# Effects of Histochrom and Emoxypin on Biophysical Properties of Electroexitable Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 2, pp. 155-159, February, 2009
Original article submitted July 24, 2008

Comparative investigation of effects of antioxidant agents histochrom and emoxypin on biophysical characteristics of isolated neurons from shell *Lymnaea stagnalis* under normal conditions and during oxidative stress was performed. Differently directed effects of these compounds on resting potential and transmembrane ion currents of neural cells under normal conditions were detected. Histochrom provides hyperpolarizing and emoxypin — depolarizing effect on neuronal membrane potential. Under conditions of oxidative stress both products possess antioxidant action. Obtained data allows coming closer to understanding of cellular-molecular mechanisms of protective action of compounds.

**Key words**: *histochrom, emoxypin, membrane potential, ion currents, isolated neurons from shell Lymnaea stagnalis*

The antioxidant agent histochrom, medicinal formulation of natural echinochrom A (2,3,5,6,8-pentahydroxy-7-ethyl-1,4-naphthoquinone), is used in ophthalmology for treatment of intraocular hemorrhages, diabetic retinopathy, dystrophy, thrombosis of central vein of retina, posttraumatic hemorrhages and for treatment of ischemic heart diseases [6]. Histochrom antioxidant action was found to appear by stopping of $Fe^{2+}$-ascorbate-induced oxidation of liposomes from egg phosphatidylcholine. In this model, histochrom ability to bound potent initiators of lipid peroxidation, $Fe^{2+}$-ions, into inactive complexes plays a crucial role in inhibition of radical reactions. Antioxidant activity was demonstrated to be provided by b-hydroxyl substituting groups in 2, 3 and 7 position of histochrom molecule [5]. However, administration of this compound had no result in complete understanding of cellular-molecular mechanisms of its action. In comparison with emoxypin (artificial 2-ethyl-6-methyl-3-hydroxypiridine), compound identical in medical indications, the number of histochrom advantages were revealed, and they can not be explained only with antioxidant properties [1,4]. The subject of this study is a comparison of effects of these medications on simple biological subjects — isolated cells.

## MATERIALS AND METHODS

Experiments were carried out on isolated neurons from shell *Lymnaea stagnalis* (age 0.5-1.5 years). Neurons were isolated with mechanical defragmentation following the enzyme treatment of pharyngeal ganglia. Before electrophysiological investigation, isolated neurons were cultivated on glass supporting plate for 18-20 hours at 6-10°C. Employed physiological solution (PS) had following content (mM): NaCl — 55, KCl — 1.6, $CaCl_2$ — 4, $MgCl_2$ — 1.5, Tris-HCl — 8 pH 7.6-7.8 without aminoacids. The sodium-free solution, containing

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0007-4888/09/1472-0196 

sucrose 120 mM was used in experiments, requiring suppression of sodium ionic conduction.

Electrophysiological investigations were carried out at 20-24ºC. Electric activity was registered by microelectrodes using firmware. Neuron reactions to compounds were represented as a diagram of membrane potential (MP) changes depending on time of incubation with investigational compound. Diagrams display mean values and standard error of mean deviation. For registration of integral ion current the micro lead approach, one of patch-clamping modifications, was employed [7].

The following medications were used: Histochrom (solution for injections 0.02%; Pacific Institute of Organic Biochemistry Far Eastern Division of the Russian Academy of Sciences) and Emoxypin-AKOS (eye drops 1% solution; OAO Sintez). Oxidative stress was induced by $Fe^{2+}$-ascorbate: ascorbic acid 100 μM (Fluka), $FeSO_4 \times 7H_2O$ 1 μM (Scharlau). Relevant aliquots of compounds were added to saline in working chamber. Differences between groups were assessed using Mann—Whitney *U* test.

## RESULTS

Firstly, was performed the determination of the dose with minimal effect on RP with no relations with their antioxidant activity (with no oxidative stress, provided by $Fe^{2+}$-ascorbate). Histrochrom provided increase in neuron RP at concentration of 3-7.5 μM. No differences were noted in cell reactions within this dose range. Not every cell possessed a reaction at histochrom concentration of 1.5 μM. According to this data, histochrom was used in concentration of 3 ìM. Emoxypin effects on neuron RP appeared to be dose dependent. Emoxypin action was investigated at concentrations of 15, 150, and 750 μM.

Histochrom affects RP at concentrations 100 fold less than emoxypin and other antioxidants do [2]. It suggests than histochrom beside antioxidant [4] possesses another protective mechanism.

Incubation of neurons in PS with histochrom led to depolarizing shift of RP, value of which in first 5-7 min reached 5-9 mV, and further the potential remained at this level for whole observation period (Fig.1, 2). For revealing the membrane mechanisms of such RP shifts the dynamics of integrated transmembrane ion currents in neuron under histochrom action was investigated. Registration of voltage-dependent ion currents was started 0.5-1.0 min after onset of cell incubation. Voltage value, generating inward current with maximal amplitude, was determined. Within 25-30 min of incubation with histochrom the significant increase in inward

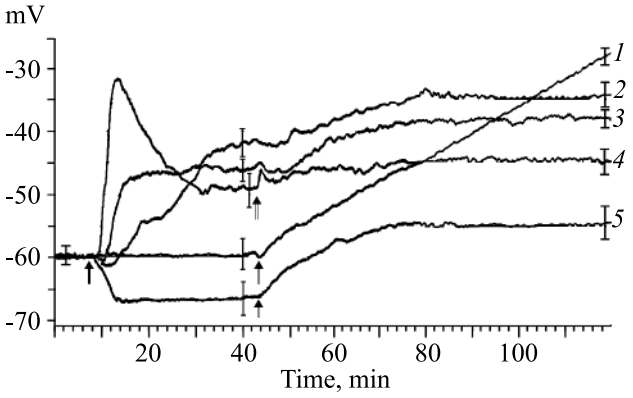


**Fig. 1**. Effects of histochrom, emoxypin and $Fe^{2+}$-ascorbate on MP of isolated neurons from *L. stagnalis* in PS. *1* — iron ascorbate (*n*=10); *2* — emoxypin, 15 ìM (*n*=9); *3* — emoxypin, 750 ìM (*n*=12), *4* — emoxypin, 150 ìM (*n*=12); *5* — histochrom (*n*=15). Single arrow: addition of emoxypin or histochrom; double arrow: addition of iron ascorbate (for all the curves).

currents was observed. Time to reach the peak value of inward currents was shortened. Thereafter, beginning with 30 min, the stabilization of inward and outward currents was observed ($p<0.001$; Fig. 3, *a*).

Inward voltage-dependent currents of neuron somatic membrane in shells are known to be multition and consist of two components — fast sodium and slow calcium currents [3]. Incubation of neurons in the solution, where sodium chloride was replaced by sucrose, is extensively used as experimental method to eliminate fast sodium component of inward currents. Histochrom was found to produce on neurons in sodium-free saline solution not hyperpolarizing, like in conventional saline solution, but depolarizing effect (Fig. 2). Histochrom increased amplitude of inward and outward currents in sodium-free solution. Increase in inward currents indicated entry of calcium ions into cell from environment, what can be responsible for depolarization shift of neuron RP ($p<0.001$; Fig. 3, *b*).

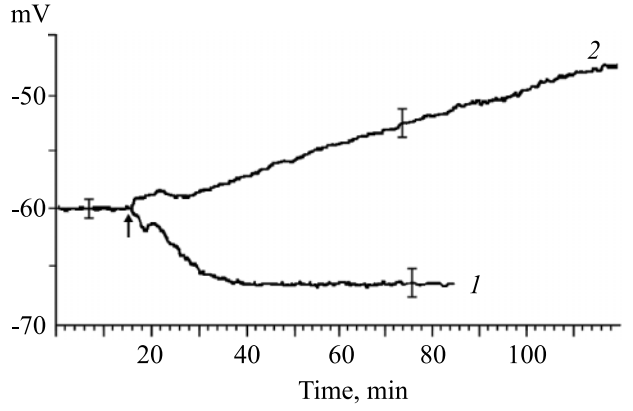


**Fig. 2**. Histochrom effects, on RP of isolated neurons from *L. stagnalis*. *1* — in PS (*n*=15); *2* — in sodium-free solution (*n*=9).

Investigation of emoxypin effects on RP and ion currents was performed in the same manner. Despite of histochrom, exposure to emoxypin in investigated concentrations was associated with short-term (2-5 min) depolarization, followed with depolarization shift of resting potential (RP) of various rate in range from 10 to 29 mV (Fig. 1). Addition of emoxypin into working chamber in concentration 15 μM was associated with slightly increase of RP (3-5 mV) within 2-5 min. Thereafter the hyperpolarization terminated and depolarization shift developed until -40-45 mV, and RP remained at with level through whole time of observation. The compound at concentration 150 μM produced high-amplitude depolarizing shift up to -30-35 mV within 2-5 min after addition, what was followed by stabilization of RP at the level of -48-50 mV. Higher emoxypin concentration (750 μM) within first 2-5 min produced increase in RP, then hyperpolarization terminated and in time interval of 5-10 min the depolarization developed up to -45-48 mV; this value was maintained throughout observational period.

Emoxypin effects on neuron transmembrane ion currents in PS were investigated at concentration 750 μM. At first 10 min emoxypin produced increase in increase and shortening of development outward current components. Thereafter (10-25 min) amplitudes of inward and outward currents declined, and 35-40 min after they are stabilized ($p<0.001$; Fig. 3, *c*). such ion current dynamics corresponds to short-term hyperpolarization changes, produced by emoxypin in concentration of 750 μM, and to long-term depolarization shift.

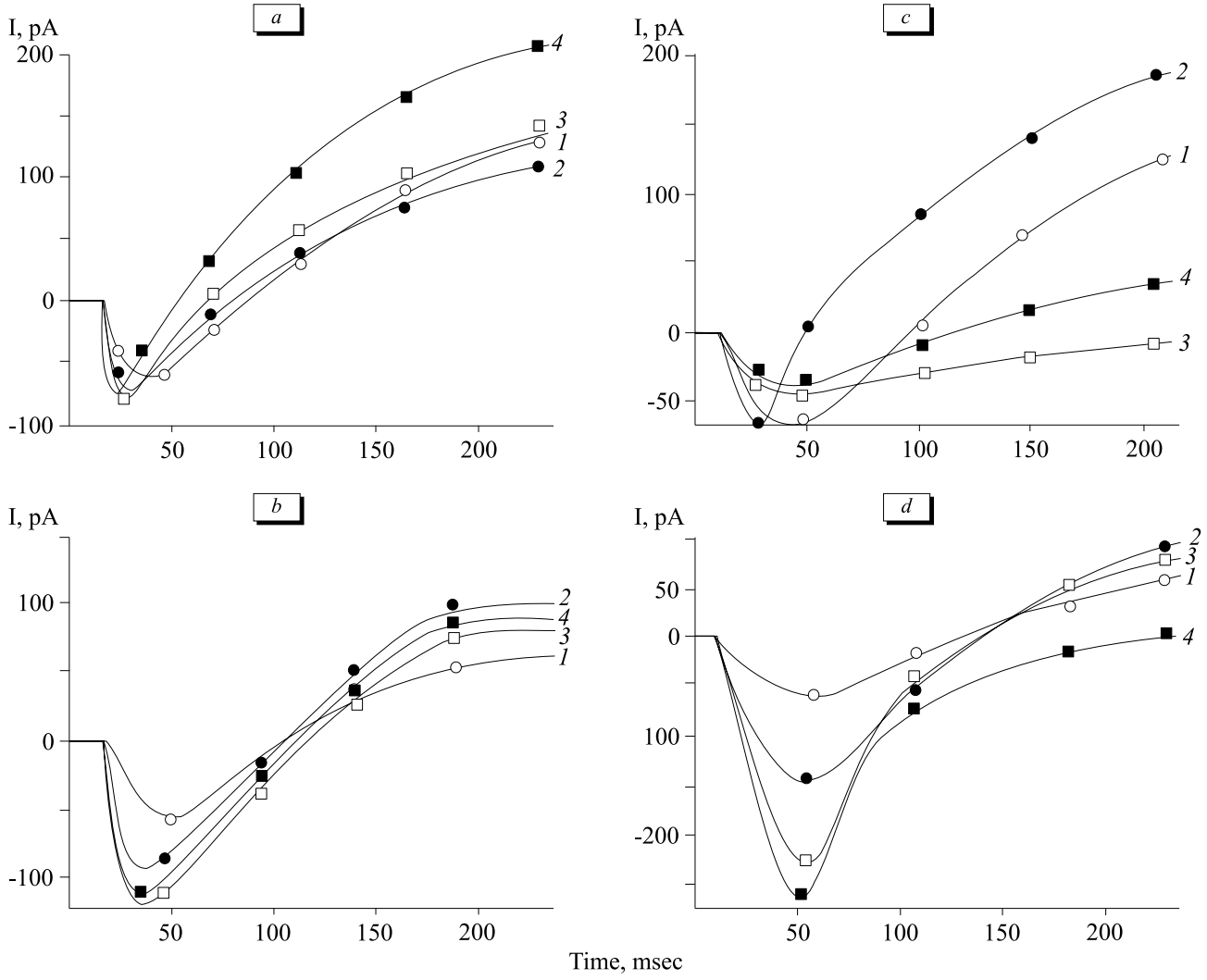


**Fig.3**. Transmembrane ion current dynamics in neurons of *L. stagnalis* in the presence of histochrom in PS (*a*; *n*=17), after addition of histochrom into sodium-free solution (*b*; *n*=16), after addition of emoxypin into PS (*c*; *n*=15), in the presence of emoxypin in sodium-free solution (*d*; *n*=12). *1* — control; *2* — 10 min after, *3* — 20 min after, *4* — 30 min after.

Emoxypin produced depolarizing shift of neuron MP both at the presence of sodium in the solution, and at the lack. In sodium-free solution during 10-20 min emoxypin-produced current changes were characterized by significant increase in inward calcium current and non significant increase in outward current. 30 min after, the amplitude of outward current stabilized at the level higher than at the baseline. Amplitude of outward currents remained to be lower than at the baseline ($p<0.001$; Fig. 3, *d*). Such ion current dynamics indicates that emoxypin produces increase in calcium entry into the cell from the environment through the voltage-gated calcium channels.

Model of $Fe^{2+}$-ascorbate-unduced lipid oxidation was employed to compare antioxidant properties of histochrom and emoxypin. Addition of $Fe^{2+}$-ascorbate into PS was associated with steadily increase in depolarizing RP shift, and 2-2.5 h later cells died (Fig. 1).

Preincubation of cells in histochrom or emoxypin solution for 30-40 min before addition of $Fe^{2+}$-ascorbate into working solution produced protective action, diminishing in different extent the $Fe^{2+}$-ascorbate-induced neuron depolarization. Preincubation of neurons in histochrom solution provided hyperpolarization in average of 5 mV. In neurons previously treated with histochrom, $Fe^{2+}$-ascorbate produced depolarizing MP shift in average of 10 mV, and RP of these cells differed from RP of intact cells in average of 5 mV (Fig. 1).

Preincubation of neurons with emoxypin in concentration 15 μM was associated with significant (in average of 15-20 mV) depolarizing RP shift in comparison with intact cells. In these cells, $Fe^{2+}$-ascorbate produced decrease in MP in average of 5 mV. Neuronal RP sustained at the level of -35 mV during time of observation (Fig. 1).

Neurons, preincubated with emoxypin (150 μM), possessed RP, shifted in average of 10-12 mV in comparison with RP of intact cells. $Fe^{2+}$-ascorbate produced nonsignificant (2-3 mV) RP sift; RP in this group of neurons stabilized at the level of -45 mV (Fig. 1).

Neurons, pretreated with emoxypin in concentration of 750 μM, possessed RP shifted in average of 12-15 mV before exposure to $Fe^{2+}$-ascorbate in comparison with RP of intact cells (Fig. 1). $Fe^{2+}$-ascorbate produced shift of MP in average of 5-7 mV; in this group of neurons, RP stabilized at the level of -38 mV.

The experiments performed revealed selective action on neuron RP of emoxypin (depolarizing) and histochrom (hyperpolarizing). Histochrom hyperpolarizing effect was found to depend on sodium and chloride ion content in the environment. In case of lack of these ions histochrom produced neuronal depolarization, and depolarizing emoxypin effect developed regardless of these two ions content in the environment. It suggests that compounds, possessing antioxidant activity, had different membrane and intracellular targets. That circumstances probably may explain the results concerning the fact that histochrom possesses protective action on intact cells dependently on ion content in the environment. Emoxypin provided significant depolarizing effect on intact cells and its protective action appeared only under conditions of oxidative stress.

On all occasions protective action of compound was characterized by non-dramatically neuron depolarization under the circumstances of oxidative stress – RP of the cells stabilized on the new level. Histochrom possessed the highest protective effect when was employed preliminary: under the circumstances of oxidative stress the non-significant depolarizing MP shift in average of 5 mV was noted.

A number of pathological states are associated with disturbances in excitability, ion homeostasis and lipid content. Ion channels of plasmolemma play a key role in regulation of these interconnected dynamic processes. Blockage of inward currents and activation of outward potassium current may produce a protective action. In some cases these cell structures appear to be the targets for compounds possessing protective properties.

The analysis of histochrom effects on ion currents and RP revealed that in normal saline environment it produces neuronal hyperpolarization. Histochrom ability to increase RP possibly causes reservation of higher ATP level in cardiomyocites under the circumstances of acute ischemia, decreases defibrillation threshold and reclaims productive heart activity during coronary artery bypass graft surgery [4,5].

This work was supported by program of Russian Academy of Sciences Presidium “Fundamental Science to Medicine” (No. 12.8) and Cross-disciplinary integration project of Siberian Division of the Russian Academy of Sciences “Scientific Basis of New Medical Preparation Development. Prospects of using of Renewable Feedstocks” (No. 54).

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