Trapping Channel Block of NMDA-Activated Responses By Amantadine and Memantine

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Blanpied, Thomas A., Faye Boeckman, Elias Aizenman, and Jon W. Johnson. Trapping channel block of NMDA-activated responses by amantadine and memantine. J. Neurophysiol. 77: 209–323, 1997. We investigated the mechanisms by which the antiparkinsonian and neuroprotective agents amantadine and memantine inhibit responses to N-methyl-D-aspartic acid (NMDA). Whole cell recordings were performed using cultured rat cortical neurons or Chinese hamster ovary (CHO) cells expressing NMDA receptors. Both amantadine and memantine blocked NMDA-activated channels by binding to a site at which they could be trapped after channel closure and agonist unbinding. For neuronal receptors, the IC₅₀'s of amantadine and memantine at –67 mV were 39 and 1.4 μM, respectively. When memantine and agonists were washed off after steady-state block, one-sixth of the blocked channels released rather than trapped the blocker; memantine exhibited “partial trapping.” Thus memantine appears to have a lesser tendency to be trapped than do phencyclidine or (5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801). We next investigated mechanisms that might underlie partial trapping. Memantine blocked and could be trapped by recombinant NMDA receptors composed of NR1 and either NR2A or NR2B subunits. In these receptors, as in the native receptors, the drug was released from one-sixth of blocked channels rather than being trapped in all of them. The partial trapping we observed therefore was not due to variability in the action of memantine on a heterogeneous population of NMDA receptors in cultured cortical neurons. Amantadine and memantine each noncompetitively inhibited NMDA-activated responses by binding at a second site with roughly 100-fold lower affinity, but this form of inhibition had little effect on the extent to which memantine was trapped. A simple kinetic model of blocker action was used to demonstrate that partial trapping can result if the presence of memantine in the channel affects the gating transitions or agonist affinity of the NMDA receptor. Partial trapping guarantees that during synaptic communication in the presence of blocker, some channels will release the blocker between synaptic responses. The extent to which amantadine and memantine become trapped after channel block thus may influence their therapeutic effects and their modulation of NMDA-receptor-mediated excitatory postsynaptic potentials.

INTRODUCTION

A large number of ions and drugs can block the channel of the glutamate receptor activated by N-methyl-D-aspartate (NMDA), and the effects of these blockers are diverse. Consider, for instance, the effects of Mg²⁺, the dissociative anesthetics phencyclidine (PCP) and ketamine, and 1-amino-3,5-dimethyladamantane (memantine). The block of the NMDA-activated channel by extracellular Mg²⁺ is critical to the normal functioning of the NMDA receptor in synaptic transmission and plasticity. However, PCP and ketamine, which also block this channel (MacDonald et al. 1991), have severe and deleterious behavioral effects in humans (Krystal et al. 1994; Luby et al. 1959), probably due to their interaction with the NMDA receptor (Javitt and Zakin 1991). Finally, memantine as well blocks the NMDA-activated channel (Bormann 1989; Chen et al. 1992), but is currently used in the treatment of Parkinson’s disease (Fischer et al. 1977), dementia (Ditzler 1991), and several movement-related disorders (e.g., Weller and Kornhuber 1991). During therapeutic use, the memantine concentrations found in cerebrospinal fluid suggest that its primary site of action is the NMDA receptor (Kornhuber and Quack 1995), and yet it appears to induce fewer and less profound effects on perception or consciousness (Ditzler 1991) than PCP or ketamine. The reasons for the surprisingly diverse behavioral effects of blockers of the NMDA-activated channel are not known. It is plausible that this variation arises in part from a diversity of mechanisms by which the channel may be blocked.

To explore in detail the range of mechanisms that may be exhibited by blockers of the NMDA-activated channel, we have examined the actions on NMDA responses of memantine and the closely related drug 1-amino-adamantan-2-amine (amantadine). Amantadine is an NMDA antagonist used for many of the same therapeutic purposes as memantine (Brenner et al. 1989; Kornhuber et al. 1993; Schwab et al. 1969). It inhibits [³H](5R,10S)-(−)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine ([³H]MK-801) binding to human brain membrane homogenates (Kornhuber et al. 1991) and reduces responses mediated by NMDA receptors with an apparent inhibition constant greater than memantine’s (Parsons et al. 1996).

We report here that channel closure and agonist dissociation is permitted while either of these drugs is bound in the NMDA-activated channel, resulting in trapping of the drug in the channel. This characteristic of the action of memantine and amantadine, which will be termed “trapping channel block” here, distinguishes them from “sequential blockers,” which prevent the channel from closing while blocked (Adams 1976; Antonov and Johnson 1996; Neher and Steinbach 1978). Sequential and trapping channel blockers differ greatly in several ways, including the dependence of their block at equilibrium on the agonist concentration and their inhibition of synaptic charge transfer. Many previously characterized blockers of the NMDA-activated channel act as trapping channel blockers, including MK-801 (Huettner
and Bean 1988), PCP, and ketamine (MacDonald et al. 1987, 1991). The molecular mechanisms for the trapping of blockers are at present unclear. The conformational changes involved in gating could sterically prevent movement of a trapping channel blocker out of the channel. On the other hand, the binding of a sequential blocker might prevent movement of the channel’s gate (Antonov and Johnson 1996). It seems likely that some drugs will exhibit a combination of these effects.

PCP and MK-801 appear to be trapped in virtually all blocked channels (Huettner and Bean 1988; Jahr 1992; Lerma et al. 1991; MacDonald et al. 1991); whether this is true of ketamine has not yet been determined. In experiments in which agonist and antagonist are simultaneously removed from the extracellular solution, we tested whether memantine becomes trapped in all channels that it blocks. We observed that memantine (and possibly amantadine) in fact was released from many of the blocked channels. This phenomenon of “partial trapping” may help to discriminate classes of drugs that otherwise have in common the mechanism of trapping channel block. In addition, partial trapping may significantly influence the effects of a blocker on synaptic transmission, because it guarantees that some channels will release the blocker between synaptic inputs.

Portions of these results have been presented elsewhere in preliminary form (Blanpied and Johnson 1994).

METHODS

Preparations and solutions

Primary neuronal cultures were prepared as described previously (Antonov et al. 1995) from enzymatically dissociated cortices of 16-day-old embryos of Sprague-Dawley rats. Cells were used for experiments after 12–40 days in culture.

The cDNAs for NMDA receptors subunits NR1, NR2A, and NR2B were subcloned into expression vectors for eukaryotic expression. NR2A and NR2B subunits were chosen for study because the mRNA for these subunits is expressed heavily both in neocortex in vivo (Watanabe et al. 1992) and in primary cultures of cortical neurons (Zhong et al. 1994). NR1-1a (nomenclature of Hollmann and Heinemann 1994) and NR2A were subcloned previously (Boeckman and Aizenman 1994, 1995) into pHCV-3 (In-vitrogen). The expression vector containing NR2B was created by ligating the 5.4-kb EcoRI fragment of pNR2B (Monyer et al. 1992) into a partial EcoRI digest of pRC/CMV. Green fluorescent protein (GFP) (Challie et al. 1994) expression was used as a marker of positive transfection in experiments for whole cell patch clamping. The 750-bp BstEII-EcoRI fragment from Tu65 (Challie et al. 1994) was subcloned into the MluI-NorI sites of the expression vector pC1 (Promega) to generate pCIGFP. NMDA-receptor subunit constructs were transiently transfected into CHO-K1 (ATCC CCL61) cells by lip-mediated gene transfer. Cells were seeded 23–25 h before transfection in medium containing Ham’s F-12 with 1 mM glutamine and 10% fetal bovine serum (CHO medium) at 3 × 10^4 cells per 35-mm well. Transfections were performed with 1.3 μg of total DNA to 6 μl of lipofectamine (GIBCO-BRL) in 1 ml of serum-free CHO medium per well for 4–5 h. Cells were transfected with a 1:4.3 ratio of marker plasmid (pCIGFP) to total NMDA subunit DNA; NMDA subunits were transfected at a 1:3 ratio of NR1 to NR2 (Ck et al. 1993). Cells then were refed with serum-containing medium. Twenty-four hours posttransfection the cells were trypsinized and replated at a 1:2 dilution onto 12-mm glass coverslips in 1 mM 5,7-dichlorokynurenic acid (RBI). CHO-K1 cells were grown at 37°C in an atmosphere of 5% CO2-95% air and maintained in CHO medium.

During experiments, cells were bathed in a control solution, which contained (in mM) 140 NaCl, 2.8 KCl, 1.0 CaCl2, and 10 Na2HPO4. pH was adjusted to 7.2 with NaOH. For all experiments, the solution also contained 0.2 mM tetrodotoxin, and in some experiments, 0.5 μM strychnine was added. The pipette solution contained (in mM) 120 CsF, 10 CsCl, 10 1,2-bis(2-aminoethoxy)ethane-N,N,N’,N’-tetraacetic acid (BAPTA), and 10 HEPES. pH was adjusted to 7.2 with CsOH. Concentrated drug stock solutions were prepared and kept frozen until use. Memantine kindly was provided by Merz (Frankfurt, Germany). 7-chlorokynurenic acid was purchased from RBI (Natick, MA), and all other chemicals were from Sigma (St. Louis, MO). For all experiments reported here, NMDA was applied at a concentration of 5 μM and was coapplied with 10 μM glycine; in descriptions of the experimental protocols, this combination of agonists is referred to as NMDA.

Whole cell recordings

Patch-clamp recordings from neurons or CHO cells were carried out at room temperature (20–25°C) on the stage of an inverted microscope (Zeiss Axiosvert 10) equipped with Hoffman Modulation Contrast optics (Modulation Optics, Greenvare, NY). Pipettes were pulled from borosilicate thin-walled glass with filaments (Clark Electromedical, Reading, UK) and had resistances of 2–5 MO. Access resistance during experiments was typically 10–15 MO and was 80% compensated in many experiments. Currents were recorded using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA), low-pass filtered at 10 kHz, digitized at 44 kHz and stored on video tape for later analysis. The liquid junction potential between the pipette and external solutions was measured to be near −7 mV, and this value has been subtracted from all holding potentials.

Drugs were applied through a five-barrel perfusion system, the outflow tubes of which were formed from square capillaries (0.7 mm OD, 0.5 mm ID; Longreach Scientific Resources, Orr’s Island, ME). The tubes were connected to reservoirs that could be raised or lowered to adjust the speed of the gravity-fed solution flow over the cell. Solenoid valves (Neptune Research, W. Cadillac, MI) were connected to the rail of the perfusion system. Portions of these results have been presented elsewhere in preliminary form (Blanpied and Johnson 1994).

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10 μM glycine to solution without agonists. The relaxation had an average time constant of ~80 ms. Solution exchange must take place more rapidly than this because the unbinding of NMDA time constant ~50 ms (Benveniste et al. 1990; Lester and Jahr 1992) also contribute to the relaxation time course. Further analysis of the rate of wash-off of higher concentrations of antagonists is given in RESULTS (see Fig. 5C) and suggests that 98–99% of a solution can be washed off within 120 ms.

**Analysis**

Data were played back through an eight-pole Butterworth filter (Model 901, Frequency Devices, Haverhill, MA) and digitized using pClamp software and a Digidata 1200 analog-to-digital converter (Axon Instruments). Low-pass filter frequencies were chosen based on the fastest primary component of the response under analysis and typically were 50–200 Hz. If the NMDA response after applications of blocker did not recover to ≥80% of control, cells were not considered for further analysis except for measurement of the time course of recovery from block by memantine.

Concentration-response relationships were analyzed using the Origin plotting and analysis program (Microcal Software, Northampton, MA) by fitting the data with the equation

$$I_B/I_{NMDA} = \frac{1}{1 + [B]/IC_{50}^B}$$

(1)

where [B] is the blocker concentration, $I_B$ is the steady-state response in the presence of blocker, $I_{NMDA}$ is the steady-state response to agonists alone, and $n_H$ represents the Hill coefficient or slope factor. If recovery or block was not complete, then $I_{NMDA}$ was calculated as the average of the response to agonists alone and the maximal response achieved after recovery. The values of $n_H$ and IC$_{50}$ that yielded the best fit were determined using the Marquardt-Levenberg least squares method to minimize the χ² value of the fit. For trapping channel block by amantadine (e.g., Fig. 1), five to seven concentrations of amantadine were tested in each cell, and the IC$_{50}$ in each cell was determined individually. For noncompetitive inhibition by memantine (e.g., Fig. 5), the data from all cells were pooled and fit simultaneously. Current relaxations during block or unblock were fit with single or double exponentials of the form $I = A_e e^{\tau s/V_m} + A_0 e^{\tau_d/V_m} + C$, where $A_e$ and $\tau_s$ are the amplitudes and time constants of the exponential components. Fitting was performed either in Origin as described above or in Clampfit (pClamp, Axon Instruments) using the Simplex method to minimize the sum of the squared errors of the fit.

The voltage dependence of inhibition was determined using the following equation

$$I_B/I_{NMDA} = \frac{1}{1 + [B]/IC_{50}^B e^{V/V_0}}$$

(2)

where $I_B$ and $I_{NMDA}$ were measured at $V_m$, $K_0$ is the apparent dissociation constant of the antagonist at $V_m = 0$ mV, and $V_0$ is the change in $V_m$ that results in an e-fold change in the apparent dissociation constant of the antagonist. Estimation of the voltage dependence of $I_B/I_{NMDA}$ was preferred to estimation of the voltage dependence of $I_B$ directly, because the $I_{NMDA}-V$ relation was outwardly rectifying in 60% of the cells used for these experiments. The rectification could cause an overestimation of the degree of voltage dependence if $V_0$ were calculated by fitting the $I_B-V$ relation assuming that the $I_{NMDA}-V$ relation is linear.

In some experiments, the initial amplitude rather than the steady-state amplitude of test responses to NMDA was measured. To do this, the test response and the response to a preceding control application were both measured at a set time after the application of agonists. This isochronic point was at the time it took the control response to reach its peak amplitude, usually 100–150 ms after the time of the NMDA application.

The models presented in the Results were numerically evaluated using the program SCoP (Simulation Resources, Berrien Springs, MI). To optimize the fit of a model to the data, parameters were adjusted in SCoP using a principal axis method to minimize a least-squares error function. All data are presented as means ± SE, and comparisons were made using two-tailed Student’s t-tests except as noted.

**RESULTS**

Voltage-dependent inhibition of NMDA-activated responses by amantadine and memantine

Responses to 5 μM NMDA + 10 μM glycine (hereafter referred to as NMDA) were reduced rapidly and reversibly by amantadine when it was coapplied at concentrations of 3–1,000 μM (Fig. 1A). Inhibition typically progressed in two phases: one fast phase (τ ~30 ms) that accounted for the majority (~85%) of the total inhibition and a second, slower phase that reached equilibrium within 10–20 s. To measure the equilibrium concentration-inhibition relation of amantadine, we applied the drug for ~20 s in the constant presence of agonists. The IC$_{50}$ of amantadine measured individually in six cells held at −67 mV was found to be 38.9 ± 4.6 μM, with $n_H = 0.99 ± 0.02$ (Fig. 1B).

The fractional inhibition produced by amantadine decreased at more positive $V_m$s (Fig. 2, A–C), as would be expected of a positively charged channel-blocking molecule. Current-voltage relations comparing the equilibrium response evoked by agonists alone and the equilibrium response after the addition of amantadine are shown in Fig. 2B. Figure 2C shows these data transformed to a plot of fractional response, and the fit of Eq. 2 to these points. From fits to the fractional response remaining in the presence of 100 μM amantadine in four cells tested at 6–9 $V_m$s, we calculated a mean $K_0$ of 261 ± 10 μM and a mean $V_0$ of 35.4 ± 1.8 mV.

The voltage dependence of inhibition by memantine is shown for one cell in Fig. 2, D and E. From fits to the data from four cells to which 5 μM memantine was applied at 5–8 $V_m$s, we calculated a mean $K_0$ of 14.2 ± 2.9 μM and a mean $V_0$ of 29.3 ± 1.0 mV. The value of $V_0$ for memantine was similar to but slightly greater than that of amantadine (P < 0.02). Given these values of $K_0$ and $V_0$, we calculated that the expected IC$_{50}$ of memantine at −67 mV is 1.4 μM. This degree of voltage dependence and the IC$_{50}$s of each drug are similar to that reported previously (Chen et al. 1992; Frankiewicz et al. 1996; Parsons et al. 1996).

The depth of the blocking sites within the membrane electric field may be estimated from the values of $V_0$ using the Woodhull model (Woodhull 1973). Although the assumptions required for application of this model may be inaccurate for the NMDA receptor, estimation of electrical depth is useful for comparisons among blockers. According to this model, these antagonists sense a substantial fraction of the field, 72% for amantadine and 87% for memantine, at their blocking site. The similarity in the degree of voltage dependence is consistent with the hypothesis that the two drugs bind to overlapping or identical binding sites within the NMDA-activated channel.

Trapping of amantadine and memantine in the NMDA-activated channel

To explore further the nature of channel block by these drugs, we tested whether they blocked according to a trapping open-channel block scheme (Lingle 1983)
FIG. 1. Concentration dependence of block by amantadine of N-methyl-D-aspartate (NMDA)-activated responses. A: currents evoked from a single cell by NMDA were reversibly blocked by amantadine coapplied at indicated concentrations. Lines above traces indicate times of drug application. B: concentration-inhibition relation for amantadine steady-state block in 6 cells to which 5 concentrations of amantadine were applied. Solid line shows best fit of Eq. 1 ($IC_{50} = 38.1 \mu M; n_H = 0.98$) to data pooled from all cells. Mean $IC_{50}$ calculated from fits to data from each cell individually was $38.9 \pm 4.6 \mu M$, with $n_H = 0.99 \pm 0.02$. Holding potential for all cells in A and B was $-67 \text{mV}$.

has been applied to explain block of NMDA-activated channels by ketamine, PCP, and MK-801 (Hueettner and Bean 1988; MacDonald et al. 1991). The two agonist binding steps shown in this model both represent NMDA binding (Benveniste and Mayer 1991; Clements and Westbrook 1994; Lester and Jahr 1992); glycine binding sites have been omitted from the model because all experiments were performed in a saturating concentration of glycine.

According to Model 1, if a channel becomes blocked and progresses to the trapped state $RB$, then it can become unblocked only after agonist rebinding. An alternative model of block is the ‘‘sequential’’ model (Adams 1976; Antonov and Johnson 1996; Neher and Steinbach 1978), in which closure of the blocked channel is not permitted and in which the unbinding of the drug therefore proceeds independently of agonist binding. We tested whether amantadine and memantine are trapping channel blockers by determining if recovery from block depended upon the presence of agonists.

FIG. 2. Voltage dependence of block by amantadine and memantine. A: currents recorded from 1 cell held at indicated voltages while NMDA and 100 $\mu M$ amantadine were applied for indicated periods. B: current-voltage relation for block by 100 $\mu M$ amantadine in a second cell. Filled circle, steady-state responses to agonists alone; open circle, steady-state responses during addition of amantadine. C: data from cell used for B replotted as fractional response. Solid line shows best fit of Eq. 2 ($K_0 = 252.2 \mu M$, and $V_0 = 39.7 \text{mV}$). D: current-voltage relation for block by 5 $\mu M$ memantine in a third cell. Filled circle, steady-state responses to agonists alone; open circle, equilibrium responses after addition of memantine. E: data from cell used for D replotted as fractional response. Solid line shows best fit of Eq. 2 ($K_0 = 8.4 \mu M; V_0 = 31.5 \text{mV}$).
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We first examined the kinetics of recovery after removal of 300 μM amantadine applied for 0.1 s in six cells held at −67 mV. These current relaxations were always better fit by two than by one exponential, and the two time constants (and relative amplitudes) were 0.17 ± 0.04 s (56 ± 8%) and 3.0 ± 0.2 s (44 ± 8%). Given these time constants, if amantadine block follows the sequential scheme, then after the wash-off of agonists, virtually all channels should be such as this would be surprising, given previous reports that many blocked channels did not trap either drug. A result that many blocked channels did not trap either drug. A result that many blocked channels did not trap either drug. A result that amantadine block follows the sequential scheme, then after the wash-off of agonists, virtually all channels should be such as this would be surprising, given previous reports that many blocked channels did not trap either drug. A result that many blocked channels did not trap either drug. A result that many blocked channels did not trap either drug. A result that the associated receptor for a much longer period of time. After a 20-s application, which produced steady-state inhibition, both agonists and amantadine were washed off with solution containing 80 μM DL- aminophosphonovaleric acid (APV). APV was included during the wash to reduce the possibility of channels opening due to contaminating agonists. The cell was perfused with this solution for 1–10 min, and then, after a brief wash of control solution (0.25–1 s to allow the dissociation of APV) (see Clements and Westbrook 1994), NMDA was reapplied. In every cell tested, the response to NMDA reapplication was initially ~25% smaller than the control response but then recovered with a time constant of ~3 s (Fig. 3, B and C). In experiments on several cells, APV also was applied for 5–30 s and washed off 0.25–1 s immediately before the first application of NMDA (Fig. 3). APV preapplied alone in this manner never reduced the subsequent response to NMDA. These experiments indicate that after block, some amantadine remained associated with the channels for very long periods of time in the absence of agonists, consistent with the trapping channel block model.

Trapping of memantine in the closed NMDA-activated channel also could be demonstrated using a similar protocol. In Fig. 4A, blockade of the response to NMDA by 50 μM memantine is shown; because the inhibition and recovery kinetics for memantine were quite slow, this concentration of memantine was chosen to produce rapid and nearly complete block. Figure 4, B–D, shows that, even after 10 min of wash in APV, a substantial proportion of the block by memantine remained, indicating that memantine also can be trapped in the NMDA channel. In addition, this figure demonstrates that virtually no escape from the “trapped” state was observed in the absence of agonists, because the degree of block remaining after 10 min was nearly identical to that remaining after only 1 min.

Our observation that complete recovery from block is agonist dependent indicates that the sequential model of block is not sufficient to account for the actions of amantadine or memantine. The existence of a blocked state, recovery from which requires the presence of agonist, can be most simply explained by the “trapping” channel block model in which channels can close and agonists unbind even while the channel is blocked.

Approach to study partial trapping

The presence of a slow phase of recovery in the experiments shown in Figs. 3B and 4, B–D, indicates that some amantadine and memantine molecules were “trapped” in closed channels (state RB) after the agonists were washed off. However, the response after reapplication of agonists clearly contained both slowly and quickly rising phases. It is tempting to conclude that the presence of a quick phase indicates that some channels did not have the drug trapped at the time of agonist reaplication. If this is true and if the amplitude of this phase reflects the proportion of channels which did not trap the drug, then Figs. 3 and 4 would indicate that many blocked channels did not trap either drug. A result such as this would be surprising, given previous reports that trapping channel blockers such as PCP and MK-801 remain trapped in nearly all blocked channels under similar circumstances (Huetter and Bean 1988; Jahr 1992; Lerma et al. 1991; MacDonald et al. 1991). Furthermore, our use of a low concentration (5 μM) of NMDA should have promoted trapping of the blocker by minimizing the proportion of blocked receptors that are liganded at steady state.

Observation of partial trapping therefore may indicate that the presence of the blocker in the channel has a significant

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influence on the state transitions of the NMDA receptor. This possibility has not previously been explored and is important for understanding the mechanism and functional consequences of channel block. We therefore investigated the possibility that partial trapping can be explained by an effect of the presence of amantadine or memantine in the channel on receptor state transitions. We first tested the plausibility of this hypothesis by determining whether Model 1 can reproduce the observed partial trapping simply by permitting the rate constants between blocked states to differ from the corresponding rate constants between unblocked states. We then examined the validity of three alternative explanations for the biphasic recovery at the time of agonist reapplication: there may be two or more binding sites at which amantadine and memantine can antagonize NMDA responses, not all of which can trap the drugs; transitions leading to trapping may not have reached equilibrium, and during longer blocker applications, trapping could progress to completion; within the almost assuredly heterogeneous population of NMDA receptors in cultured cortical neurons, partial trapping may be observed because some subtypes of receptors are unable to trap the drugs.

**Kinetic model of block by amantadine and memantine**

To determine whether Model 1 alone can account for observations such as those in Figs. 3 and 4, we simulated these experiments by numerically solving a kinetic model based on Model 1. This model was designed primarily to illustrate the essential features of the blocking scheme, and in order to limit the number of free parameters, numerous states that are thought to be available to NMDA receptors have been omitted. For instance, the model includes no desensitized states, only one open state, and requires binding of exactly two agonist molecules for opening. Undoubtedly, such simplifications severely restrict the ability of the model to account for many aspects of receptor function. However, we have found that even this minimal model could reproduce many features of blockade by amantadine and memantine, including the partial trapping shown in Figs. 3 and 4.

Results of these simulations are shown in Figs. 3 and 4 as solid lines overlaying the physiological data points, and the rate constants which were used to produce this output are listed in Table 1. These simulations demonstrate that Model 1 can reproduce two important aspects of the data presented here. First, in both the simulations and current records, the slow phase of recovery following the reapplication of agonists (beginning at arrowheads in Figs. 3B and 4, B–D) did not start from the current amplitude observed during steady state block. Second, both the simulations and the current records exhibit multieponential kinetics of blockade and recovery in the continuous presence of agonist. There are also notable differences between the current traces and fits; these differences will be considered in DISCUSSION.

In the simulated block by memantine, the initial jump in current after agonist reapplication suggests that trapping was not complete, and we confirmed that this was in fact the case. The simulated fractional occupancy of the trapped state RB was 45% during the steady state immediately before agonist reapplication, substantially less than the total fraction of channels that were blocked in the presence of antagonist (94%). The amplitude of the quickly rising phase of the
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TABLE 1. Rate constants used to fit Model 1 to the responses shown in Figs. 3 and 4

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<thead>
<tr>
<th></th>
<th>Memantine</th>
<th>Amantadine</th>
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<tr>
<td>(k_{m1}), (\mu M^{-1}) s(^{-1})</td>
<td>2</td>
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</tr>
<tr>
<td>(k_{m2}), s(^{-1})</td>
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<td>40</td>
</tr>
<tr>
<td>(\beta), s(^{-1})</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>(\alpha), s(^{-1})</td>
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<td>130</td>
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<tr>
<td>(k_{c1}), (\mu M^{-1}) s(^{-1})</td>
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<tr>
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<td>(k_{m2}'), s(^{-1})</td>
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During the optimization of the fit to block by memantine, \(k_1\), \(k_2\), \(\beta\), \(\alpha\), \(\alpha'\), \(k_{m1}'\), and \(k_{m2}'\) were allowed to vary. The values of \(k_0\) and \(k_{m0}\) were set based on previous reports (Benveniste and Mayer 1991; Benveniste et al. 1990; Clements and Westbrook 1994). \(\alpha\) and \(\beta\) were fixed based on the mean burst duration of NMDA-activated channels (Antonov and Johnson 1996; Gibb and Colquhoun 1992) and a maximal open probability of NMDA-activated channels during whole cell recordings of 0.03–0.04 (Rosenmund et al. 1995). The model also included a baseline holding current value and a scaling factor to account for the number of channels in the cell. Both these parameters were fixed for each cell so as to reproduce the response to agonists alone. Based on the expectation that \(k_0\) should vary little among blockers of similar structure, the \(k_0\) for amantadine was fixed at the value obtained from the fit to block by memantine. Some data points were not used in calculating the error of the fit: 200 ms after the beginning of each NMDA or blocker application, and the baseline current level except for the first 5 s between the washoff of blocker and the reaplication of agonists.

The simulated response was 48% of the control response amplitude, a value close to the percentage of blocked channels which had not become trapped.

On the other hand, the trapping of amantadine was not accurately reflected in the amplitude of the initial jump in current following agonist reaplication, as determined through use of the simulations. In the simulated experiment shown in Fig. 3B, the response to the reaplication of agonists rose quickly to 80% of the control amplitude and recovered slowly thereafter. Our initial interpretation of this observation was that the drug remained trapped in only 20% of the channels. In fact, the trapping of amantadine was much more substantial: 97% of blocked channels trapped the drug.

In general, we found that the quickly rising phase of the response to the reaplication of agonists reflects channels that had not trapped the drug only if the time course of recovery from block is much slower than the time course of response activation. To determine whether this is the case with memantine, we examined in detail the kinetics of the recovery from block by 20 \(\mu M\) memantine at \(-67\) mV (Fig. 4E). In 12 of 13 cells, the current relaxations were better fit by two than one exponential, and the two time constants were 5.26 ± 1.10 s (31 ± 4% of the recovery amplitude) and 64.9 ± 20.0 s. Even the faster of these is substantially slower than either the time constant of solution exchange of our perfusion system (~30 ms) or of the onset of the control response to NMDA + glycine (time to peak 50–200 ms). We concluded that the relative amplitude of the recovery from block by memantine that proceeds slowly in experiments such as those shown in Fig. 4, B–D, can be taken as a measure of the proportion of channels that had trapped memantine.

Noncompetitive inhibition of NMDA-activated responses by amantadine and memantine

One clear premise of Model 1 is that the blocker has a single binding site, accessible only when the channel is open. However, if a second site existed at which amantadine or memantine could bind to inhibit NMDA responses but at which they could not become trapped, action at this site might reduce steady-state occupancy at the site of trapping channel block. Such an effect might interfere with our ability to determine whether memantine was trapped in all channels that were blocked. We tested the assumption that these drugs have only one site of action and found that both memantine and amantadine can inhibit responses to NMDA when the channel is closed, through access to a distinct, low-affinity binding site. As shown below, due to the low affinity of this interaction, the trapping of either drug in the channel should be nearly unaffected by action at this second site.

This effect on the closed channel is demonstrated in Fig. 5A, which shows responses from a cell to which NMDA was applied after application of either APV alone or APV + 1.00 \(\mu M\) amantadine. The APV or APV + amantadine were washed off 250 ms before the applications of NMDA. Nevertheless, the initial response to the application of NMDA that was preceded by amantadine was considerably reduced, indicating that amantadine antagonized the response by acting when the channel was closed. Figure 5B shows responses from a cell to which 50 \(\mu M\) memantine + APV were applied in the absence of NMDA or glycine. Applications of NMDA before and 1 s after the application of memantine indicate that memantine acting on the closed channel inhibited the subsequent response to NMDA. Because the recovery from this type of inhibition by amantadine was quite fast, we concentrated in the following experiments on characterizing the inhibition by memantine.

The kinetics of recovery from inhibition in the absence of agonists resembled the kinetics of recovery from trapping channel block. We therefore were concerned that the inhibition shown in Fig. 5, A and B, could have resulted artifically from trapping channel block. It is unlikely that this inhibition was due to block of channels activated by contaminating agonists because nearly all experiments on closed channel inhibition were performed in the presence of 80 \(\mu M\) dl-APV. In addition, when similar experiments were performed without APV or in the presence of 10 \(\mu M\) 7-chlorokynurenic acid, no difference in the degree of inhibition was observed. However, it is possible that the inhibition shown in Fig. 5 could have resulted from incomplete washoff of the antagonist before reaplication of agonists. As a stringent test, we measured the rate of dilution of high concentrations of APV or \(\text{Mg}^{2+}\) (100–1,000 times their \(\text{IC}_{50}\)) in a protocol similar to that used to examine the closed-channel inhibition by the adamantanes (Fig. 5C). In 10 cases examined using either APV or \(\text{Mg}^{2+}\), the mean time required for a 50- to 500-fold dilution was 117 ± 10 ms. For the experiments with memantine presented here, we used primarily concentrations of 50–100 times the open channel \(\text{IC}_{50}\) and a wash duration of 1 s between memantine and agonist applications. For amantadine, because its unbinding time constant was considerably faster than memantine’s, we used a 250- to 500-ms wash, but generally used...
FIG. 5. Noncompetitive inhibition of NMDA-activated responses by memantine and amantadine. 

A: 2 responses from a cell to which either 80 μM APV alone (left) or 1 mM amantadine + APV (right) was applied in absence of agonists. APV or APV + amantadine were washed off, and agonists then were reapplied after a 250-ms wash in control solution. Response preceded by APV + amantadine was transiently reduced. 

B: response from a cell held at −67 mV to which 50 μM memantine was applied in absence of agonists and in presence of APV. After memantine application, there was a 1-s wash in control solution, and then agonists were reapplied; resulting response was transiently reduced. 

C: same cell as in Fig. 4. C: solution exchange rate was stringently tested by washing off 1.4 mM Mg²⁺ concentration-inhibition relation for memantine applied in absence of agonists at Dsolution, and then agonists were reapplied; resulting response was transiently reduced. 

D: response from a cell held at 67 mV to which 50 μM memantine was applied in absence of agonists and in presence of APV. After memantine application, there was a 1-s wash in control solution, and then agonists were reapplied; resulting response was transiently reduced. 

Each trace shows average of 5 responses; 3 protocols were delivered in an intermixed order. Twenty-millisecond wash reveals that although onset of the current was delayed while [Mg²⁺] fell to below saturating levels, current recovered to ~80% of control within ~100 ms. Thus within this time, [Mg²⁺] must have fallen to well below 7 μM, a 200-fold dilution. However, complete wash-off followed a biphasic time course, and remaining Mg²⁺ appeared to take ~1 s to wash away. Results consistent with these were also obtained washing off 2 mM DL-APV or 3.5 mM Mg²⁺.

**Two sites of action of amantadine and memantine**

The two types of inhibition of NMDA responses produced by each drug appear to result from actions at two different sites. As shown in Figs. 3 and 4, complete recovery from trapping channel blockade required the presence of agonists. Thus if noncompetitive inhibition by memantine or amantadine were mediated by binding at the site of trapping channel block, then the drugs should be trapped at this site while the channel is closed and agonists are unbound. As shown in Fig. 6A, however, complete recovery from noncompetitive inhibition was observed even in the absence of agonists. Agonist-independent recovery was observed in each of four
PARTIAL TRAPPING OF CHANNEL BLOCKERS

FIG. 6. Evidence that noncompetitive inhibition and channel block occur at different sites. A: memantine bound at the noncompetitive site is not trapped. Left shows noncompetitive inhibition by 100 \( \mu M \) memantine + APV and complete recovery during periodic applications of agonist. Gap preceding final application of NMDA represents 40 s. Right shows that complete recovery from an identical application of memantine also occurred in the absence of agonist. B: voltage dependence of noncompetitive inhibition. Responses from 1 cell to which 1,000 \( \mu M \) memantine + 80 \( \mu M \) APV were applied using protocol shown in Fig. 4B for 60 s at indicated \( V_m \)s. C: voltage dependence of noncompetitive inhibition compared to that of trapping channel block. \( I_{\text{Mem}}/I_{\text{NMDA}} \) measured in cell shown in A is plotted. Solid line is best fit of Eq. 2 (\( K_b = 1.04 \) mM; \( V_0 = 67.2 \) mV). Dashed line predicts amount of trapping channel block in this cell that would have been produced by memantine at a concentration that would cause same fractional block at 0 mV. Equation that describes dashed line is 

\[
I_{\text{Mem}}/I_{\text{NMDA}} = 1/[1 + 13.7/(14.2 \cdot e^{-V_m/29.3})].
\]

cells tested in this manner, and was also observed for inhibition by amantadine (\( n = 3 \)).

Also consistent with action at two different binding sites, the voltage dependence of noncompetitive inhibition by memantine differed from that of trapping channel block. The responses shown in Fig. 6B are from one cell to which 1 mM memantine + APV were applied for 60 s, as in Fig. 5B, while the cell was held at three different \( V_m \)s. The fractional block plotted as a function of \( V_m \) (Fig. 6C) shows that the degree of inhibition decreased at more positive potentials. From fits of Eq. 2 to such plots from six cells to which either 50, 300, or 1,000 \( \mu M \) memantine was applied for 60 s at 4–6 \( V_m \)s, we calculated a mean \( K_b \) of 1.2 ± 0.5 mM and a mean \( V_0 \) of 56.3 ± 3.5 mV. As illustrated in Fig. 6C, the voltage dependence of noncompetitive inhibition was significantly less than that found for trapping channel block by memantine or amantadine (\( P < 0.005 \) compared with block by memantine or amantadine). With this method, the measured voltage dependence of noncompetitive antagonism will be overestimated if any spontaneous channel openings allowed trapping channel block to occur during the antagonist application. Amantadine noncompetitive inhibition was also voltage dependent, but the effect was not quantified due to the high concentrations required to produce inhibition >50%.

Can the noncompetitive action of memantine explain its incomplete trapping at the channel blocking site? Although we do not know how the two sites interact, we can use a simple model to estimate the fraction of channels that would be unavailable for block (and therefore that could not trap memantine) if occupation of the two sites were mutually exclusive. If simultaneous occupation of both sites were possible, the effect of the noncompetitive site on trapping would be less than we will calculate here. The model can be represented by the reaction scheme \( \text{RB} \leftrightarrow \text{R} + \text{memantine} \leftrightarrow \text{RN} \), where \( \text{RB} \) is the receptor with the channel blocked by memantine (apparent dissociation constant \( K_b \)) and \( \text{RN} \) is the receptor noncompetitively inhibited by memantine (apparent dissociation constant \( K_N \)). Using the data presented above, at \(-67\) mV, \( K_b = 1.4 \) \( \mu M \) and \( K_N = 179 \) \( \mu M \). The model predicts that the percentage of channels in state \( \text{RB} \) at equilibrium is 97.3% in the absence of noncompetitive binding and 96.5% when noncompetitive binding is permitted. Thus, because of the difference in the apparent affinity of memantine for the two sites, noncompetitive inhibition would have a negligible effect on trapping of memantine. We nevertheless attempted to minimize the potential influence of noncompetitive inhibition on drug trapping in subsequent experiments by using a relatively hyperpolarized holding potential (\(-97\) mV) and a lower concentration of memantine (20 \( \mu M \)). Because the voltage dependence of trapping channel block by memantine is greater than that of noncompetitive inhibition, the difference between their apparent dissociation constants increases with hyperpolarization.
Partial trapping of memantine at equilibrium

To investigate whether partial trapping of memantine was due to slow progression of channels from the open-blocked into the trapped state, we next determined the steady-state proportion of channels in which the drug is trapped. We examined the time course of accumulation of channels in the trapped state by applying memantine in the presence of NMDA for various lengths of time (Fig. 7A). At the end of each application, the agonists and memantine were washed off at the same time, and APV was applied for 2–3 min. The fraction of channels that trapped the drug then was determined with a subsequent test application of NMDA. Any inhibition of the initial response to the test application in this protocol must be due to channels that had been in the trapped state.

After short applications of memantine, the response was substantially inhibited, but very few channels trapped the drug. The onset of block of the whole cell current was well fit by a double exponential function (n = 5). The first exponential (τ = 85 ± 2 ms) accounted for the majority (74 ± 2%) of the total inhibition, and the second slow phase approached equilibrium with τ = 5.8 ± 1.6 s. A 2-s application of memantine thus inhibited the response by 80.1 ± 2.6%; however, the response subsequently tested after the period of APV application initially was reduced only by 15.8 ± 4.0% compared with the original response (n = 5). Longer applications of memantine revealed that blocked channels slowly accumulated in the trapped state after an approximately single exponential time course with τ = 10.0 ± 1.1 s, as shown for one cell in Fig. 7B. Even after long applications in which both the current and the degree of blocker trapping had reached a steady-state level, the fraction of channels that trapped memantine was much less than the fraction that had been blocked. Figure 7C illustrates that memantine applied for 60 s produced 97.8 ± 0.5% block, yet only 84.0 ± 2.2% of the channels trapped the drug (n = 6). These values were significantly different (P < 0.005), indicating that 14.2 ± 1.9% of blocked channels did not trap the memantine. Additional experiments with 20 μM memantine revealed that at −67 mV, 19.7 ± 2.1% of blocked channels released rather than trapped the blocker (n = 7). The results at −67 and −97 mV were not significantly different (P > 0.08), and, taken together, suggest that in this voltage range, roughly one-sixth of channels blocked by memantine released the drug rather than trap it.

Time course of amantadine trapping

We next determined the time course with which channels accumulated in the trapped state when blocked with amantadine, using an approach similar to that used with memantine (Fig. 8A). However, recovery from block by amantadine, unlike recovery from block by memantine, included a large, rapid component (see also Fig. 3A). This component could not be distinguished from the fast phase of response activation after reapplication of agonist (see Fig. 3C). Thus during the fast phase of response activation, some channels that had trapped amantadine may have had time to unblock. Therefore, our approach should characterize reliably the time course of the approach to steady state trapping, but the proportion of channels that trap amantadine cannot be determined without additional information. In this cell, accumulation of channels in the trapped state progressed with an apparent τ = 3.9 s (Fig. 8B). In six cells tested with 100–300 μM amantadine at −97 mV, the amplitude of the slow phase of recovery increased with a time constant of 8.7 ± 2.7 s, a value not different from that calculated for memantine. On average, 300 μM amantadine applied for 60 s at −97 mV blocked 95.5 ± 0.6% of the response, and the amplitude of the slow phase of recovery was 31.0 ± 2.8% of the control amplitude (Fig. 8C).
Partial trapping of memantine within a homogeneous receptor population

The cortical neurons used in these experiments are likely to contain a heterogeneous population of NMDA receptors (Zhong et al. 1994). Thus it is possible that all of these subtypes of receptors can be blocked by memantine and amantadine but that incomplete trapping was observed only because one or more subtypes do not trap the drugs. To test the hypothesis that a homogeneous population of receptors cannot exhibit partial trapping of a blocker, we examined block by memantine of recombinant NMDA receptors expressed in CHO cells. Cells were transiently transfected with mRNA for NR1-1a and either the NR2A or NR2B subunit. Except for possible differences in NR1/NR2 stoichiometry, this method should produce a uniform population of receptors.

We first determined the steady state level of memantine trapping in NR1-1a + NR2A receptors using a protocol identical to that used on the cortical neurons. We applied memantine for various durations, as in Fig. 7, and found that the proportion of receptors in which memantine became trapped reached steady state within 10 s, similar to but slightly faster than in the cortical neurons. We therefore used 60-s applications to measure the amount of trapping obtained in these receptors. Figure 9A shows a response evoked in one CHO cell by application of NMDA and block of that response by 20 μM memantine. In five cells, the current in the presence of memantine under these conditions was nearly indistinguishable from the baseline holding current, indicating that memantine almost completely blocked the response. After the wash-off of memantine and NMDA, the reaplication of agonists showed that memantine could be trapped in receptors of this type, because the response was initially reduced but then recovered in the presence of agonists. To measure the fraction of channels that had trapped the drug, we fit a double exponential function to the recovery and extrapolated this curve back to the time of agonist application. The amplitude of the fitted curve at this point was taken to represent the proportion of channels that had not trapped the memantine. In several cortical neurons tested, this analysis gave results very similar to those obtained by measuring, as for Figs. 7 and 8, the initial amplitude of the response to the reaplication of agonists. In principle, the method of fitting exponentials to the response is more accurate. However, in the cortical neurons, Ca²⁺-dependent inactivation (Legendre et al. 1993) made it impractical to apply NMDA for a single period that was long enough to observe complete recovery from block by memantine. Instead, recovery from block was hastened typically by applying NMDA while holding the cell at a more depolarized V_m. Thus it was impossible in most neurons to measure the extent of trapping by fitting exponentials to the recovery phase. In the CHO cells, recoveries essentially were unaffected by Ca²⁺-dependent inactivation, possibly because the CHO cells tended to have smaller responses than did the neurons, and recovery was therefore taken to completion in the continuous presence of NMDA. In five CHO cells, the amplitude of the fitted curve at the time of agonist application was 17.2 ± 3.3% of the control response to agonists. Thus although nearly all receptors were blocked under these conditions, memantine became trapped in only 83% of the NR1-1a + NR2A receptors.

We performed similar experiments on CHO cells expressing only receptors composed of the NR1-1a + NR2B subunits (Fig. 9B). As in receptors that contained the NR2A subunit, the proportion of receptors in which memantine became trapped reached steady state within ~10 s. In five cells, 20 μM memantine applied for 60 s nearly completely blocked the response to NMDA, as it had in cells expressing NR1-1a + NR2A. A double exponential function again was used to fit the response to the reaplication of agonists. The amplitude of the fitted curve at the time of agonist application was 100% of the control response to agonists and extrapolated this curve back to the time of agonist application. The amplitude of the fitted curve at this point was taken to represent the proportion of channels that had not trapped the memantine. In several cortical neurons tested, this analysis gave results very similar to those obtained by measuring, as for Figs. 7 and 8, the initial amplitude of the response to the reaplication of agonists. In principle, the method of fitting exponentials to the response is more accurate. However, in the cortical neurons, Ca²⁺-dependent inactivation (Legendre et al. 1993) made it impractical to apply NMDA for a single period that was long enough to observe complete recovery from block by memantine. Instead, recovery from block was hastened typically by applying NMDA while holding the cell at a more depolarized V_m. Thus it was impossible in most neurons to measure the extent of trapping by fitting exponentials to the recovery phase. In the CHO cells, recoveries essentially were unaffected by Ca²⁺-dependent inactivation, possibly because the CHO cells tended to have smaller responses than did the neurons, and recovery was therefore taken to completion in the continuous presence of NMDA. In five CHO cells, the amplitude of the fitted curve at the time of agonist application was 17.2 ± 3.3% of the control response to agonists. Thus although nearly all receptors were blocked under these conditions, memantine became trapped in only 83% of the NR1-1a + NR2A receptors.

![Figure 8](http://jn.physiology.org/)

**FIG. 8.** Time course of amantadine trapping. A: responses from 1 cell held at −97 mV to which 100 μM amantadine was applied for either 2 s (left) or for 60 s (right). Response was blocked 89% by short application and 92% by longer application. Protocol was same as in Fig. 5, except that APV was applied for 1 min. Arrowheads mark initial amplitude of response at time of application of NMDA. After 2 s of block, amplitude of slow phase of recovery was only 4% of I_{NMDA}, whereas after longer exposure, it was 23%. Breaks in data represent 50 s. Open circle and filled circle measurements plotted in B. B: open circle, degree of block produced by each of 6 separate applications of amantadine given in random order to a single cell. Dashed line is a fit to these data of a single exponential with τ = 3.82 s. Shortest amantadine application was 1 s. Filled circle, initial amplitude of test response evoked after wash-off of APV, and solid line shows a single exponential fit to these data with τ = 3.80 s. Sixty-second application was sufficient to produce steady-state block and trapping. C: summary of data from 8 cells held at −97 mV to which 300 μM amantadine was applied for 2 or 60 s. Open bars show fractional steady-state current during application of NMDA and memantine, and hatched bars show initial amplitude of response evoked after wash-off of APV. * Significantly less than initial response after wash at this application duration (P < 0.005). # Significantly less than following the 2-s application (P < 0.005).
reapplication indicated that memantine escaped from 12.0 ± 2.9% of the blocked channels, rather than become trapped in all of them. Thus even in a population of receptors containing just one variant of NR1 and one type of NR2 subunit, memantine becomes trapped in only a fraction of the blocked channels. The proportion of blocked channels in which memantine was trapped was not different in neurons or CHO cells expressing NR2A or NR2B (1-way ANOVA, \( F = 1.1, P > 0.3 \)).

**DISCUSSION**

After block of NMDA-activated channels by amantadine and memantine, channel closure and agonist unbinding can result in trapping of the antagonist in the channel. The observation that trapping occurs indicates that there must be no direct transition between states R and RB, consistent with Model 1. Although trapping of these drugs does occur, memantine appears macroscopically to be trapped only partially. Partial trapping was demonstrated by washing away blocker and agonists after block and trapping had reached steady state; memantine was observed to unbind from roughly one-sixth of blocked channels rather than becoming trapped in all of them. Such partial trapping may be due to an effect of drug binding on channel gating or agonist affinity. The feasibility of this hypothesis was demonstrated with simulations of a kinetic model that reproduced partial trapping. Alternative explanations of partial trapping were investigated and rejected. First, the time course was measured over which channels accumulate in the trapped state in the presence of NMDA and memantine, and it was found that memantine was trapped partially even at steady state. Second, memantine and amantadine each inhibited responses to NMDA by acting noncompetitively at a site distinct from the site of trapping channel block; however, the low affinity of this action made it unlikely to influence the degree of trapping. Third, memantine was trapped partially also in recombinant NMDA receptors composed purely of NR1-1a and NR2A or NR2B subunits, indicating that the heterogeneity of cortical NMDA receptors did not underlie partial trapping.

We conclude that memantine and perhaps amantadine, when bound in the channel, greatly alter the gating of the channel or its interaction with NMDA.

**Mechanisms of partial trapping**

If the state transitions of channels blocked by amantadine were the same as those of unblocked channels, release of the blocker from substantially fewer channels would have been expected in experiments such as shown in Fig. 7. When agonists and antagonists were removed after steady-state block, unblock is likely to have occurred only from fully liganded receptors. This suggests that 2.9% of blocked channels were liganded fully at the steady state preceding the solution exchange, a much greater proportion than expected for unblocked channels in the presence of 5 \( \mu \text{M} \) NMDA. Furthermore, this difference may be underestimated because unblock is likely under these conditions only if the blocker unbinding rate is not slow compared with the channel closing and agonist unbinding reactions. In fact, we found that the fastest component of memantine unblock (\( \tau \approx 5 \text{ s} \)) was much slower than channel gating and NMDA unbinding in the absence of blocker (\( \tau \approx 80 \text{ ms} \) in our experiments) (see Benveniste et al. 1990; Lester and Jahr 1992). In addition, the results of the simulations shown here predict the dissociation rate constant of memantine (\( \approx 7 \text{ s}^{-1} \)) to be considerably slower than the closing rate constant of unblocked channels (\( \approx 130 \text{ s}^{-1} \)) and the dissociation rate constant of the first molecule of NMDA (\( 80 \text{ s}^{-1} \)).

There are a number of ways in which binding of memantine could increase the probability that a channel remains fully liganded. For instance, memantine binding could decrease receptor affinity for NMDA. Alternatively, memantine could alter the equilibrium between open and closed liganded channels, drawing channels to the open state. Although these are straightforward interpretations within the
context of Model 1, it is also possible that memantine modu-
lates occupancy of desensitized states not included in the
model. At present, we cannot confidently determine the ef-
fects that memantine has on channel state transitions. How-
ever, our simulations of Model 1 demonstrated that partial
trapping arose primarily because blocked channels had a
greater affinity for NMDA. Although memantine greatly al-
tered the rates of channel gating, the simulated steady-state
open probability of blocked channels in the presence of 5
μM NMDA was only 6% higher than that of unblocked
channels.

The partial trapping of memantine functionally distin-
guishes it from block by PCP or MK-801, which remain
trapped in nearly all blocked channels under analogous con-
ditions (Huettner and Bean 1988; Jahr 1992; Lerma et al.
1991; MacDonald et al. 1991). It is conceivable that PCP
has the same effect on channel gating and agonist unbind-
ing as does memantine, but that memantine has the addi-
tional, functional consequence that it is trapped only partially
simply because it unbinds more quickly. Although our data do
not directly address this issue, we used further simulations
of Model 1 to explore the trapping of a hypothetical blocker
and to test possible differences between PCP and memantine.

We supposed that the only difference between PCP (IC₅₀
~140 nM) (Lerma et al. 1991) and memantine (~1.4 μM)
might be a 10-fold difference in kᵣ; that is, this fictional
blocker had the effects of memantine but the affinity of PCP.

To determine the extent to which such a blocker would be
trapped, we simulated its application in a protocol identical
to that shown in Fig. 4, at a concentration 10 times lower
than used for memantine (to obtain the same steady state
fractional block). After the blocker and agonist were re-
moved, occupancy of state RB rose to much higher levels
(5 times fewer channels unblocked rather than trapped); that
is, the fractional trapping of this blocker was more nearly
complete. Thus by this functional measure, the drug ap-
peared to act much more as does PCP. In another simulation,
we determined that if a blocker’s kᵣ and kᵫ were those pre-
dicted by the model of memantine’s action, but its effect on
agonist binding is eliminated, then the trapping of the blocker
is complete. Based on these simulations, we conclude that
both a drug’s kinetics and its effects on channel gating or
agonist binding are important in determining the extent to
which it is trapped.

The simulations in Figs. 3 and 4 differ from the current
traces in two notable respects. First, there are some discrep-
ancies in the time course of blockade and recovery. These
discrepancies may be due to omission from the model of
closed states such as desensitized states. Second, all sets of
parameters that produced adequate simulations of block by
memantine also predicted a transient, inward “tail” current
at the time the blocker and agonist were washed off. We
examined the current relaxations following the removal of
NMDA and memantine after equilibrium block, and saw no
evidence of tail currents. The current relaxation time course
was not substantially different from that expected based on
relaxations after the wash-off of agonist alone, and there
was never a peak greater than the steady state current level
during block.

Tail currents have been observed after wash-off of tetrabu-
tylammonium (Koshelev and Khodorov 1992) or 9-amino-
acridine (Benveniste and Mayer 1995) and are thought to
result from the requirement that blocked channels must pass
through the open state to unblock. In our experiments, it is
striking that even cells such as shown in Fig. 4 that trapped
memantine in only a small proportion of channels still
showed no tail current. These observations imply that there
may be a long-lived blocked state from which unblocking
is more likely than trapping. In this case, only few channels
at a time would pass through the open state, and the tail
current barely would be detectable. Alternatively, there may
be a route from blocked states to unblocked states other
than through the open state. In terms of Model 1, the only
possibilities are direct transitions from state A₂RB to A₂R
or AR to AR (Benveniste and Mayer 1995), although there
may exist additional routes through states not represented in
this model.

Evidence from this study and from numerous others sug-
gests that the degree to which different blockers are trapped
can vary through a continuum. Some blockers of NMDA-
activated channels, such as IEM-1857 (Antonov and John-
on 1996) and 9-aminoacridine (Benveniste and Mayer
1995), either cannot be trapped or are trapped very infre-
cently; some blockers, such as memantine, exhibit substan-
tial but partial trapping; some blockers, such as PCP and
MK-801, appear to be fully trapped. There may be more
than one mechanism by which a blocker can influence the
degree to which it is trapped. Comparison of a variety of
adamantane derivatives that block the NMDA-activated
channel has suggested that blockers with an elongated struc-
ture can sterically inhibit channel closure and presumably
therefore prevent trapping (Antonov and Johnson 1996).
The models used here suggest that trapping also can be
affected if the presence of blocker in the channel causes a
more general change in receptor conformation that allosteri-
cally alters agonist binding.

Noncompetitive inhibition by amantadine and memantine

The noncompetitive form of inhibition by amantadine and
memantine differed from the trapping channel block in three
important respects: as a noncompetitive inhibitor, each drug
exhibited a lower affinity, a less steep voltage dependence,
and an inability to become trapped. These three differences
are most simply explained by the presence of two different
binding sites on the NMDA receptor-channel complex for
amantadine and memantine.

On the basis of the present experiments, we cannot deter-
mine whether the site of noncompetitive inhibition is in the
channel or is outside the channel and inhibits current flow
allosterically. It seems unlikely that the binding site is in the
channel accessible through a hydrophilic pathway when the
gate is closed, because one would expect the recovery from
inhibition at this site to have been faster than observed,
given the low affinity of each drug. Alternatively, because
amantadine easily passes into phospholipid bilayers (Duff
et al. 1993), the site might be reached via a hydrophobic
route. In this case, the relatively slow recovery kinetics from
this mode of inhibition could reflect a slow exit of the antago-
nist from the membrane lipid. If the competitive site can
be reached through the membrane, it should be accessible
from either the intracellular or extracellular solution. To test
this possibility, we included 100–1,000 μM amantadine in the pipette solution. NMDA-activated currents recorded under these conditions were not visibly different from control currents in size or voltage dependence (Blanpied and Johnson 1994). This argues against a hydrophobic location of the noncompetitive site. However, even 1,000 μM amantadine may not have been a sufficient intracellular concentration to cause significant noncompetitive inhibition, or the intracellular concentration of amantadine at the locations of the activated receptors may have been lower than that in the pipette. Localization of the site of noncompetitive inhibition will require further experiments.

Functional implications

Access to a trapped state may contribute to the effectiveness of amantadine and memantine as antiparkinsonian (Brenner et al. 1989; Fischer et al. 1977; Schwab et al. 1969) or neuroprotective (Chen et al. 1992; Seif el Nasr et al. 1990; Weller et al. 1993) agents by increasing their equilibrium affinity. Whether a blocker is a sequential or trapping channel blocker will not affect its microscopic Kₒ, which by definition depends only on the rates of interaction with the open channel (Kₒ = K⁺/K₋). However, the blocker's macroscopic unbinding rate and therefore also its IC₅₀, decreases with increasing fractional occupancy of blocked states from which the blocker cannot unbind. Thus the observation that memantine and amantadine can be trapped implies that they inhibit NMDA responses more effectively than they would if channel closure were prevented while they are bound. During daily treatment for Parkinson’s disease, cerebrospinal fluid concentrations of amantadine and memantine are estimated to reach 10 μM (Brenner et al. 1989; Kornhuber et al. 1995) and 0.3 μM (Kornhuber and Quack 1995), respectively. These concentrations are sufficient to act at NMDA receptors, given the IC₅₀s reported here.

Amantadine and memantine are practical for therapeutic use because they appear to induce fewer and less profound effects on perception or consciousness (Ditzler 1991) than other NMDA channel blockers that can be trapped in the channel, such as PCP (Luby et al. 1959) and ketamine (Krystal et al. 1994). It has been proposed (Chen et al. 1992; Rogawski 1993) that some NMDA channel blockers may be safer because they effectively inhibit overstimulation of NMDA receptors by high tonic levels of glutamate, for instance during ischemia, while sparing synaptic responses. At least two features of blockers of NMDA-activated channels may relate to this hypothesis. First, faster kinetics may be correlated with increased clinical safety (Rogawski 1993). This idea generally is supported by the sequence of unblocking rate constants (from slowest to fastest: MK-801 < PCP < ketamine < memantine < amantadine) (Blanpied and Johnson 1993; Chen et al. 1992; MacDonald et al. 1991; Parsons et al. 1996; and data presented here). Second, partial trapping may be important in determining the effects of a trapping channel blocker during repetitive synaptic transmission. A drug that is trapped in nearly all blocked channels will cause a relatively large fraction of channels to accumulate in the trapped state. However, a drug that is identical except for being partially trapped would cause fewer channels to accumulate in the trapped state; a portion of the channels that become blocked during a synaptic response would release the drug rather than trap it during the period between synaptic inputs. Therefore the properties of memantine may ensure that during its use a substantial fraction of channels remain available for synaptic activation. If the mechanism of channel block influences a drug’s psychotomimetic effects, then the therapeutic utility of memantine and perhaps amantadine may be enhanced by a tendency to be only partially trapped.

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