PHYSIOLOGY

Specific Features of Molecular Postsynaptic Excitation Processes of Different Sensory Modalities in Edible Snail Neurons

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Stimulations of different sensory modalities result in selective activation of second messengers and genes controlled by them in defensive behavior command neurons (LPl-1 and RPl-1) in edible snail. Excitation induced by stimulation of chemoreceptors of snail head results in cAMP accumulation in command neurons and induction of associated transcriptional factors of immediate early gene C/EBP, whereas regulating mechanisms of another sensory input mediating excitation after tactile head stimulation, involve protein kinase C and associated transcriptional factor SRF. These findings reflect specific features of postsynaptic processes providing excitation of different sensory modalities converging to defensive behavior command neurons in edible snails.

Key Words: *neuron; cAMP; protein kinase C; C/EBP, SRF, and zif268 transcriptional factors*

On the basis of previous experimental data, specificity of postsynaptic processes activated by stimulations of different sensory modalities in edible snail neurons were discussed [8]. These data confirmed the hypothesis proposed by P. K. Anokhin on specificity of neurochemical mechanisms providing integrative activity of neurons [2]. At present, postsynaptic processes are usually considered in the terms of general reactions of CNS neurons resulting from activation of membrane proteins, cyclic nucleotides, calcium ions, inositides, protein kinase, and nuclear DNA genes [13,14]. At the same time, molecular and informational specificity of postsynaptic processes in neurons during the formation of systemic organism activity remains poorly studied despite their fundamental importance for the development of the neuronal plasticity problem.

Here we evaluated the involvement of second messengers and transcriptional factors C/EBP (CAAT/enhancer binding protein), SRF (serum response factor), and zif268 in postsynaptic processes mediating stimulations of different sensory modalities in edible snail command neurons.

MATERIALS AND METHODS

The experiments were performed on LPl1 and RPl1 belonging to defensive behavior command neurons of edible snail (*Helix lucorum*). These neurons participate in induction and maintenance of nociceptive sensitization, a simple form of learning [4]. Receptive fields of LPl1 and RPl1 include whole skin surface of the body. However, there is a special zone on the snail

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head, where sensory stimulation induces maximum cell response with minimum latency.

Semi-intact preparations of snails were used. Nociceptive sensitization was induced by triple application of concentrated quinine (600 μl, 10%) to the skin of snail head, the interval between applications was 15 min. Chemical (weak 0.25-0.50% quinine solution in volume of 600 μl) and tactile stimulations were applied to the head and the middle part of the foot. Tactile stimulation was applied with an electromechanic device [5,6]. The responses evoked by sensory stimulation were assessed by the area (mV×sec) under the curve of slow excitatory postsynaptic potentials (sEPSP) indicative for command neurons. The test compounds (cAMP, protein kinase C inhibitors polymyxin B and chelerythrine, oligonucleotides to C/EBP, SRF, zif268) were applied to neurons or injected intracellularly through a glass micropipette using compressed air or microionophoresis. The data were averaged and expressed in percents from baseline values. Standard error of the mean was estimated. Significance of differences was assessed by Student's *t* test.

RESULTS

Three sensitizing stimulations of snail's head resulted in intensification of synaptic responses to chemical and mechanical sensory stimulations; the phenomena developed 1.5 h after the start of sensitization training and persisted for more than 24 h. On minutes 120-150 and after the start of training, neuronal responses to sensory stimulations stabilized, herewith sEPSP areas in responses to mechanical stimulations of the head and foot and to application of weak quinine solution to the head exceeded the baseline values by 57±12, 38±7, and 101±18%, respectively. Triple sensitizing stimulation of snail's foot (nonspecific receptor zone for neurons LPl1 and RPl1) led to significantly less pronounced facilitation compared to that after head sensitization, which persisted for more than 24 h after habit formation.

These results indicate that the formation of nociceptive sensitization in edible snail is accompanied by specific long-term changes in the efficiency of synaptic transmission in LPl1 and RPl1 neurons. The intensity and duration of changes in neuronal responses to sensory stimulation depended on their informational magnitude, modality (tactile or chemical stimulation), and on application site.

The hypothesis about possible participation of second messengers in the mechanisms of neuronal synaptic plasticity was proposed at the early period of corresponding investigations. The mechanisms of neuronal excitation were found to involve various second messengers: cAMP, calcium/calmodulin, protein kinase C, mitogen-activated protein kinase (MAPK), *etc*. [13,14]. Synaptic stimulation induces a cascade of molecular events, including activation of protein kinase A, MAPK, protein kinase C, calcium/calmodulin-dependent protein kinase, and protein synthesis [13,14]. Protein kinases activate protein synthesis on pre-existing mRNAs in activated synapses [12]. Protein kinase A and MAPK, and also proteins synthesized in synapses retrogradely enter the nucleus, where they regulate activity of some transcriptional factors, *e.g.* CREB (cAMP-response element-binding protein) [10,12,13]. These factors express early response genes (including C/EBP, activatin factor, ubiquitin hydrolase) and late response genes [10,13]. Synthesized mRNAs are transported anterogradly and induce long-term facilitation of synaptic transduction.

Studies on mammalian brains also revealed numerous facts indicating mediation and intracellular processing of incoming excitations, including activation of second intracellular messengers and protein kinases, processes of phosphorylation and protein synthesis, and activation of gene transcription [1,2,9,13,14].

We identified neurochemical substrates (up to individual genes) involved in integrative neuronal activity.

We found that after formation of sensitization in snail LPl1 and RPl1 neurons, the modality- and site-specific facilitation of synaptic transduction was observed, which was mediated by second messengers and neurotransmitters. cAMP injections into LPl1 and RPl1 neurons (11 neurons) resulted in response facilitation (by 40-50% compared to baseline values persisting for 2-4 h) only after chemical stimulation of snail head. The effects of cAMP were specific, since the responses of the same neurons to mechanical stimulation of head and foot remained unchanged.

The development of sensitization under conditions of intracellular injection of protein kinase C inhibitors polymyxin B and chelerythrine into LPl1 neurons (n=31) resulted in complete and specific inhibition of response facilitation to head stimulation. The inhibitors did not affect facilitation of the reactions of these neurons to mechanical stimulation of the foot or chemical stimulation of the head.

Acquisition of new habits is associated with the expression of early response genes controlled by pre-existing transcriptional factors activated by extracellular signals through protein kinase cascades. Protein products of "early" genes can act as transcriptional factors capable for regulating expression of the effector gene. C/EBP is one of the "early" genes, its rapid and short-term expression is induced by extracellular stimuli. Some members of C/EBP family are activated by cAMP-dependent CREB transcription regulators. C/EBP can bind with ERE (enhancer responses element) of "early" gene c-fos and with ERE-sequence

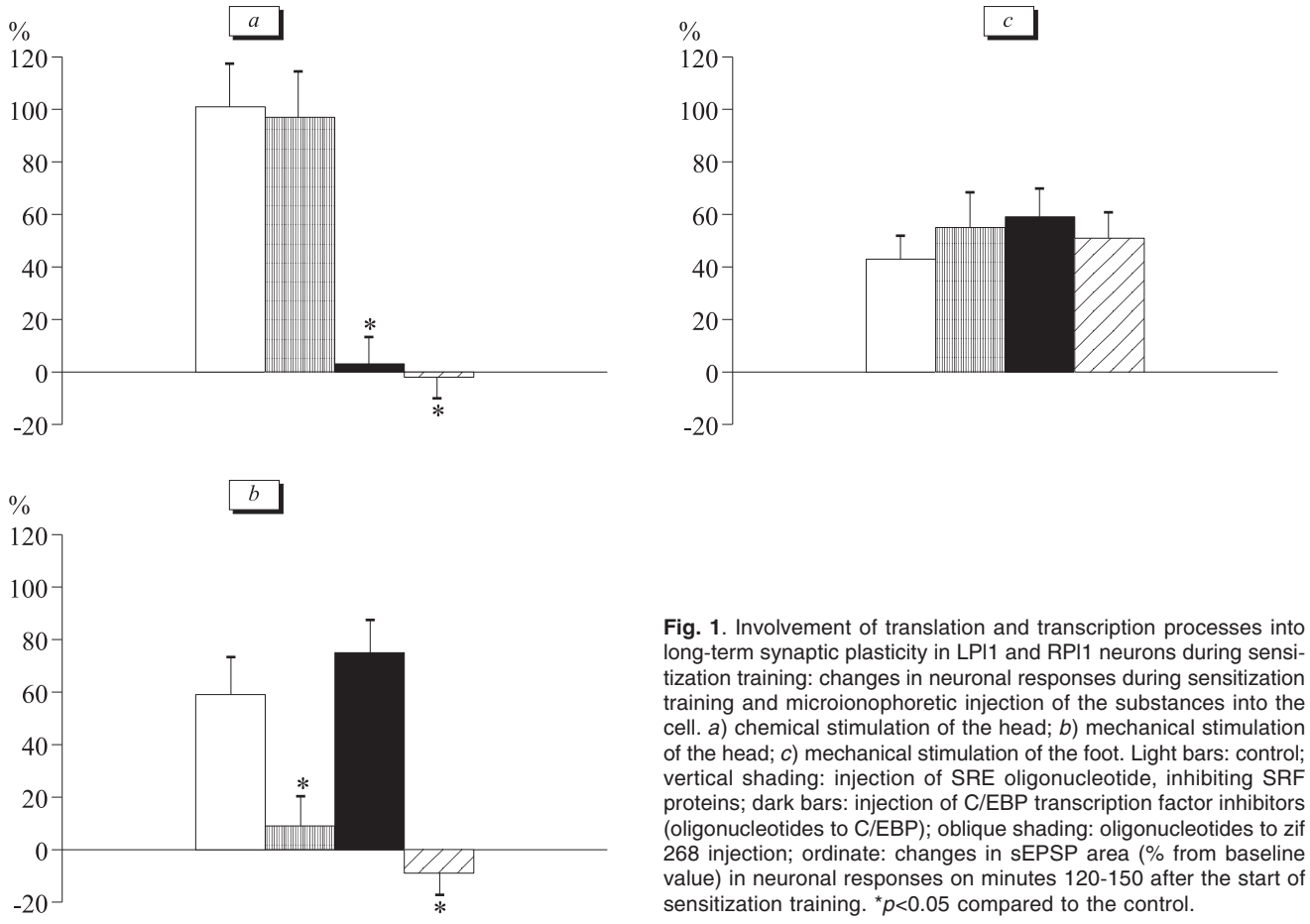

Fig. 1. Involvement of translation and transcription processes into long-term synaptic plasticity in LPl1 and RPl1 neurons during sensitization training: changes in neuronal responses during sensitization training and microionophoretic injection of the substances into the cell. *a*) chemical stimulation of the head; *b*) mechanical stimulation of the head; *c*) mechanical stimulation of the foot. Light bars: control; vertical shading: injection of SRE oligonucleotide, inhibiting SRF proteins; dark bars: injection of C/EBP transcription factor inhibitors (oligonucleotides to C/EBP); oblique shading: oligonucleotides to zif 268 injection; ordinate: changes in sEPSP area (% from baseline value) in neuronal responses on minutes 120-150 after the start of sensitization training. *$p<0.05$ compared to the control.

of some "late" genes [10,13]. Taking these data into consideration, one may assume that cAMP in LPl1 neurons activates CREB-dependent genes, *e.g.* C/EBP, involved in neuronal mechanisms of long-term memory formation in various species [10,15].

We investigated the role of C/EBP in post-synaptic excitatory mechanisms in LPl1 neurons using ERE mammalian double-stranded oligonucleotides and BS2 ApC/EBP from *Aplysia*, C/EBP inhibitor proteins, and antisense c/EBP mRNA [7]. Sensitization training during intracellular injection of C/EBP inhibitors into LPl1 neurons (n=16) led to inhibition of facilitation of their response to chemical stimulation of the head (Fig. 1). At the same time, facilitation of the response of these neurons to tactile stimulation of the head and foot did not differ from that in neurons of control sensitized animals (n=12).

SRF is a transcriptional factor, whose phosphorylation and activity depends from protein kinase C [11]. Promoters of some genes, including immediately early response genes, such as c-fos, fosB, junB, zif268 [11], include SRF-binding DNA sequence (serum response element, SRE), which serves as an assembly station for multiprotein complexes, including SRF dimer and proteins of ternary complex factor [11].

We found that injection of double-stranded SRE oligonucleotide inhibiting SRF protein into LPl1 neurons (n=17) during sensitization training led to inhibition of response facilitation to tactile stimulation of snail head (Fig. 1) [5]. Facilitation of the responses to chemical stimulation of the head or mechanical stimulation of the foot in these neurons did not differ from that in neurons of control sensitized animals (n=14).

Injection of antisense zif268 mRNA into LPl1 neurons (n=12) during sensitization training inhibited response facilitation to chemical and mechanical stimulation of the head [6]. Facilitation of the responses to mechanical stimulation of the foot in theses neurons did not differ from that in neurons of control animals (n=12; Fig. 1).

Our investigations showed that mechanisms of sensitization development in snails selectively involve different second messengers and genes regulated by them. cAMP and cAMP-dependent transcriptional factors of immediate early gene C/EBP were found to be involved in induction of long-term facilitation in sensory neuronal inputs from chemical receptors of the head, while protein kinase C and dependent transcriptional factor SRF are involved in the regulation of another sensory input of neurons LPl1 and RPl1, from

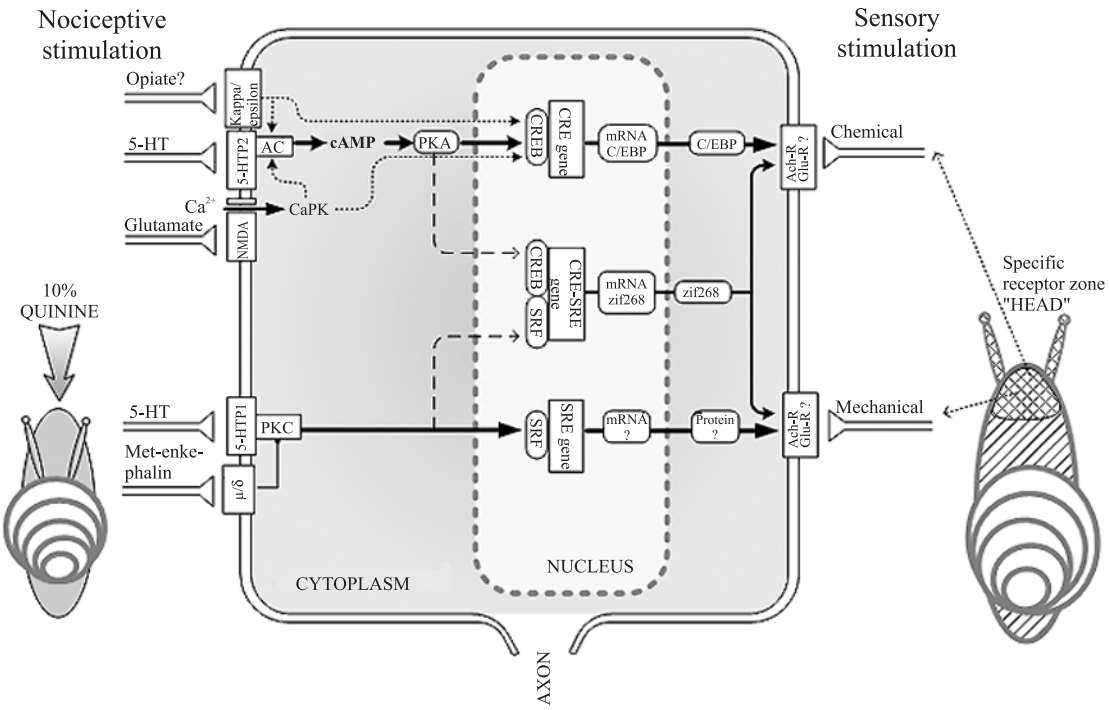

Fig. 2 . Scheme of specific molecular-genetic postsynaptic processes induced by heterogeneous sensory stimulation in LPl1 neuron in edible snail. 5-HT (5-hydroxytryptamine): serotonin receptor; NMDA (N-methyl-D-aspartate): glutamate receptor; AC: adenylyl cyclase; PKA: protein kinase A; PKC: protein kinase C; CaPK: calcium-dependent protein kinase; Ach-R: acetylcholine receptor; Glu-R: glutamate receptor.

chemical receptors of the head (Fig. 2). Moreover, both sensory inputs from snail head are regulated by "early" gene zif268.

Preservation of informational significance of heterogenic excitations coming to neurons during its postsynaptic processing is a fundamental principle of systemic information processing in the brain and its integration in individual neuronal cells [2]. We studied possible mechanisms of intraneuronal information processing with preservation of its sensory significance. Stimuli converging to the neuron induce specific neurochemical reactions in the cytoplasm and nucleus. Second messengers activate various transcriptional factors and genes, which leads to synthesis of synapse-specific mRNA and proteins responsible for long-term modification of the synapse. This chemical "projection" of synaptic connections to neuronal genome was postulated as a central element of the hypothesis on integrative neuronal activity [2]. The described mechanism of preservation and processing of information converging to neurons seems to involve not individual synaptic connections, but certain groups of synapses. However, one can assume that some types of learning, *e.g*. classical conditioning, require more complicated and delicate postsynaptic molecular mechanisms for mediating and processing. For example, it is quite possible that highly specific relationship between certain genes and synapses can include neurospecific protein metabolism [3]. These questions require further investigations.

Summing the data obtained, we conclude that preservation of informational magnitude of excitations converging to individual neurons is based on interrelated and specific postsynaptic neurochemical processes.

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