The Transient Stage of Long-Term Synaptic Facilitation in Defensive Behavior Command Neurons in Sensitized Snails

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Experiments on common snails showed that three exposures to sensitizing stimuli (10% quinine applied to the snail’s head every 15 min) induced synaptic facilitation in defensive behavior command neurons LP11 and RP11, with facilitation of responses to sensory stimuli lasting more than 24 h. Application of single stimuli produced transient synaptic facilitation and was expressed in responses to tactile stimulation of the head for about 1 h and in responses to dilute quinine for 3 h. Serotonin and cAMP imitated stimulus-specific transient synaptic facilitation. These substances facilitated the responses of neurons LP11 and RP11 to test stimulation of the head without producing changes in the responses to stimulation of other areas of skin on the animal’s body. Calmodulin antagonists and glutamate NMDA receptor antagonists inhibited sensitization-induced synaptic facilitation in command neurons. Expression of transient synaptic facilitation depended on protein synthesis—it was suppressed by anisomycin and cycloheximide. It is suggested that transient synaptic facilitation during the acquisition of sensitization is associated with activation of translation/transcription processes and subsequent synthesis of specific short-lived protein molecules with selectively regulate the synaptic “inputs” of command neurons LP11 and RP11 from their specific skin innervation zones on the snail’s head.

KEY WORDS: Learning, sensitization, memory stages, neuron, plasticity, synaptic facilitation.

The most widely accepted division of memory is into short-term and long-term stages [12, 13]. The short-term stage of memory has been shown to last a few minutes or hours and is associated with synaptic plasticity of neurons in the brain, this being controlled by covalent modifications of protein molecules which have already been synthesized in nerve cells. At the same time, long-term changes in behavior and synaptic plasticity in learning persist for more than 24 h and depend on the synthesis of new protein and RNA. Long-term memory had then be subdivided into a transient early stage, which is sensitive to interference, and a more stable late stage [11, 12].

The molecular-cellular mechanisms of the early stages of memory have been studied in most detail in the marine mollusk Aplysia. One simple type of behavior—sensitization of the siphon or gill withdrawal reflex in Aplysia—is accompanied by facilitation of synaptic connections between the sensory and motor neurons innervating these parts of the body [12]. Recent studies on Aplysia [14] showed that there is an intermediate stage of synaptic facilitation between the short-term and long-term stages of facilitation of sensorimotor connections in sensitized animals. In terms of its onset time, this intermediate stage corresponds to the transient memory stage at the behavioral level; it lasts 3-6 and has a number of characteristic molecular features. Its expression involves three molecular mechanisms: covalent modification of protein molecules and the processes of translation and transcription [14]. It is important to note that the translation-dependent component of synaptic facilitation is not blocked by...
transcription inhibitors. This property of intermediary synaptic facilitation significantly distinguishes it from long-term synaptic facilitation, which is completely suppressed by both translation inhibitors and transcription inhibitors [14].

Serotonin induces the intermediate stage of facilitation of sensorimotor synapses in *Aplysia* [12, 14]. In sensory neurons, serotonin activates the enzyme adenylate cyclase, leading to increases in intracellular cAMP levels and subsequent activation of protein kinase A. It has been suggested that the catalytic subunits of protein kinase A activate the synthesis of proteins which act on transcriptional and translation processes [14]. The direct effects of the catalytic subunits of protein kinase A on translational processes may represent one of the mechanisms underlying the expression of the transcription-independent component of intermediate synaptic facilitation. The proteins synthesized are involved in the mechanism facilitating neurotransmitter release from presynaptic terminals of sensory neurons [12].

We have previously reported that the acquisition of nociceptive sensitization in the common snail leads to short-term and long-term facilitation of defensive reflexes consisting of withdrawal of the anterior part of the foot, as well as to typical changes in electrical activity in defensive behavior command neurons LPll and RPll [3, 6, 9]. In particular, the short-term stage of nociceptive sensitization is accompanied by depolarization of the plasma membrane and increases in plasma membrane excitability. At the same time, long-term sensitization is characterized not only by changes in electrogenic membrane properties, but also by selective facilitation of the synaptic “inputs” of command neurons, which lasts more than 24 h [6]. Later studies showed that the initial stage of long-term synaptic facilitation in command neurons is sensitive to various disrupting influences, including translation inhibitors [4] and that this stage can last no more than a few hours [5]. In accordance with these criteria, we described this stage of synaptic facilitation as transient and suggested that it seems to be identical to the intermediate stage of facilitation of the sensorimotor arc in *Aplysia*.

This report presents results obtained in our studies of the molecular-cellular mechanisms of induction and retention of the transient stage of synaptic facilitation in defensive behavior command neurons LPll and RPll in the common snail during acquisition of nociceptive sensitization.

**METHODS**

Experiments were conducted using the common snail *Helix lucorum*. Neurophysiological experiments were performed using semi-intact snail preparations as described previously [2]. Before surgery, snails were anesthetized by cooling with an ice:water mixture for 30-40 min. Snails' shells were removed and the anterior part of the foot, excluding the cephalic end, was cut in the midline. Snails were then placed in a bath filled with paraffin and a silicone ring of volume 200 μl was attached around the paraglottal complex of ganglia (i.e., the CNS); this ring permitted application of substances exclusively to the CNS.

Experiments were performed on cells LPll and RPll, which are defensive behavior command neurons in the snail [2]. These command neurons have identical receptive field sizes, these include the entire cutaneous surface of the snail's body [2]. However, the structures of the receptive fields are different. Each of the command neurons has a specific zone, test stimulation of which produces a maximal response from the cell with the shorter latent period. It is important to note that the specific zones of the command neuron receptive fields coincide with the ecologically important parts of the body—the head and the opening of the pulmonary cavity. Stimulation of non-specific zones produces smaller responses from the command neurons, and these have relatively long latent periods. The specific cutaneous zone for neurons LPll and RPll is located on the snail's head.

Bioelectrical activity was recorded from neurons LPll and RPll using glass microelectrodes filled with 2 M KCl, with tip diameters of less than 1 μm and resistance of about 30 MΩ. Potentials were amplified with an MEZ-8101 amplifier and displayed on a VC10 oscilloscope and an RJG-4024 pen recorder (Nihon Kohden, Japan).

Sensitization was developed using 10% quinine HCl, which was applied to the skin of the anterior part of the snail's head, once or 2-3 times with 15-min intervals.

Neuron responses to sensory stimuli were tested using dilute quinine (0.25-0.5%) and tactile stimuli. Quinine (600 μl) was applied for 30 sec to the anterior part of the snail's head, between the ommatophores. The animal's head was washed with physiological saline 2 min after application ended. Tactile stimulation was applied to the head, the middle part of the foot, and the mantle ridge using an electromechanical device.

Responses produced by test sensory stimuli were assessed in terms of the area (in relative units)