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Research Report

Memantine selectively blocks extrasynaptic NMDA receptors in rat substantia nigra dopamine neurons

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ABSTRACT

Recent studies suggest that selective block of extrasynaptic N-methyl-D-aspartate (NMDA) receptors might protect against neurodegeneration. We recorded whole-cell currents with patch pipettes to characterize the ability of memantine, a low-affinity NMDA channel blocker, to block synaptic and extrasynaptic NMDA receptors in substantia nigra zona compacta (SNC) dopamine neurons in slices of rat brain. Pharmacologically isolated NMDA receptor-mediated EPSCs were evoked by electrical stimulation, whereas synaptic and extrasynaptic receptors were activated by superfusing the slice with NMDA (10 μM). Memantine was 15-fold more potent for blocking currents evoked by bath-applied NMDA compared to synaptic NMDA receptors. Increased potency for blocking bath-applied NMDA currents was shared by the GluN2C/GluN2D noncompetitive antagonist DQP-1105 but not by the high-affinity channel blocker MK-801. Our data suggest that memantine causes a selective block of extrasynaptic NMDA receptors that are likely to contain GluN2C/2D subunits. Our results justify further investigations on the use of memantine as a neuroprotective agent in Parkinson's disease.

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1. Introduction

Loss of dopamine neurons in the substantia nigra zona compacta (SNC) is responsible for the cardinal symptoms of Parkinson's disease. Molecular mechanisms that may contribute to dopamine cell death include mitochondrial dysfunction, dysregulation of calcium homeostasis, oxidative stress, and intracellular accumulation of abnormal proteins, and both genetic and environmental factors predispose to toxicity by these mechanisms (Semchuk et al., 1993; Bossy-Wetzel et al., 2008; Hardy, 2010). Excessive glutamate receptor stimulation has long been suspected as another toxic mechanism, in part because calcium influx through N-methyl-D-aspartate (NMDA) receptor/ion channels can increase the risk of

Abbreviations: CNQX, 6-cyano-7-nitro-quinoxalone; EPSC, excitatory postsynaptic current; NMDA, N-methyl-D-aspartate; SNC, substantia nigra zona compacta; TTX, tetrodotoxin

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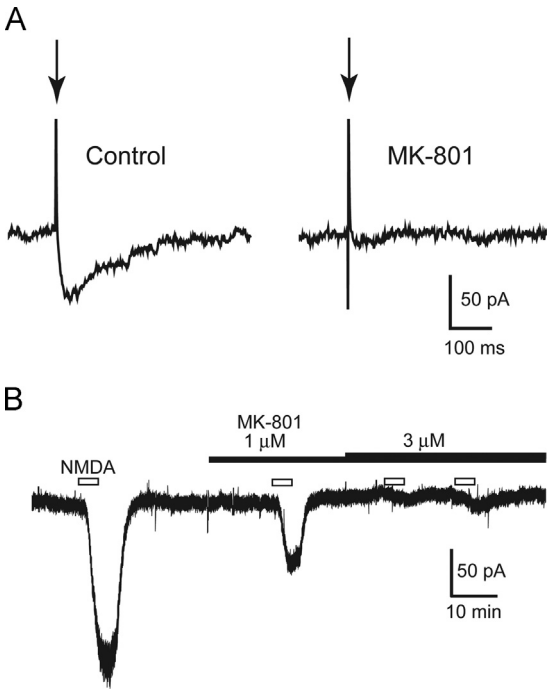

Fig. 1 – NMDA receptor-mediated currents are evoked by synaptic stimulation or bath application of NMDA. (A) Synaptic NMDA currents are evoked by electrical stimulation in the presence of picrotoxin and CNQX. NMDA EPSCs were completely blocked by MK-801 (10 μM). Arrows indicate stimulus artifacts. (B) Whole-cell currents are evoked by superfusing the slice with NMDA (10 μM). Currents are markedly reduced by MK-801. Bath application of NMDA activates all available synaptic and extrasynaptic receptors.

calcium-dependent oxidative stress (Hara and Snyder, 2007). As a result, NMDA blocking agents have been explored at length in preclinical models of Parkinson's disease, but with mixed results. Moreover, non-selective blocking agents such as MK-801 cause unacceptable behavioral and cognitive side effects that make their clinical use untenable (Ellison, 2014; Andiné et al., 1999). Thus, lack of consistent efficacy and a high level of unacceptable adverse effects have limited the feasibility of using NMDA receptor blocking agents as possible neuroprotective agents.

However, recent studies suggest that the subcellular distribution of NMDA receptors may influence their biological action, and some receptor subtypes may be more relevant for neuroprotection than others (Xu et al., 2009; Hardingham and Bading, 2010). Hardingham et al. (2002) reported that NMDA receptors located within the synapse facilitate the expression of neurotrophic factors, whereas extrasynaptic NMDA receptors facilitate pathways leading to cell death. Moreover, selective block of extrasynaptic NMDA receptors has been shown to increase cell survival in several models of neurodegeneration (Baron et al., 2010; Milnerwood et al., 2010; Rush and Buisson, 2014). Amongst subtypes of NMDA receptor, those containing GluN2C or GluN2D subunits have been shown to exist predominantly in extrasynaptic locations (Harney et al., 2008; Groc et al., 2009; Costa et al., 2009). Moreover, SNC dopamine neurons express these subtypes of receptor (Ishii et al., 1993; Jones and Gibb, 2005; Standaert et al., 1994). Although development of GluN2C/2D receptor antagonists is ongoing, a low-affinity NMDA channel blocker, memantine, has been shown to have a relatively high affinity for binding to NMDA receptors that contain GluN2D subunits (Wrighton et al., 2008; Parsons et al., 1999). Furthermore, memantine has been shown to block NMDA currents in rodent SNC dopamine neurons (Giustizieri et al., 2007). Moreover, memantine and its close relative amantadine are currently in clinical use and are well-tolerated. Although memantine and amantadine reportedly produce mild clinical improvement in some symptoms of Parkinson's disease (Moreau et al., 2013; Parkes et al., 1970), there are still unanswered questions about the actions of memantine in SNC dopamine neurons. Moreover, neither agent has been thoroughly evaluated for possible neuroprotective actions in Parkinson's disease.

The present study used whole-cell patch-clamp recordings of SNC dopamine neurons to test the hypothesis that memantine preferentially blocks extrasynaptic NMDA receptors in slices of rat midbrain. We compared the ability of memantine to block currents evoked by synaptic activation of NMDA receptors to currents evoked by bath application of NMDA, which activates both synaptic and extrasynaptic receptors. Concentration–response curves for memantine were compared with those for the GluN2C/2D receptor antagonist DQP-1105 and the non-selective high-affinity channel blocker MK-801. Our results suggest that memantine causes a selective block of extrasynaptic NMDA receptors that are likely to contain GluN2C/2D receptor subunits.

2. Results

2.1. *Memantine selectively blocks bath-applied NMDA currents*

Fig. 1A shows a typical NMDA EPSC that was evoked in a dopamine neuron by a bipolar stimulation electrode. The EPSC, which is mediated by synaptic NMDA receptors, was completely blocked by MK-801 (10 μM). In contrast, NMDA receptors that are located both synaptically and extrasynaptically were activated by bath application of NMDA. Fig. 1B shows that inward currents evoked by bath application of NMDA were also markedly reduced by MK-801 applied by superfusion. Slices were superfused with a concentration of NMDA (10 μM) that produced approximately the same amp; litude of inward current as was evoked by synaptic stimulation.

We proceeded to test the ability of memantine to block NMDA currents evoked by synaptic stimulation and bath-applied NMDA. Because the effects of channel blocking agents are slow to reverse, concentrations of memantine (and other blocking agents) were increased in a cumulative fashion. Furthermore, only one neuron was studied in each brain slice. The scatter plot in Fig. 2A shows the concentration-dependent effect of memantine on NMDA EPSC amplitude in one SNC neuron. Note that significant reduction in EPSC amplitude began upon raising the memantine concentration to 100 μM.