BIOPHYSICS AND BIOCHEMISTRY

Donepezil Eliminates Suppressive Effects of β-Amyloid Peptide (1-42) on Long-Term Potentiation in the Hippocampus

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β-Amyloid peptide 1-42 in a concentration of 200 nM impairs induction of long-term posttetanic potentiation of population spike in CA1 pyramidal neurons in rat hippocampal slices. Application of donepezil, a drug used for the treatment of Alzheimer disease, in a concentration of 1 μM eliminates the suppressive effect of β-amyloid peptide 1-42 on long-term posttenanic potentiation in the hippocampus.

Key Words: *β-amyloid peptide; donepezil; long-term potentiation; hippocampus*

Alzheimer disease is a prevalent neurodegenerative disorder characterized by severe memory deficit and other cognitive defects. Memory deficit at the early stages of the disease is associated with functional changes in neuronal synaptic contacts, whereas extensive neurodegerative brain lesions were found at later stages [13]. One of the major pathogenesis factor of Alzheimer disease is β-amyloid peptide (β-AP). This peptide is present in high concentrations in the cerebrospinal fluid of patients with Alzheimer disease; postmortem examination also reveals aggregates of this peptide in the form of characteristic plaques in the brain tissue [8]. Under experimental conditions, β-AP in submicromolar concentrations impairs synaptic transmission in glutamate synapses [10], while in micromolar concentration it induces apoptotic neurodegeneration [4].

The mechanisms underlying disturbances of synaptic transmission caused by subapoptotic doses of β-AP attract much attention. Experiments showed that β-AP inhibits basal neurotransmission in glutamatergic synapses [10], impairs induction of long-term posttetanic potentiation (LTP) [14,15], and enhances induction of long-term synaptic depression [3] in the hippocampus. The mechanisms of these effects of β-AP are little studied. It was assumed that complicated cascade of biochemical reactions results in imbalance in glutamate receptor phosphorylation processes and the number of these receptors in the post-synaptic membrane decreases [1].

The question arises, whether the drugs used for the treatment of Alzheimer disease can correct disturbances in glutamatergic transmission caused by β-AP. These properties were found in two antiamnestic agents, memantine (NMDA-receptor antagonist) and huperzine A (acetylcholinesterase inhibitor) [15]. These agents were shown to reverse the inhibitory effects of β-AP on LTP in the hippocampus.

Here we evaluate the capacity of donepezil also used for the treatment of Alzheimer disease to modulate LTP inhibition caused by β-AP.

MATERIALS AND METHODS

Experiments were performed on hippocampal slices from young male Wistar rats weighing 80-110 g. Immediately after preparation, the slices were placed

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into registration chamber and perfused with modified Ringer solution containing (in mM) 124 NaCl, 3 KCl, 2.5 $CaCl_2$, 2.5 $MgSO_4$, 1.25 Na_2HPO_4, 26 $NaHCO_3$, and 10 D-glucose continuously saturated with carbogen (5% CO_2) and heated to 29-30°C. Registration of electric activity was started 1.5-2 h after slice preparation. Focal responses in the CA1 pyramidal field induced by individual rectanglular pulses (0.1 msec, 1/15 sec) applied to stratum radiatum were recorded with a glass microelectrode filled with 1.5 M NaCl (resistance 2-5 mΩ). Stimulus intensity was selected so, that the peak response amplitude reflecting integrated spike response of pyramidal neurons (pop-spike) constituted one-half of its maximum value. Pop-spike potentiation was induced by high-frequency stimulation (HFS, 100 Hz, 1 sec) through the same electrodes and with the same stimulus intensity. Only one HFS was performed on each slice. Fifteen minutes before HFS, the flow system was switched to the second reservoir with solution containing the test compounds in the specified concentration. Reverse switch was performed 5 min after HFS. Changes in reactivity of pyramidal neurons were estimated by deviations of the pop-spike amplitude from the mean value determined from 15-min recording under baseline conditions (before flow switch).

Concentrated water solutions of β-AP (1-42) (Sigma) were stored as frozen microdoses and were brought up to the needed concentration with perfusion medium before application. For preparation of donepezil solution, Aricept tablets (Pfizer) containing 5 mg donepezil hydrochloride were used. The obtained solution was filtered through a 0.45-μ filter (Schleicher and Schul). Stock donepezil solution was stored in deionized water at 4°C. Solutions with needed concentration were prepared immediately before application. Each slice was used to test only one donepezil concentration.

The obtained results were expressed as means and errors of the mean ($M \pm m$). The data were processed statistically using Mann–Whitney test and Student's t test.

RESULTS

According to published data, HFS of Schaffer collaterals produces long-lasting facilitation of the evoked potential in CA1 hippocampal area, its magnitude and duration depended on HFS intensity [3,14,15]. In our experiments, standard HFS (100 Hz, 1 sec) resulted in the pop-spike LTP: 30 min after tetanus, its amplitude increased to 139±8% (n=5; Figs. 1-3). Slice perfusion with 1 μM solution of antiamnestic agent donepezil did not significantly affect reactivity of hippocampal neurons, which was seen from the lack of significant changes in pop-spike amplitude. At the same time, donepezil produced a trend toward LTP intensification. Mean pop-spike amplitude after 15-min perfusion of the slices with donepezil solution (before HFS) was 90±9% and 30 min after HFS it increased to 158±13% (n=5). However, these values did not significantly differ from the corresponding values, obtained in normal physiological solution (100±6% before HFS and 139±8% after HFS).

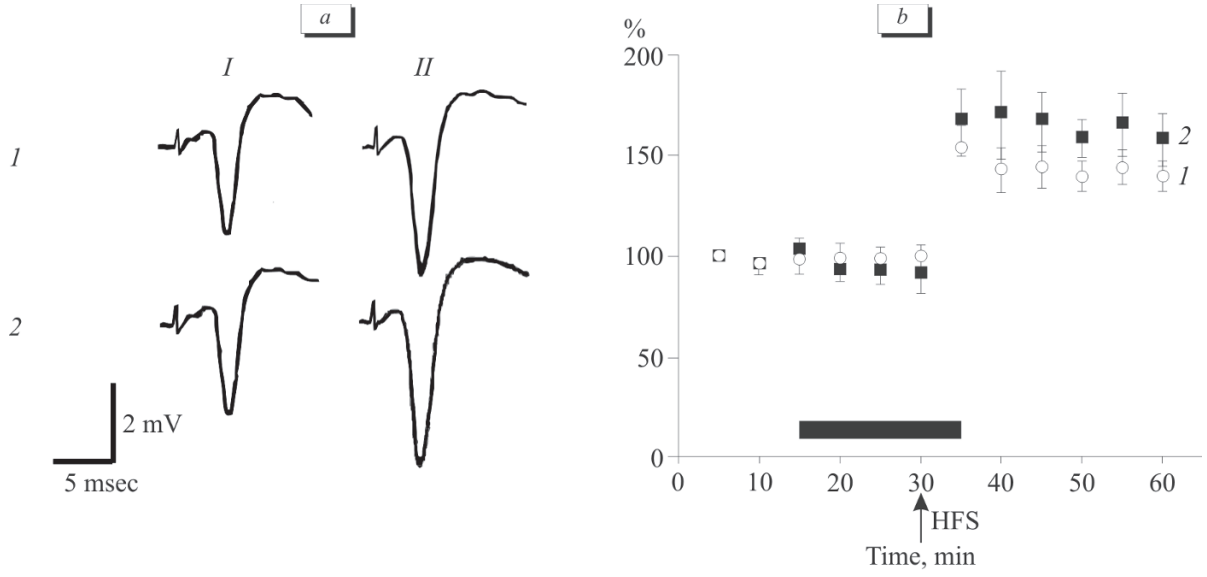

Fig. 1. Donepezil produces a trend toward LTP intensification in the hippocampus. *a)* pop-spike record before (*I*) and 30 min after HFS (*II*) from hippocampal slice perfused with normal physiological solution (*1*) and from another slice perfused with solution containing 1 μM donepezil (*2*); *b*) dynamics of amplitude of integrated pop-spike hippocampal slices during perfusion with control solution (*1*; n=5) and solution containing 1μM donepezil (*2*; n=5). Arrow: time of HFS, heavy line: time of donepezil application.

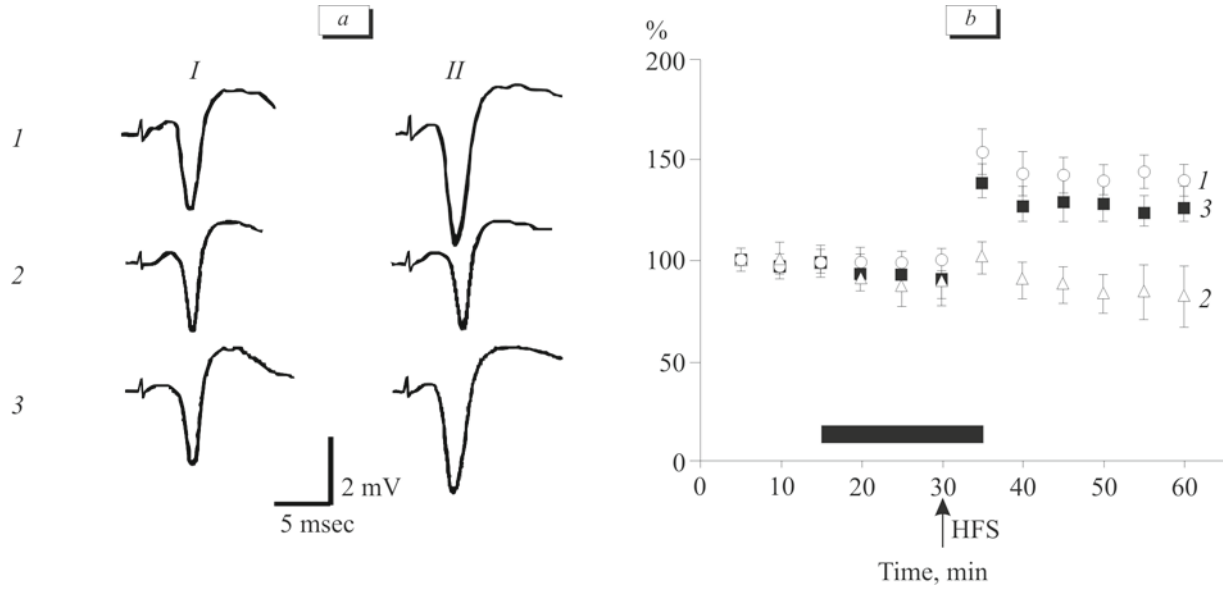

Fig. 2. Elimination of inhibitory effects of β-AP on LTP with donepezil. *a*) pop-spike record before (*I*) and 30 min after HFS (*II*) from three different hippocampal slices; the first slice was perfused with normal physiological solution (*1*), the second was exposed to 200 nm β-AP for 20 min (*2*), and the third was exposed to 200 nM β-AP+1 μM donepezil (*3*); *b*) dynamics of amplitude of integrated pop-spike hippocampal slices during perfusion with control solution (*1*; *n*=5), solution containing 200 nm β-AP, (*2*; *n*=5), and 200 nM β-AP+1 μM donepezil (*3*; *n*=5). Arrow: time of HFS, heavy line: time of preparation exposure in solution.

Perfusion with solution containing 200 nM β-AP 1-42, did not significantly affect the baseline pop-spike characteristics, but impaired LTP induction in hippocampal slices after HFS (Fig. 2). Mean pop-spike amplitude after 15-min perfusion of slices with β-AP solution (before HFS) and 30 min after HFS was 87±10 and 82±15%, respectively (*n*=5). The latter significantly differed from the control ($p<0.01$; Fig. 3).

When 200 nM β-AP and 1μM donepezil were applied simultaneously, donepezil eliminated the suppressive effects of β-AP on LTP induction. The mean pop-spike amplitude was 91±11% after 15-min combined application of these solutions, and 127±9% 30 min after HFS (*n*=5). Latter value significantly surpassed the corresponding parameter obtained in β-AP solution (with error probability $p<0.05$; Fig. 3).

We found that donepezil eliminates suppressive effects of β-AP on LTP in the hippocampus. This effect was observed with donepezil concentration of 1 μM, which is close to its therapeutic doses [9]. These findings attest to a of new possible mechanism of the therapeutic action of donepezil and extents our knowledge about this action, which include intensification of acetylcholine transmission through acetylcholinesterase inhibition or increase in the number of nicotinic receptors [5,12] and neuroprotection [4]. Apart from acetylcholinesterase, molecular targets of donepezil are NMDA-receptors [7], σ_1-receptors [2,6], voltage-gated K^+- and Ca^{2+}-channels [11].

Mechanisms of donepezil restitution of LTP disturbed by β-AP require special investigations. Standard explanation (acetylcholinesterase inhibition) cannot be used for the observed effect, because afferent fibers forming acetylcholine terminals on CA1 interneurons are cut during slice preparation and, consequently, acetylcholine transmission does not participate in pop-spike generation. Voltage-gated channels cannot also be regarded as possible donepezil target, since they bind donepezil in concentrations higher than 1 μM [11]. NMDA receptors and/or σ_1 receptors seem to be appropriate molecular targets for donepezil, because their activation can affect plasma Ca^{2+} level and

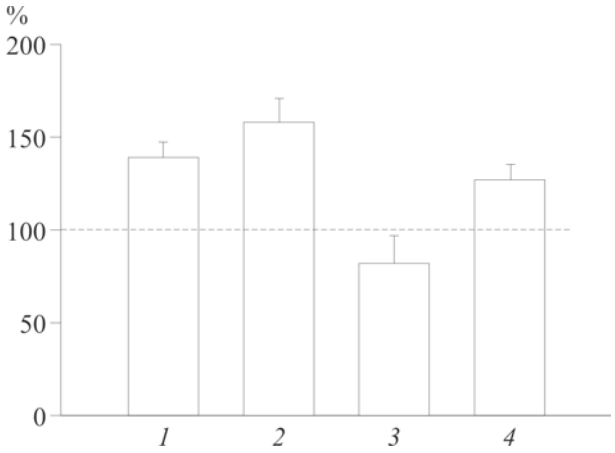

Fig. 3. Mean amplitude of pop-spike recorded 30 min after HFS from slices perfused before HFS with normal physiological solution (*1*), solution containing 1 μM donepezil (*2*), 200 nM β-AP (*3*), and 200 nM β-AP+1 μM donepezil (*4*). Dotted line: control response in the beginning of the experiment (100%).

phosphorylation processes. Additional argument for σ_1 receptor participation in the observed effects of donepezil appeared from the results of behavioral experiments, where donepezil eliminated amnesia induced by β-AP and σ_1 receptor antagonist BD1047 inhibited the effect of donepezil [6].

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