Effect of Interleukin-1β on NMDA-induced $^{45}Ca^{2+}$ Uptake by Synaptosomes of Rat Brain Cortex

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The effect of interleukin-1β on presynaptic NMDA receptors was evaluated by studying NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes from rat brain cortex. Interleukin-1β inhibited $^{45}Ca^{2+}$ uptake by synaptosomes. Our results indicate that interleukin-1β modulates presynaptic NMDA receptors and is probably involved in the regulation of synaptic transmission in the central nervous system.

Key Words: *interleukin-1β; presynaptic NMDA receptors; synaptosomes; $^{45}Ca^{2+}$ uptake*

The polypeptide interleukin-1β (IL-1β) produces various effects on the central nervous system (CNS). For example, IL-1β mediates nervous excitation and sleep, affects secretion of adrenocorticotropic hormone, plays a role in the acute phase response to damage and infection, and modulates synaptic transmission in CNS [7,8,10,11]. Histochemical studies showed that IL-1β is localized in neurons. Endogenous IL is probably synthesized in astrocytes and microglia [4].

IL-1 has no direct effect on L-glutamate release from synaptosomes in rat striatum under conditions of KCl-induced depolarization. Moreover, IL-1β does not directly modulate Ca^{2+} uptake by synaptosomes upon treatment with KCl [2]. Taking into account published data that Il-1β modulates synaptic transmission, it can be hypothesized that the effects of this substance are mediated by presynaptic mechanisms [12].

Presynaptic NMDA receptors are a possible target for the effect of IL-1β. Published data show that IL-1β modulates activity of postsynaptic NMDA receptors [13]. Previous studies showed that presynaptic NMDA receptors modulate synaptic transmission in glutamatergic [9], cholinergic [3], and dopaminergic synapses [6]. IL-1β probably affects presynaptic NMDA receptors and, therefore, modulates synaptic transmission in various neurotransmitter systems of CNS

The effect of IL-1β on presynaptic NMDA receptors was evaluated by studying $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex.

MATERIALS AND METHODS

Synaptosomes were isolated from rat brain cortex by the standard method of Hajos [5]. Experiments were performed with the cerebral cortex of newborn Wistar rats (day 9 of life). For accumulation of a radioactive label, P_2 fraction of synaptosomes was suspended in incubation buffer A. The buffer contained 132 mM NaCl, 5 mM KCl, and 5 mM HEPES (pH 7.4, protein concentration 1.5-2.0 mg/ml). $^{45}Ca^{2+}$ uptake by synaptosomes was stimulated by glutamate (200 μM) [1] or NMDA (200 μM NMDA and 5 μM glycine). After 3-min incubation with NMDA receptor agonist at 37°C, uptake was blocked by filtering of the mixture through GF/B fiberglass filters (Whatman). The samples were washed 3 times with cold buffer solution B containing 145 mM KCl, 10 mM Tris, and 54 mM Trilon B (pH 7.4) and studied on a liquid scintillation β-counter. The measurements were performed in 4 parallel

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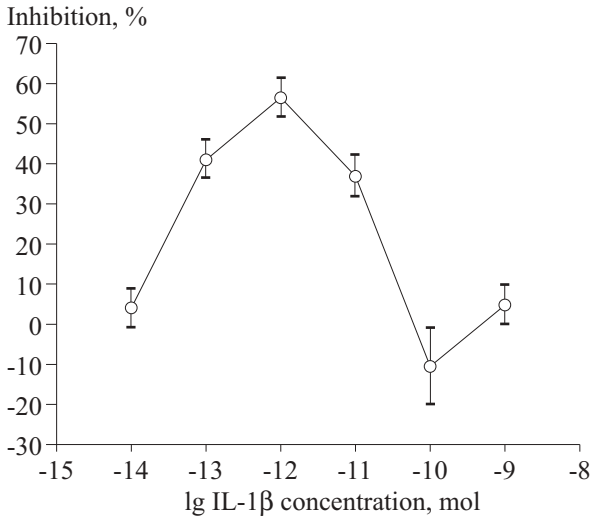

Fig. 1. Effect of IL-1β on NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes from rat brain cortex. Ordinate: inhibition of $^{45}Ca^{2+}$ uptake by synaptosomes of the cerebral cortex (%).

samples (3-4 independent experiments). The amount of $^{45}Ca^{2+}$ accumulated in synaptosomes was calculated as the difference between the concentration of radioactive label in the presence and absence of uptake stimulator NMDA. It was expressed in percents of the control (100%).

RESULTS

We studied the effect of IL-1β in concentrations of 10^{-14}-10^{-9} M on $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex upon stimulation with 200 μM NMDA. IL-1β in concentrations of 10^{-13}-10^{-11} M inhibited NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex ($p<0.05$). IL-1b in a concentration of 10^{-12} M most significantly inhibited $^{45}Ca^{2+}$ uptake by synaptosomes (56.5%, $IC_{50}=4.47\times10^{-13}$ M). IL-1β in concentrations of 10^{-14}, 10^{-10}, and 10^{-9} M had little effect on $^{45}Ca^{2+}$ uptake by synaptosomes. The dependence of NMDA-induced $^{45}Ca^{2+}$ uptake by synaptosomes on IL-1β concentration was described by a bell-shaped curve (Fig. 1).

IL-1β inhibited glutamate-induced $^{45}Ca^{2+}$ uptake by synaptosomes of rat brain cortex. Treatment with IL-1β in a concentration of 10^{-11} M decreased glutamate-induced $^{45}Ca^{2+}$ uptake by synaptosomes by 10.3%.

Our results indicate that IL-1β modulates function of NMDA receptors upon activation with excitatory amino acids. One of the mechanisms of modulation of synaptic transmission in CNS can be associated with the effect of IL-1β on presynaptic NMDA receptors and regulation of transmitter release in neurotransmitter systems. It can be hypothesized that the effects of IL-1β on synaptic transmission are realized via various receptors, including other subtypes of presynaptic glutamate receptors.

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