Effect of Corticotropin-Like Intermediate Lobe Peptide on Presynaptic and Postsynaptic Glutamate Receptors and Postsynaptic GABA Receptors in Rat Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 147, No. 3, pp. 291-294, March, 2009
Original article submitted July 10, 2008

We studied the effect of corticotropin-like intermediate lobe peptide (CLIP) on presynaptic NMDA receptors and postsynaptic GABA, NMDA, and AMPA receptors in rat brain. CLIP inhibited presynaptic and postsynaptic NMDA receptors, but potentiated postsynaptic GABA and AMPA receptors. Our results indicate that CLIP modulates function of ionotropic receptors for glutamate and GABA.

Key Words: *CLIP; receptors for GABA, AMPA, and NMDA; synaptosomal $^{45}Ca^{2+}$ uptake*

Corticotropin-like intermediate lobe peptide (CLIP) is a fragment of ACTH (18-39). This compound was detected in various regions of the brain and, particularly, in nerve fibers [3,4]. CLIP initiates and increases paradoxical sleep, while ACTH has no effect on sleep [11]. The phase of paradoxical sleep (or phase of rapid eye movement) is important for memory consolidation [12]. Restraint stress is followed by a considerable increase in blood CLIP concentration (by 56%) and induction of sleep in rats [2]. As differentiated from ACTH, CLIP increases the amplitude of the population potential in the hippocampus (by 200%) that results in enhanced neuronal excitability [9].

GABA receptors play an important role in sleep [5,6]. Moreover, AMPA and NMDA glutamate receptors are involved in learning and memory [7]. Here we studied a direct effect of CLIP on postsynaptic receptors for GABA, AMPA, and NMDA and presynaptic receptors for NMDA that modulate the release of transmitters from presynaptic endings.

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MATERIALS AND METHODS

Activation of the receptor recognition site is directly or indirectly related to function of ion channels, opening of channels, and increased flux through the neuronal membrane. Hence, the activity of glutamate receptors may be evaluated biochemically from a change in transmembrane calcium flux [13]. $^{45}Ca^{2+}$ uptake in synaptosomes of rat brain cortex was studied upon stimulation with NMDA. Cerebral cortical synaptosomes were isolated from newborn Wistar rats (9-10 days of life) by the standard method. The brain was homogenized (glass-Teflon) in a 10-fold volume of cold sucrose (0.32 M, 900 rpm). The homogenate was centrifuged at 1500*g* for 10 min. The supernatant was centrifuged at 10,000*g* for 20 min. For accumulation of radioactive label, the P_2 fraction of synaptosomes was suspended in incubation buffer A of 132 mM NaCl, 5 mM KCl, 5 mM HEPES, and 10 mM glucose (pH 7.4, protein concentration 1.5-2.0 mg/ml). Calcium concentration in the final volume was 1.25 mM

0007-4888/09/1472-0319

(1.4 mCi/ml). NMDA (200 mM NMDA and 5 mM glycine) was used to stimulate $^{45}Ca^{2+}$ uptake in synaptosomes. After 3-min incubation with NMDA at 37°C, $^{45}Ca^{2+}$ uptake was stopped by filtration through GF/B fiberglass filters (Whatman). Threefold washing was conducted with cold buffer solution B of 145 mM HEPES, 10 mM Tris, and 5.4 mM Trilon B (pH 7.4). All measurements were performed in 3-4 parallel samples (2-4 independent experiments). The samples were analyzed on a liquid scintillation β-counter.

The specific uptake of $^{45}Ca^{2+}$ in synaptosomes was calculated as follows:

$$K_{(43/21)}=[(Ca_4-Ca_3)/(Ca_2-Ca_1)]\times100\%,$$

where Ca_1 is $^{45}Ca^{2+}$ uptake in the control (without NMDA and CLIP); Ca_2 is $^{45}Ca^{2+}$ uptake in the control upon stimulation by NMDA (without CLIP); Ca_3 is $^{45}Ca^{2+}$ uptake in the presence of CLIP (without NMDA); and Ca_4 is $^{45}Ca^{2+}$ uptake in the presence of NMDA and CLIP.

Electrophysiological studies were performed on the culture of rat hippocampal neurons. Hippocampal neurons were obtained from newborn rats (1-2 days of life) by the method of trypsinization and pipetting. The suspension of cells in the culture medium (3 ml) was placed into a 6-well plate (Nunc) or petri dishes. The glasses coated with poly-L-lysin were put in petri dishes. The concentration of cells was 2.5×10^{-6}-5×10^{-6} cells/ml. The culture medium consisted of minimum Eagle's medium and DME/F12 medium (1:1) and contained 10% fetal bovine serum, 2 mM glutamine, 50 mg/ml gentamicin, 15 mM glucose, and 20 mM KCl (pH 7.0-7.4). The plates were maintained in a CO_2 incubator at 37°C and 100% humidity. Cytosine arabinoside at a concentration of 10-20 mM/ml was added on day 2-3 of culturing. Glucose (1 mg/ml) was added to the medium after 6-7 days of culturing. Otherwise, the medium was replaced (depending on the next series).

Transmembrane fluxes were induced by application of NMDA, GABA, or kainic acid (KA). They were recorded electrophysiologically by the whole-cell patch-clamp technique on an EPC-9 device (HEKA). The substances were applied by the method of rapid superfusion. Since AMPA causes a strong and rapid desensitization of AMPA receptors, these receptors were activated by KA [8].

The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

$^{45}Ca^{2+}$ uptake in synaptosomes of rat brain cortex upon stimulation with NMDA is related to the activation of glutamate NMDA receptors. NMDA-induced $^{45}Ca^{2+}$ uptake in synaptosomes was shown to decrease after treatment with the following inhibitors of NMDA receptors: MK-801 (IC_{50}~1 mM), CPP (IC_{50}~100 mM), memantine (IC_{50}~0.4 mM), and Mg^{2+} (IC_{50}~100 mM). Therefore, NMDA activates ionotropic NMDA receptors in the P_2 fraction of cerebral cortical synaptosomes in rats [1]. CLIP at low concentrations (10^{-14}-10^{-11} M) had an inhibitory effect on NMDA-induced $^{45}Ca^{2+}$ uptake in synaptosomes of rat brain cortex (IC_{50}~5.6×10^{-13} M). The inhibition of $^{45}Ca^{2+}$ uptake was most significant under the influence of CLIP at a concentration of 10^{-11} M. CLIP at a higher concentration did not modulate NMDA-induced $^{45}Ca^{2+}$ uptake in synaptosomes (Fig. 1). These data indicate that CLIP affects presynaptic NMDA receptors in the P_2 fraction of cerebral cortical synaptosomes in rats.

An electrophysiological study by patch-clamp technique showed that CLIP at various concentrations (10^{-14}-10^{-8} M) causes blockade of NMDA-induced fluxes in the culture of rat hippocampal neurons ($p\leq0.05$). The inhibition of postsynaptic NMDA receptors was most pronounced after treatment with CLIP at a concentration of 10^{-11} M (inhibition ~60%, Fig. 2).

CLIP had a complex action on KA-induced fluxes in hippocampal neurons from rat brain. CLIP at a concentration of 10^{-14} M causes blockade of KA-induced fluxes (by 16%). However, increasing the concentration of CLIP (10^{-13}-10^{-8} M) was accompanied by potentiation of AMPA-receptors on hippocampal neurons from rat brain (Fig. 2). The potentiation of KA-induced fluxes reached maximum under the influence of CLIP at a concentration

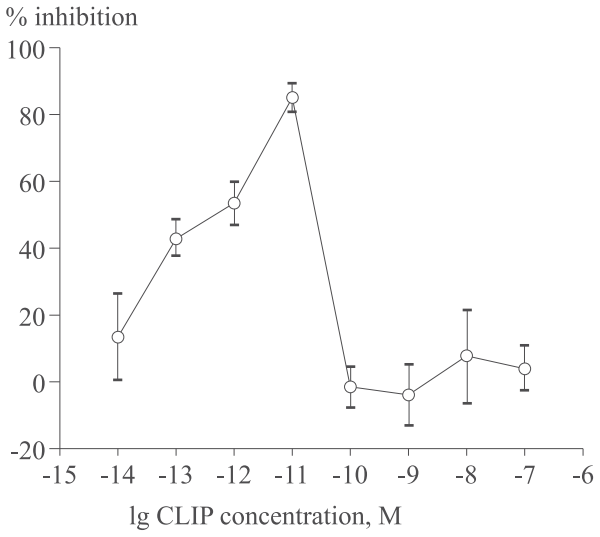

Fig. 1. Effect of CLIP on synaptosomal $^{45}Ca^{2+}$ uptake in rat brain cortex upon stimulation with NMDA. Control, 100%.

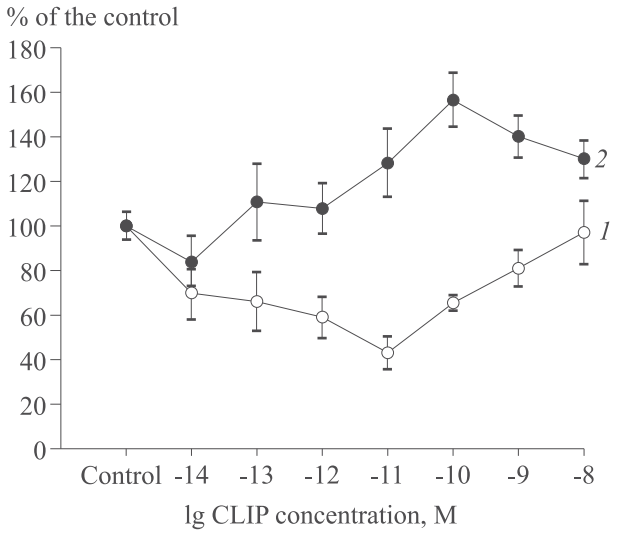

Fig. 2. Effect of CLIP on NMDA-induced (*1*) and KA-induced fluxes (*2*) in the culture of rat hippocampal neurons.

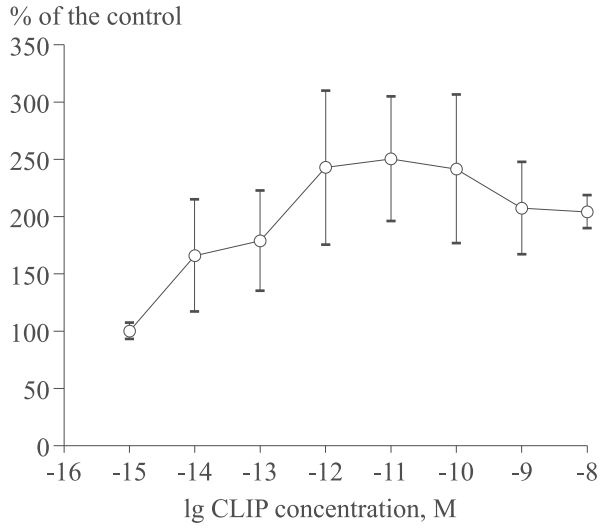

Fig. 3. Effect of CLIP on GABA-induced fluxes in the culture of rat hippocampal neurons.

of 10^{-10} M (156% compared to the control). These differences were statistically significant at a CLIP concentration of 10^{-11}-10^{-8} M ($p \leq 0.05$).

CLIP at concentrations of 10^{-14}-10^{-8} M had a strong potentiating effect on GABA receptors in rat brain hippocampus. The potentiation of GABA-induced fluxes was most pronounced under the influence of CLIP at a concentration of 10^{-11} M (250.3% of the control, Fig. 3). These differences were statistically significant at a CLIP concentration of 10^{-12}-10^{-8} M ($p \leq 0.05$).

CLIP had a similar effect on presynaptic and postsynaptic NMDA receptors (Figs. 1 and 2). The inhibition of synaptosomal $^{45}Ca^{2+}$ uptake upon stimulation with NMDA and blockade of NMDA-induced fluxes are mediated by similar mechanisms. Moreover, these processes occur in the same range of CLIP concentrations. The maximum inhibitory effect was achieved at a CLIP concentration of 10^{-11} M. However, the inhibition of presynaptic NMDA receptors was not observed after treatment with CLIP at a concentration of 10^{-10} M. By contrast, blockade of NMDA-induced fluxes in postsynaptic receptors was shown to decrease under the influence of CLIP at this concentration. The inhibition of postsynaptic NMDA receptors was completely abolished at a higher concentration of CLIP (10^{-8} M). It is important that CLIP has a potentiating effect on AMPA receptors. The CLIP-induced potentiation of AMPA receptors is probably related to an activating effect of this substance on memory [11]. Blockade of NMDA receptors was not observed with an increase in the concentration of CLIP. These processes probably contribute to memory consolidation, which involves NMDA receptors.

Much attention is paid to studying the stimulatory effect of ACTH fragments on cognitive function and memory. Published data show that some fragments of ACTH are most potent in improving the cognitive activity [10]. It is difficult to evaluate the role of CLIP in cognitive improvement, since this neuropeptide has a soporific effect. It should be emphasized that the CLIP-induced phase of paradoxical sleep has a major role in memory consolidation. Moreover, CLIP has an activating effect on hippocampal neurons. These data indicate that CLIP is involved in the improvement of memory consolidation.

Our results suggest that an activating effect of CLIP on AMPA receptors contributes to memory consolidation during paradoxical sleep. Sleep is related to the potentiation of GABA receptors.

This work was supported by the International Science and Technology Center (grant No. 2704).

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