Effect of Chronic Administration of Chlorpromazine on Electrical Parameters of Command Neurons in Edible Snail

T. Kh. Gainutdinova, A. V. Meshchaninova, V. V. Andrianov, D. I. Silant'eva, and Kh. L. Gainutdinov

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Electrophysiological experiments showed that chronic administration of neuroleptic chlorpromazine depolarized plasma membrane and decreased the threshold of action potential generation in command neuron of defense behavior of edible snail and motoneurons of pneumostome closure reflex. The effects of chlorpromazine on electrical parameters of the identified neurons can be explained by its serotonin-depleting action.

Key Words: *central inspiratory activity; subretrofacial area; hypercapnia*

Neuroleptic chlorpromazine (CP, aminazine) is widely used in clinical practice [5]. This agent blocks central adrenergic and dopaminergic receptors and decreases abnormally enhanced tone of the dopaminergic system in the brain [6,8,10]. Long-term administration of CP significantly modulates parameters of motor activity: ciliary and muscle locomotion, opening rate of the lung cavity orifice, and coordinate packing of ovules in embryonic membranes [11,14]. These changes are partially or completely prevented by administration of serotonin (5-HT) or its precursors [9,11]. The decrease in 5-HT level under the effect of CP was also reported. The therapeutic effects of neuroleptics can be explained by depletion of neuronal dopamine and 5-HT, which improves the balance of dopamine and 5-HT in disturbed brain areas.

Modeling of 5-HT deficiency in the nervous system of edible snail with neurotoxin 5,6-dihydroxytriptamine (5,6-DHT) is accompanied by membrane depolarization and reduction of action potential threshold for defensive behavior command neurons [3]. We assumed that CP could also affect the parameters of electrical activity of certain neurons. Our aim was to study the effects of CP on electrical activity of the identified snail neurons assessed by resting potential, spike threshold, and critical depolarization.

Kazan Physicotechnical Institute, Kazan Research Center, Russian Academy of Sciences. ***Address for correspondence:*** gainutdinov@mail.knc.ru. Kh. L. Gainutdinov

MATERIALS AND METHODS

The experiments were carried out on mature edible snails *Helix Lucorum* of Crimean population *Pulmonata* and *Gastropoda*. The snails had similar weight and size. They were kept in small groups in aquariums at room temperature, high humidity, and food *ad libitum*. Prior to the experiments, the snails were active at least for 3 weeks; thereafter they were randomized into control and experimental groups.

During 8 days, the experimental snails were daily treated with CP (3 mg/kg, 0.1 ml), which was injected into the internal cavity of the sinus node region. The control snails received the same volume of physiological saline.

Electrical parameters were recorded in isolated preparations of snail nervous system. Before surgery, the animals were anesthetized by cooling in water-ice mixture for 15-30 min. The preparation of the nervous system consisted of neural ring, which included the subglottal complex of ganglia (pleural, parietal, pedal, and visceral ganglia. The isolated nervous system was placed into a solution containing (in mM): 80 NaCl, 4 KCl, 10 $CaCl_2$, 5 $MgCl_2$, 5 $NaHCO_3$ (alternatively, 5 mM Tris HCl), pH 7.6-7.8. The peripharyngeal ring

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was fixed to the bottom of the experimental chamber with tungsten staples. Then the connective shell covering the neurons was removed with forceps and microscalpel. In some cases, this process was preceded by pronase treatment.

On the next day after termination of injections, we recorded resting potential, spike threshold, and critical depolarization of the defensive behavior command neurons (LPa3, RPa3, LPa2, and RPa2, [2], n=48) and pneumostome closing (B3, B5, and B7) and opening motoneurons (B8, B9, and B11, n=66). The measurements were carried out using intracellular glass microelectrodes (5-30 MΩ) filled with 2.5 M KCl saline. Resting potential was determined as the point between spikes where changes in membrane potential were minimal. The threshold of action potential was determined as the difference between resting potential and the point where the rising rate of action potential (the first derivative of potential) attained a critical value of 1 V/sec. Since command neurons are silent under normal conditions, they were stimulated in isolated preparation with depolarizing electric current delivered to the cells via the recording microelectrode (minimal current triggering 2-3 spikes). Stimulation was performed with an internal stimulator of microelectrode amplifier MC-01M. In addition, this stimulator counterbalanced the microelectrode tip potential. Parameters of spontaneous activity of motoneurons were also recorded.

The results were analyzed statistically using Student's t test and Mann—Whitney U test.

RESULTS

In snails, CP decreased the membrane potential of defensive behavior command neurons (Fig. 1, *a*, *1*). In addition, this agent decreased the threshold of action potential of defensive behavior command neurons (Fig. 1, *b, 1*) and the membrane potential in motoneurons that control the pneumostome closure reflex (Fig. 1, *a*, *2*). There were no significant differences in the threshold of action potential of these motoneurons in control and experimental snails (Fig. 1, *b*, *2*). CP produced no significant effect on electrical parameters of motoneurons controlling opening of the pneumostome (Fig. 1, *a*, *3* and *b*, *3*). Significant changes of critical depolarization in response to CP were observed only in the pneumostome closure reflex motoneurons (Table 1).

An important feature of the effect of the test neuroleptic is blockade of dopamine receptors, which decreases abnormally enhanced tone of the dopaminergic system [6,8,10]. By contrast, CP significantly decreases the content of 5-HT in the nervous tissue [9,11]. As a transmitter, 5-HT plays a key role in the peripheral and central nervous systems in many animal species [4,15]. This agent is involved in the regulation of various vital activities in mollusks (defensive behavior, food-procuring behavior, locomotion, reproduction, and circadian rhythms) [2,7]. Injection of 5-HT accelerates locomotion in mollusks without lengthening their sole [13]. Application of 5,6- and 5,7-DHT (neurotoxic analogs of 5-HT selectively destroying serotonin terminals) is an important experimental tool in studies of the role of 5-HT in the regulation of different behavioral patterns [12]. We showed that injection of 5,6-DHT depolarized the defensive behavior command neurons in edible snail and decreased their threshold potential, which can be explained by the effect of 5-HT on the inputs of these neurons [3]. Snail behavior immediately after injection of 5,6-DHT is comparable with that after injection of 5-HT [3,13] disturbing associative learning and long-term sensitization of defensive behavior [1-3]. Injections of CP

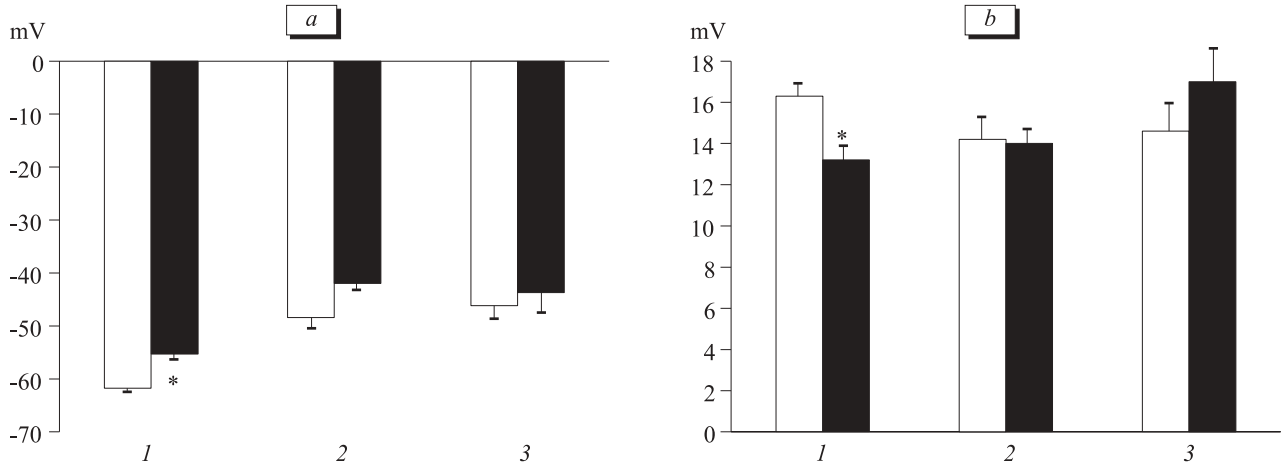

Fig. 1. Effect of chlorpromazine (3 mg/kg for 8 days, solid bars) and physiological saline (light bars, control) on resting (*a*) and threshold (*b*) membrane potential of defensive behavior command neurons (*1*) and motoneurons of pneumostome closure (*2*) and opening (*3*) reflex in snails. *p<0.01 compared to the control.

TABLE 1. Effect of Chlorpromazine on Critical Depolarization of Different Neuron Types in Edible Snail ($M \pm m$)

Group	Command neurons	B3, B5, B7
Control	-45.5±1.0 (n=10)	-34.2±1.8 (n=15)
CP	-42.1±1.5 (n=20)	-28.0±1.2* (n=25)

Note. *p<0.05 compared to the control. n: number of snails.

enhanced excitability of identified neurons of defensive behavior, which manifested in depolarization shift of membrane potential and decrease in the threshold potential. There are data on changes in spontaneous activity of some other snail neurons towards moderation of the discharge rate [9]. Similar depolarization shift and decrease in the threshold potential were observed in command neurons of edible snail during chronic administration of 5,6-DHT [3], which probably attests to pronounced synaptic input in defensive behavior command neurons mediated by the serotonergic neurons. In addition, CP depolarized the pneumostome closure reflex motoneurons, which belong to the defensive reflex arch. Thus, the effects of CP on electrical parameters of the identified neurons could be explained by CP-induced deficiency of 5-HT.

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