PHYSIOLOGY

Interaction of Acetylcholinesterase Inhibitor Donepezil with Ionic Channels of the Neuronal Membrane

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The effects of donepezil on voltage-dependent Ca^{2+}- and low-threshold K^+-current were studied on isolated molluscan neurons using two-electrode voltage clamp technique. Donepezil reduced the amplitude of voltage-dependent Ca^{2+}-current (IC_{50}=7.9 μM) and shifted the current-voltage relationships toward hyperpolarization. Donepezil in low concentration (5 μM) increased, while in higher concentrations (≥10 μM) decreased the low-threshold K^+-current.

Key Words: *calcium channels; potassium channels; donepezil; molluscan neurons*

Donepezil, an efficient inhibitor of acetylcholine esterase, is widely used in the therapy of Alzheimer's disease [11]. In addition to acetylcholine esterase, other molecular targets of donepezil were found, including K^+- [1,14,15] and Na^+-channels [14] and some receptors as NMDA [9] and sigma-1 receptors [7].

An important feature of donepezil is its neuroprotective effect described on various models. Application of donepezil in concentrations of 0.1-10.0 μM protects neurons from oxygen-glucose deprivation [2], glutamate neurotoxicity [12], and NMDA-toxicity [3]. Of particular interest is the potency of donepezil to prevent the death of neurons triggered by β-amyloid peptide (βA) [4,8], because this peptide is considered to play the key role in the pathogenesis of Alzheimer disease. The mechanisms of βA-toxicity include activation of some types of K^+-channels [6,13] and high-threshold Ca^{2+}-channels [10]. Potassium antagonist tetraethylammonium [13] and calcium antagonist nifedipine [5] reduced βA-toxicity. It can be hypothesized that the neuroprotective effect of donepezil is mediated by blockade of K^+- and Ca^{2+}-channels. The block of potassium channels with donepezil was demonstrated on rat [14,15] and molluscan [1] neurons. However, the effects of donepezil on voltage-dependent Ca^{2+}-current and low-threshold K^+-current (I_A) are still unknown. Our aim was to examine these effects on molluscan neurons.

MATERIALS AND METHODS

The experiments were carried out on isolated neurons from edible snail *Helix pomatia*. The cells were isolated without proteolytic treatment of the ganglion. The neurons were voltage clamped with two 12-14-MΩ microelectrodes filled with potassium citrate (2 M). The currents and potentials were recorded with an MEZ-7101 microelectrode amplifier and a CEZ-1100 voltage clamp amplifier (Nihon Kohden). The data were recorded using a 4-channel RJG pen recorder. Calcium currents were recorded at holding potential of -60 mV. For measuring I_A, the membrane was hyperpolarized to -130 mV. The potential-dependent Ca^{2+}- and K^+-currents were activated by depolarizing pulses. Leakage current was measured by hyperpolarizing pulses shifting

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the membrane potential from -60 mV to -80 mV and further on to -120 mV with 10-mV steps. The current-voltage curves were plotted after subtraction of leakage current. During K^+-current measurements the external solution contained (in mM): 100 NaCl, 4 KCl, 5 $CaCl_2$, 4 $MgCl_2$, 3 $NaHCO_3$, 5 Tris-Cl (pH 7.6). During Ca^{2+}-current measurements we used a sodium-free solution containing (in mM): 4 KCl, 10 $CaCl_2$, 4 $MgCl_2$, 95 TEA, 5 4-AP, 5 Tris-Cl (pH 7.6). Tetraethylammonium bromide (TEA) and 4-aminopyridine (4-AP) were used to block the potassium channels. Aricept tablets (Pfizer) containing 5 mg donepezil were dissolved in the control solution, which was filtered and added to the experimental chamber under stop-flow conditions.

The data were analyzed by unpaired *t* test using Prism 3.0 software (GraphPad).

RESULTS

The high-threshold calcium current I_{Ca} was measured with 150-500 msec depolarizing pulses. The maximum I_{Ca} was usually recorded at membrane potential of 30 mV. The current-voltage curve was plotted with the potential stepped by 10 mV in the range from -30 to +60 mV. Extracellular application of donepezil (1-100 μM) induced rapid dose-dependent and reversible (upon washout) inhibition of I_{Ca} (*n*=6/6). This effect attained a maximum 1-3 min after application and completely disappeared after 6-8-min washout.

Figure 1, *a* shows current-voltage curves of I_{Ca} in the control solution and in the presence of donepezil (5 and 10 μM) recorded in one of the cells. Apart from the decrease in maximum of I_{Ca}, done-

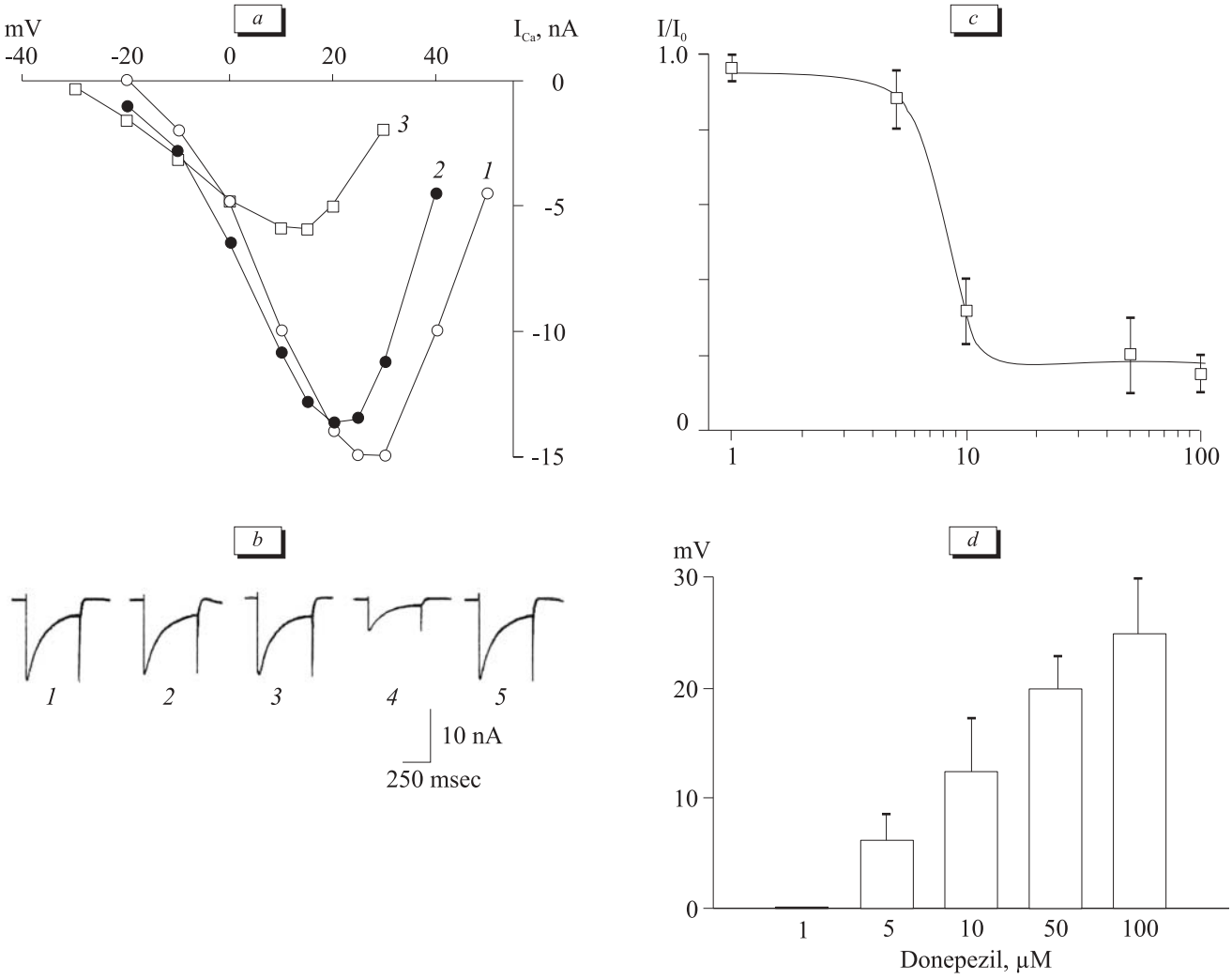

Fig. 1. Inhibitory effect of donepezil on potential-dependent calcium current in molluscan neurons. *a*) current-voltage plot of the peak amplitude of the inward current under control conditions (*1*) and in the presence of donepezil in concentrations of 5 μM (*2*) and 10 μM (*3*). *b*) calcium currents in control solution (*1*), in the test solutions with donepezil in concentrations of 5 μM (*2*) and 10 μM (*4*), and after washout with the control solution (*3*,*5*). *c*) dose-dependence of inhibitory action of donepezil on the peak amplitude of calcium current. *d*) dose-dependence of donepezil-induced shift of current-voltage relations of calcium channels.

pezil caused a left shift of the current-voltage curve. In the presence of 5 and 10 μM donepezil these shifts were 10 and 15 mV, respectively. The drug did not affect the kinetics of activation and inactivation of the calcium current (Fig. 1, *b*). The holding potential was -60 mV and the currents were recorded during depolarizing pulses shifting the potential to 30 mV in control solution and to 20 and 15 mV in the presence of 5 and 10 μM donepezil, respectively. The pulse duration was 500 msec. The mean amplitudes of I_{Ca} at different donepezil concentrations are shown (n=6, Fig. 1, *c*). When applied in a concentration of 10 μM, donepezil decreased I_{Ca} by 68±11% (p<0.001). The effect attained a maximum of 82±10% at 100 μM (p<0.001). The experimental points were fitted by Boltzmann equation with IC_{50}=7.9 μM and Hill coefficient 1.1. The dependence of the shift of the current-voltage curve on donepezil concentration is presented (Fig. 1, *d*). The current-voltage relations were shifted by 25±5 mV at 100 μM donepezil (p<0.001).

The effects of donepezil on I_A were examined in the next series of the experiments. This current was inactivated at resting potential and its activation requires preliminary hyperpolarization. For construction of the current-voltage curves, the holding potential was set at -130 mV and then the membrane potential was shifted from -80 mV to -20 mV (step 10 mV) with test 150-500-msec pulses (Fig. 2, *a*). The larger stimuli were not used to avoid

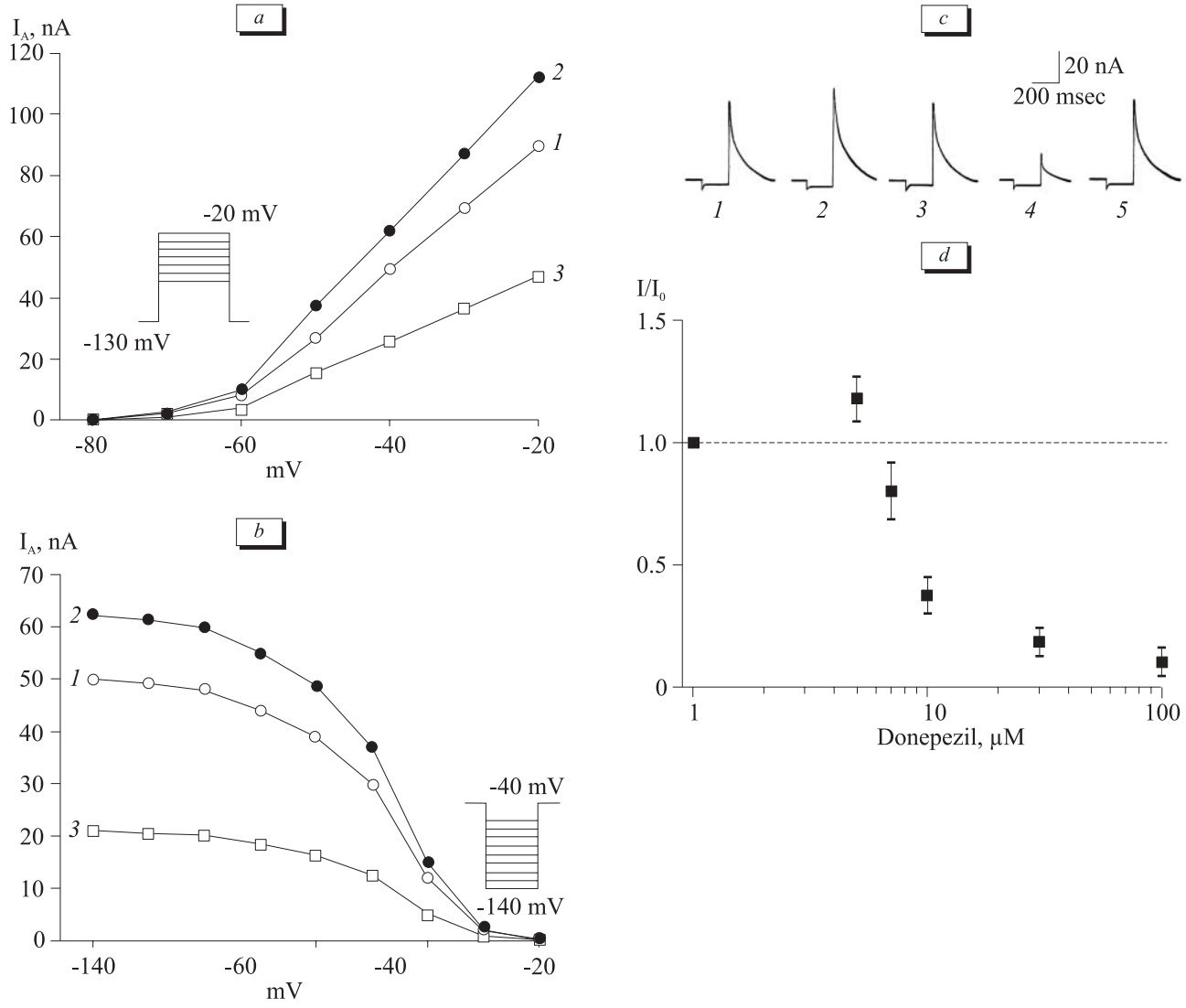

Fig. 2. Dual effect of donepezil on low-threshold fast potassium current. *a*) current-voltage plot of the peak amplitude of outward current in control solution (*1*) and in the presence of donepezil in concentrations of 5 μM (*2*) and 10 μM (*3*). *b*) stationary inactivation of I_A in the same cell. *c*) outward currents in control solution (*1*), in the test solutions with donepezil in concentrations of 5 μM (*2*) and 10 μM (*4*), and after washout with the control solution (*3*, *5*). *d*) dose-dependence of donepezil action on the peak amplitude of I_A current measured at -40 mV after the hyperpolarizing pulse shifting the membrane potential to -130 mV for 200 msec (n=5).

activation of high-threshold potassium current. The threshold of I_A current was about -70 mV. To examine the stationary inactivation of this current, the holding potential was set at -40 mV. The stimulation protocol consisted of a 200 msec hyperpolarizing step shifting the membrane potential to the region from -120 to -60 mV with the step of 10 mV. I_A was recorded at the holding potential of -40 mV just after termination of the hyperpolarizing pulse (Fig. 2, *b*, *c*). These experiments showed that low concentration of donepezil (5 μM) inxreased I_A, while higher concentrations (≥7 μM) inhibited it. The dual effect of donepezil was observed with both stimulation protocols yielding the current-voltage and stationary inactivation plots. The dose-dependent plot of the peak I_A is given in Fig. 2, *d*. When applied in a concentration of 5 μM, donepezil increased the mean peak value of I_A by 16±9%, while 10 μM of this drug decreased it by 60±6% ($p<0.001$). The maximum inhibitory effect of donepezil was 92±5% at 100 μM ($p<0.0001$).

Thus, this study showed that micromolar concentrations of cholinesterase inhibitor donepezil change I_{Ca} and I_A in molluscan neurons.

The rates of the development (1-3 min) and washout (6-8 min) of donepezil effects on potential-dependent calcium current suggest direct interaction of the drug with the channel protein. Hill coefficient of calcium blockade was near 1, which attests to the absence of interaction between the binding sites. The block of I_{Ca} was accompanied by a shift of the current-voltage plot towards hyperpolarization, which probably resulted from changes in surface charge on the membrane produced by the drug-channel interaction.

The study also showed that donepezil produces a dual effect on I_A: increases it at low concentrations and blocks at high ones. There are published reports on the blockade of I_A in rat neurons with donepezil [14]. In contrast to our study, where donepezil blocked I_A by more than 50% at 10 μM, the cited paper reported IC_{50}=249 μM [14]. This disagreement can be explained by, first, differences in A-channels in mollusks and rats, and second, considerable differences of the routine patch-clamp from the two-electrode voltage clamp technique employed in this study. In the patch-clamp experiments dialysis of neurons can change phosphorylation of the channel protein and, consequently, the affinity of agonist binding sites. The fine microelectrodes used in our experiments produced less damage to the cytoplasm and provide better physiological conditions for channel function.

The mechanisms underlying potentiation of I_A by low doses of donepezil are still unclear. Probably, this effect depends on metabolism and for this reason it was now revealed before [14].

The novel features of donepezil action, which modulate the work of Ca^{2+}-channels and low-threshold K^+-channels enlarge our view on the pharmacological profile of this drug and help to gain better insight into its neurotropic protective effects.

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