Mg²⁺ Imparts NMDA Receptor Subtype Selectivity to the Alzheimer's Drug Memantine

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N-Methyl-D-aspartate receptors (NMDARs) mediate interneuronal communication and are broadly involved in nervous system physiology and pathology (Dingledine et al., 1999). Memantine, a drug that blocks the ion channel formed by NMDARs, is a widely prescribed treatment of Alzheimer's disease (Schmitt, 2005; Lipton, 2006; Parsons et al., 2007). Research on memantine's mechanism of action has focused on the NMDAR subtypes most highly expressed in adult cerebral cortex, NR1/2A and NR1/2B receptors (Cull-Candy and Leszkiewicz, 2004), and has largely ignored interactions with extracellular Mg²⁺ (Mg_o²⁺). Mg_o²⁺ is an endogenous NMDAR channel blocker that binds near memantine's binding site (Kashiwagi et al., 2002; Chen and Lipton, 2005). We report that a physiological concentration (1 mM) of Mg_o²⁺ decreased memantine inhibition of NR1/2A and NR1/2B receptors nearly 20-fold at a membrane voltage near rest. In contrast, memantine inhibition of the other principal NMDAR subtypes, NR1/2C and NR1/2D receptors, was decreased only ~3-fold. As a result, therapeutic memantine concentrations should have negligible effects on NR1/2A or NR1/2B receptor activity but pronounced effects on NR1/2C and NR1/2D receptors. Quantitative modeling showed that the voltage dependence of memantine inhibition also is altered by 1 mM Mg_o²⁺. We report similar results with the NMDAR channel blocker ketamine, a drug used to model schizophrenia (Krystal et al., 2003). These results suggest that currently hypothesized mechanisms of memantine and ketamine action should be reconsidered and that NR1/2C and/or NR1/2D receptors play a more important role in cortical physiology and pathology than previously appreciated.

Introduction

N-Methyl-D-aspartate receptors (NMDARs) are excitatory glutamate receptors that exhibit high calcium (Ca²⁺) permeability and voltage-dependent channel block by extracellular Mg²⁺ (Mg_o²⁺) (Dingledine et al., 1999), properties of fundamental physiological and pathological importance. Channel block by Mg_o²⁺ reduces Ca²⁺ influx at membrane voltages near rest, but is relieved during neuronal excitation (Dingledine et al., 1999). Ca²⁺ influx through NMDARs induced by synaptic activity is required for many types of synaptic plasticity, and underlies some forms of learning and memory. Excessive Ca²⁺ influx may result in excitotoxic cell death, one of many NMDAR-mediated processes hypothesized to play a role in neurodegenerative and neuropsychiatric disorders (Dingledine et al., 1999; Krystal et al., 2003; Moghaddam and Jackson, 2003).

Functional NMDARs are composed of 4 subunits and usually contain NR1 and one or more of the four NR2 (NR2A–NR2D) subunits (Dingledine et al., 1999). NR2 subunit expression is developmentally and regionally regulated (Monyer et al., 1994; Cull-Candy and Leszkiewicz, 2004). The identity of the NR2 subunit(s) in an NMDAR strongly influences receptor properties, including agonist affinity, deactivation kinetics, single-channel

conductance, Ca²⁺ permeability, and channel block by Mg_o²⁺ (Dingledine et al., 1999; Cull-Candy and Leszkiewicz, 2004). Of particular relevance here, NMDAR inhibition by Mg_o²⁺ is considerably weaker in NR1/2C and NR1/2D receptors than in NR1/2A or NR1/2B receptors (Kutsuwada et al., 1992; Monyer et al., 1994; Kuner and Schoepfer, 1996).

Several drugs of clinical importance, including memantine, act by binding in the channel of NMDARs at a site that overlaps with the Mg_o²⁺ blocking site (Kashiwagi et al., 2002; Chen and Lipton, 2005) (see Fig. 1A). Memantine block exhibits voltage dependence; the concentration of memantine that inhibits NMDAR responses by 50% (the IC₅₀) increases with depolarization (Rogawski and Wenk, 2003; Johnson and Kotermanski, 2006; Parsons et al., 2007). Memantine slows the cognitive decline associated with Alzheimer's disease and represents a departure in Alzheimer's disease therapy from previously developed medications that enhance cholinergic transmission (Schmitt, 2005; Lipton, 2006). Considerable evidence indicates that the therapeutic effects of memantine derive predominantly from NMDAR inhibition (Rogawski and Wenk, 2003; Schmitt, 2005; Lipton, 2006; Parsons et al., 2007). However, it appears paradoxical that inhibition of NMDARs slows memory loss associated with Alzheimer's disease, since NMDAR activation is essential for memory formation (Dingledine et al., 1999). Hypotheses proposed to explain the therapeutic actions of memantine include slowing of neuronal loss due to excitotoxic overactivation of NMDARs and correction of an excitation-inhibition imbalance (Rogawski and Wenk, 2003; Schmitt, 2005; Johnson and Kotermanski, 2006; Lipton, 2006; Parsons et al., 2007). Here, we investigate the possibility that physiological Mg_o²⁺ modifies the inhib-

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itory properties of NMDAR channel blockers, conferring on them properties that may help explain the clinical utility of memantine.

Materials and Methods

Cell culture and transfection. Experiments were performed on the HEK293T mammalian cell line. Cells were transfected using Lipofectamine (Invitrogen) (Qian and Johnson, 2006), with cDNA for the NR1-1a [GenBank accession number (ACCN) X63255, in pcDM8] subunit and either the NR2A (ACCN M91561, in pcDM8), NR2B (ACCN M91562, in pcDNA1), NR2C (ACCN M91563, in pcDNA1), or NR2D (ACCN L31612, in pcDM8) subunit. Enhanced green fluorescent protein (eGFP) cDNA was cotransfected as a marker of successful transfection.

Electrophysiology. Whole-cell patch-clamp recordings were performed with an Axopatch-1D amplifier ~24 h after transfection. For experiments in 1 mM Mg^{2+} , cells with bright eGFP fluorescence (associated with larger NMDAR responses) were chosen. Recording electrodes of 2–6 M Ω resistance were filled with an internal solution consisting of the following (in mM): 125 CsCl, 10 BAPTA, and 10 HEPES; pH adjusted to 7.20 $\pm$ 0.05 with CsOH; osmolality 275 $\pm$ 10 mmol/kg. Series resistance was compensated 80–95%.

External solutions consisted of the following (in mM): 140 NaCl, 2.8 KCl, 1 CaCl₂, and 10 HEPES (and 1 MgCl₂ when indicated); pH adjusted to 7.20 $\pm$ 0.05 with NaOH; osmolality adjusted to 290 $\pm$ 10 mmol/kg with sucrose. Solutions were applied to cells by a seven-barrel fast perfusion system. NMDARs were activated by 1 mM glutamate and 100 μ M glycine. Correction for the –6 mV junction potential was applied to all data.

Data analysis. Data were low-pass filtered at 100 Hz and analyzed using Clampfit 9.2 (Axon Instruments). Currents averaged during 500 ms time windows were used for all baseline current and steady-state NMDAR-mediated current measurements. Concentration-inhibition curves were fit using the following equation: I_{Mem}/I_{Con} or $I_{Ket}/I_{Con} = 1/(1 + ([B]/IC_{50})^{n_H})$, where I_{Mem} and I_{Ket} are steady state currents in agonists plus blocker(s), I_{Con} is steady-state current in agonists alone, $[B]$ is concentration of memantine or ketamine, and n_H is the Hill coefficient.

Results

Identification of an inhibitory drug's site(s) of action requires knowledge of its IC_{50} at potential targets under physiological conditions. The median value of many published measurements of memantine's IC_{50} for NMDARs at voltages near rest is ~1 μ M (Johnson and Kotermanski, 2006; Parsons et al., 2007). Memantine has been found to exhibit only weak NMDAR subtype selectivity, with an IC_{50} for NR1/2A receptors ~2- to several-fold higher than for NR1/2B, NR1/2C, and NR1/2D receptors (Dravid et al., 2007; Parsons et al., 2007). Use of these data to evaluate memantine's interactions with NMDARs under physiological conditions suffers from a potentially critical oversight: endogenous Mg^{2+} may compete with or otherwise affect memantine binding. Previous estimates of memantine affinity, selectivity, and voltage dependence were performed in 0 Mg^{2+} , possibly because of difficulty in measuring memantine inhibition of the small NMDAR responses observed in physiological Mg^{2+} . However, evidence for overlap of memantine and Mg^{2+} binding sites (Kashiwagi et al., 2002; Chen and Lipton, 2005), and hindrance of memantine binding by Mg^{2+} (Sobolevsky et al., 1998) suggest that physiological Mg^{2+} could powerfully influence memantine inhibition of NMDARs. If Mg^{2+} and memantine compete for binding in the channel, then the memantine IC_{50} should increase in Mg^{2+} . We examined memantine inhibition of NMDARs in a physiological $[Mg^{2+}]$ (1 mM) (see supplemental material, available at www.jneurosci.org).

We compared memantine inhibition of whole-cell currents recorded at –66 mV from HEK293T cells transfected with cDNAs encoding the NR1 and either the NR2A, NR2B, NR2C, or

NR2D subunit in 0 or 1 mM Mg^{2+} . NMDAR responses were activated with a saturating [glutamate] (1 mM) and [glycine] (100 μ M) to avoid possible agonist concentration dependence of memantine inhibition (Lipton, 2006) (but see Parsons et al., 2007). Results of experiments in 0 Mg^{2+} confirmed that memantine exhibits little NMDAR subtype selectivity. Memantine IC_{50} s for all NMDAR subtypes were between 0.5 and 1 μ M (Fig. 1B,C, Table 1), with the IC_{50} for NR1/2A receptors slightly higher than for other receptor subtypes.

At voltages near rest, 1 mM Mg^{2+} strongly inhibits NMDAR responses, especially responses mediated by NR1/2A or NR1/2B receptors. Under conditions similar to those used here (all conditions same except 30 μ M NMDA plus 10 μ M glycine were used as agonists; 10 mM EGTA replaced 10 mM BAPTA; holding voltage = –65 mV), 1 mM Mg^{2+} inhibited NMDAR responses by: NR1/2A, 94.5 $\pm$ 0.7% (n = 6); NR1/2B, 96.0 $\pm$ 1.5% (n = 5); NR1/2C, 78.6 $\pm$ 0.5% (n = 4); NR1/2D, 77.8 $\pm$ 2.2% (n = 5) (R. J. Clarke and J. W. Johnson, unpublished observations). Use of transfected HEK293T cells with large NMDAR-mediated currents nevertheless permitted accurate measurement of memantine concentration-inhibition curves in 1 mM Mg^{2+} . The memantine IC_{50} was greatly influenced in a subtype-selective manner by 1 mM Mg^{2+} (Fig. 1D,E, Table 1): memantine concentration-inhibition curves were right-shifted by factors of 16.8 (NR1/2A), 18.2 (NR1/2B), 3.1 (NR1/2C), and 3.3 (NR1/2D). Memantine thereby acquires in 1 mM Mg^{2+} a 5.9- to 8.3-fold selectivity for NR1/2C and NR1/2D receptors over NR1/2A and NR1/2B receptors.

We also investigated the effects of Mg^{2+} on NMDAR inhibition by ketamine, another channel blocker of broad clinical and pathological significance that exhibits kinetics and IC_{50} values similar to memantine's. Ketamine is an important tool in schizophrenia research because it is psychotomimetic in healthy adults, exacerbates symptoms in schizophrenics (Krystal et al., 2003), and is used to generate animal models of the disease (Moghaddam and Jackson, 2003). Ketamine also is used as a pediatric and veterinary anesthetic and may be useful as a treatment for conditions including neuropathic pain (Annetta et al., 2005). Ketamine has been reported to be largely unselective among NMDAR subtypes (Yamakura et al., 1993; Dravid et al., 2007). However, as with memantine, nearly all studies of ketamine IC_{50} and selectivity were performed in 0 Mg^{2+} ; when interactions have been examined, NMDAR inhibition by ketamine was reported to be both reduced (MacDonald et al., 1991) and augmented (Liu et al., 2001) by Mg^{2+} .

The effects of 1 mM Mg^{2+} on ketamine IC_{50} resembled the effects on memantine inhibition (Fig. 2, Table 1): ketamine concentration-inhibition curves were right-shifted by factors of 16.2 (NR1/2A), 16.4 (NR1/2B), 2.3 (NR1/2C), and 3.6 (NR1/2D). Thus, the powerful effects of Mg^{2+} are not specific to memantine, a conclusion also supported by previous data indicating that the ketamine analog phencyclidine interacts competitively with Mg^{2+} when inhibiting NMDAR responses (Lerma et al., 1991). Because of the structural dissimilarity of memantine and ketamine, the results presented here suggest that NMDAR channel-blocking drugs generally interact competitively with Mg^{2+} .

The principal therapeutic effects of NMDAR channel blockers have been hypothesized to take place in moderately depolarized neurons with weakened inhibition by Mg^{2+} (Rogawski and Wenk, 2003; Lipton, 2006; Parsons et al., 2007). Thus, effects of Mg^{2+} on the voltage dependence of NMDAR channel blockers may be of great importance. Because NMDAR inhibition by

Mg^{2+} is more strongly voltage dependent than inhibition by memantine or ketamine (see supplemental material, available at www.jneurosci.org), voltage may have complex effects on competition between Mg^{2+} and memantine or ketamine. We used a model to investigate the voltage dependence of memantine and ketamine inhibition in Mg^{2+} . The model was based on simple competition (two blockers cannot bind in the channel simultaneously) and used previously published data and models to estimate the voltage dependence of inhibition (see supplemental material, available at www.jneurosci.org). We first compared model predictions to our measurements at -66 mV. Experimentally measured Mg^{2+} -induced increases of memantine and ketamine IC_{50} s for NR1/2A and NR1/2B receptors were in good quantitative agreement with predictions of the competition model (Table 1, compare values in Exp IC_{50} and Model IC_{50} columns). Measured Mg^{2+} -induced increases of memantine and ketamine IC_{50} s for NR1/2C and NR1/2D receptors were lower than model predictions by a factor of ~ 2 (Table 1). This discrepancy suggests that binding of Mg^{2+} is not fully competitive with memantine or ketamine binding at NR1/2C or NR1/2D receptors. A possible explanation is the relatively fast rate of Mg^{2+} permeation of NR1/2D (and presumably NR1/2C) receptors (Qian and Johnson, 2006), which may enhance memantine or ketamine access to the channel of NR1/2C and NR1/2D receptors in Mg^{2+} .

To predict the voltage dependence of memantine and ketamine IC_{50} s in 1 mM Mg^{2+} , we modified the model so its predictions agreed with the IC_{50} values measured at -66 mV. We modified the model by calculating “equivalent” $[Mg^{2+}]$ s for each blocker and receptor subtype. An equivalent $[Mg^{2+}]$ of 1 mM would indicate that the measured memantine or ketamine IC_{50} in 1 mM Mg^{2+} equaled the IC_{50} predicted by the model. An equivalent $[Mg^{2+}]$ of <1 mM would indicate that the measured IC_{50} was lower than predicted by simple competition (that is, 1 mM Mg^{2+} right-shifted the concentration-inhibition curve less than expected). The calculated equivalent $[Mg^{2+}]$ s (range: 0.24 – 0.88 mM) are given in supplemental material, available at www.jneurosci.org. Voltage dependencies of memantine and ketamine IC_{50} s were predicted (Fig. 3) using the competition model with equivalent $[Mg^{2+}]$ s. To test the accuracy of competition model predictions, we measured memantine and ketamine IC_{50} s for NR1/2A and NR1/2D receptors also at -26 mV. All IC_{50} s at -26 mV were within a factor of 2 of competition model predictions (Fig. 3; supplemental Table, available at www.jneurosci.org as supplemental material), supporting model accuracy. Furthermore, the competition model correctly predicted the direction of

Figure 1. Effects of Mg^{2+} and NMDAR subunit composition on memantine inhibition. **A**, Schematic of an NMDAR showing the four subunits of a functional receptor (left) and transmembrane structure of an NR1 and an NR2 subunit (right). The approximate channel blocking region of Mg^{2+} , memantine, and ketamine (filled black circle with +) is shown near the asparagines (filled red circles) involved in blocker binding. **B**, **D**, Overlay of NR1/2A (black) and NR1/2D (red) receptor current traces recorded from transfected HEK293T cells in 0 (**B**) and 1 mM (**D**) Mg^{2+} during application of the indicated memantine concentrations ($[Mem]$). Peak inward NR1/2A receptor currents here and in Figure 2 are truncated. Current noise in Figures 1 and 2 appears lower in Mg^{2+} because Mg^{2+} block shifts noise to higher (more heavily filtered) frequencies. Experiments similar to those shown in **A** and **B** were performed with NR1/2B and NR1/2C receptors (examples not shown). **C**, **E**, Memantine concentration-inhibition curves recorded in 0 (**C**) and 1 mM (**E**) Mg^{2+} from the indicated NMDAR subtypes. Gray shaded areas represent estimated extracellular memantine concentration range (0.5 – 1 μM) during therapeutic treatment (Parsons et al., 2007).

change of IC_{50} between -26 and -66 mV in all cases: with both memantine and ketamine, IC_{50} was lower at -26 mV for NR1/2A receptors, but lower at -66 mV for NR1/2D receptors (supplemental Table, available at www.jneurosci.org as supplemental material).

Both model and data show that the voltage dependence of memantine IC_{50} in 1 mM Mg^{2+} differs strikingly from voltage dependence in 0 Mg^{2+} (Fig. 3A). Positively charged extracellular channel blockers typically inhibit less effectively with membrane voltage depolarization. In contrast, over a range of 10 's of mV around resting voltage in Mg^{2+} , memantine inhibition of all NMDAR subtypes was predicted to moderately increase (IC_{50} s to

Table 1. Effect of Mg^{2+} on memantine and ketamine IC_{50} s at -66 mV

Drug	$[Mg^{2+}]$ (mM)	NMDAR subtype										
		NR1/2A		NR1/2B		NR1/2C		NR1/2D		Ratio 2A/2B		
		Exp IC_{50}	Model IC_{50}	Exp IC_{50}	Model IC_{50}	Exp IC_{50}	Model IC_{50}	Exp IC_{50}	Model IC_{50}	Ratio 2A/2C	Ratio 2A/2D	Ratio 2A/2B
Memantine	0	0.80 ± 0.07		0.57 ± 0.09		0.52 ± 0.02		0.54 ± 0.06		1.54	1.48	
	1	13.4 ± 1.3	16.5	10.4 ± 0.4	11.8	1.61 ± 0.06	3.36	1.76 ± 0.06	3.49	8.32	7.61	
Ketamine	0	0.33 ± 0.01		0.31 ± 0.02		0.51 ± 0.01		0.83 ± 0.02		0.65	0.40	
	1	5.35 ± 0.34	6.82	5.08 ± 0.02	6.41	1.18 ± 0.04	3.30	2.95 ± 0.02	5.37	4.53	1.81	

All IC_{50} values are in μM . Exp (Experimental) IC_{50} s (IC_{50} values measured by fitting concentration-inhibition curves; see Materials and Methods) are given as mean ± SE (estimated during nonlinear curve fitting by Origin) and are based on measurements from 3–4 cells for each condition. For information on Model IC_{50} values, see supplemental material (available at www.jneurosci.org). Each Ratio is the IC_{50} for NR1/2A receptors divided by the IC_{50} for the indicated NMDAR subtype.

Figure 2. Effects of Mg^{2+} and NMDAR subunit composition on ketamine inhibition. **A, C**, Overlay of NR1/2A (black) and NR1/2D (red) receptor current traces recorded in 0 (**A**) and 1 mM (**C**) Mg^{2+} during application of the indicated concentrations of (+/–)ketamine ([Ket]). Similar experiments were performed with NR1/2B and NR1/2C receptors (examples not shown). **B, D**, Ketamine concentration-inhibition curves recorded in 0 (**B**) and 1 mM (**D**) Mg^{2+} from the indicated NMDAR subtypes.

decrease) with depolarization. The IC_{50} of memantine decreases with depolarization because the voltage dependence of inhibition is greater for Mg^{2+} than for memantine. The effects of Mg^{2+} on the voltage dependence of ketamine IC_{50} s (Fig. 3B) parallel results with memantine.

Discussion

The above results reveal that Mg^{2+} has a powerful effect on the IC_{50} , selectivity, and voltage dependence of clinically relevant NMDAR channel blockers. The IC_{50} increases take place in a critical concentration range. The estimated free extracellular memantine concentration in the brain of patients during typical treatment regimes is ~ 0.5 – $1 \mu M$ (Parsons et al., 2007). The similarity of the therapeutic memantine concentration and its NMDAR IC_{50} measured in 0 Mg^{2+} has been critical in identifying memantine's site of action. Because of memantine's weak NMDAR subtype selectivity in 0 Mg^{2+} (Dravid et al., 2007; Parsons et al., 2007) and the high expression of NR2A and NR2B subunits in cortex (Cull-Candy and Leszkiewicz, 2004), research generally has focused on memantine interactions with NR2A- and/or NR2B-containing receptors (Blanpied et al., 1997; Kashi-

wagi et al., 2002; Chen and Lipton, 2005; Parsons et al., 2007). Our data indicate that memantine is unlikely to act therapeutically by inhibiting NR1/2A or NR1/2B receptors: in physiological Mg^{2+} and at voltages near rest, the memantine IC_{50} s for NR1/2A and NR1/2B receptors is over tenfold higher than therapeutic brain concentrations (Fig. 1, Table 1). The therapeutic utility of memantine has been hypothesized to be related to its weak selectivity for NR2C- and NR2D-containing receptors (observed in 0 Mg^{2+}) (Rogawski and Wenk, 2003; David et al., 2006). Our data extend this hypothesis by showing that 1 mM Mg^{2+} enhances the selectivity of memantine (and ketamine, and probably other channel blockers) for NR1/2C and NR1/2D receptors through two mechanisms: first, the Mg^{2+} IC_{50} is higher for NR1/2C and NR1/2D than NR1/2A and NR1/2B receptors; second, Mg^{2+} competes less effectively with memantine or ketamine binding to NR1/2C and NR1/2D than to NR1/2A and NR1/2B receptors (compare Exp IC_{50} with Model IC_{50} in Table 1). These data suggest that NR2C- and/or NR2D-containing NMDARs are likely sites of therapeutic memantine action.

In 1 mM Mg^{2+} , depolarizations around resting voltage were predicted to decrease moderately memantine's and ketamine's IC_{50} s for all four NMDAR subtypes (Fig. 3) (data for NR1/2B and NR1/2C not shown). Measured IC_{50} s at -26 mV supported the accuracy of the model (Fig. 3; supplemental Table, available at www.jneurosci.org as supplemental material). The reversal by Mg^{2+} of the voltage dependence of memantine block is consistent with the hypothesis that memantine may act therapeutically by preferentially inhibiting NMDARs of depolarized neurons (Rogawski and Wenk, 2003; Lipton, 2006; Parsons et al., 2007). The decrease of IC_{50} with depolarization also might permit therapeutic memantine concentrations to mediate an appreciable inhibition of NR1/2A and NR1/2B receptors in depolarized neurons. However, the predicted minimum memantine IC_{50} s for NR1/2A ($4.9 \mu M$ at -24 mV) and NR1/2B ($3.6 \mu M$ at -23 mV) receptors are well above memantine's therapeutic concentration range and are at quite depolarized voltages. Consistent with model predictions, the NR1/2A receptor IC_{50} measured at -26 mV is $6.34 \mu M$ (supplemental Table, available at www.jneurosci.org as supple-

Figure 3. Model predictions of Mg^{2+} and NMDAR subtype effects on voltage dependence of memantine and ketamine inhibition. **A**, **B**, Modeled voltage dependence of memantine (**A**) and ketamine (**B**) IC_{50} s in 0 and 1 mM Mg^{2+} , corrected for incomplete competition between memantine or ketamine and Mg^{2+} (by using equivalent $[Mg^{2+}]_s$). For clarity, only plots for NR1/2A (which resemble plots for NR1/2B) and for NR1/2D (which resemble plots for NR1/2C) receptors are shown. Points at -66 and -26 mV show measured IC_{50} values in 0 (filled circles) and/or 1 mM (open circles) Mg^{2+} . The two points at -26 mV in **B** are nearly identical. For details of model and calculation of equivalent $[Mg^{2+}]_s$, see supplemental material (available at www.jneurosci.org).

mental material). Thus, it remains unlikely that NR1/2A or NR1/2B receptors are principal sites of memantine action.

Memantine also inhibits other receptors with low IC_{50} s, including nicotinic acetylcholine receptors (nAChRs) (Oliver et al., 2001; Maskell et al., 2003; Aracava et al., 2005; Parsons et al., 2007). It was argued that inhibition of nAChRs is unlikely to be the basis of memantine's therapeutic utility in Alzheimer's disease (Johnson and Kotermanski, 2006; Parsons et al., 2007) because other Alzheimer's disease treatments augment cholinergic transmission. However, especially given the increased memantine IC_{50} s for NMDARs reported here, a contribution to memantine's clinical utility by actions at other receptors is difficult to exclude (see below).

The clinical effects of memantine and ketamine suggest that preferential inhibition of NR2C- and/or NR2D-containing NMDARs can strongly impact cognitive function. NR2C and NR2D subunits are broadly expressed in the adult mammalian brain, including in hippocampus, cortex, and thalamus, brain regions hypothesized to be involved in Alzheimer's disease and/or schizophrenia. A possible consequence of inhibition of

NR2D-containing NMDARs is preferential reduction of tonic NMDAR-mediated pyramidal cell currents, which may be carried by extrasynaptic NR1/2D receptors (Le Meur et al., 2007). Alternatively, inhibition of NR2D-containing receptors could selectively reduce excitation of a subset of inhibitory neurons that highly express the NR2D subunit (Monyer et al., 1994) (see supplemental material, available at www.jneurosci.org), an effect that may contribute to the clinical utility of memantine. In Alzheimer's disease, amyloid- β accumulation (especially in excitatory pyramidal neurons) (D'Andrea and Nagele, 2006) can cause internalization of NMDARs (Snyder et al., 2005) and preferential loss in cortex of excitatory terminals (Bell and Claudio Cuello, 2006). Memantine could partially counterbalance an amyloid- β -induced reduction of cortical excitation by preferentially antagonizing NMDARs on inhibitory interneurons; if this suggestion is correct, then more selective inhibition of NR1/2D receptors may hold therapeutic promise. The clinical utility of memantine may be enhanced by its inhibition of α -7 nAChRs [reported memantine IC_{50} ranges from 0.34 (Aracava et al., 2005) to 5 μM (Maskell et al., 2003)], receptors that participate in amyloid- β -induced NMDAR internalization (Snyder et al., 2005). Ketamine shows a similar (although weaker) preferential inhibition of NR1/2D and especially NR1/2C receptors (Fig. 2) that is predicted to reverse at moderately depolarized voltages (Fig. 3B), properties that suggest more complex subtype selectivity. Ketamine's selectivity for NR1/2C and NR1/2D receptors at voltages near rest also may lead to cortical disinhibition, a process hypothesized to be responsible for ketamine's ability to induce a schizophrenia-like psychotic state (Greene, 2001; Homayoun and Moghaddam, 2007; Lisman et al., 2008). These ideas emphasize the importance of understanding the roles played by NR2C and NR2D subunits in brain function, and the mechanisms that underlie the diverse clinical actions of NMDAR channel blockers.

References

- Annetta MG, Iemma D, Garisto C, Tafani C, Proietti R (2005) Ketamine: new indications for an old drug. *Curr Drug Targets* 6:789–794.
- Aracava Y, Pereira EF, Maelicke A, Albuquerque EX (2005) Memantine blocks α 7* nicotinic acetylcholine receptors more potently than N-methyl-D-aspartate receptors in rat hippocampal neurons. *J Pharmacol Exp Ther* 312:1195–1205.
- Bell KF, Claudio Cuello A (2006) Altered synaptic function in Alzheimer's disease. *Eur J Pharmacol* 545:11–21.
- Blanpied TA, Boeckman FA, Aizenman E, Johnson JW (1997) Trapping channel block of NMDA-activated responses by amantadine and memantine. *J Neurophysiol* 77:309–323.
- Chen HS, Lipton SA (2005) Pharmacological implications of two distinct mechanisms of interaction of memantine with N-methyl-D-aspartate-gated channels. *J Pharmacol Exp Ther* 314:961–971.
- Cull-Candy SG, Leszkiewicz DN (2004) Role of distinct NMDA receptor subtypes at central synapses. *Sci STKE* 2004:re16.
- D'Andrea MR, Nagele RG (2006) Targeting the α 7 nicotinic acetylcholine receptor to reduce amyloid accumulation in Alzheimer's disease pyramidal neurons. *Curr Pharm Des* 12:677–684.
- David HN, Anseau M, Lemaire M, Abraïni JH (2006) Nitrous oxide and xenon prevent amphetamine-induced carrier-mediated dopamine release in a memantine-like fashion and protect against behavioral sensitization. *Biol Psychiatry* 60:49–57.
- Dingledine R, Borges K, Bowie D, Traynelis SF (1999) The glutamate receptor ion channels. *Pharmacol Rev* 51:7–61.
- Dravid SM, Erreger K, Yuan H, Nicholson K, Le P, Lyuboslavsky P, Almonte A, Murray E, Mosely C, Barber J, French A, Balster R, Murray TF, Traynelis SF (2007) Subunit-specific mechanisms and proton sensitivity of NMDA receptor channel block. *J Physiol* 581:107–128.
- Greene R (2001) Circuit analysis of NMDAR hypofunction in the hippocampus, in vitro, and psychosis of schizophrenia. *Hippocampus* 11:569–577.

- Homayoun H, Moghaddam B (2007) NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J Neurosci* 27:11496–11500.
- Johnson JW, Kotermanski SE (2006) Mechanism of action of memantine. *Curr Opin Pharmacol* 6:61–67.
- Kashiwagi K, Masuko T, Nguyen CD, Kuno T, Tanaka I, Igarashi K, Williams K (2002) Channel blockers acting at N-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. *Mol Pharmacol* 61:533–545.
- Krystal JH, D'Souza DC, Mathalon D, Perry E, Belger A, Hoffman R (2003) NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development. *Psychopharmacology (Berl)* 169:215–233.
- Kuner T, Schoepfer R (1996) Multiple structural elements determine subunit specificity of Mg^{2+} block in NMDA receptor channels. *J Neurosci* 16:3549–3558.
- Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M (1992) Molecular diversity of the NMDA receptor channel. *Nature* 358:36–41.
- Le Meur K, Galante M, Angulo MC, Audinat E (2007) Tonic activation of NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus. *J Physiol* 580:373–383.
- Lerma J, Zukin RS, Bennett MV (1991) Interaction of Mg^{2+} and phencyclidine in use-dependent block of NMDA channels. *Neurosci Lett* 123:187–191.
- Lipton SA (2006) Paradigm shift in neuroprotection by NMDA receptor blockade: memantine and beyond. *Nat Rev Drug Discov* 5:160–170.
- Lisman JE, Coyle JT, Green RW, Javitt DC, Benes FM, Heckers S, Grace AA (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* 31:234–242.
- Liu HT, Hollmann MW, Liu WH, Hoenemann CW, Durieux ME (2001) Modulation of NMDA receptor function by ketamine and magnesium: Part I. *Anesth Analg* 92:1173–1181.
- MacDonald JF, Bartlett MC, Mody I, Pahapill P, Reynolds JN, Salter MW, Schneiderman JH, Pennefather PS (1991) Actions of ketamine, phenylcyclidine and MK-801 on NMDA receptor currents in cultured mouse hippocampal neurones. *J Physiol* 432:483–508.
- Maskell PD, Speder P, Newberry NR, Bermudez I (2003) Inhibition of human $\alpha 7$ nicotinic acetylcholine receptors by open channel blockers of N-methyl-D-aspartate receptors. *Br J Pharmacol* 140:1313–1319.
- Moghaddam B, Jackson ME (2003) Glutamatergic animal models of schizophrenia. *Ann N Y Acad Sci* 1003:131–137.
- Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH (1994) Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529–540.
- Oliver D, Ludwig J, Reisinger E, Zoellner W, Ruppersberg JP, Fakler B (2001) Memantine inhibits efferent cholinergic transmission in the cochlea by blocking nicotinic acetylcholine receptors of outer hair cells. *Mol Pharmacol* 60:183–189.
- Parsons CG, Stöffler A, Danysz W (2007) Memantine: A NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system - too little activation is bad, too much is even worse. *Neuropharmacology* 53:699–723.
- Qian A, Johnson JW (2006) Permeant ion effects on external Mg^{2+} block of NR1/2D NMDA receptors. *J Neurosci* 26:10899–10910.
- Rogawski MA, Wenk GL (2003) The neuropharmacological basis for the use of memantine in the treatment of Alzheimer's disease. *CNS Drug Rev* 9:275–308.
- Schmitt HP (2005) On the paradox of ion channel blockade and its benefits in the treatment of Alzheimer disease. *Med Hypotheses* 65:259–265.
- Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P (2005) Regulation of NMDA receptor trafficking by amyloid- β . *Nat Neurosci* 8:1051–1058.
- Sobolevsky AI, Koshelev SG, Khodorov BI (1998) Interaction of memantine and amantadine with agonist-unbound NMDA- receptor channels in acutely isolated rat hippocampal neurons. *J Physiol* 512:47–60.
- Yamakura T, Mori H, Masaki H, Shimoji K, Mishina M (1993) Different sensitivities of NMDA receptor channel subtypes to non-competitive antagonists. *Neuroreport* 4:687–690.