Comparative Study of Action Mechanisms of Dimebon and Memantine on AMPA- and NMDA-Subtypes Glutamate Receptors in Rat Cerebral Neurons

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Dimebon in low concentrations potentiated activity of AMPA-receptors in rat cerebellar Purkinje neurons, while memantine produced only an insignificant potentiation in a small group of these cells. In cortical neurons of rat brain memantine efficiently blocked NMDA-induced currents in dimebon-insensitive neurons. By contrast, its effect was far weaker in neurons, where the blocking action of dimebon on NMDA-receptors was most pronounced. It was hypothesized that the differences in the effects of memantine and dimebon are determined by their interaction with different sites of NMDA-receptors.

Key Words: *Alzheimer disease; memantine; dimebon; action mechanism; glutamate receptors*

Dimebon and memantine exhibit cognitive-stimulating properties and produce a positive effect in patients with Alzheimer disease (AD) [1,7]. Memantine blocks a site in the luminal pore of NMDA receptor-channel complex in a potential-dependent manner. It was hypothesized that this blockade markedly improves the signal-to-noise ratio for Ca^{2+} entry into neurons, which explains potent cognitive-stimulating effects of memantine in AD patients [6]. The possibility of potentiation of AMPA-receptor activity with memantine was reported. Modulation of glutamate receptors in CNS is an important component of the mechanism of cognitive-stimulating effect of dimebon [3]. *In vivo* experiments showed that dimebon blocks NMDA-induced seizures [1]. Moreover, dimebon blocks voltage-operated calcium channels in cerebellar granular neurons [2].

Here we compared mechanisms of action of memantine and dimebon on ionotropic glutamate (NMDA and AMPA) receptors in neurons of mammalian brain.

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MATERIALS AND METHODS

Experiments were carried out on cortical neurons (effects on NMDA receptors) and Purkinje cerebellar cells isolated from 7-17-day-old rats. The whole-cell currents were recorded under voltage clamp conditions [5] with an EPC-9 (HEKA) apparatus. The substances were applied using rapid superfusion technique. AMPA-receptors were activated with kainic acid (KA). The data were processed using Pulsefit software (HEKA). IC_{50} were presented as $M \pm m$. Application of KA induced inward membrane currents with usual S-shaped dose-dependence. When perfusion saline was replaced with saline containing various concentrations of dimebon, electrical parameters of the membrane remained unchanged. KA was added to the perfusion saline containing various dimebon concentrations. The amplitudes of KA-induced membrane currents were compared with reference amplitudes obtained without dimebon.

RESULTS

The effect of dimebon on AMPA-receptors was examined on 36 neurons. It was established that dimebon

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exerts a complex and ambiguous effect on these receptors, including potentiation of AMPA receptor responses. In various neurons, dimebon increased KA-induced currents by 10-22% starting from the concentration of 0.2 μM. In a concentration of 0.5 μM increased dimebon the currents by 45-55%. Further increase in dimebon concentration produced no additional increment of KA-induced currents. In dimebon concentration range of 1-20 μM, potentiation of KA-induced currents was 20-70% (42% on the average). Further increase in dimebon concentration produced less pronounced potentiation of these currents, and in a dimebon concentration range of 40-50 μM the responses were equal or even below (by 6%) the control. Potentiation of KA-induced currents in the presence of dimebon as described by a bell-shaped curve (Fig. 1, *a*).

The changes of KA-induced currents during washout attest to dual action of dimebon (potentiation and inhibition of KA-induced currents) starting from certain concentrations. After the start of washout, potentiation was preserved within 1-2 min only when dimebon was used in concentrations of 0.2-0.5 μM, then the amplitude of KA-induced currents returned to normal. In a dimebon concentration range of 1-10 μM washout rapidly restored the responses within 1-2 min. When dimebon was applied in concentrations of 20-50 μM, washout decreased the currents below the control values. This decrease was dose-dependent and reached 60% at dimebon concentration of 50 μM. The amplitude returned to the initial (control) values only after 8-10-min washout. These data suggest that dimebon produced a dual effect on KA-induced currents of

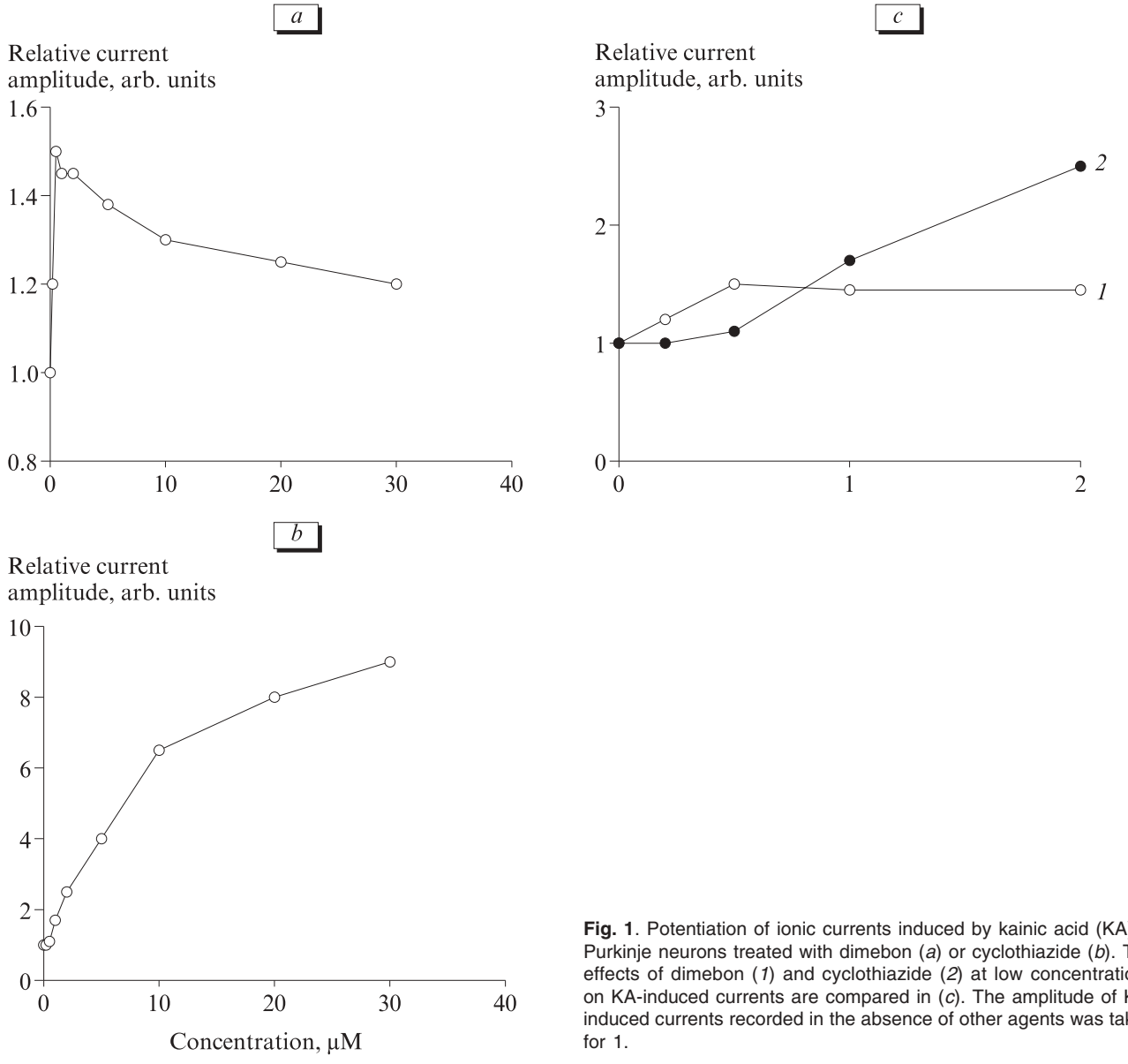

Fig. 1. Potentiation of ionic currents induced by kainic acid (KA) in Purkinje neurons treated with dimebon (*a*) or cyclothiazide (*b*). The effects of dimebon (*1*) and cyclothiazide (*2*) at low concentrations on KA-induced currents are compared in (*c*). The amplitude of KA-induced currents recorded in the absence of other agents was taken for 1.

AMPA receptor-channel complexes: potentiation and inactivation. The nature of the latter effect remains unclear. It can be hypothesized that these processes are induced by dimebon in specific concentration ranges.

For comparison, we examined the effect of cyclothiazide (CTZ), a standard positive modulator of AMPA receptors, applied in the same concentrations (0.2-50.0 μM). CTZ produced significant and dose-dependent potentiation of KA-induced currents. The effect of CTZ appeared at the lowest concentration of 0.5 μM, which potentiated KA-induced currents by 5-15%. At a concentration of 1 μM potentiation was 15-25%. Potentiation increased with increasing CTZ concentration and varied from 400 to 1000% depending on test cells and KA concentration (Fig. 1, *b*).

Thus, there are essential differences in the effects of CTZ and dimebon on AMPA-receptors. Both agents potentiate KA-induced currents, but the potentiating effect of dimebon was observed at lower concentrations in comparison with that of CTZ (Fig. 1, *c*). The increase in CTZ concentration induced further S-shaped potentiation of the currents, which could far surpass the control values. By contrast, the increase in dimebon concentration leads to inactivation of AMPA receptors, which limits possible potentiation of KA-induced currents.

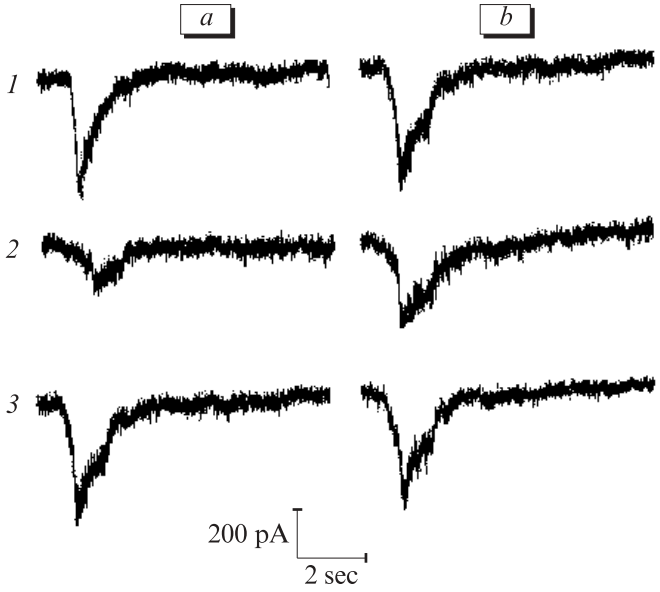

Fig. 2. Effect of dimebon (*a*) and memantine (*b*) on NMDA-induced currents in group 1 neurons. *1*) control; *2*) dimebon (10 μM) or memantine (20 μM); *3*) 3 min after washout.

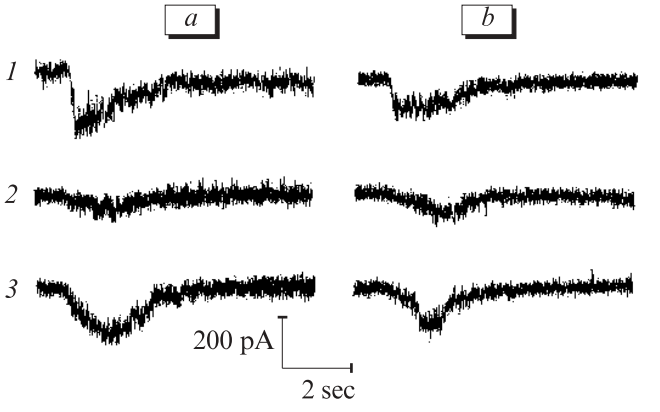

Fig. 3. Effect of memantine (*a*) and dimebon (*b*) on NMDA-induced currents in group 2 neurons. *1*) control; 2) memantine (2 μM) or dimebon (50 μM); 3) 3 min after washout.

It should be noted that memantine modulated AMPA-receptors only in some Purkinje neurons (n=28). In 17 neurons memantine in a concentration of 0.5-20.0 μM produced no apparent effect, while in 11 cells it slightly potentiated KA-induced currents. The degree of this potentiation varied from 6 to 55%. The effect did not depend on memantine concentration and was not obligatorily reproduced during the repeated applications of this neurotransmitter. The character of memantine action on AMPA receptors is difficult to describe. The observed peculiarities can be determined by different properties of AMPA-receptors (subunits) in individual cells.

Dimebon blocked NMDA-induced currents (n=32). According to the character of this blockade, we subdivided cortical neurons into two groups. In group 1 neurons (n=6) IC_{50} varied from 6 to 10 μM (7.7±1.9 μM, Fig. 2, *a*). In group 2 neurons (n=26) IC_{50} varied from 50 to 90 μM (73±21 μM, Fig. 3, *b*). In some neurons of the second group (n=14) dimebon (10-50 μM) decreased NMDA-induced currents by 15-25%. However, no dose-dependence was observed in this concentration range. This dependence was revealed only at dimebon concentrations >50 μM. Blockade of NMDA-induced currents occurred after preliminary application of dimebon. The amplitude of responses after several repeated applications of NMDA did not vary, which attests to the absence of use-dependent inhibition (dependence of an effect from previous action of an agonist). The responses in group 1 neurons very rapidly recovered during washout with physiological saline (within 1-2 min). In group 2 neurons, this recovery took more time (probably because of higher concentrations of dimebon).

Memantine also blocked NMDA-induced currents (n=23), and this blockade differed from that caused by dimebon. In group 2 neurons (n=18) memantine efficiently blocked these currents. In this case, IC_{50} varied from 0.9 to 2.0 μM (mean 1.4±0.4 μM, Fig. 3, *a*). In group 1 neurons (n=5) the blocking effect of memantine was less pronounced: IC_{50} varied from 12 to 18 μM (mean 15.0±3.2 μM, Fig. 2, *b*). In this study group 2 neurons prevailed. In contrast to dimebon, the effect of memantine was characterized by a weak use-dependence. The response to the second application of NMDA in memantine-treated cells was significantly

lower than the response to the first application of the transmitter. During washout the amplitudes of NMDA-induced currents recovered slower. Application of memantine during washout promoted this recovery, which again attests to the use-dependent nature of memantine blockade.

Thus, despite certain similarities, the effects of dimebon and memantine are characterized by some peculiarities. These agents block NMDA receptor-channel complexes, but they probably act upon different channel and/or receptor subunits. Our data confirm the view that memantine acts as an open-channel blocker of NMDA receptor. In contrast to memantine, dimebon probably affects the polyamine site of NMDA-receptor located on NR2B subunit, which is also the target for histamine [4]. It can be hypothesized that dimebon, being an antagonist to H1-histamine receptor, can modulate activity of NMDA-receptors by binding to this site. Thus, the effect of dimebon in low concentrations is most pronounced in neuronal population with high concentration of NR2B subunits. Probably, at higher concentration dimebon acts on any neuron as uncompetitive antagonist of NMDA-receptors.

Dimebon acts as a positive modulator of AMPA receptors only at low concentrations. This is quite a favorable clinical feature of dimebon, which attests to the absence of excitotoxicity of this agent at high concentrations. By contrast, the effect of memantine on AMPA-receptors was unstable, and its ability to potentiate AMPA-induced currents was observed only in some neurons.

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