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Short Communication

Memantine Agonist Action at Dopamine $D2^{High}$ Receptors

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ABSTRACT Memantine is reported to improve symptoms in moderate cases of Alzheimer's disease and Parkinson's disease, but is also known to trigger psychosis in some Parkinson patients. Because these clinical features suggested a possible dopamine component of memantine action, we measured the potency of memantine on the functional high-affinity state of dopamine D2 receptors, or $D2^{High}$. Using [^{3}H]domperidone to label D2 receptors, the memantine dissociation constant at $D2^{High}$ was 917 ± 23 nM for rat striatal D2 receptors and 137 ± 19 nM for human cloned D2Long receptors. The memantine dissociation constant for striatal *N*-methyl-D-aspartate (NMDA) receptors labeled by [^{3}H]MK 801 was 2200 ± 400 nM. Memantine stimulated the incorporation of [^{35}S]GTP-γ-S into D2-expressing Chinese Hamster Ovary cells with a dissociation constant of 1200 ± 400 nM. Memantine, between 200 and 2000 nM, directly acted on $D2^{High}$ to inhibit the release of prolactin from isolated anterior pituitary cells in culture. Because the memantine potencies at NMDA receptors and dopamine $D2^{High}$ receptors are of a similar order of magnitude, it is likely that the clinical features of memantine can be attributed to its action at both types of receptors. **Synapse 62:149–153, 2008.**

INTRODUCTION

It has been reported that memantine (20 mg per day) provides functional and cognitive benefit to moderately diseased Alzheimer patients (Reisberg et al., 2003), reducing the rate of decline over 24 weeks as tested by a Daily Living scale and caregiver input (Reisberg et al., 2006). In addition, memantine has been reported to improve tremor and overall signs and symptoms of Parkinson's disease, especially in milder and early stages of the disease (Merello et al., 1999; Schneider et al., 1984). However, in four Parkinson patients memantine had little if any motor improvement but triggered psychosis in three of the patients (Riederer et al., 1991a,b).

These memantine clinical features of cognitive enhancement, mild motor improvement, and risk of psychosis suggest that there may be a dopamine agonist component to the action of memantine.

Although memantine is commonly considered to be an NMDA (*N*-methyl-D-aspartate) antagonist (Bresink et al., 1996; Chen et al., 1992; Parsons et al., 1993, 1999), memantine is known to elicit dopamine action indirectly (Peeters et al., 2003; Shearman et al., 2006) as well as direct stimulation of dopamine receptors that cause hyperactivity and circling (Costall and Naylor, 1975). It is possible, for example, that the properties of memantine may be similar to those of phencyclidine and ketamine which have similar potencies for the NMDA receptor and the high-affinity state of the dopamine D2 receptor (Kapur and Seeman, 2001; Seeman and Lasaga, 2005; Seeman et al., 2005). The present experiments, therefore, were done specifically to test this point.

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MATERIALS AND METHODS

Binding of [^{3}H]ligands to rat striata and to the human D2 clone

The striata were removed from rat brains (Sprague-Dawley) and stored at −70°C until used. The striata were homogenized in buffer (4 mg frozen tissue per ml buffer) using a teflon-glass homogenizer (with the piston rotating at 500 rpm) and 10 up and down strokes of the glass container. The buffer contained 50 mM Tris-HC1 (pH 7.4 at 20°C), 1 mM EDTA, 5 mM KCl, 1.5 mM $CaC1_2$, 4 mM $MgC1_2$, and 120 mM NaCl. The homogenate was not washed, centrifuged, or preincubated because previous work found that 30–50% of the D2 receptors were lost by these procedures (Seeman et al., 1984).

The NMDA and dopamine D2 receptors in the rat striatum were measured with [^{3}H]MK 801 (23 Ci/mmol [PerkinElmer Life Sciences, Boston, MA]; final concentration of 6–10 nM in the incubation tube) and [^{3}H]domperidone (1.2–2 nM final concentration; custom synthesized as [phenyl-^{3}H(N)]domperidone (68 Ci/mmol); PerkinElmer Life Sciences, Boston, MA; Seeman et al., 2003), respectively. Each incubation tube (12 × 75 mm, glass) received, in the following order, 0.5 ml buffer, with or without a final concentration of 200 μM GN (guanilylimidodiphosphate), and with or without a final concentration of 100 μM phencyclidine (to define specific binding to the NMDA receptors), or 10 μM S-sulpiride (to define nonspecific binding to the dopamine D2 receptors), 0.25 ml [^{3}H]ligand, and 0.25 ml of tissue homogenate. The tubes, containing a total volume of 1 ml, were incubated for 2 h at room temperature (20°C), after which the incubates were filtered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-presoaked glass fiber filter mats (Whatman GF/C). After filtering the incubate, the filter mat was rinsed with buffer for 15 s (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (7 ml, 16 × 54 mm; Valley Container, Bridgeport, CO). The minivials received 4 ml each of scintillant (Research Products International, Mount Prospect, IL), and were monitored 6 h later for tritium in a Beckman L5000 scintillation spectrometer at 55% efficiency. The specific binding of each [^{3}H]ligand was defined as total binding minus that in the presence of either 100 μM phencyclidine or 10 μM S-sulpiride for NMDA and D2 receptors, respectively. The competition data were analyzed as previously described (Seeman et al., 1984); the program provided two statistical criteria to judge whether a two-site fit was better than a one-site fit, or whether a three-site fit was better than a two-site fit.

The human cloned dopamine D2Long receptor, expressed in Chinese Hamster Ovary (CHO) cells, was used, as previously described (Liu et al., 2000).

The drug-induced incorporation of [^{35}S]GTP-γ-S (1,250 Ci/mmol; final concentration of 0.2 nM) was measured as previously described (Kapur and Seeman, 2001).

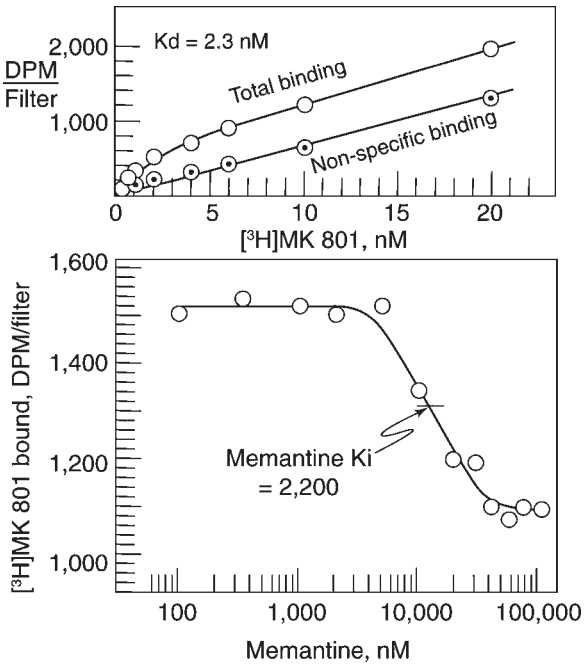

Fig. 1. Top: Saturation of [^{3}H]MK 801 (17.1 Ci/mmol) to NMDA receptors in a homogenate of rat striatum. Representative experiment. The Kd was 2.3 nM and the density, Bmax, was 14.6 pmol/g of original wet striatum. Bottom: The competition between memantine and 10 nM [^{3}H]MK 801 yielded a memantine Ki of 2200 nM. Nonspecific binding (top and bottom Figs.) was defined by the presence of 100 μM phencyclidine.

The effect of memantine on the release of prolactin from rat isolated anterior pituitary cells in primary tissue culture was measured as previously reported (Seeman and Lasaga, 2005), except that the final measurement of rat prolactin was done by means of ELISA kits obtained from MD Biosciences (St. Paul, MN) and using the procedure recommended by the manufacturer. The anterior pituitary culture has 45–50% lactotrophs, 20% somatotropes, with the remainder being tyrotropes, corticotropes, and gonadotropes.

Memantine (3,5-dimethylamantadine hydrochloride) was purchased from Sigma-Aldrich (St. Louis, MO).

RESULTS

The competition between memantine and [^{3}H]MK 801 yielded a memantine dissociation constant of 2200 ± 400 nM (average ± s.e.; $N = 3$) at the NMDA receptors in the striatal tissue (Fig. 1, bottom), using a dissociation constant of 2.3 nM for [^{3}H]MK 801 binding to striatal NMDA receptors (Fig. 1, top).

The competition between memantine and [^{3}H]domperidone yielded a memantine dissociation constant of 917 ± 23 nM (average ± s.e.; $n = 4$) at the high-affinity state of dopamine D2 receptors, $D2^{High}$, an example of which is shown in Figure 2. All the high-affinity D2

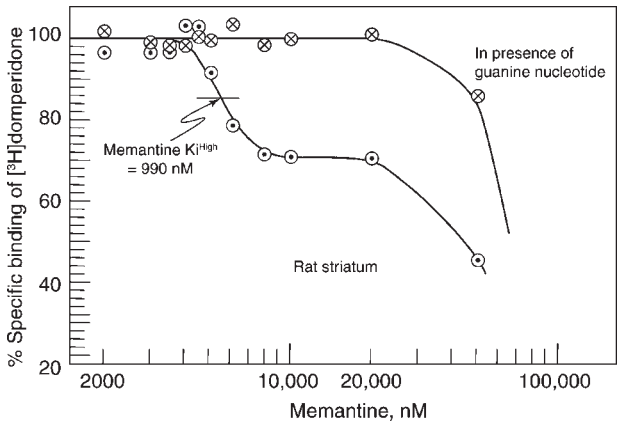

Fig. 2. Competition between memantine and [^{3}H]domperidone for dopamine D2 receptors in rat striatal homgenate. The memantine dissociation constant at the high-affinity state of D2, or D2^{High}, was 990 nM in this representative experiment. The presence of 200 μM GN converted all the D2 receptors with high affinity for mematine into low affinity for memantine. Nonspecific binding was defined by the presence of 10 μM S-sulpiride.

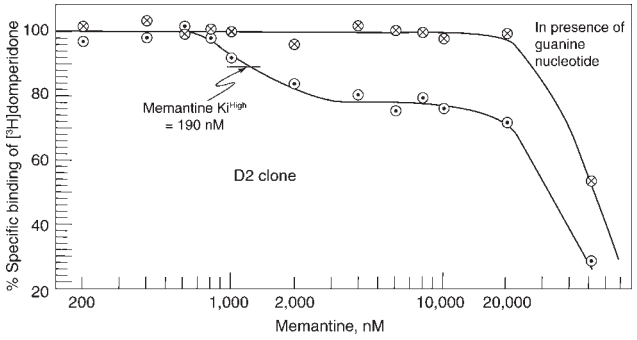

Fig. 3. Competition between memantine and [^{3}H]domperidone for human cloned dopamine D2Long receptors in CHO cells. The memantine dissociation constant at the high-affinity state of D2, or D2^{High}, was 190 nM in this representative experiment. The presence of 200 μM GN converted all the D2 receptors with high affinity for mematine into low affinity for memantine. Nonspecific binding was defined by the presence of 10 μM S-sulpiride.

receptors, D2^{High}, were converted to low-affinity D2 receptors, D2$_{Low}$, in the presence of 200 μM guanilylimidodiphsophate (Fig. 2).

Using the human cloned D2Long receptor, the memantine dissociation constant was 137 ± 19 nM (average ± s.e.; n = 4) at D2^{High} (Fig. 3), and here, too, all the D2^{High} receptors were converted to D2$_{Low}$ receptors in the presence of guanine nucleotide (Fig. 3).

To obtain an index of the functional potency of memantine at the dopamine D2 receptors, the effect of memantine was tested on the incorporation of [^{35}S]GTP-γ-S into the D2-containing CHO cells. The memantine dissocation constant for this stimulating action, Ks, was 1200 ± 400 nM (average ± s.e., n = 4), while that for dopamine itself was 350 ± 100 nM (average ± s.e., n = 4) (Fig. 4).

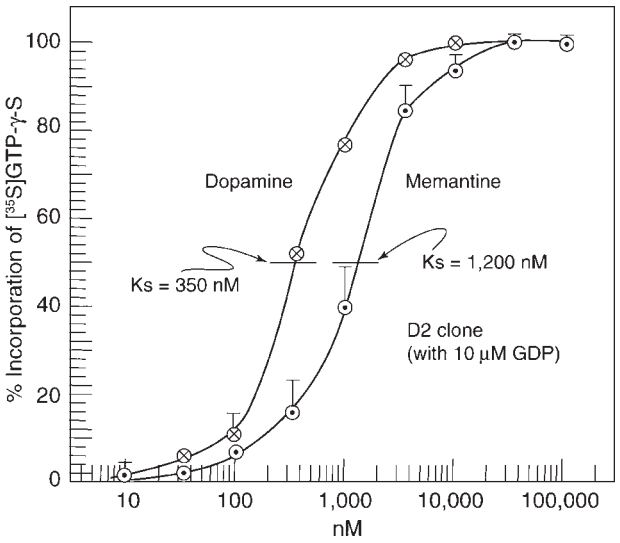

Fig. 4. Memantine and dopamine stimulated the incorporation of [^{35}S]GTP-γ-S into CHO cells containing human cloned dopamine D2Long receptors. The dissociation constant for stimulation was taken as the concentration stimulating the incorporation by 50%.

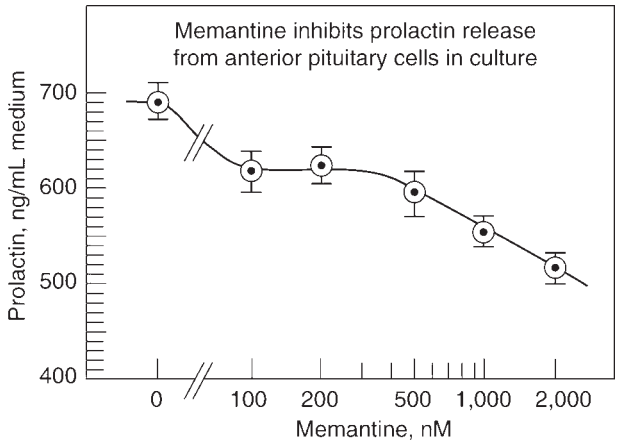

Fig. 5. Memantine, between 200 and 2000 nM, inhibited the release of prolactin after 24 h from lactotropes within the rat isolated anterior pituitary cells in primary tissue culture. Similar results occurred after 4 h, but with greater variation. Vertical bars indicate s.e.m. (n = 6 to 7 independent measurements per concentration).

A second index of the physiological potency of memantine at dopamine D2 receptors was its action on the dopamine D2^{High} receptor that controls the release of prolactin from anterior pituitary cells (George et al., 1985). Memantine, between 200 and 2000 nM, was effective in inhibiting the release of prolactin from the lactotropes in the rat isolated anterior pituitary cells after 24 h, as shown in Figure 5.

DISCUSSION

Although the memantiine dissociation constant at the NMDA receptors was 2200 nM (Fig. 1), the memantine dissociation constant at the dopamine D2^{High}

receptor was between 137 and 917 nM (Figs. 2–4), with physiological action between 200 and 2000 nM at the pituicyte $D2^{High}$ receptor to inhibit the release of prolactin.

It appears, therefore, that the memantine potency at the dopamine $D2^{High}$ receptor has similar or greater potency than that of mematine at the NMDA receptor. This may have considerable clinical relevance, considering that the functional state of the dopamine D2 receptor is the high-affinity state, $D2^{High}$ (Bickford-Wimer et al., 1990; Fujita et al., 1985; George et al., 1985; Grigoriadis and Seeman, 1985; Olianas and Onali, 1987).

The memantine dissociation constants of 1200–2200 nM at the NMDA receptors (Figs. 1 and 4) are in agreement with the memantine values of 500–1000 nM at rat recombinant NMDA receptors (Bresink et al., 1996), of ~3,000 nM on cultured hippocampal neurons (Parsons et al., 1993), of 600–1200 nM on open-channel block of NMDA receptors (Chen et al., 1992), and of therapeutic concentrations of 500 nM (Kornhuber and Quack, 1995; Kornhuber et al., 1989, 1995) in healthy patients (Periclou et al., 2006), using the value of 45% for memantine binding to plasma proteins (RxList, 2006).

Because the present results indicate that the clinical serum concentrations of 500–1000 nM memantine have a dopamine agonist action at $D2^{High}$ receptors (Figs. 4 and 5), it would be expected that patients on memantine would have a lower level of serum prolactin. However, memantine (20 mg/day) did not lower the serum prolactin in a study of 15 older men (Hergovich et al., 2001) or in 49 elderly individuals (MRZ-9402). Because the memantine potency on NMDA receptors and dopamine $D2^{High}$ receptors is of the same order of magnitude (see also Parsons et al., 1999), the lack of memantine action on serum prolactin may arise because of a balanced and simultaneous action on NMDA and D2 receptors, because glutamate pathways modify the release of prolactin (Login, 1990; Pampillo et al., 2002). Moreover, the potential prolactin-lowering action of memantine may only be detected clinically during the first hour after ingestion because of the low daily dose of 10–20 mg per patient.

In conclusion, it is possible that the clinical features of memantine, including the risk of psychotic events, may arise from its combined antagonist action at NMDA receptors and its agonist action at dopamine $D2^{High}$ receptors.

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