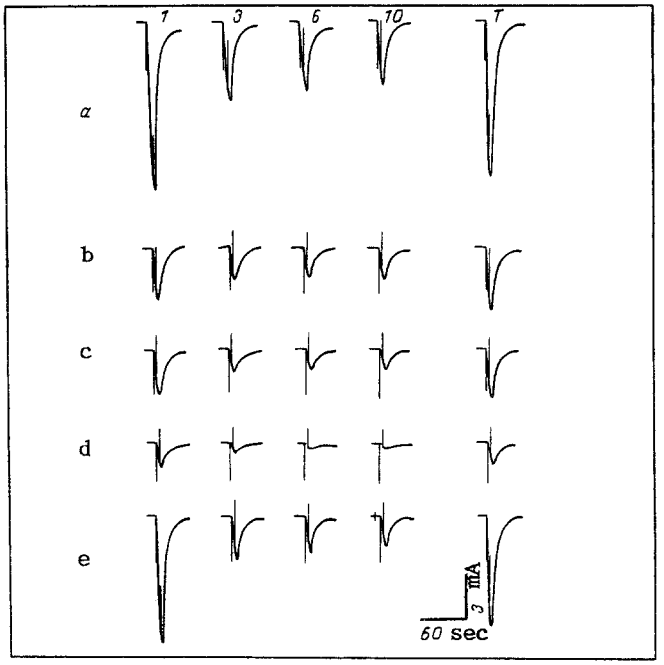

Fig. 2. Opposite short- and long-latency effects of 15-HETE on extinction of ACh-current of LPa3 neuron. Traces of inward current (downward deflection) in response to first, third, sixth, and 10th repeated iontophoretic applications of ACh (738 nA, 3 sec) with an interval of 60 sec, and also testing applications of ACh from the same iontophoretic pipet 10 min after end of repetitive stimulation. a) In Ringer's solution of normal composition; b-d) after injection of 15-HETE (8 μM) into chamber with preparation after 25 min (b), 65 min (c) and 95 min (d); e) after rinsing out compound with Ringer's solution for 25 min. Holding potential 75 mV. Calibration: current 3 nA, time 60 sec.

all neurons (Fig. 1b); the curves 2 illustrate the effects of 15-HETE with increasing exposure. 15-HETE (8 μM) weakened extinction of neuron LPa3 after 25 min by 21.3 ± 1.6% ($p < 0.01$), after 65 min weakening of extinction was less marked at 5.8 ± 0.9 ($p < 0.01$), and after 95 min extinction was strengthened by 13.6 ± 1.2% ($p < 0.01$). after rinsing for 30 min to remove the eicosanoid the extinction curve remained at the same level as in the previous experiment ($p > 0.05$), demonstrating strengthening of extinction by 12.0 ± 0.8% compared with the control ($p < 0.01$).

The results indicate that 15-HETE gives rise to two opposite effects on extinction of the ACh-current of the test neurons, depending on the length of exposure to the compound. The short-latency action (from 10 to 60-80 min) consists of weakening of extinction, the long-latency action (after 60-80 min) is manifested as strengthening of extinction of the ACh current. The action of 15-HETE is irreversible. 15-HETE probably is involved in the regulation of plasticity of acetylcholine receptors of the RPa3 and LPa3 neurons of *Helix lucorum*. The short-latency effect of 15-HETE may be connected with its known inhibition by 5-LO [2, 5] and 12-LO [6], since inhibitors of lipoxygenases weaken extinction of the ACh-current [1]. The long-latency effect of 15-HETE may be connected with its direct action. Most probably the two opposite actions of 15-HETE are triggered simultaneously, but the effect due to blocking of lipoxygenases is broader, i.e., it inhibits the action of a whole range of eicosanoids, formed during oxidation of arachidonic acid by 5-LO and 12-LO, and it is therefore stronger than the direct effect of 15-HETE. If under these circumstances the indirect action of 15-HETE is shorter than its direct effect, the direct action is manifested after the indirect. Consequently, the possibility cannot be ruled out that the direct and indirect actions of 15-HETE arise simultaneously, but because of the greater strength and shorter duration of the effect mediated through blocking of lipoxygenases, the direct action of 15-HETE is masked in the initial stage by the oppositely directed indirect effect, and comes to light when that effect ends.

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DETECTION OF THE SEROTONIN-MODULATED PROTEIN FRACTION AND ITS ROLE IN ORGANIZATION OF PASSIVE AVOIDANCE BEHAVIOR IN RATS

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Mediators are known to initiate many intracellular metabolic events in nerve cells, including regulation of the genetic apparatus [2, 9, 14]. For some mediators and, in particular, for serotonin, functional activity has been shown to depend on their control over protein synthesis [11-13]. Most investigations, however, have dealt with the study of the role of mediators in the regulation of total protein synthesis, although there are grounds for considering that they may have a selective action on protein metabolism [5].

The aim of this investigation was to study the effect of serotonin and noradrenalin on metabolism of individual water-soluble protein fractions in the cerebral cortex and to investigate the role of these fractions in passive avoidance behavior in rats.

EXPERIMENTAL METHOD

The effect of mediators on protein metabolism was studied in the following way. Experiments were carried out on male rats weighing 200-280 g. Under pentobarbital anesthesia an area of the skull with the dura mater was removed from the experimental animals above the occipital cortex of the brain bilaterally, and for 40 min a 10^{-3} M solution of serotonin in physiological saline (n = 12), and in another series 10^{-3} M noradrenalin solution (n = 10) was applied for 40 min. Physiological saline was applied to the control animals. After decapitation of the rats, water-soluble proteins were extracted from the occipital regions of the cerebral cortex in 0.01 M phosphate buffer solution (pH 7.2) containing 0.2 M NaCl, and these were fractionated by disk-electrophoresis under nondenaturing conditions in polyacrylamide gel as described by Davis

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