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Effects of the Serotonin Precursor 5-Hydroxytryptophan and the Neurotoxic Analog 5,7-Dihydroxytryptamine on the Formation of a Conditioned Defensive Reflex in the Common Snail

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We report here our studies on the effects of the metabolic serotonin precursor 5-hydroxytryptophan and the neurotoxic analog 5,7-dihydroxytryptamine on the acquisition of a conditioned defensive reflex and post-training electrophysiological parameters of defensive behavior command neurons in snails. Injections of 5-hydroxytryptophan into snails via the sinus node led to acceleration of the acquisition of the conditioned defensive reflex. Snails injected with 5,7-dihydroxytryptamine did not form the conditioned reflex; subsequent injection of 5- hydroxytryptophan prevented this blockade. While 5-hydroxytryptophan prevented the action of 5,7-dihydroxytryptamine at the behavioral level, no such effect was seen at the level of the electrical properties of command neurons.

KEY WORDS: learning, conditioned reflex, serotonin, 5-hydroxytryptophan, 5,7-dihydroxytryptamine.

Serotonin is one of the most widely distributed and best studied transmitters in the nervous system [13, 18]. Studies of the functionality of serotonin have demonstrated its important role in the activity of the central nervous system and the mechanisms of learning and memory [1–5, 15, 21].

Many studies of the role of the serotoninergic system use application or injection of serotonin or its metabolic precursor 5-hydroxytryptophan (5-HTP) [2, 13, 15]. Administration of 5-hydroxytryptophan, the immediate precursor in the biological synthesis of this amine, is the most effective way of increasing serotonin levels. The pharmacological effects of 5-HTP consist of significant increases in serotonin levels at 30 min, reaching a peak at 1 h, lasting several hours, and completely ending by 1 day [10]. Specific impairments to the operation of the serotoninergic system are efficiently induced by use of the neurotoxic serotonin analogs 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-HTM), which lead to degeneration of serotoninergic terminals and significant decreases in the serotonin concentration in the CNS [17, 19, 20].

Extensive experimental evidence has now accumulated demonstrating the relationship between serotonin levels in the nervous system and learning ability [1–3, 12, 15, 18, 21]. These include studies of the effects of the serotonin precursor 5-HTP and the neurotoxins 5,6- and 5,7-HTM used separately. However, the literature contains no reports as to which serotonin pool is affected by the neurotoxins 5,6- and 5,7-HTM or how the serotonin precursor acts in this situation. We have therefore studied the effects of the serotonin precursor 5-HTP and the neurotoxin 5,7-HTM on the acquisition of a conditioned defensive reflex on the electrical properties of defensive behavior command neurons in trained animals.

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TABLE 1. Mean Values of Electrical Properties of Defensive Behavior Command Neurons

Snail groups and subgroups	Membrane potential (V_m), mV	Action potential generation threshold (V_t), mV	Action potential amplitude (V_s), mV
Control	−59.6 ± 1.1 (n = 9)	16.6 ± 0.4 (n = 7)	62.2 ± 1.9 (n = 9)
Control + 5-HTP	−58 ± 1.5 (n = 5)	17.9 ± 1.1 (n = 7)	59.2 ± 4.1 (n = 6)
PS + PS + CDR	−55.3 ± 1.3 (n = 15)*	13.9 ± 0.7 (n = 9)*	55.8 ± 2.9 (n = 9)
PS + 5-HTP + CDR	−57.2 ± 1.2 (n = 15)	15.3 ± 0.5 (n = 15)	61.6 ± 2.1 (n = 15)
HTM + PS + CDR	−54.2 ± 1.7 (n = 5)*	13.5 ± 1 (n = 4)*	58.9 ± 2.2 (n = 7)
HTM + 5-HTP + CDR	−5.1 ± 1.4 (n = 15)*	14.5 ± 0.5 (n = 18)*	58.6 ± 1.8 (n = 21)

Note. *Significant difference from control group, $p < 0.005$.

METHODS

Experiments were performed using mature *Helix lucorum* snails of uniform weight and size, which remained active for two weeks. A conditioned defensive reflex (CDR) consisting of closing of the pneumostoma was developed using a previously developed scheme [9]. The conditioned stimulus (CS) consisted of tapping on the shell, which in normal conditions does not induce a defensive reflex in snails. The unconditioned stimulus (UCS) consisted of a jet of air directed into the pneumostoma, which induces an unconditioned pneumostoma-closing reaction. A total of 60 combinations of CS and UCS were presented per day. Complete closure of the pneumostoma I response to the CS was recorded as a positive reaction. The CDR was regarded as acquired if the animal gave positive reactions to 30 CS in a row. The number of combinations required for training was evaluated.

Serotonin was depleted by injection of 5,7-dihydroxytryptamine (Sigma, USA) at a dose of 20 mg/kg into the sinus node. Neurotoxin was dissolved in 0.1 ml of physiological saline (PS) for common snails supplemented with 0.1% ascorbic acid as antioxidant. Physiological saline for snails contained 78 mM NaCl, 4.5 mM KCl, 10 mM $CaCl_2$, 6.7 mM $MgCl_2$, and 4.5 mM $NaHCO_3$, pH 7.6–7.8. The metabolic precursor of serotonin, 5-HTP, was given at a dose of 10 mg/kg dissolved in 0.1 ml of PS. Some snails received injections of PS (controls) at the same volumes and times as used in experimental series. Development of the CDR was started five days after injections of 5,7-HTM or PS. Each group was divided into two subgroups; snails of one subgroup received injections of 5-HTP 1 h before training sessions, the other subgroup receiving PS injections. Thus, the CDR was developed in four subgroups: 1) PS + 5-HTP + + CDR; 2) PS + PS + CDR; 3) HTM + 5-HTP + CDR; 4) HTM + PS + CDR. In addition, electrophysiological experiments used two control subgroups: one consisting of intact snails and one consisting of snails after injections of 5-HTP.

After the behavioral part of the studies was completed, the electrical properties of defensive behavior command neurons, i.e., LPa3, RPa3, LPa2, and RPa2, were recorded. Electrophysiological experiments used isolated snail central nervous system preparations. Animals were cooled in a water/ice bath for 30 min before preparation. Measurements were made using intracellular glass microelectrodes with resistance 5–25 MΩ. Experiments recorded the following parameters of the electrical activity of neurons: membrane resting potential (V_m), action potential generation threshold (V_t), and action potential amplitude (V_s). A computerized recording system was used. Results were analyzed statistically using Student's t test and the Mann–Whitney U test. Mean values and standard errors are presented ($M \pm SEM$).

RESULTS

The CDR was acquired (n = 5 snails) after presentation of 330 combinations (Fig. 1). Daily injection of 5-HTP into the sinus node (n = 7) accelerated acquisition of the conditioned defensive reflex (acquisition after 260 combinations). Administration of the neurotoxin 5,7-HTM via the sinus node prevented learning (n = 3) (Fig. 2). This experiment reproduced our previous data obtained using the neurotoxin 5,6-HTM to block the acquisition of a conditioned reflex [3]. At the same time, daily injections of 5-HTP after injections of the neurotoxin 5,7-HTM into the sinus node restored the animals' ability to learn (n = 6). As 5-HTP promotes the synthesis of serotonin in the nervous system, it can be suggested that 5-HTP prevents blockade of learning by 5,7-HTM. This indicates that blockade of the acquisition of the conditioned reflex is associated with decreases in serotonin levels rather than with the destruction of serotoninergic synaptic terminals or the toxic actions of 5,7-HTM.

Recording of the electrical properties of defensive behavior command neurons yielded the following results: as compared with intact snails (control group), neurons in control snails (the PS + PS + CDR group), as demonstrated previously in our laboratory [5], showed a depolarization shift in V_m and a decrease in V_t (Table 1). Previous studies have also demonstrated that injections of 5,7-HTM into intact snails led to greater shifts in membrane potential and action potential threshold towards depolarization than seen after training of snails injected with PS [3, 4]. After the training procedure, snails previously injected with 5,7-HTM showed no further depolarization or decreases in action potential thresholds.

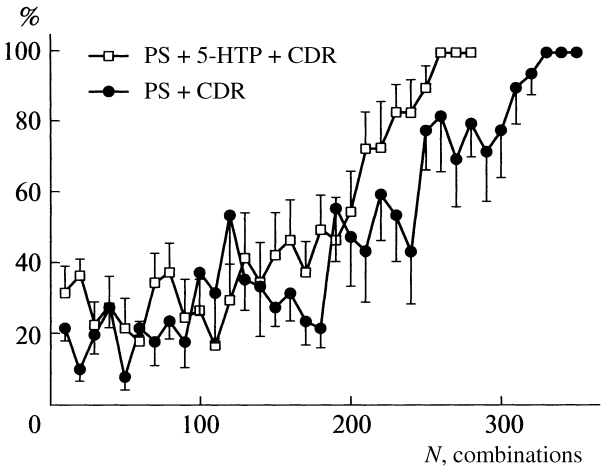

Fig. 1. Effects of injections of 5-HTP on the acquisition of a conditioned defensive reflex (CDR) in common snails. The abscissa shows the number of combinations of the conditioned and unconditioned stimuli and the ordinate shows the number of positive responses per 10 combinations of the conditioned and unconditioned stimuli (%). Standard errors of the mean are shown. PS = physiological saline, 5-HTP = 5-hydroxytryptophan.

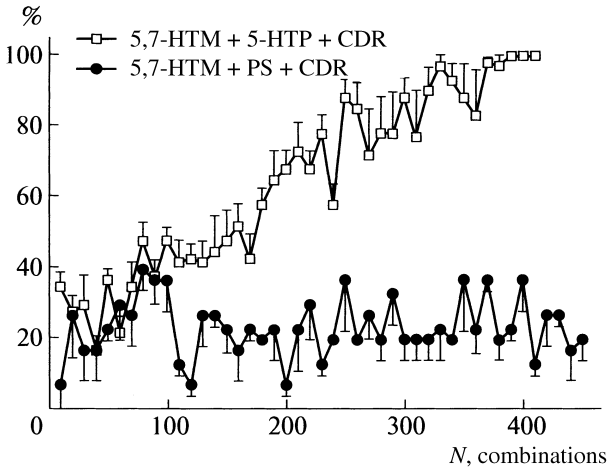

Fig. 2. Effects of injections of 5,7-HTM and 5-HTP on the acquisition of a conditioned defensive reflex in common snails. The abscissa shows the number of combinations of the conditioned and unconditioned stimuli and the ordinate shows the number of positive responses per 10 combinations of the conditioned and unconditioned stimuli (%). Standard errors of the mean are shown. 5,7-HTM – 5,7-dihydroxytryptamine.

Injections of 5-HTP to control snails produced no significant changes in the electrical properties of neurons. Training after injection of 5-HTP produced only a small (insignificant) depolarization shift in V_m and an insignificant increase in V_t as compared with controls. In snails trained after injection of 5-HTP and 5,7-HTM via the sinus node, there were significant reductions in V_m and V_t to the level seen in snails injected with 5,7-HTM only (Table 1).

DISCUSSION

Studies of the mechanisms of learning and memory have led to new experimental approaches in investigations of the neurotransmitter and modulatory effects of serotonin and the mechanisms by which these systems are involved in behavioral plasticity [12, 14, 18]. The use of the neurotoxic serotonin analogs 5,6- and 5,7-HTM and the serotonin precursor 5-HTP constitutes an important experimental approach for studies of the mechanisms of the involvement of serotonin in the learning and control of various types of behavior [4, 13, 16].

The studies reported here show that administration of the serotonin synthesis precursor 5-HTP into snails accelerated acquisition of a conditioned reflex, but had no effect on the electrical properties of command neurons. This result appears to be linked with the fact that serotonin, which plays a key role in defensive behavior in mollusks [6, 12], is involved in the reflex reinforcement system, thus accelerating learning. At the same time, the membrane correlates of learning [5] are independent of the rate of acquisition of the conditioned reflex but are determined by the acquisition or not of the conditioned reflex.

We also found, as previously [3, 4], that the neurotoxin 5,7-HTM induces a depolarization shift in membrane potential. This series of experiments did not provide a satisfactory answer to the question of why use of the neurotoxin 5,7-HTM induced a depolarization shift in membrane potentials.

We demonstrated that the effects of both 5-HTP and 5,7-HTM are not directly related to the excitability of command neurons after formation of the conditioned reflex. While at the behavioral level, 5-HTP prevented the action of 5,7-HTM, 5-HTP had no such effect at the level of the electrical properties of command neurons. Studies of the effects of transient 5,7-HTM-induced serotonin deficit on learning showed that serotonin depletion prevented acquisition of the conditioned reflex, as in studies reported by Balaban et al. [1, 2]. We have also obtained this result previously [3]. In continuation of our previous studies, we conducted experiments on the simultaneous effects of injections of 5-HTP and 5,7-HTM via the sinus node on the formation of a CDR. These experiments showed that daily 5-HTP injections via the sinus node before training sessions with the neurotoxin 5,7-HTM restored the ability of the animals to learn. At the same time, the electrical properties of command neurons (membrane and threshold potentials) did not return to initial – there was no more than a tendency. The difference in the effects of these substances appears to result from the fact that the neurotoxin acts predominantly (and perhaps only) at the level of serotoninergic terminals, while the effects of neurotoxins on membrane potential (in this

case of command neurons) also depends on receptors on the bodies of command neurons, as demonstrated by Pivovarov [11]. This suggestion is consistent with results obtained by Sakharov et al. [7, 8].

CONCLUSIONS

1. Daily injections of the serotonin precursor 5-HTP to snails via the sinus node accelerated the formation of a conditioned defensive reflex.

2. Administration of the neurotoxin 5,7-HTM to snails via the sinus node blocked the acquisition of the conditioned defensive reflex, while daily injections of 5-HTP via the sinus node after administration of the neurotoxin 5,7-HTM restored the animals' learning ability.

3. Injections of 5-HTP into snails via the sinus node produced no changes in the electrical properties (membrane and action potential threshold) of command neurons in either intact or trained snails.

4. Snails trained after injection of 5-HTP and 5,7-HTM via the sinus node produced significant reductions in membrane and action potential threshold potentials to the levels seen in snails injected with 5,7-HTM only.

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