Modulatory Serotonin Receptors on the Soma of Command Neurons in Edible Snail

A. S. Pivovarov and V. L. Nistratova

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Local application of serotonin (5-hydroxytryptamine, 5-HT) to the soma of command neurons LPa2, LPa3, PPa3, and PPa2 of edible snail *Helix lucorum* reversibly decreased acetylcholine-induced inward current in these neurons. NAN-190 and methiothepin, antagonists of 5-HT_1 serotonin receptors, prevented this modulatory effect of 5-HT. By contrast, LY-53.857, ICS-205.930, and SDZ-205.557, antagonists of 5-HT_2, 5-HT_3, and 5-HT_4 serotonin receptors, respectively, produced no effect on the modulatory effect of serotonin. The data confirm the presence of modulator 5-HT_1 serotonin receptor on the soma of command neurons of *Helix pomatia.*

Key Words: *serotonin, acetylcholine, serotonin antagonists, neurons, Helix lucorum*

Monoamine serotonin (5-hydroxytryptamine, 5-HT) was found in the nervous system of edible snail [4,12]. In this organism 5-HT probably plays a role of a humoral factor, which exerts a neuromodulator effect on command neurons responsible for defensive behavior. The somas of these neurons are surrounded by a network of serotonin-containing fibers that have no specialized synaptic membranes [13]. Command neurons are sensitive to serotonin, which increases the amplitude of orthodromic excitatory postsynaptic potential (EPSP) [1,2,8,9]. The presence of 5-HT in the extracellular medium is a prerequisite for the development of a long-term potentiation of EPSP in the command neuron caused by its intracellular tetanization [6]. 5-HT modulates the extrasynaptic cholinoreceptors of the command neurons: it potentiates or attenuates acetylcholine-induced depolarization and inward current [3].

At present, seven types of serotonin receptors are described [10]. Our aim was to determine the type of serotonin receptors mediating the modulator effect of 5-HT on previously identified extrasynaptic cholinoreceptors of the command neurons of edible snail, which control its defensive behavior [7].

Department of Higher Nervous Activity, M. V. Lomonosov Moscow State University

MATERIALS AND METHODS

Experiments were carried out on command neurons LPa2, LPa3, PPa2, and PPa3 [5], which control defensive behavior of *Helix lucorum* (Crimean population of edible snail). The isolated ganglia with these neurons were examined under the stop-flow conditions with physiological saline containing (in mM): 100 NaCl, 4 KCl, 10 $CaCl_2$, 4 $MgCl_2$, 10 Tris-HCl (pH 7.5-7.7).

The transmembrane ionic currents in command neurons were measured by two-electrode voltage clamp technique with virtual ground. The currents were recorded in a computer using CONAN 3.0 software. Resting potential and membrane resistance were -63.20±1.05 mV and 3.23±0.26 MΩ, respectively.

Local acetylcholine (Ach) was applied ionophoretically on the dorsal surface of neuron soma from a glass micropipette filled with 1 M acetylcholine chloride (Sigma) in distilled water.

5-HT hydrochloride (Sigma) was locally applied to a neuron under a pressure developed by a KM-2 piston injector. 5-HT (5 mM) was dissolved in physiological saline immediately before use. Before application, the solution was visually controlled in air, where it formed a droplet similar in size to the target cell with a diameter of 0.2-0.3 mm. The volume of

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injectate varied from 4 to 14 nl. The internal diameter of the injection micropipette tip was 4.25-10.60 μ. The duration of each application was 5 or 10 sec; volume rate varied from 0.4 to 2.8 nl/sec.

Five antagonists of serotonin receptors were used: NAN-190 hydrochloride, Sigma (antagonist of 5-HT_{1A} receptors); methiothepin mesylate, Sigma (antagonist of 5-$HT_{1E/1F}$ receptors); LY-53.857 maleate, RBI (antagonist of 5-HT_2 receptors); ISC-205.930, ICN (antagonist of 5-HT_3 receptors); SDZ-205.557 hydrochloride, RBI (antagonist of 5-HT_4-receptors). The antagonists were dissolved in physiological saline and applied with a microsyringe directly into the perfusion chamber with isolated ganglia.

The data were processed statistically using Student, Wilcoxon, Van-der-Varden, and Mann—Whitney tests and presented as means and SEM. Calculations were performed with STADIA 6.2 and Statistica 5.0 software.

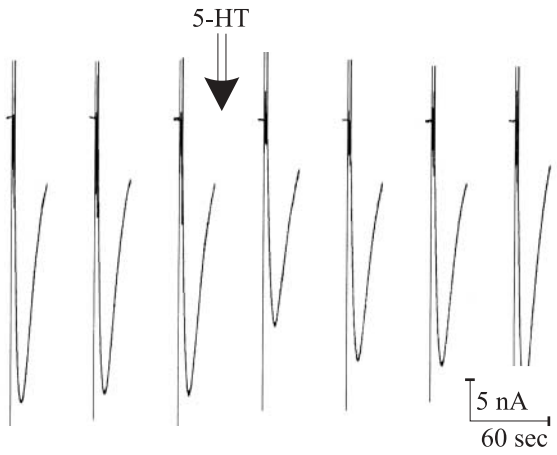

Fig. 1. Serotonin-induced inhibition of acetylcholine-triggered inward currents (Ach-current) in LPa3 command neuron. Ionic currents induced by Ach applied repetitively with a 5 min interval are shown. The arrow shows application of serotonin (duration 5 sec). Holding potential -75 mV.

RESULTS

In the control, application of physiological saline to neuron soma (n=11) produced no significant changes in the amplitude of Ach-triggered currents in LPa2, LPa3, PPa3, and PPa2 cells: the postapplication current was 101.03±5.83% of the control value. By contrast, 5-HT reversibly decreased the amplitude of Ach-triggered inward current (Ach-current) according to Wilcoxon, Van-der-Varden, and Mann—Whitney tests (n=44, p<0.00001). The maximum effect was observed 20-300 sec after the end of application (Fig. 1). The amplitude of Ach-current was 66.57±2.53% of the control value.

These findings attest to the existence of extrasynaptic 5-HT modulator receptors on the soma of command neurons LPa2, LPa3, PPa3, and PPa2 of *Helix lucorum*.

Six experimental series were carried out to assess the sensitivity of extrasynaptic modulatory receptors to antagonists of four basic types of 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_3, and 5-HT_4). The first series included two successive identical applications of 5-HT with an interval of 50-80 min. (The second application controlled the change of 5-HT action during repeated use). Series II-VI consisted of three successive applications of 5-HT: before action of 5-HT antagonist, during its application and after washout. The duration of antagonist exposure before serotonin application was 25-45 min. In all series, the preparation was rinsed for 10-15 min with physiological saline 15 min after 5-HT application.

In control series (n=9) the second application of 5-HT did not differ from the first one in the efficiency of Ach-current suppression, the difference between amplitude decrements being only -0.56±2.35%. The changes in the efficiencyof serotonin-induced inhibition of Ach-currents produced by 5-HT antagonists were compared with the control effect of the second application of serotonin in control series.

NAN-190 (30-50 μM), an antagonist of 5-HT_{1A} receptors, prevented the effect of serotonin (n=7). The difference between the maximum effects of serotonin before and during application of the blocker was -10.60±4.34% (p=0.039, 0.05, 0.025, and 0.021 according to Student, Mann—Whitney, Wilcoxon, and Van-der-Varden tests, respectively). The effect of NAN was reversible, because the difference between the maximum effects of serotonin before application of the blocker and after washout was -0.48±3.36% (p=0.044 and p=0.037 according to Wilcoxon and Van-der-Varden tests, correspondingly).

Methiothepin (30-50 μM), an antagonist of 5-$HT_{1E/1F}$ receptors, also prevented the effect of serotonin (n=7). The mean difference between the maximum effects of serotonin before and during application of the blocker was -20.07±5.72% (p=0.005, 0.023, 0.011, and 0.01 accordingly to Student, Mann—Whitney, Wilcoxon, and Van-der-Varden tests, respectively). The effect of methiothepin was irreversible, because the mean difference between the maximum effects of serotonin before application of the blocker and after washout was -8.67±8.09% (p=0.223, 0.112, and 0.105 according to Mann—Whitney, Wilcoxon and Van-der-Varden tests, correspondingly).

LY-53.857 (50 μM), an antagonist of 5-HT_2 receptors, did not prevent the effect of serotonin (n=7). The mean difference between the maximum effects of serotonin before and during application of the blocker was 10.39±4.18% (p=0.058, 0.101, 0.05, and 0.067

according to Student, Mann—Whitney, Wilcoxon, and Van-der-Varden tests, respectively).

ICS-205.930 (10-50 μM), an antagonist of 5-HT_3 receptors, did not modify the effect of serotonin (n=7). The mean difference between the maximum effects of serotonin before and during application of the blocker was -5.93±4.28% (p=0.205, 0.224, 0.112, and 0.098 according to Student, Mann—Whitney, Wilcoxon, and Van-der-Varden tests, respectively).

SDZ-205.557 (50 μM), an antagonist of 5-HT_4 receptors, did not modify the effect of serotonin (n=7). The mean difference between the maximum effects of serotonin before and during application of the blocker was -4.27±2.75% (p=0.201, 0.266, 0.133, and 0.111 according to Student, Mann—Whitney, Wilcoxon, and Van-der-Varden tests, respectively).

These data showed that the modulatory serotonin receptors on the soma of command neurons LPa2, LPa3, PPa3, and PPa2 belong to type 5-HT_1 receptors. 5-HT modulates potential-dependent calcium current in non-identified neurons of *Helix lucorum* via the same receptor [11].

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