Effects of Preliminary Administration of Haloperidol in Low Doses on the Effects of Haloperidol on Behavioral Reactions and Command Neuron Membrane Potential in Edible Snail

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We studied the effects of preliminary administration of haloperidol in low doses on changes in motor activity of edible snail and in electrical properties of defensive behavior command neurons induced by chronic administration of haloperidol. The rate of locomotion decreased after injections of haloperidol preparations (C6, C12, C30, C200 and a mixture C12+C30+C200) for 3 days. Similar changes were observed after 3 days of haloperidol administration. Haloperidol preparations in low doses produced a modulating effect on the decrease in locomotion rate and hyperpolarization of command neurons in edible snails caused by chronic exposure to haloperidol: the decrease in locomotion rate caused by chronic haloperidol treatment was prevented by preliminary injection of haloperidol in low doses C6, C12 and C30; the depolarizing shift of command neuron membrane potential was also abolished after consecutive injection of the same haloperidol preparations C6, C12 and C30.

Key Words: *haloperidol; locomotion; defensive reactions; neuron; preconditioning*

Investigation of mechanisms of action of pharmacological compound is of special interest, since they determine the field of application, indications, and contraindications [5]. A great variety of mechanisms can modulate physiological response. They include, transmitter co-modulation within one synapse and influences providing preliminary depolarization and increase in calcium conductance within the presynaptic terminal. Such modification occurs during preconditioning, *i.e.* exposure to mild hypoxia before exposure to severe hypoxia. A neuroprotective effect of low glutamate doses was demonstrated during preconditioning for toxic effects of high doses of glutamate on spinal, cortical and cerebellar neurons [9,12]; low doses of cycloheximide also affected neuron survival in glutamate solution [10].

Neuroleptics, *e.g.* haloperidol (Hal), form an extensive group of so-called psychotropic compounds acting predominantly on psychic functions and emotional state. They eliminate fear, anxiety, and emotional strain and are used for the treatment of neurotic disorders, predominantly in severe dysfunctions of the nervous system [5]. The nature of the therapeutic action of Hal is still the subject of active research [6,11].

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The effects of low doses of bioactive substances attract much recent attention [2,4,7]. Interesting results were obtained when basic substance was administered in combination with diluted form (low doses) [8]. Recent experiments on isolated preparation of *Helix lucorum* nervous system revealed two types of neurons responding by different ways to application of antibodies to Ca-binding protein S100 (AB-S100): the frequency of action potentials decreased in B1 and B17 neurons and increased in neurons B4 and B6 [1,8]. It was found that these changes in the main properties of the neuronal membrane induced by AB-S100 in physiological doses can be prevented by preincubation (preconditioning) of these "simple systems" with the same antibodies in low doses (dilution 10^{-12}). The mechanism of this effect is not completely clear and requires further investigations at all levels of organization of the neuronal system.

Here we studied possible action of Hal in low doses on the effects of chronic Hal injections on the behavior of edible snail *Helix lucorum* and on electric parameters of identified neurons.

MATERIALS AND METHODS

Experiments were carried out on terrestrial pulmonate gastropod *Helix lucorum (Gastropoda, Pulmonata)* from the Crimean population. Ten groups were formed: 8 experimental groups (6 groups received injections of Hal in low doses for 3 days, 1 group received physiological saline (PS) and 1 group received distilled water, and then Hal were administered to all groups for 7 days) and 2 control groups (PS and Hal injection for 7 days). Each group consisted of 15 animals and more. PS for edible snail had the following content: 80 mM NaCl, 4 mM KCl, 10 mM $CaCl_2$, 5 mM $MgCl_2$, and 5 mM $NaHCO_3$.

Experiments were performed using Hal (Sigma) in a dose of 1 mg/kg snail weight diluted in 0.1 ml PS. The test preparations were low doses of Hal in different dilutions: C3, C6, C12, C30, C200 and a mixture C12+C30+C200 dissolved in 0.1 ml distilled water. All experimental series were based on chronic Hal administration during 7 days preceded by preliminary injections of Hal in low doses for 3 days. All injections were performed into the sinus node area using disposable syringes.

Snail locomotion was evaluated by the distance passed by the snail over one minute on vertical glass wall of the terrarium [6]. On the next day after the last injection, membrane potentials of defensive behavior command neurons LPa3, RPa3, LPa2, and RPa2 [3] were recorded on a computer via an analog-to-digital converter. Since it was impossible to make adjustment for the effect of preparation administration for 3 days

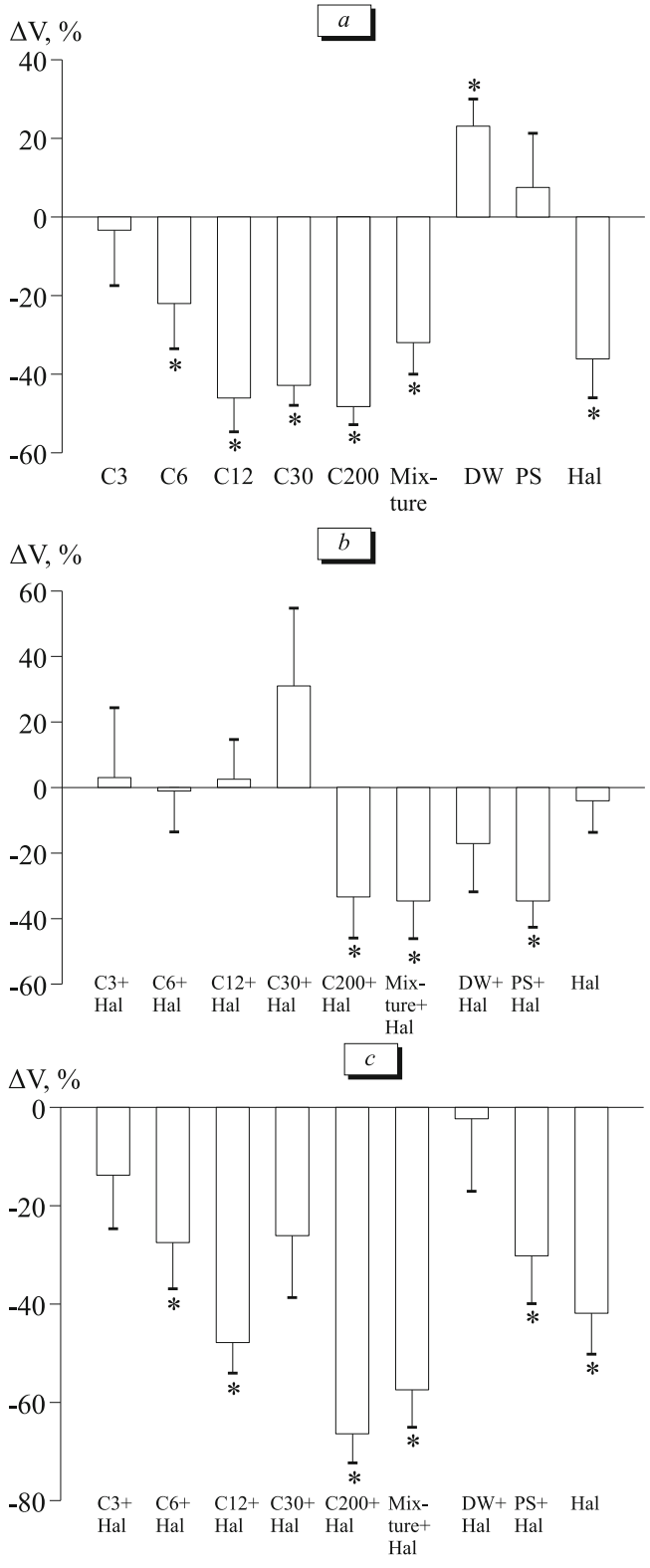

Fig. 1. Changes in snail locomotion rate after 3-day administration of Hal preparation (*a*) with subsequent chronic 7-day Hal administration (*b*) and combined effect of Hal preparation administration and subsequent chronic Hal injection (*c*). Ordinate: for *a* and *c*: % of baseline locomotion rate, for *b*: % of locomotion speed after Hal preparations. Here and on Fig. 2: DW: distilled water; mixture: C12+C30+C200. *$p<0.05$ compared to PS (*a*), to DW+Hal (*b, c*).

for the assessment of the effects produced by the test agents on the electric properties of command neuron, the values of membrane potential in different series were directly compared at the end of the study.

The results were statistically proceeded using Mann–Whitney *U* test and Student *t* test, and expressed as the mean and standard error of the mean (*M*±*SEM*).

RESULTS

Chronic exposure to Hal decreased locomotion rate in edible snail; we previously established the optimal time for Hal administration: 7 days [6]. Thus, at the end of exposure (day 8), locomotion rate decreased ~1.5 fold (Fig. 1, *c*).

Changes in locomotion speed were observed after injection of Hal preparations for 3 days: it decreased after administration of preparations C6, C12, C30, C200, and mixture C12+C30+C200 (Fig. 1, *a*). Similar changes were revealed after 3 days of Hal administration. Interesting, C3 preparation did not reduce locomotion rate. Then Hal was administered for 7 days to all experimental groups receiving low doses of Hal and control groups receiving physiological saline and distilled water. Hal did not reduce locomotion rate in groups pretreated with preparations C3, C6, C12, and C30 (Fig. 1, *b*). In groups receiving other preparations, the rate of locomotion decreased by ~35%, similarly as in the control group pretreated with PS. Considering combined effect of Hal preparations and subsequent chronic administration of Hal, one may conclude that preliminary injections of C3 and C30 preparations abolished the effect of Hal on locomotion speed reduction in edible snail (Fig. 1, *c*). At the same time, the effect of Hal was enhanced after administration of C200 and C12+C30+C200 mixture and not affected after injection of C6 and C12 preparations.

Measurement of electrical characteristic in defensive behavior command neurons in Hall series showed, that chronic Hal administration results in hyperpolarization of command neuron. Resting potential was shifted to -59.8±0.8 mV after 7 days of Hal injections, in comparison with -58.6±0.7 mV in snail received PS (Fig. 2). Unlike chronic Hal administration, when membrane hyperpolarization develops in command neurons, administration of C6, C12 and C30 preparations followed by Hal injections results in depolarizing shift of the membrane potential in comparison with the group receiving Hal only (Fig. 2). The effects of other preparation were insignificant.

Thus, analysis of the effects of Hal preparation in different dilutions was performed. Hal preparations in low doses produced a modulating action on reduction of locomotion rate in edible snail and on hyperpolarization of command neurons caused by chronic administration of Hal.

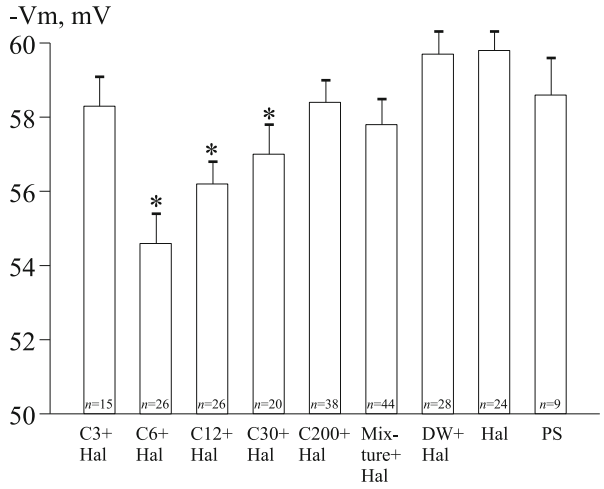

Fig. 2. Membrane potential (-Vm) of command neurons after 3-day administration of Hal preparations in low doses followed by 7-day administration of Hal. **p*<0.05 in comparison with Hal and DW+Hal.

Preconditioning (neuroprotective) effect of glutamate in low doses against its high-dose toxicity was demonstrated on neurons of the spinal cord, cortex, and cerebellum [9,12], the same effect was found for low doses of cyclohexemide for neuronal survival in glutamate solution [10]. The mechanism of this effect of low doses is a very important problem. Thus, the neuroprotective effect of low doses NMDA was shown to be mediated by activation of synaptic and extrasynaptic NMDA receptors finally increasing neuronal excitability [12]. The protective effect of low doses of antibodies to Ca^{2+}-binding protein S100 manifests in modulation of the effects of the main S100-Ab solution on specific neuronal structures, particularly on channels of inward and outward current (primary Ca^{2+}-channels), but not only in modulation of the membrane and threshold potentials and duration of action potential, which emphasizes the genesis of the main phenomenon and points to the location of its mechanism at the level of ion channels, which participate in action potential generation and provide calcium ion influx to the cell [1].

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