PHYSIOLOGY

Protein Kinase C is Selectively Involved in the Mechanisms of Long-Term Synaptic Plasticity

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Sensitization produced by intracellular administration of a specific protein kinase C inhibitor chelerythrine selectively inhibits synaptic facilitation in the response of LPl1 neurons to tactile sensory stimulation of snail head. Synaptic facilitation in the response to chemical stimulation of the head or tactile stimulation of the foot did not differ from that observed in neurons of control sensitized animals. Our findings suggest that protein kinase C plays a key role in the induction of long-term genetic regulation of sensory input in command neurons from mechanoreceptors on the head.

Key Words: *mollusk; nociceptive sensitization; long-term synaptic plasticity; protein kinase C*

The formation of long-term memory is associated with changes in the efficiency of specific synaptic contacts between nerve cells in the brain (synaptic plasticity), which depends on activity of the genome [6]. Induction of long-term synaptic plasticity involves secondary intracellular transmitters, including protein kinase C. Their effects on the genome are mediated by regulatory transcription factors [6,8,9]. Experiments on *Aplysia* neurons and mammalian hippocampal cells revealed a mechanism of long-term synaptic plasticity [6,8] not involving secondary intracellular synaptic transmitters, genes, and transcription products. Consolidation of new skills is accompanied by selective activation of a synaptic tag in activated neuronal synapses. This mechanism mediates local recognition and cooptation of transcription products synthesized in the neuron body during learning and transported to synapses [7]. Local protein synthesis provides a specific change in functional and morphological characteristics of activated synapses.

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Our previous experiments on snail command LPl1 and RPl1 neurons responsible for defensive behavior showed that nociceptive sensitization (simple form of learning) is accompanied by selective involvement of cAMP and cAMP-dependent transcription factors C/EBP (CAAT/enhancer binding protein) in the induction of long-tern synaptic plasticity of neuronal synaptic input from chemoreceptors on the head [1,4].

We demonstrated that sensitization with a relatively specific protein kinase C inhibitor polymyxin B selectively suppresses facilitation in the response of RPl1 and LPl1 neurons to tactile stimulation of the head during long-term sensitization. However, this inhibitor had no effect on facilitation in the response produced by tactile stimulation of the foot or chemical stimulation of the head [2]. These data indicate that polymyxin B has a selective effect relative to modality of sensory stimulation and site of stimulation in the snail body. It should be emphasized that systemic application of polymyxin B to central snail ganglia did not allow us to identify nerve cells in the system of defensive behavior that serve as a target for this inhibitor. Besides this, it remains unclear whether polymyxin B can produce a specific effect. This inhibitor

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could modulate not only protein kinase C, but also other types of protein kinases.

Here we studied the effect of specific protein kinase C inhibitor chelerythrine on long-term synaptic plasticity of snail defensive behavior command LPl1 neurons upon intracellular administration of an inhibitor during sensitization.

MATERIALS AND METHODS

Experiments were performed on a semi-intact preparation of *Helix lucorum* snails using standard electrophysiological methods [5]. Before surgery the animal was anesthetized by cooling in a mixture of water and ice for 30-40 min. Sensitization was produced by 3-fold application of 100 μl concentrated quinine hydrochloride (10%) to the skin on the snail head at 15-min intervals. The neuronal response to sensory stimulation was tested with 0.5% quinine solution and tactile stimulation. Quinine (600 μl) was applied to the anterior surface of the head for 30 sec. Tactile stimulation was applied to the head or middle part of the foot using an electromechanical device. The test stimulus was delivered before and 120-150 min after sensitization. Our previous studies showed that synaptic facilitation in LPl1 neuron reaches a constant level 120-150 min after sensitization and remains unchanged for more than 24 h [1]. We measured the area of slow excitatory postsynaptic potentials (sEPSP, arb. units) in the response to sensory stimulation.

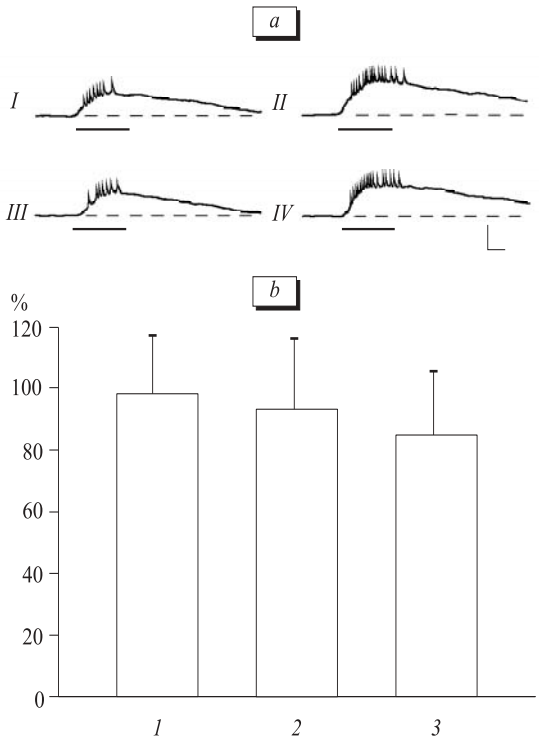

Fig. 1. Changes in the neuron LPl1 response produced by chemical stimulation of the snail head upon nociceptive sensitization and treatment with a protein kinase C inhibitor chelerythrine. Horizontal line: time of application of 0.5% quinine solution. Here and in Figs. 2 and 3: (*a*) neuronal response to chemical stimulation of the head under basal conditions (*I*, *III*) and 120 min after the first nociceptive stimulation (*II*, *IV*). Neuronal response in control sensitized snails to intracellular injection of 0.05% DMSO (*I*, *II*); neuronal response in animals sensitized during intracellular administration of chelerythrine (*III*, *IV*). Calibration, 5 mV/20 sec. (*b*) Summary diagram: (*1*) neuronal response 120-150 min after sensitization of snails with no intracellular injections (control) or upon intracellular administration of 0.05% DMSO (*2*) and chelerythrine (*3*). Ordinate: change in the area of a slow excitatory postsynaptic potential (sEPSP) in the response to sensory stimulation (relative to the pre-sensitization value, %).

Protein kinase C inhibitor chelerythrine chloride (Sigma) was dissolved in dimethylsulfoxide (DMSO, Sigma) and diluted with a solution containing 0.5 M KCl and 10 mM Tris-chloride (pH 7.6). Final concentrations of chelerythrine and DMSO were 200 μM and 0.05%, respectively. A microelectrode was filled with chelerythrine solution and introduced intracellularly immediately before injection. Chelerythrine solution was infused 10-15 min before sensitization. This microelectrode was removed from the cell 10 min after sensitization. The solution was administered intracellularly on a Neuro Phore BH-2 device (Medical System Corporation) under compressed-air pressure (1-2 kg/cm^2). The volume of infused solution was 2-5% of volume. Study substances were not infused to control LPl1 neurons. Otherwise, the solution of 0.05% DMSO not containing chelerythrine was applied intracellularly to control LPl1 neurons.

The data were standardized relative to presensitization parameters (100%), averaged, and expressed in percents of the baseline level. Standard error was calculated. The significance of differences was estimated by Student's *t* test.

RESULTS

Application of 0.5% quinine to the mollusk's head was followed by sEPSP generation in LPl1 neuron (389±47 arb. units, *n*=37, Fig. 1). The area of sEPSP in the neuronal response to tactile stimulation of the head and middle part of the foot was 236±25 (*n*=43) and 152±19 arb. units (*n*=36), respectively (Figs. 2 and 3).

The area of sEPSP in the response of snail LPl1 neurons to chemical stimulation of the head surpassed the baseline level 120-150 min after sensitization (Fig. 1): no injection of study substances, by 97±19% (*n*=14); intracellular administration of 0.05% DMSO, by 93±24% (*n*=11); and injection of chelerythrine, by 85±21% (*n*=12). The area of sEPSP in the response to tactile stimulation of the middle part of the foot increased by 38±9 (*n*=12), 40±12 (*n*=10), and 52±18% (*n*=14), respectively (Fig. 2). The area of sEPSP in the response of LPl1 neurons to tactile stimulation of the

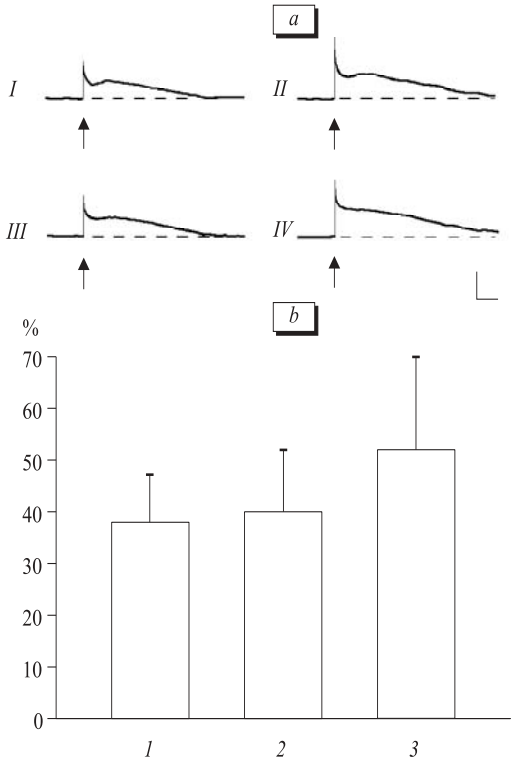

Fig. 2. Neuron LPl1 response to tactile stimulation of the middle part of the snail foot during sensitization upon chelerythrine treatment. Here and in Fig. 3: arrow, tactile stimulation.

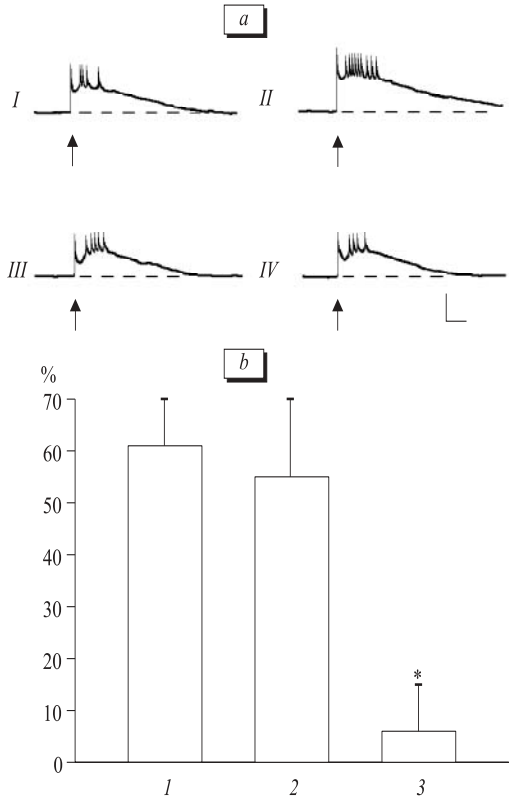

Fig. 3. Responses of Neuron LPl1 to tactile stimulation of the snail head during sensitization upon chelerythrine treatment. *p<0.001 compared to the area of sEPSP in neurons of control sensitized snails.

head changed 120-150 min after sensitization: no injection of study substances, by 61±9 (n=14), intracellular administration of 0.05% DMSO, by 55±15 (n=10), and injection of chelerythrine, by 6±9% (n=19, Fig. 3).

These data show that during sensitization after intracellular administration of chelerythrine, facilitation of the responses of LPl1 neurons to tactile stimulation of the foot and chemical stimulation of head did not differ from that in control neurons (p>0.05). Synaptic facilitation of neuronal response to tactile stimulation of the head was abolished after sensitization upon chelerythrine treatment. The area of sEPSP did not differ from the baseline level, but was much lower compared to control neurons (p<0.001).

Our findings suggest that sensitization after application of chelerythrine to LPl1 neurons specifically suppresses facilitation in the response to tactile stimulation of the head during long-term sensitization. Polymyxin B had a selective effect relative to modality of sensory stimulation and site of stimulation in the snail body. It should be emphasized that chelerythrine was introduced into the test neurons. This inhibitor displays high specificity for protein kinase C. Sensitization is probably accompanied by activation of protein kinase C in command neurons. This enzyme initiates induction of long-term specific facilitation in neuronal synaptic input from mechanoreceptors on the snail head.

Our previous studies showed that synaptic facilitation in LPl1 and RPl1 neurons develops 1.5 h after sensitization and is suppressed by inhibitors of protein and RNA synthesis [1,3]. It can be hypothesized that RNA and protein molecules whose synthesis depends on protein kinase C are involved in selective long-term synaptic facilitation in the neuronal response to tactile stimulation of the head. Experiments with the acquisition of new skills in mammals, mollusks, and other animals showed that protein kinase C plays a role in the mechanisms of induction of long-term synaptic facilitation depending on protein synthesis [6,8,9]. There are no data that protein kinase C is specifically involved in the mechanisms of induction of long-term plasticity in certain synaptic contacts in the same nerve cell characterized by specific morphological, functional, or neurochemical properties.

Taking into account previous results, we hypothesized that long-term plasticity of various synaptic inputs in the same command neurons responsible for snail defensive behavior is regulated by different molecular and genetic mechanisms during sensitization. For example, in command neurons LPl1 and RPl1 the system of cAMP and cAMP-dependent transcription regulators C/EBP induce the synthesis of RNA and proteins that selectively regulate synaptic input in nerve cells from mechanoreceptors on the snail head [1, 4]. Our findings suggest that protein kinase C plays a

key role in the induction of long-term genetic regulation of sensory input in command neurons from mechanoreceptors of the head.

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