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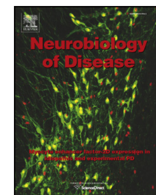

Effects of memantine on the excitation-inhibition balance in prefrontal cortex

Nadezhda V. Povysheva [a,*], Jon W. Johnson [a,b,*]

[a] Department of Neuroscience, and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260, United States
[b] Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260, United States

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ABSTRACT

Memantine is one of the few drugs currently approved for treatment of Alzheimer's disease (AD). The clinical effects of memantine are thought to be associated with inhibition of NMDA receptors (NMDARs). Surprisingly, other open-channel NMDAR blockers have unacceptable side effects that prevent their consideration for AD treatment. One of the mechanisms proposed to explain the therapeutic benefits of memantine involves preferential decrease of excitatory drive to inhibitory neurons in the cortical circuitry and consequent changes in balance between excitation and inhibition (E/I). In this study we addressed effects of memantine on E/I balance in the prefrontal cortex (PFC). We found that a moderate concentration of memantine shifted E/I balance away from inhibition in the PFC circuitry. Indeed, memantine decreased the frequency and amplitude of spontaneous inhibitory postsynaptic currents in pyramidal neurons while leaving spontaneous excitatory postsynaptic currents unaffected. These circuitry effects of memantine were occluded by the competitive NMDAR inhibitor AP-5, and thus are associated with NMDAR inhibition. We also found that memantine decreased feed-forward disynaptic inhibitory input to pyramidal neurons, which is thought to be mediated by parvalbumin (PV)-positive interneurons. Accordingly, memantine caused a greater decrease of the amplitude of NMDAR-mediated synaptic responses in PV-positive interneurons than in pyramidal neurons. Finally, memantine reduced firing activity in PV-positive interneurons while increasing firing in pyramidal neurons. This study elucidates a novel mechanism of action of memantine associated with shifting of the E/I balance away from inhibition in neocortical circuitry, and provides important insights for AD drug development.

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1. Introduction

Alzheimer's disease (AD) is a devastating brain disorder that heavily burdens the aging American population. Although a daunting challenge, creating new AD therapeutics can be aided by better understanding of the mechanisms of action of existing AD drugs.

Memantine has been used to treat AD and other dementias for more than two decades. Although memantine binds to a number of receptors, it is generally believed that the therapeutic effects of memantine are mainly associated with *N*-methyl-D-aspartate receptor (NMDAR) channel block (Lipton, 2006, Parsons et al., 2007, Johnson et al., 2015). There is no general agreement on how NMDAR channel block by memantine slows cognitive decline in AD patients. Several mechanisms of memantine action have been proposed, the majority of which are based on memantine's neuroprotective action. The neuroprotective effect of memantine could result from prevention of excitotoxicity due to excessive NMDAR excitation in pathological brain conditions (Lipton, 2006), or from preferential inhibition of extrasynaptic NMDARs (Leveille et al., 2008, Xia et al., 2010) based on the hypothesis that extrasynaptic NMDAR activation can lead to cell death (Hardingham and Bading, 2010) (but see (Wroge et al., 2012)). However, there is evidence arguing against therapeutic effects of memantine based solely on neuroprotection, e.g. the lack of beneficial effects in early-stage AD, and the rapidity of memantine's effects (Johnson and Kotermanski, 2006).

The ability of memantine to slow cognitive decline in AD patients has also been proposed to result from partial correction of an AD-induced alteration of cortical excitation/inhibition (E/I) balance (Schmitt, 2005). A delicate balance of excitatory and inhibitory elements in the cortex is essential to circuit function, and disturbances of this balance can lead to pathological conditions (Homayoun and Moghaddam, 2007, Haider and McCormick, 2009). There is strong

Abbreviations: AD, Alzheimer's disease; NMDAR, *N*-methyl-D-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; E/I, excitation/inhibition; EGFP, enhanced green fluorescent protein; PFC, prefrontal cortex; sEPSCs, spontaneous excitatory postsynaptic currents; sIPSCs, spontaneous inhibitory postsynaptic currents; mIPSC, miniature inhibitory postsynaptic currents; dIPSCs, disynaptic inhibitory postsynaptic currents; moEPSCs, monosynaptic excitatory postsynaptic currents; PV, parvalbumin; TTX, tetrodotoxin; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline; ACSF, artificial cerebrospinal fluid.

* Corresponding authors at: Department of Neuroscience, University of Pittsburgh, A210 Langley Hall, Pittsburgh, PA 15260, United States.
E-mail addresses: nvp1@pitt.edu (N.V. Povysheva), jjohnson@pitt.edu (J.W. Johnson).

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evidence that the E/I balance is shifted away from excitation in AD. A decrease in cortical activity (Rombouts et al., 2000) and PFC hypometabolism in the prefrontal cortex (PFC) (Schroeter et al., 2012) were reported in AD patients. Data from AD postmortem brains indicate that, whereas excitatory pyramidal neurons in the PFC are prone to neurodegeneration (Hof and Morrison, 2004), PFC inhibitory neurons that express the Ca^{2+} binding protein parvalbumin (PV) are spared (Hof et al., 1991). In transgenic AD model mice, despite substantial neuronal loss in the PFC, no changes in PV-positive and calretinin-positive interneurons were detected (Lemmens et al., 2011). There is substantial loss of cortical spines, the principal site of excitatory synaptic input onto pyramidal neurons, in both the neocortex and hippocampus of AD patients (Cochran et al., 2014). Note, however, that some data do not support decreased excitation in AD. For instance, in the parietal cortex and hippocampus of mice expressing human amyloid precursor protein, nonconvulsive seizure activity resulting from an aberrant increase in network excitability was detected (Palop et al., 2007), and increased excitability of hippocampal CA1 pyramidal neurons was detected in APP/PS1 AD model mice (Siskova et al., 2014).

In this study we explore the hypothesis that memantine can shift the E/I balance in cortical circuitry away from inhibition (Johnson and Kotermanski, 2006) by preferentially reducing NMDAR-mediated excitation of inhibitory neurons. This hypothesis is based on two observations: (1) in physiological Mg^{2+}, memantine at therapeutic concentrations preferentially inhibits NMDARs that contain the GluN2C or GluN2D subunits (Kotermanski and Johnson, 2009); (2) in adult cortex and hippocampus, the GluN2D subunit is preferentially expressed in inhibitory neurons (Monyer et al., 1994, Standaert et al., 1996). If this hypothesis is correct, then memantine at a concentration that preferentially inhibits GluN2C and GluN2D-containing NMDARs should be more effective at reducing the action potential frequency of inhibitory neurons than of excitatory neurons. To test this prediction we explored in mouse PFC the effects of 10 μM memantine on inhibitory and excitatory synaptic inputs to pyramidal neurons, on NMDAR-mediated synaptic inputs to PV-positive interneurons, and on synaptically-activated action potentials in pyramidal neurons and PV-positive interneurons.

2. Materials and methods

Experiments were performed on PFC slices from 3 to 7 month old CB6-Tg(Gad1-EGFP)G42jh/J mice of either sex, which express enhanced green fluorescent protein (EGFP) in PV-positive interneurons (http://jaxmice.jax.org/strain/007677.html). All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and approved by our Institutional Animal Care and Use Committee. Mice were deeply anesthetized with chloral hydrate and decapitated. The brain was quickly removed and immersed in ice-cold preoxygenated artificial cerebrospinal fluid (ACSF). Coronal slices containing PFC were made as previously described (Povysheva et al., 2006). Throughout experiments, ACSF (31–32 °C, perfused with a 95% O_2/5% CO_2) of the following composition was used (in mM): 126 NaCl, 2.5 or 10 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 24 $NaHCO_3$, 10–20 glucose; pH ~7.3.

2.1. Electrophysiological recordings

Whole-cell recordings were performed from layer 2–3 neurons visualized by IR-DIC videomicroscopy as previously described (Povysheva and Johnson, 2012). Pyramidal neurons were identified by their apical dendrites and triangular somata. Interneurons were recognized based on EGPF green fluorescence. Patch electrodes (5–10 MΩ open-tip resistance) were filled with a solution containing (in mM): 105 Cs-gluconate, 2 $MgCl_2$, 10 NaCl, 10 HEPES, 10 phosphocreatine, 4 ATP-Mg, 0.3 GTP, and 10 BAPTA; pH 7.25. Alexa hydrazide 568 (0.075%; Molecular Probes, Eugene, OR) was added to the solution for morphological identification of the recorded neurons. Voltage and current recordings were performed with a Multi-Clamp 700A amplifier (Axon Instruments, Union City, CA). Signals were filtered at 2 kHz and acquired at a sampling rate of 10 kHz using Clampex 10.2 software (Molecular Devices Corporation, Sunnyvale, CA). Access resistance typically was 10–20 MΩ and remained relatively stable during experiments (≤30% increase). Corrections were made for liquid junction potential (−13 mV). We used: gabazine (10 μM) to inhibit $GABA_A$ receptors; 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline (NBQX; 20 μM) to inhibit kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs); D-2-amino-5-phosphopentanoic acid (AP-5; 50 μM) to inhibit NMDARs; tetrodotoxin (TTX; 0.5 μM) to inhibit voltage-gated Na^+ channels; memantine (10 μM). Gabazine, NBQX, and AP-5 were purchased from Ascent Scientific LTD (Bristol, UK); TTX and memantine from Sigma (St. Louis, MO).

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded at +12 mV, spontaneous excitatory post-synaptic currents (sEPSCs) were recorded at −70 mV, both in ACSF with elevated K^+ (10 mM). Miniature inhibitory postsynaptic currents (mIPSCs) were recorded at +12 mV in TTX. We found that sIPSCs and sEPSCs were stable in the absence of memantine for the typical duration of experiments. A bipolar stimulating electrode placed on the border of white matter and layer 6 (Povysheva et al., 2006) was used to evoke NMDAR EPSCs, sequences of feed-forward monosynaptic and disynaptic IPSCs (Fig. 4), and trains of EPSPs and synaptically-activated action potentials (Fig. 5). NMDAR EPSCs were recorded at −70 mV, and feed-forward monosynaptic and disynaptic IPSCs were recorded at −50 mV, both in elevated K^+. Trains of EPSPs and synaptically-activated action potentials were evoked by five stimuli delivered at 25 Hz in elevated K^+; 14–18 trains with an intertrain interval of 10 s were used for each cell in each condition.

2.2. Electrophysiological, morphological, and statistical data analysis

Membrane properties and morphology of neurons were analyzed as previously described (Povysheva et al., 2006). Spontaneous and miniature events were analyzed using the MiniAnalysis Program (Synaptosoft, Decatur, GA) as previously described (Povysheva and Johnson, 2012). Amplitude of evoked synaptic responses was measured on averaged traces as the most positive (for IPSC) or the most negative (for EPSC) current value compared to baseline current using Clampfit. Charge per sIPSC or sEPSC was the averaged area of spontaneous currents above or below mean baseline current; mean current was mean charge per spontaneous current multiplied by spontaneous current frequency. Spike probability for each stimulus number in a train was calculated as the total number of stimulation-evoked spikes divided by the total number of stimuli across all stimulus trains given to a cell in each condition. Two-tailed paired *t*-test was used for paired comparisons and ANOVA for correlated samples followed by Tukey post hoc test was used for group comparisons using Excel (Microsoft Corp., Redmond, WA). Values are presented as mean ± SEM.

3. Results

3.1. Memantine reduces inhibition of pyramidal neurons

First we assessed memantine's effects on inhibitory input to pyramidal neurons, the cell type that is the main source of excitation in PFC circuitry. All pyramidal neurons studied exhibited typical physiological phenotype including large action potential amplitude, strong adaptation (Fig. 1A, *left*), and typical morphology (triangular cell body, pronounced apical dendrite, etc.; Fig. 1A, *right*). To isolate sIPSCs, pyramidal neurons were held at +12 mV (Cossart et al., 2001), a voltage far from the reversal potential of $GABA_A$ receptor responses, but close to that of glutamatergic responses, which were undetectable at +12 mV: all visible events were abolished by gabazine (Fig. 1B). The

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