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Donepezil in low micromolar concentrations modulates voltage-gated potassium currents in pyramidal neurons of rat hippocampus

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ABSTRACT

Donepezil is a cholinesterase inhibitor widely used for the treatment of Alzheimer's disease. Voltage-gated K^+-channels are discussed as possible targets for the drug, but the results obtained by different authors are contradictory. In the present study performed on pyramidal cells isolated from rat's hippocampus, we investigated the effect of donepezil on delayed rectifier K^+-current ($I_{K(DR)}$) and transient outward K^+-current ($I_{K(A)}$) using patch-clamp technique. The inhibitory effect of donepezil on $I_{K(DR)}$ was found in all the cells tested, but its strength varied in different cells. Two groups of neurons were differing in their sensitivity to donepezil: more sensitive (IC_{50} = 8.9 μM) and less sensitive (IC_{50} = 114.9 μM). The effect of the drug on $I_{K(DR)}$ was rapid, reversible and voltage-dependent, increasing with depolarization. Donepezil modulated $I_{K(A)}$ in two different ways: in some cells it suppressed the current with the IC_{50} value of 23.4 μM, while in other cells it augmented the current with a bell-shaped dose–response curve. Maximal (about twofold) enhancement of $I_{K(A)}$ amplitude was caused by 10 μM donepezil. Augmentation of $I_{K(A)}$ increased with membrane depolarization. Our results show for the first time that voltage-dependent potassium channels in mammals' neurons are effectively modulated by low micromolar concentrations of donepezil.

1. Introduction

Acetylcholinesterase (AChE) inhibitor donepezil is widely used for the treatment of Alzheimer's disease (AD) [1,2]. Besides cognitive benefits in AD, other effects of donepezil observed in clinical and preclinical investigations include: reduction of the number of falls in patients with Parkinson disease [3]; attenuation of neuronal damage and cognitive deficits after global cerebral ischemia [4,5]; improvement of cognitive impairment in schizophrenia and psychotic depression [6]. Accumulative evidence suggests that action of donepezil is not confined to AChE inhibition in the brain, and some other targets for the drug's action are currently discussed [7]. Voltage-gated potassium channels as possible targets for donepezil attract attention of investigators because those channels dysfunction is believed to play a role in AD pathogenesis. The mechanisms of beta-amyloid-induced neurodegeneration were shown to involve an abnormal rise in activity of plasma membrane K^+-channels followed by cellular K^+-loss and an induction of apoptotic cascade [8,9]. The effects of donepezil on voltage-gated potassium current were studied in different models [5,10,11]. The results of the above studies appear to be contradictory and there is not enough clarity about this issue. In our previous work conducted on molluscan neurons [10], we have described the inhibitory effect of donepezil on delayed rectifier K^+-current ($I_{K(DR)}$) and its stimulatory effect on transient K^+-current ($I_{K(A)}$). The data that the drug can affect K^+-currents in low micromolar concentrations allowed us to consider the involvement of K^+-channels into the mechanisms(s) of the drug therapeutic action. Strong inhibitory effect of donepezil (IC_{50} = 7.6 μM) on Kv2.1 potassium channels was observed by Yuan et al. [5] in Kv2.1 transfected HEK293 cell line. At the same time, Yu and Hu [11], who performed the experiments in rat hippocampal neurons, have found the inhibitory effect of donepezil on both $I_{K(DR)}$ and $I_{K(A)}$ with rather high values of IC_{50}: 78 and 249 μM, accordingly. The authors have concluded that the blocking effect of donepezil on the voltage-gated potassium channels is unlikely to contribute to the clinical benefits to patients with AD. We decided to conduct our own investigation of the effects of donepezil on voltage-gated potassium currents in rat hippocampal neurons. In contrast to Yu and Hu [11], we have added ATP into a recording pipette because a decrease in cellular ATP level might cause disturbances in phosphorylation processes and change sensitivity of ionic channels to the antagonists [12]. We also slightly modified the protocol of stimulation of K^+-currents (see Section 3.1). The effects of donepezil on voltage-gated K^+-currents in rat hippocampal neurons observed in this work appear to be similar to our previous data obtained

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in molluscan neurons [10], but do not coincide with the data reported by Yu and Hu [11].

2. Materials and methods

2.1. Cell preparation

All procedures were performed in accordance with the institutional guidelines on the care and use of experimental animals set by the Russian Academy of Sciences. Wistar rats (11–14 days old) were decapitated, slices 200–500 μm thick were cut with a razor blade and incubated at room temperature for at least 2 h. The incubation solution consisted of (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 2 $MgSO_4$, 25 $NaHCO_3$, 1.3 NaH_2PO_4, and 10 D-glucose at pH 7.4. The saline was continuously stirred and bubbled with carbogen (95% O_2 + 5% CO_2). Neurons were dissociated from CA3 regions of hippocampus by a vibrating fused glass pipette with a spherical tip [13]. The large pyramidal-shaped neurons were chosen for study. The dissociation procedure was carried out in the following saline (in mM): 140 NaCl, 3 KCl, 3 $CaCl_2$, 5 $MgCl_2$, 10 D-glucose, 10 HEPES hemisodium, pH 7.4.

2.2. Whole-cell patch-clamp technique

Voltage-clamp recording was obtained using the whole-cell configuration of the patch-clamp technique [14]. Patch pipettes had resistances of 2–3 MΩ and were filled with (in mM): 40 KF, 100 KCl, 0.1 $CaCl_2$, 1 EGTA, 10 HEPES, 2 Na_2ATP, 3 $MgCl_2$, pH 7.3. The external solution contained (in mM): 140 NaCl, 3 KCl, 3 $CaCl_2$, 3 $MgCl_2$, 10 HEPES, 10 D-glucose and 0.001 tetrodotoxin at pH 7.4. Experiments were performed at room temperature using a List EPC-7 patch-clamp amplifier. Tight seals (>1 GΩ) were obtained during the recordings. Leak currents were small and could be estimated directly from hyperpolarizing pulses. Current recordings were not corrected for leak and capacitive components. The holding potential was maintained at −70 mV. Data were collected and analyzed using home-made software and stored on a computer disk.

2.3. Reagents

Donepezil solution was prepared by dissolving a tablet of Aricept (Pfizer) containing 5 mg of donepezil hydrochloride in distillated water. The liquid was filtered using membrane filter, so that insoluble ingredients (corn starch, microcrystalline cellulose, magnesium stearate, talk) were filtered out. Lactose was the main soluble ingredient of the inert filling material. Control experiments with lactose were carried out (see Section 3.8). Donepezil solution was stored as 1 mM stock at 4 °C and was freshly dissolved in external solution for each experiment. Other chemicals were purchased from Sigma.

2.4. Data analysis and statistics

Voltage protocol and data analysis for $I_{K(DR)}$ and $I_{K(A)}$ are described in Section 3.1. All data were analyzed using the Prism 3.0 (GraphPad) software, and expressed as mean ± SEM. Significant differences between groups were assessed by paired Student's *t*-test. The criterion for significance was $p < 0.05$ in all the analyses. The IC_{50} values for donepezil inhibition of voltage-gated K^+-current were determined using the equation [15]: $I/I_0 = 1 - [\max/(1 + (IC_{50}/C)^n)]$, where I_0 and I are current amplitudes measured in control solution and in the presence of donepezil, max is the maximum inhibition attainable, C is the concentration of donepezil in the external solution, IC_{50} is the half-maximal inhibitory concentration and n is the slope factor (Hill coefficient).

3. Results

3.1. Isolation of $I_{K(DR)}$ and $I_{K(A)}$

Total voltage-gated K^+-current in rat hippocampal pyramidal neurons contains two main components, the transient outward K^+-current ($I_{K(A)}$) and the delayed rectifier K^+-current ($I_{K(DR)}$) [16,17]. In our experiments, total K^+-currents (I_{Total}) were activated with a 200 ms depolarizing test pulse from a holding potential of −70 to +50 mV, in 10 mV increments, following a conditioning pre-pulse to −110 mV (Fig. 1A). $I_{K(DR)}$ was isolated by using the same voltage protocol as for total K^+-currents, but with a 100 ms interval to −50 mV after the pre-pulse which was inserted to inactivate $I_{K(A)}$. The currents obtained at the end of depolarizing pulse were referred to as $I_{K(DR)}$ (Fig. 1B). We used a 100 ms interval to −50 mV after the pre-pulse *vs* a 50 ms interval used by other authors [11,17] in order to separate the pure $I_{K(DR)}$ to the full extent. $I_{K(A)}$ was obtained by subtracting the $I_{K(DR)}$ traces from the total outward K^+-current. The peak of the subtracted currents was referred to as $I_{K(A)}$ (Fig. 1C).

3.2. Two types of $I_{K(DR)}$: sensitive and non-sensitive to low micromolar concentrations of donepezil

The sensitivity of $I_{K(DR)}$ to low micromolar concentrations of donepezil varied from cell-to cell so that the cells tested may be divided into two groups. In half of the cells examined (n = 12/24), a

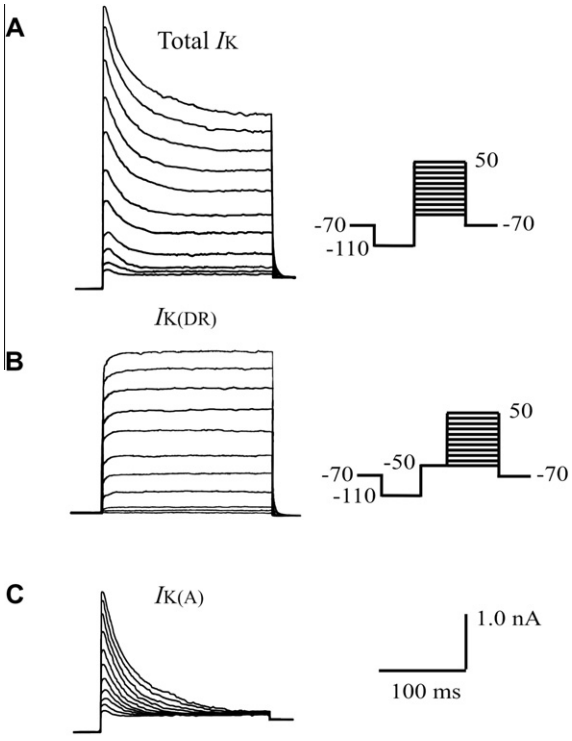

Fig. 1. Voltage-gated K^+-current components in a hippocampal pyramidal neuron. Holding potential was at −70 mV. (A) Total outward K^+-current recorded during 200 ms depolarizing pulses from −50 to +50 mV in 10 mV steps following a hyperpolarizing pre-pulse of 150 ms to −110 mV. (B) $I_{K(DR)}$ recorded with a similar protocol, except that a 100 ms interval at −50 mV was inserted after the pre-pulse. Currents recorded at the end of the depolarizing pulse were referred to as $I_{K(DR)}$. (C) Isolated $I_{K(A)}$, obtained by subtraction of current traces in (B) from those in (A). The peak of the subtracted currents was referred to as $I_{K(A)}$.

noticeable and reversible inhibition of $I_{K(DR)}$ in the presence of 1 μM donepezil was observed, while threshold concentration for other cells was 10 μM or higher (n = 12/24). These two subtypes of $I_{K(DR)}$ were referred to as $I_{K(DR)1}$ and $I_{K(DR)2}$. There were no noticeable differences in size or shape of the cells from these two groups. The inhibitory effect of donepezil on both $I_{K(DR)1}$ and $I_{K(DR)2}$ was rapid, reversible and dose-dependent. The effect occurred over 1–3 min, reached a steady state by ~5 min and returned to near control values within 3–6 min upon removal of donepezil. The Fig. 2B (left) illustrates dose dependence of donepezil action on $I_{K(DR)1}$ and $I_{K(DR)2}$ measured at +50 mV. Maximal effect for $I_{K(DR)1}$ reached 83.2 ± 8.7% at 300 μM donepezil, and half-maximal inhibitory concentration (IC_{50}) and the slope factor (Hill coefficient) were calculated as 8.9 ± 3.6 μM and 0.7 ± 0.1, respectively. The IC_{50} value and Hill coefficient for $I_{K(DR)2}$ were calculated as 114.9 ± 12.2 μM and 0.96 ± 0.11, respectively (Fig. 2B). The Fig. 2A (left) shows the original current traces of $I_{K(DR)1}$ before and after the addition of different concentrations of donepezil.

3.3. Dual effect of donepezil on $I_{K(A)}$

In contrast to $I_{K(DR)}$, $I_{K(A)}$ was not changed in the presence of 1 μM donepezil. The higher concentrations of the drug (5–100 μM) were found to cause two different kinds of effect: in some cells donepezil suppressed the peak amplitude of the current (n = 5/17), and in other cells it augmented the current (n = 12/17). So, we have arranged the cells into two groups which were referred to as $I_{K(A)1}$ and $I_{K(A)2}$, accordingly. There were no noticeable differences in size or shape of the cells from these two groups. Such parameters as the peak amplitude and the rate of inactivation appeared to be different in these groups. The mean values of amplitude and half-decay time (τ) at +50 mV for $I_{K(A)1}$ were 1.8 ± 0.4 nA and 21.5 ± 3.8 ms, whereas these values for $I_{K(A)2}$ were on the average 0.9 ± 0.3 nA and 10.5 ± 1.9 ms. Donepezil reversibly suppressed $I_{K(A)1}$. Maximal effect reached 71.4 ± 7.2% with 300 μM donepezil, and the IC_{50} value and Hill coefficient were calculated as 23.4 ± 8.1 μM and 1.5 ± 0.48, respectively. In contrast to $I_{K(A)1}$, donepezil suppressed $I_{K(A)2}$ at 300 μM only whereas it potentiated this current at concentration of 5–100 μM (Fig. 2), so the dose–response curve was bell-shaped. Maximal increase of the $I_{K(A)2}$ amplitude reached 196.1 ± 30.4% and was observed in the presence of 10 μM donepezil (p < 0.01, n = 8). In the presence of 300 μM donepezil, the amplitude decreased to 53.3 ± 14.5% (p < 0.01, n = 7). The stimulatory effect on $I_{K(A)2}$ disappeared completely after 10–15 min washing in half of the cells tested (6/12), while only partial recovery was observed in the remaining cells.

3.4. The effects of donepezil on the voltage dependence of the steady-state activation of $I_{K(DR)1}$

The inhibitory effect of low concentrations (1–50 μM) of donepezil on $I_{K(DR)1}$ was voltage-dependent increasing progressively with depolarization. In contrast, the inhibitory effect of 300 μM donepezil on $I_{K(DR)1}$ was voltage-independent, and effect of 100 μM was voltage-dependent only in half of the cells tested. Fig. 3 illustrates the influence of 10 and 300 μM donepezil on $I_{K(DR)1}$ at different potentials. The average values of the amplitude of the current at +50 mV in control and in the presence of 10 μM donepezil were 3.1 ± 0.3 and 1.7 ± 0.2 nA (p < 0.01, n = 6), while there was no significant difference between corresponding currents recorded at 0 mV (1.1 ± 0.2 *vs* 0.8 ± 0.1 nA, n = 6). The amplitudes of $I_{K(DR)1}$ were converted to conductance (G), as described in [15], and the normalized conductance was fitted with Boltzmann function (Fig. 3C). The values of half-maximal activation for $I_{K(DR)1}$ ($V_{1/2}$) were −6.4 ± 1.8 mV in control, −13,4 ± 1.0 mV in the presence of 10 μM donepezil (p < 0.05, n = 6), and −7.1 ± 2.2 mV in the presence of 300 μM donepezil (n = 5). Thus, 10 μM donepezil caused a hyperpolarizing shift of the steady-state activation curve of about −7 mV, and 300 μM donepezil did not alter the voltage dependence of $I_{K(DR)1}$ activation.

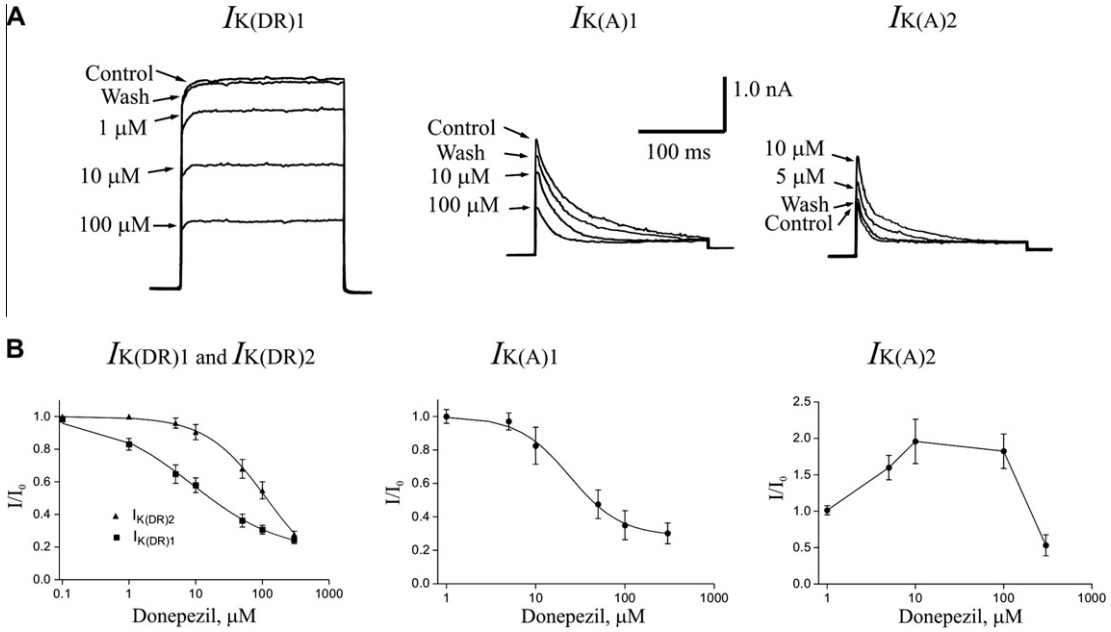

Fig. 2. Dose dependence of donepezil effects on $I_{K(DR)}$ and $I_{K(A)}$ measured at +50 mV. (A) Superimposed current traces obtained at +50 mV in control solution, 5 min after application of donepezil and after washout of the drug. Two subtypes of $I_{K(DR)}$ were identified: more sensitive to donepezil ($I_{K(DR)1}$) and less sensitive ($I_{K(DR)2}$). Current traces of $I_{K(DR)1}$ are presented, and current traces of $I_{K(DR)2}$ are not shown. Two different effects of donepezil on $I_{K(A)}$ was observed in different cells: a suppression ($I_{K(A)1}$) and an enhancement ($I_{K(A)2}$). Both effects are shown. (B) Concentration–response curves for the action of donepezil on $I_{K(DR)1}$, $I_{K(DR)2}$, $I_{K(A)1}$ and $I_{K(A)2}$. Data were fitted with the Hill equation (see section 2. 4) for $I_{K(DR)1}$, $I_{K(DR)2}$ and $I_{K(A)1}$. The direct lines between data points were drawn for $I_{K(A)2}$.

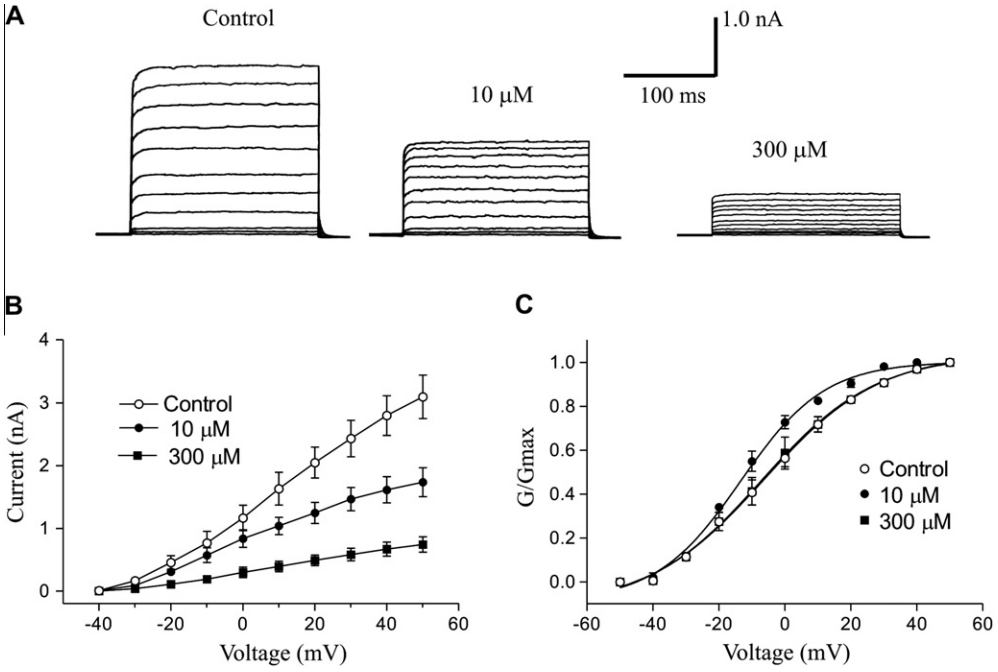

Fig. 3. Effects of donepezil on current–voltage (*I–V*) curves and the voltage dependence of $I_{K(DR)1}$ activation. (A) The original current traces of $I_{K(DR)1}$ before and after addition of 10 or 300 μM donepezil. (B) The effects of 10 and 300 μM donepezil on the *I–V* curves. (C) The effects of 10 and 300 μM donepezil on the steady-state activation curves. The amplitudes of $I_{K(DR)1}$ were converted to conductance and normalized to maximal conductance. Normalized data points were fitted with the Boltzmann equation. 10 μM donepezil caused a hyperpolarizing shift of the steady-state activation curve of $I_{K(DR)1}$ of about −7 mV, and 300 μM donepezil did not alter the voltage dependence of $I_{K(DR)1}$ activation.

3.5. Voltage-independence of the inhibitory effect of donepezil on $I_{K(A)1}$

The inhibitory effect of the drug on $I_{K(A)1}$ was voltage-independent at all concentrations tested. No shift of the steady-state activation curve was observed in the presence of donepezil. The values of $V_{1/2}$ was −9.4 ± 4.4 mV in control and −9.5 ± 3.9 mV in the presence of 100 μM donepezil (n = 5) (not shown).

3.6. Potential-dependent augmentation of $I_{K(A)2}$ by donepezil

The stimulatory effect of low doses of donepezil (5–100 μM) on $I_{K(A)2}$ was observed at positive potentials only and increased progressively with membrane depolarization. In contrast, inhibitory effect of high concentration of the drug (300 μM) was voltage-independent. Fig. 4 shows the average *I–V* curve and the normalized *G–V* relationship for $I_{K(A)2}$ in control solution and in the presence of 10 μM or 300 μM donepezil. The values of $V_{1/2}$ was −11.2 ± 3.7 mV in control, −19.6 ± 4.6 mV ($p < 0.05$, n = 6) in the presence of 10 μM donepezil, and −12.9 ± 4.8 mV in the presence of 300 μM donepezil (n = 5). Thus, a depolarizing shift of the steady-state activation curve of about 8 mV was caused by 10 μM donepezil, and no shift was observed in the presence of 300 μM donepezil.

3.7. No changes in donepezil effects in Ca^{2+}-free solution

In Ca^{2+}-containing medium, the contamination of voltage-gated K^+-current with voltage-gated Ca^{2+}-current and Ca^{2+}-dependent K^+-current might occur. We compared the effects of donepezil on voltage-gated outward current in Ca^{2+}-containing and Ca^{2+}-free solution in the same cells (n = 8). In Ca^{2+}-free solution, the amplitude of control outward currents changed at +50 mV not more than by 10%, and the effects of donepezil were similar to those observed in Ca^{2+}-containing medium (not shown). The results suggest that observed donepezil induced changes in voltage-gated outward currents were really caused by the changes in voltage-gated K^+-channels functioning.

3.8. Control experiments with lactose

Tablets of "Aricept" contain both insoluble and soluble ingredients as inert filling materials. While insoluble ingredients were filtered out, soluble ingredients contaminated donepezil solution. Lactose was the main soluble ingredient of the inert filling material. We measured voltage-gated K^+-current of rat hippocampal neurons in the presence of lactose (ChemMed, Russia) in the wide range of concentrations, and found no noticeable effects.

4. Discussion

In the present work, we studied the influence of donepezil on $I_{K(DR)}$ and $I_{K(A)}$ in rat pyramidal neurons isolated from CA3 hippocampal regions. In our experiments, the sensitivity of $I_{K(DR)}$ to donepezil varied from cell to cell, so that the cells examined may be arranged into two groups: more sensitive ($I_{K(DR)1}$) and less sensitive ($I_{K(DR)2}$) to donepezil. Such a variability in sensitivity of $I_{K(DR)}$ to donepezil looks understandable taking into account that the family of DR-channels in hippocampal neurons consists, at least, of seven members built with various proteins [16]. The effects of donepezil on $I_{K(A)}$ also varied in different cells. We observed both a suppression and an augmentation of this current by donepezil in different cells. As is the case with $I_{K(DR)}$, a diversity in the properties of different A-channels may underlie this distinction [16]. Our results indicating the ability of donepezil to modulate in low micromolar concentrations the voltage-gated K^+-currents in rat hippocampal neurons agree with the results of Yuan et al. [5] obtained in Kv2.1 transfected HEK293 cell line. The value of IC_{50} for the inhibition of $I_{K(DR)}$ with donepezil was found to be 7.6 μM in that study. At the same time, our results do not coincide with the data reported by Yu and Hu [11] who performed the

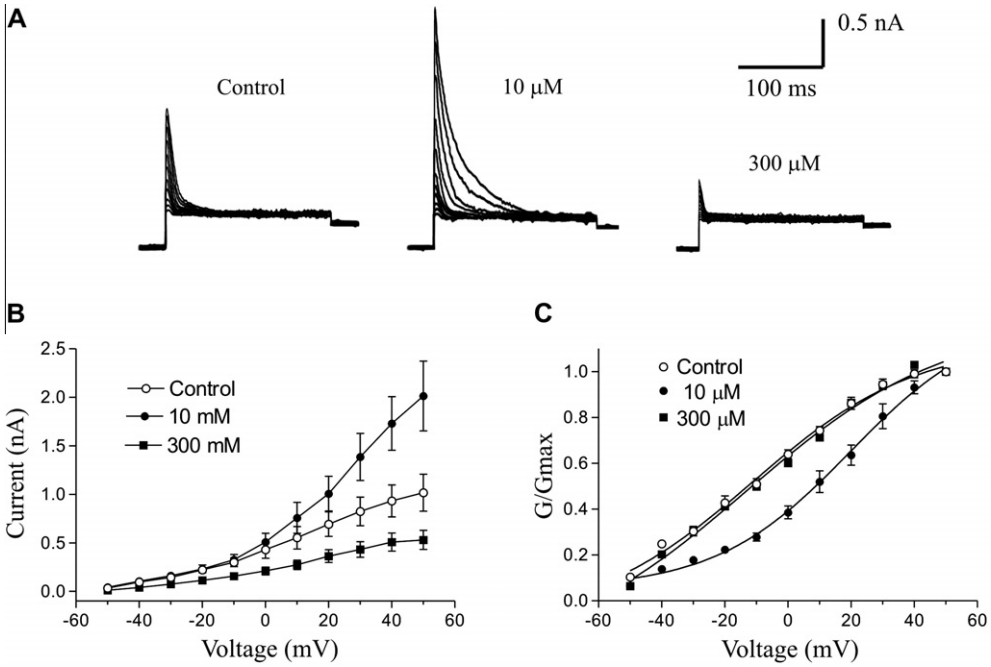

Fig. 4. Potential-dependent augmentation of $I_{K(A)2}$ by donepezil. (A) The original current traces of $I_{K(A)2}$ before and after addition of 10 and 300 μM donepezil. (B) The effects of 10 and 300 μM donepezil on the *I–V* curves. (C) The effects 10 and 300 μM donepezil on the steady-state activation curves. The amplitudes of $I_{K(A)2}$ were converted to conductance and normalized to maximal conductance. Normalized data points were fitted with the Boltzmann equation. A depolarizing shift of the steady-state activation curve of about 8 mV was caused by 10 μM donepezil, and no shift was observed in the presence of 300 μM donepezil.

experiments in rat hippocampal neurons and found that the inhibitory effects of donepezil on both $I_{K(DR)}$ and $I_{K(A)}$ require high micromolar concentrations (IC_{50} = 78 and 249 μM, accordingly). The reason of this discrepancy is not clear. Some variations in the methods used, such as the presence of ATP in the recording pipette, some differences in the protocol of stimulation and postnatal age of the rats, may, at least in part, explain this discrepancy.

Our results outlined in the present paper resemble, in general, our previous findings obtained in molluscan neurons which have shown that donepezil inhibited $I_{K(DR)}$ with IC_{50} = 8.0 μM and had a dual effect on $I_{K(A)}$: an augmentation by 5 μM and a suppression by higher doses [10]. However, despite that general similarity, a number of differences can be found between donepezil action on K^+-currents in rat neurons and in molluscan neurons. The main difference is that the effects in molluscs were voltage-independent while in rats this independence was observed only at high concentration of the drug (300 μM). Lower donepezil concentrations caused voltage-dependent inhibition of $I_{K(DR)1}$ and voltage-dependent augmentation of $I_{K(A)2}$. Voltage-dependence of donepezil effects in rats allows us to think that their mechanisms are more complicated than those in molluscan neurons. The latter was supposed to involve donepezil binding with the external mouth of K^+-channels [18]. From the extracellular side, the molecules of the antagonist can't enter the channel's pore deeply because of the position of selective filter. In the present study, such a simple explanation can work for the following voltage-independent effects of donepezil: inhibition of $I_{K(A)1}$ by all donepezil concentrations, and inhibition of $I_{K(DR)1}$ and $I_{K(A)2}$ by 300 μM of the drug. But voltage-dependent effects need some other explanations. One of the possible explanations involves the penetration of charged molecules of a blocker into the channel's pore from the intracellular side. The point is that donepezil molecules can exist in both uncharged and charged form, because the amino group of donepezil can be protonated at physiological pH. The uncharged donepezil can cross the lipid membrane and reach the internal mouth of K^+-channel. In cytoplasm, the substance molecules may be protonated, and positively charged ions may enter deeply into the channel pore in potential-dependent manner. Such a blockade might explain voltage-dependent inhibition of $I_{K(DR)}$ by donepezil. The voltage-dependent augmentation of $I_{K(A)2}$ can also be explained by the drug interaction with internal mouth of K^+-channel. Indeed, under physiological conditions, voltage-gated K^+-channels are known to be blocked by internal cations (Na^+, Mg^{2+}, polyamines) in a voltage-dependent manner [19,20]. An attenuation of such a blockade will cause a voltage-dependent increase in K^+-current, and donepezil may augment $I_{K(A)}$ just due to this mechanism.

Some reasons allow us to think that therapeutical doses of donepezil may modulate voltage-gated K^+-currents in patients' brain. Plasma level of donepezil in the patients receiving effective doses (10 mg) of donepezil hydrochloride per day corresponds approximately to 122 nM [21]. Additionally, taking into account that in rodents the concentration of donepezil in the brain was estimated to be 6–9 times higher than in the plasma [22], it is conceivable to hypothesize that donepezil concentration in human brain can reach the level of 1 μM. Finally, there are reasons to suppose that, in AD brain, donepezil affects K^+-currents stronger than in our experiments. Literature data show that beta-amyloid increases membrane expression of K^+-channels and, as a result, dramatically intensifies voltage-gated K^+-currents [8,9]. Under the circumstances, blocking effect of classical antagonist of K^+-channels, tetraethylammonium, was found to be enhanced [8]. Similarly, the neurons in AD brain may response to donepezil stronger than those untreated with beta-amyloid.

Our results are in line with the literature data concerning the properties of other AChE inhibitors, such as galantamine [23] and rivastigmine [15]. These drugs were shown to block voltage-gated K^+-currents in low micromolar concentrations, therefore one may consider the involvement of this effect in their therapeutic action.

Sum up, it may be said, that the regulation of voltage-gated K^+-currents of rat hippocampal neurons by low micromolar concentrations of donepezil reconfirms a possibility of contribution of

K^+-channels modulation to the mechanisms of the drug therapeutic action.

Acknowledgments

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References

[1] R.S. Doody, D.S. Geldmacher, M.R. Farlow, Y. Sun, M. Moline, J. Mackell, Efficacy and safety of donepezil 23 mg versus donepezil 10 mg for moderate-to-severe Alzheimer's Disease: a subgroup analysis in patients already taking or not taking concomitant memantine, Dement. Geriatr. Cogn. Disord. 33 (2012) 164–173.

[2] R. Howard, R. McShane, J. Lindesay, C. Ritchie, A. Baldwin, R. Barber, et al., Donepezil and memantine for moderate-to-severe Alzheimer's disease, N. Engl. J. Med. 366 (2012) 893–903.

[3] K.A. Chung, B.M. Lobb, J.G. Nutt, F.B. Horak, Effects of a central cholinesterase inhibitor on reducing falls in Parkinson disease, Neurology 75 (2010) 1263–1269.

[4] D. Min, X. Mao, K. Wu, Y. Cao, F. Guo, S. Zhu, et al., Donepezil attenuates hippocampal neuronal damage and cognitive deficits after global cerebral ischemia in gerbils, Neurosci. Lett. 510 (2012) 29–33.

[5] H. Yuan, W.P. Wang, N. Feng, L. Wang, X.L. Wang, Donepezil attenuated oxygen–glucose deprivation insult by blocking Kv2.1 potassium channels, Eur. J. Pharmacol. 657 (2011) 76–83.

[6] T. Niitsu, M. Iyo, K. Hashimoto, Sigma-1 receptor agonists as therapeutic drugs for cognitive impairment in neuropsychiatric diseases, Curr. Pharm. Des. 18 (2012) 875–883.

[7] N.R. Relkin, Beyond symptomatic therapy: a re-examination of acetylcholinesterase inhibitors in Alzheimer's disease, Expert. Rev. Neurother. 7 (2007) 735–748.

[8] A. Pannaccione, F. Boscia, A. Scorziello, A. Adornetto, P. Castaldo, R. Sirabella, et al., Up-regulation and increased activity of KV3.4 channels and their accessory subunit MinK-related peptide 2 induced by amyloid peptide are involved in apoptotic neuronal death, Mol. Pharmacol. 72 (2007) 665–673.

[9] H.B. Yu, Z.B. Li, H.X. Zhang, X.L. Wang, Role of potassium channels in Abeta(1–40)-activated apoptotic pathway in cultured cortical neurons, J. Neurosci. Res. 84 (2006) 1475–1484.

[10] E.I. Solntseva, J.V. Bukanova, E. Marchenko, V.G. Skrebitsky, Donepezil is a strong antagonist of voltage-gated calcium and potassium channels in molluscan neurons, Comp. Biochem. Physiol. Part C 144 (2007) 319–326.

[11] B. Yu, G.-Y. Hu, Donepezil blocks voltage-gated ion channels in rat dissociated hippocampal neurons, Eur. J. Pharmcol. 508 (2005) 15–21.

[12] T. Yang, H. Kanki, D.M. Roden, Phosphorylation of the *IK*s channel complex inhibits drug block, Circulation 108 (2003) 132–134.

[13] V.S. Vorobjev, Vibrodissociation of sliced mammalian nervous tissue, J. Neurosci. Meth. 38 (1991) 145–150.

[14] O.P. Hamill, A. Marty, E. Neher, B. Sakmann, F.J. Sigworth, Improved patch-clamp techniques for high-resolution current-recording from cells and cell-free membrane patches, Pflugers Arch. 391 (1981) 85–100.

[15] Y.P. Pan, X.H. Xu, X.L. Wang, Rivastigmine blocks voltage-activated K^+ currents in dissociated rat hippocampal neurons, Br. J. Pharmacol. 140 (2003) 907–912.

[16] G.A. Gutman, K.G. Chandy, S. Grissmer, M. Lazdunski, D. McKinnon, L.A. Pardo, et al., International union of pharmacology. LIII. Nomenclature and molecular relationships of voltage-gated potassium channels, Pharmacol. Rev. 57 (2005) 473–508.

[17] J.F. Storm, Potassium currents in hippocampal pyramidal cells, Prog. Brain Res. 83 (1990) 161–187.

[18] E.I. Solntseva, J.V. Bukanova, E.V. Marchenko, A.V. Rossokhin, V.G. Skrebitsky, The binding of donepezil with external mouth of K^+ channels of molluscan neurons, Cell. Mol. Neurobiol. 29 (2009) 219–224.

[19] A.N. Lopatin, C.G. Nichols, Internal Na^+ and Mg^{2+} blockade of DRK1 (Kv2.1) potassium channels expressed in Xenopus oocytes, J. Gen. Physiol. 103 (1994) 203–216.

[20] A.N. Lopatin, E.N. Makhina, C.G. Nichols, Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification, Nature 372 (1994) 366–369.

[21] S.L. Rogers, R.S. Doody, R.S. Mohs, L.T. Friedhoff, Donepezil improves cognition and global function in Alzheimer disease: a 15-week, double-blind, placebo-controlled study, Donepezil Study Group, Arch. Intern. Med. 158 (1998) 1021–1031.

[22] T. Kosasa, Y. Kuriya, K. Matsui, Y. Yamanishi, Inhibitory effect of orally administered donepezil hydrochloride (E2020), a novel treatment for Alzheimer's disease, on cholinesterase activity in rats, Eur. J. Pharmacol. 389 (2000) 173–179.

[23] Y.P. Pan, X.H. Xu, X.L. Wang, Galantamine blocks delayed rectifier, but not transient outward potassium current in rat dissociated hippocampal pyramidal neurons, Neurosci. Lett. 336 (2003) 37–40.