Impact of Amyloid-β Peptide (1-42) on Voltage-Gated Ion Currents in Molluscan Neurons

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Different types of voltage-gated ion currents were recorded in isolated neurons of snail *Helix pomatia* using the two-microelectrode voltage-clamp technique. Application of amyloid-β peptide (1-42, 1-10 μM) in the bathing solution did not change delayed rectifier K^+-current and leakage current, but enhanced inactivation of Ca^{2+}-current and blocked Ca^{2+}-dependent K^+-current.

Key Words: *amyloid-β peptide; Ca^{2+} channels; K^+-channels; mollusk neurons*

Amyloid-β peptide (Aβ) is believed to play a key role in the pathogenesis of Alzheimer disease. It is shown that Aβ modulates the function of voltage-gated Ca^{2+}- and K^+-channels of surface neuronal membrane [2-6,8-13,15]. Three mechanisms of this modulation are described: 1) direct interaction of Aβ with channel proteins; 2) indirect effect of Aβ on channel proteins via modulation of their phosphorylation; 3) activation of the genome and increase in the density of membrane channel proteins. Despite numerous studies in this field, many aspects remain unexplored. In particular, the impact of Aβ on inactivation of Ca^{2+}-channels and conductance of Ca^{2+}-activated K^+-channels is poorly understood and is the objective of this study.

MATERIALS AND METHODS

Experiments were conducted on isolated neurons of *Helix pomatia* snail using the two-microelectrode voltage-clamp technique. Microelectrodes were filled with 2M potassium citrate solution, electrode resistance was 12-14 MΩ. MEZ-7101 microelectrode amplifier and a CEZ-1100 voltage clamp amplifier (Nihon Kohden) were used. Voltage-gated Ca^{2+}- and K^+-currents were recorded during depolarizing stimuli. Leakage current was measured during the corresponding hyperpolarizing stimuli. Reference solution for measuring K^+-current contained 100 mM NaCl, 4 mM KCl, 5 mM $CaCl_2$, 4 mM $MgCl_2$, 3 mM $NaHCO_3$, and 5 mM tris-Cl (pH 7.6). For measuring Ca^{2+}-current, sodium-free solution (4 mM KCl, 10 mM $CaCl_2$, 4 mM $MgCl_2$, 95 mM TEA, 5 mM 4-AP, and 5 mM tris-Cl, pH 7.6) was used containing K^+-channel antagonists tetraethylamminoum (TEA) and 4-amino-pyridine (4-AP). Concentrated aqueous solutions of Aβ (1-42) (Sigma) were stored frozen in the form of microdoses. Before the experiment, Aβ was thawed and just before the applying adjusted to the desired concentration with perfusing medium.

Statistical analysis was performed using Prism 3.0 (GraphPad). Statistical significance of results was estimated by unpaired Student's *t* test.

RESULTS

Ca^{2+}-current was activated by 200-msec depolarizing test stimuli every 2 min from the holding potential (V_h) of -60 mV to values lying in the range from -30 to 60 mV with 10 mV steps. The maximum current was usually generated at a potential of 30 mV. The inward Ca^{2+} current peaked after 22±9 msec and then decayed in a mono- or biexponential manner. This decline, according to published data, reflects voltage-gated and Ca^{2+}-dependent inactivation of Ca^{2+}-channels [1]. In our experiments, the half-decay time (τ) of Ca^{2+}-current at 30 mV was 116±24 msec.

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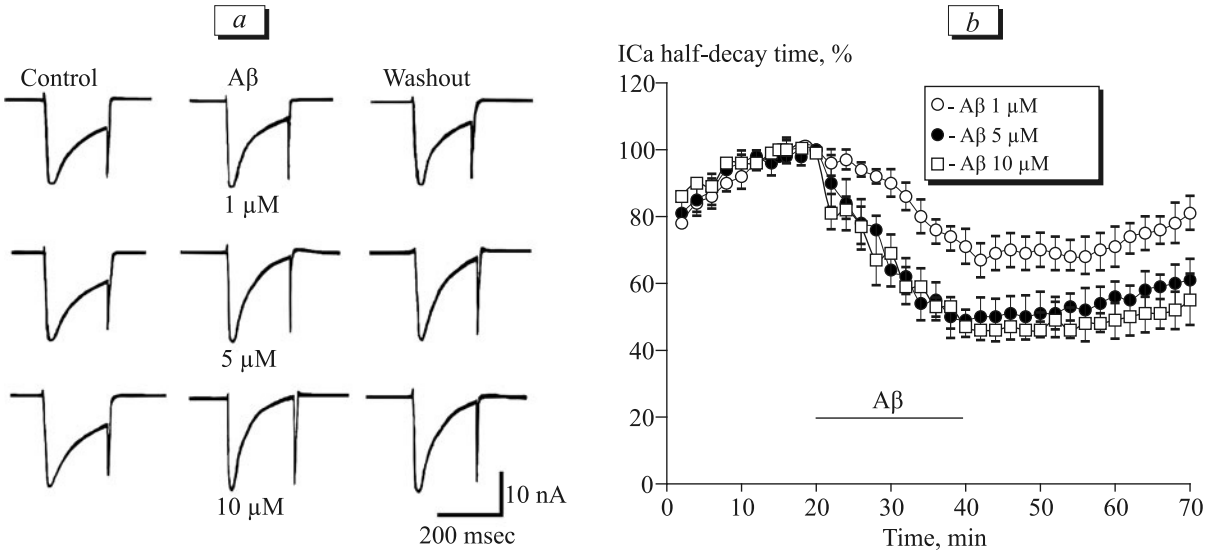

Fig. 1. Acceleration of decline in voltage-gated Ca^{2+}-current under the influence of Aβ (1-42). *a)* Ca^{2+}-current recorded in three different cells during the test stimulus shifting the membrane potential from -60 mV to 30 mV, in the control, in the presence of various concentrations of Aβ, and after washout cells from Aβ; *b)* time course of the mean half-decay time (τ) of Ca^{2+}-current in the presence of Aβ in a concentration of 1 (*n*=5), 5 (*n*=6) and 10 μM (*n*=5).

Aβ was added to the bathing solution to concentrations of 1, 5 or 10 μM for 20 min and then the cell was washed with the control solution for 30 min. It was found that Aβ did not significantly affect the peak amplitude of I_{Ca} and did not shift the current-voltage characteristics (CVC) of the inward current

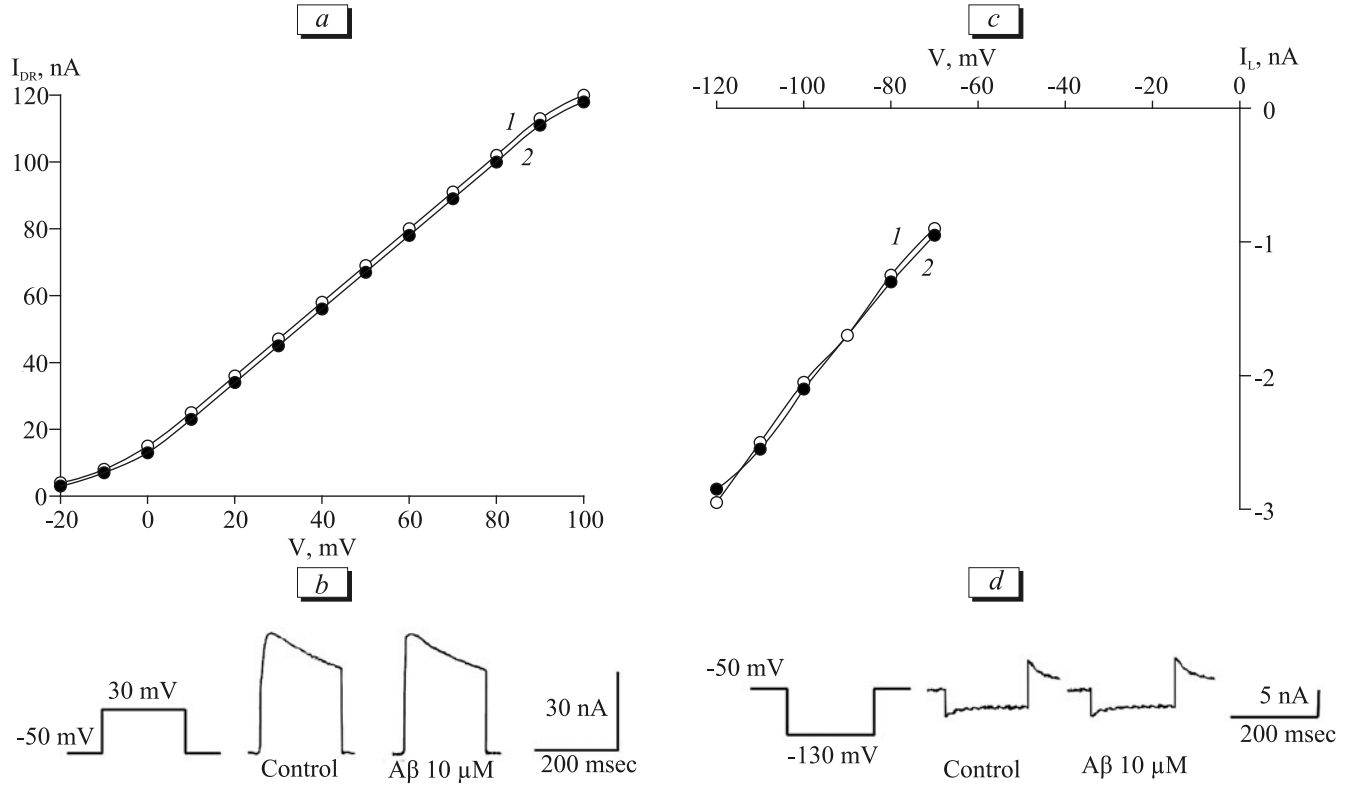

Fig. 2. The absence of changes in delayed rectifier K^+-current (I_{DR}) and leakage current (I_L) under the influence of Aβ (1-42). *a*) CVC of I_{DR} in the control (*1*) and in the presence of 10 μM Aβ (*2*); *b*) I_{DR} record in a single cell during the test stimulus shifting the membrane potential from -50 to 30 mV in the control and in the presence of 10 μM Aβ: the test stimulus and the corresponding record of the current; *c*) CVC of I_L in the control (*1*) and in the presence of 10 μM Aβ (*2*); *d*) I_L record in a single cell during the test stimulus shifting the membrane potential from -50 to -130 mV in the control and in the presence of 10 μM Aβ: the test stimulus and the corresponding record of the current.

along the voltage axis. At the same time it considerably accelerated I_{Ca} decay, *i.e.* reduced τ, which attested to enhanced inactivation of Ca^{2+}-channels under the influence of Aβ (Fig. 1, *a*, *b*). The effect of Aβ on τ of Ca^{2+}-current was dose- and time-dependent. After 20-min application of 1 μM Aβ, τ decreased to 71±5% of the control value (n=5). The corresponding values of τ in experiments with application of 5 and 10 μM Aβ were significantly lower (p<0.05): 49±3% (n=6) and 47±3% (n=5), respectively. Washout with control solution for 30 min led to only partial recovery of τ.

Voltage-gated K^+-current was studied in 13 isolated neurons in normal Ringer's solution. This current was activated with depolarizing stimuli that shifted the membrane potential from V_h -50 mV to values lying in the range from -30 to 100 mV with 10 mV steps. In the majority of cells (9 of 13), CVC of outward K^+-current was a smooth curve and activation and inactivation kinetics of the current was similar at different potentials. The time of activation at 30 mV was 21±3 msec and current decay over 200 msec varied on different cells in the range of 15-30%. These characteristics and high sensitivity of the current to 1-5 mM TEA suggest that this current represents a delayed rectifier K^+-current (I_{DR}) [7]. In the same cells, the leakage current (I_L) was measured by applying hyperpolarizing stimuli shifting the membrane potential from V_h -50 mV to values lying in the range from -80 to -130 mV with 10 mV steps. Aβ was added to the bathing solution for 20 min in concentrations of 1, 5, and 10 μM. It was found that none of the specified concentrations of Aβ caused significant changes in I_{DR} or I_L (Fig. 2, *a*, *b*).

In 4 of 13 cells, total K^+-current apart from IDR contained Ca^{2+}-dependent K^+-current ($I_{K(Ca)}$). In these cells, CVC of the outward K^+-current was described by a N-like curve with an additional peak at 30 mV (Fig. 3, *a*). In Ca-free solution, this additional maximum disappeared and CVC was presented by a smooth curve. The kinetics of the current generated at 30 mV differed from that generated at 100 mV (Fig. 3, *c*).

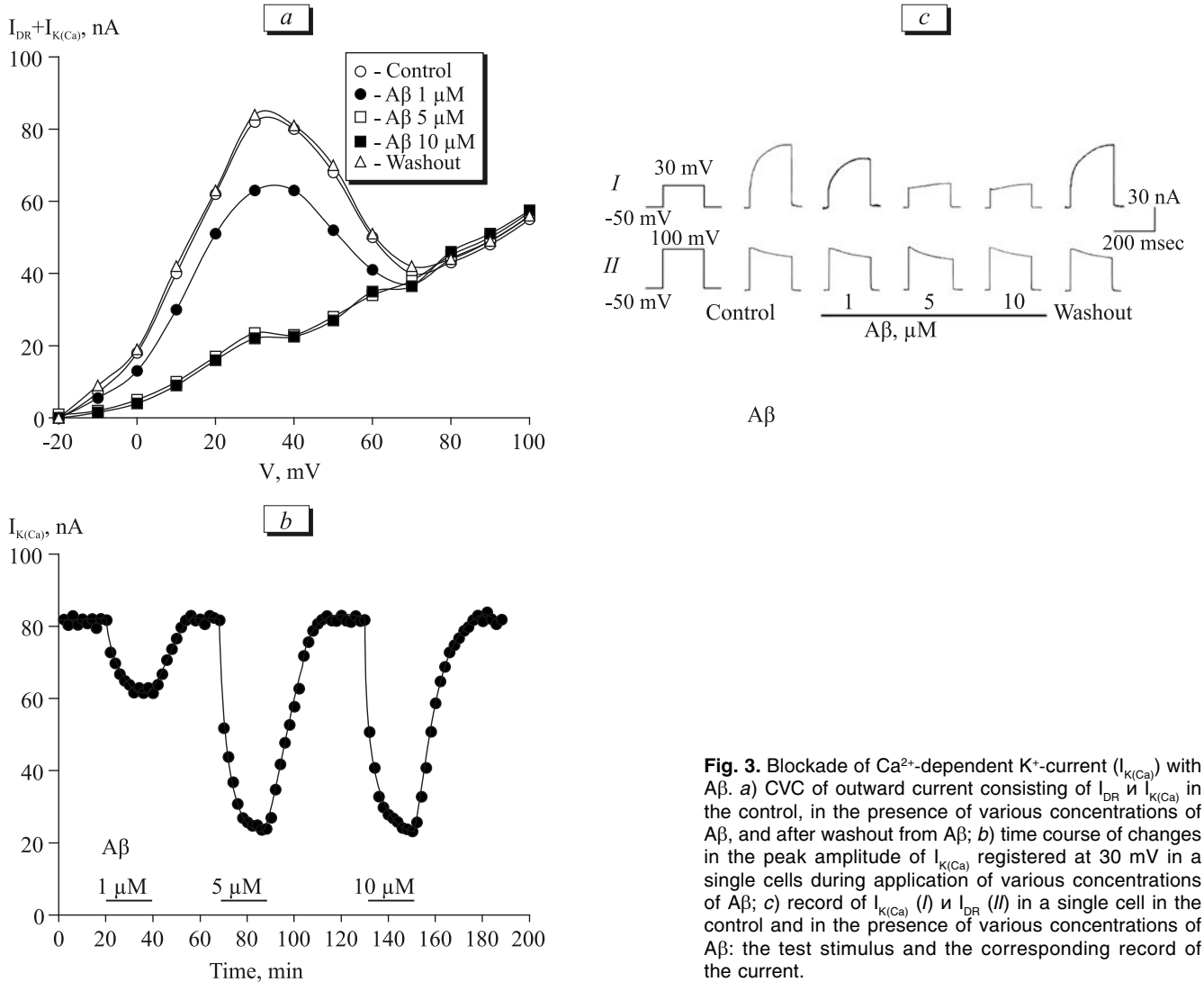

Fig. 3. Blockade of Ca^{2+}-dependent K^+-current ($I_{K(Ca)}$) with Aβ. *a*) CVC of outward current consisting of I_{DR} и $I_{K(Ca)}$ in the control, in the presence of various concentrations of Aβ, and after washout from Aβ; *b*) time course of changes in the peak amplitude of $I_{K(Ca)}$ registered at 30 mV in a single cells during application of various concentrations of Aβ; *c*) record of $I_{K(Ca)}$ (*I*) и I_{DR} (*II*) in a single cell in the control and in the presence of various concentrations of Aβ: the test stimulus and the corresponding record of the current.

The mean activation time of the current at 30 and 100 mV was 162±12 and 19±4 msec, respectively. Thus, as $I_{K(Ca)}$, and I_{DR} could be studied on the same cell, when measuring the current at 30 and 100 mV, respectively.

Application of 1-10 μM Aβ to the bathing solution produced different effects on the two types of current. In the presence of Aβ the current remained unchanged at 100 mV and was significantly inhibited at 30 mV (Fig. 3 *a*, *b*). The inhibitory effect of Aβ on $I_{K(Ca)}$ was dose-dependent, rapid, and completely reversible upon washout with the control solution for 20-30 min (Fig. 3, *b*). The mean values of the peak amplitude of $I_{K(Ca)}$ decreased in the presence of 1, 5, and 10 μM Aβ to 75±11, 25±7, and 24±6% of the control values, respectively.

We described two new electrophysiological effect of Aβ: acceleration of the I_{Ca} decay and a decrease in amplitude of $I_{K(Ca)}$. These findings suggests that Aβ can enhance inactivation of voltage-gated Ca^{2+}-channels and decrease the conductivity of Ca^{2+}-dependent K^+-channels. At the same time, our experiments showed that Aβ does not appreciably affect delayed rectifier K^+-channels and the leakage channels. A possible mechanism of the observed $I_{K(Ca)}$ inhibition in the presence of Aβ can obviously be enhanced inactivation of I_{Ca}, because $I_{K(Ca)}$ depends on Ca^{2+} entry into the cell through voltage-gated Ca^{2+}-channels. However, in our opinion the effect of Aβ on these two types of channels occurs through two independent mechanisms, which is seen from different dynamics of the development and disappearance (upon washout) of Aβ effects on $I_{K(Ca)}$ and I_{Ca}. Fast kinetics of the development and disappearance of Aβ effects on $I_{K(Ca)}$ suggests a direct interaction between the peptide and Ca^{2+}-dependent K^+-channels. The inhibitory effect of Aβ on A-type K^+-current was described previously [2,6]. We showed that Aβ can also inhibit $I_{K(Ca)}$.

In contrast to $I_{K(Ca)}$, the effect of Aβ on I_{Ca} had slow kinetics of development and washout, which suggests that the effect is mediated by metabolic processes. According to published data, calcineurin (protein phosphatase 2B) enhances Ca^{2+}-dependent inactivation of Ca^{2+}-channels in mollusk neurons [1]. The authors demonstrated that calcineurin not only accelerates I_{Ca} decay, but also reduces its peak amplitude. In our view, calcineurin activation explains increased I_{Ca} inactivation, since Aβ has documented ability to activate calcineurin [14].

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