



# Potency, voltage-dependency, agonist concentration-dependency, blocking kinetics and partial untrapping of the uncompetitive N-methyl-D-aspartate (NMDA) channel blocker memantine at human NMDA (GluN1/GluN2A) receptors

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## ABSTRACT

Both the clinical tolerability and the symptomatic effects of memantine in the treatment of Alzheimer's disease have been attributed to its moderate affinity ( $IC_{50}$  around  $1 \mu\text{M}$  at  $-70 \text{ mV}$ ) for NMDA receptor channels and associated fast, double exponential blocking/unblocking kinetics and strong voltage-dependency. Most of these biophysical data have been obtained from rodent receptors. Some substances show large species-specific differences, so using human rather than rodent receptors and tissue may highlight important differences in the effects of drugs. In the present study we compared the potency of memantine, ketamine and (+)MK-801 in binding to NMDA receptors in post-mortem human cortical tissue and to antagonize intracellular  $\text{Ca}^{2+}$  responses of human GluN1/GluN2A receptors expressed in HEK-293 cells. In addition, the biophysical properties of memantine and ketamine were compared using patch clamp recordings from these cells.

Memantine was confirmed to be a moderate affinity ( $IC_{50}$  at  $-70 \text{ mV}$  of  $0.79 \pm 0.02 \mu\text{M}$ , Hill =  $0.92 \pm 0.02$ ), strongly voltage-dependent ( $\delta = 0.90 \pm 0.09$ ) uncompetitive antagonist of human GluN1/GluN2A receptors. Moreover, the rapid double exponential blocking kinetics (e.g. at  $10 \mu\text{M}$  – onset  $\tau_{\text{fast}} = 273 \pm 25 \text{ ms}$  (weight 69%), onset  $\tau_{\text{slow}} = 2756 \pm 296 \text{ ms}$ , offset  $\tau_{\text{fast}} = 415 \pm 82 \text{ ms}$  (weight 38%) offset  $\tau_{\text{slow}} = 5107 \pm 1204 \text{ ms}$ ) and partial untrapping (around 20%) previously reported for memantine on rodent receptors were confirmed for human receptors. Ketamine showed similar potency ( $IC_{50}$  at  $-70 \text{ mV}$  of  $0.71 \pm 0.03 \mu\text{M}$ , Hill =  $0.84 \pm 0.02$ ) but somewhat less pronounced voltage-dependency ( $\delta = 0.79 \pm 0.04$ ), slower, single exponential kinetics (ketamine:  $k_{\text{on}} = 0.15 \pm 0.05 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.22 \pm 0.05 \text{ s}^{-1}$  c.f. memantine following normalization  $k_{\text{on}} = 0.32 \pm 0.11 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.53 \pm 0.10 \text{ s}^{-1}$ ) and was fully trapped.

The present data closely match previously reported data from studies in rodent receptors and suggest that the proposed mechanism of action of memantine in Alzheimer's disease as a fast, voltage-dependent open-channel blocker of NMDA receptors can be confirmed for human NMDA receptors.

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## 1. Introduction

Memantine (1-amino-3,5-dimethyl-adamantane) is registered in over 60 countries worldwide, amongst them the USA and Europe, for the treatment of moderate to severe Alzheimer's disease. Both the clinical tolerability and the symptomatic effects of memantine have been attributed to its moderate affinity ( $IC_{50}$  around  $1 \mu\text{M}$  at  $-70 \text{ mV}$ ) for NMDA receptor channels and associated fast double exponential blocking/unblocking kinetics and strong

voltage-dependency (Rogawski, 1993; Parsons et al., 1993, 1999; Johnson and Kotermanski, 2006). These functional properties have been characterized and confirmed by numerous groups using whole-cell patch clamp recordings from primary cultures of rat hippocampal and rat cortical neurones as well as from rat NMDA receptors expressed heterologously in HEK-293 cells (Parsons et al., 1993, 1995, 1996, 1998; Bresink et al., 1996; Chen and Lipton, 1997; Blanpied et al., 1997; Sobolevsky and Koshelev, 1998; Sobolevsky et al., 1998; Losi et al., 2006).

How these biophysical properties account for the better therapeutic efficacy/safety of memantine compared to other channel blockers such as (+)MK-801, phencyclidine and ketamine has been a matter of considerable scientific debate and there are several

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theories, which will be discussed in more detail later. However, the reported underlying biophysical properties underlying all such theories are consistent and are detailed below.

Memantine blocks and unblocks open NMDA receptor channels with double exponential kinetics in a voltage- and use-dependent manner, meaning that it can only gain access to the channel in the presence of agonist and remains largely trapped in the channel following removal of agonist (Parsons et al., 1993). The amplitude and speed of the fast component of block increase with memantine concentration. In contrast, the speed of fast unblock remains constant but its weight (relative to the slow component) decreases with memantine concentration (Frankiewicz et al., 1996; Bresink et al., 1996; Blanpied et al., 1997; Sobolevsky et al., 1998; Sobolevsky and Koshelev, 1998). Moreover, the predominant effect of depolarization is to increase dramatically the weight of the faster recovery time-constant due to the voltage-dependence of the blockade (Frankiewicz et al., 1996; Bresink et al., 1996; Parsons et al., 1998). These data indicate that memantine binds to at least two sites within the channel (Sobolevsky and Koshelev, 1998; Sobolevsky et al., 1998).

It has further been suggested that the equilibrium blockade of NMDA receptor channels is dependent upon the concentration of agonist (Lipton, 2006). This assumption is supported neither by the blocking model proposed by Blanpied et al. (1997) nor by our own recent data which highlighted, for us, the technical problems in performing such studies, in particular regarding the kinetics of blockade (Gilling et al., 2007).

In addition to strong voltage-dependency and fast blocking kinetics, memantine appears to have a lesser tendency to be trapped in NMDA receptor channels than do ketamine, phencyclidine or (+)MK-801 (Blanpied et al., 1997; Sobolevsky and Koshelev, 1998; Johnson and Kotermanski, 2006). This difference has been partly attributed to the faster kinetics of memantine. Receptors blocked by memantine retain agonist and thereby open and release memantine following removal of both agonist and memantine from the extracellular solution. This partial untrapping was reported to be less pronounced for higher affinity compounds as their slower unblocking kinetics do not allow them to leave the channel quickly enough following agonist removal.

One major caveat for the translation of such *in vitro* functional findings to the *in vivo* human situation is that almost all studies were performed on cultures, clones or slices from rodent receptors/CNS tissue. There are very limited data available for human NMDA receptors. Memantine did indeed displace [<sup>3</sup>H]MK-801 from NMDA receptor channels in post-mortem human cortical tissue with a similar potency to that observed in these studies in receptors/tissue from rodents (Kornhuber et al., 1989). However, these studies provided no information on the functional biophysical properties of this interaction with human NMDA receptors and also bear the unfortunate caveat that such studies were made on receptors under no influence of membrane potential because they were performed in isolated membrane fragments. This fact begs the question, was it perhaps fortuitous that the potency of a voltage-dependent NMDA receptor channel blocker was similar at human NMDA receptors examined in this manner?

The only data that we are aware of on the functional blockade of human NMDA receptors by memantine is the study of Ferrer-Montiel et al. (1998) where memantine blocked human GluN1/GluN2A expressed in *Xenopus* oocytes with an IC<sub>50</sub> of 220 nM at –100 mV. This fivefold greater potency of memantine than observed in the binding experiments (Kornhuber et al., 1989) on human receptors indicates that these caveats in the interpretation of binding data might be justified.

*In vitro* data are often used to predict *in vivo* effects of compounds, especially those to be used in a clinical situation, and it

is therefore important that the assays used represent the therapeutic situation as closely as possible. Most *in vitro* experiments are performed using cells and tissue derived from rodent sources for practical reasons, but the use of cloned receptors has led to the increased possibility of testing substances upon human proteins. Some substances show large species-specific differences, so using human rather than rodent receptors and tissue may highlight important differences in the effects of drugs.

Here, we wished to confirm certain *in vitro* functional/biophysical properties of memantine, ketamine and (+)MK-801 upon human NMDA receptors, characteristics which have previously been reported by this and other groups on rodent receptors (for examples, see Chen et al., 1992; Parsons et al., 1993, 1995; Bresink et al., 1996).

In this study, we were able to obtain human brain tissue for use in radioligand binding assays to confirm the findings reported by Kornhuber et al. (1989). Furthermore, we used human GluN1/GluN2A NMDA receptor subunits expressed in the human cell line, HEK-293 for functional studies. By using these cells, it was possible to measure the effect of antagonist upon NMDA-induced Ca<sup>2+</sup> influx into the cells using a FLIPR device by detecting changes in intracellular Ca<sup>2+</sup> levels. In addition, the effects on human GluN1/GluN2A receptors were studied using electrophysiological patch clamp techniques allowing faster perfusion of the cells compared to two electrode voltage clamp recordings made from *Xenopus* oocytes and therefore better resolution of the current blocking kinetics.

Specifically, several issues were addressed: 1, the potency of channel blockers in binding to NMDA receptors in post-mortem human cortical tissue expressing NMDA receptors; 2, their potency to antagonize intracellular Ca<sup>2+</sup> responses of human GluN1/GluN2A receptors expressed in HEK-293 cells; 3, the potency, kinetics and voltage-dependency of block by memantine and ketamine at human GluN1/GluN2A receptors using patch clamp experiments; 4, partial trapping of memantine/ketamine by human GluN1/GluN2A receptors and 5, the agonist concentration-dependency of blockade of human GluN1/GluN2A receptors by memantine using different concentrations of the endogenous agonist glutamate.

Some of this data has previously been published in poster form (Gilling et al., 2006).

## 2. Methods

### 2.1. Binding experiments

Male Sprague–Dawley rats (200–250 g) were decapitated and their brains removed rapidly. Frozen human cortical tissue was kindly provided by Prof. P. Riederer of the University of Würzburg, Germany, and was treated in the same manner as the rodent tissue.

The cortex was dissected and homogenized in 20 volumes of ice-cold 0.32 M sucrose using a glass-Teflon homogenizer. The homogenate was centrifuged at 1000 × g for 10 min. The pellet was discarded and the supernatant centrifuged at 20,000 × g for 20 min. The resulting pellet was re-suspended in 20 volumes of distilled water and centrifuged for 20 min at 8000 × g. The supernatant and the buffy coat were then centrifuged at 48,000 × g for 20 min in the presence of 50 mM Tris–HCl, pH 8.0. The pellet was re-suspended and centrifuged two to three more times at 48,000 × g for 20 min in the presence of 50 mM Tris–HCl, pH 8.0. All centrifugation steps were carried out at 4 °C. After re-suspension in 5 volumes of 50 mM Tris–HCl (pH 8.0) the membrane suspension was frozen rapidly at –80 °C.

On the day of assay, the membranes were thawed and washed four times by re-suspension in 50 mM Tris–HCl (pH 8.0) and centrifugation at 48,000 × g for 20 min and finally re-suspended in 50 mM Tris–HCl (pH 7.4). The amount of protein in the final membrane preparation (250–500 µg/ml) was determined according to the method of Lowry (Lowry et al., 1951). Incubations were started by adding (<sup>3</sup>H)-(+)-MK-801 (23.9 Ci/mmol, 5 nM, Dupont NEN) to vials with glycine (10 µM), glutamate (10 µM), and 125–250 µg protein (total volume of 0.5 ml) and various concentrations of the agents, and left at room temperature for 120 min. Non-specific binding was determined by the addition of unlabeled (+)-MK-801 (10 µM). Incubations were terminated using a Millipore filter system. The samples were rinsed twice

with 4 ml of ice-cold assay buffer over glass fibre filters (Schleicher & Schuell) under a constant vacuum. Following separation and rinsing, the filters were placed into scintillation liquid (5 ml Ultima Gold) and the radioactivity retained by the filters was determined with a conventional liquid scintillation counter (Hewlett Packard, Liquid Scintillation Analyser).

The  $K_d$  of [ $^3\text{H}$ ](+)-MK-801 was determined to be 4.1 nM by Scatchard analysis and was used according to the Cheng Prussoff relationship to calculate the affinity of displacers as  $K_i$  values.

## 2.2. HEK-293 cell culture and transfection

HEK-293 cells were transfected with human GluN1 and GluN2A subunits and were then cultivated in Dulbecco's Modified Eagle's Medium (DMEM, Biochrom) supplemented with 10% heat inactivated foetal calf serum (FCS, Sigma), 2 mM Glutamax (Invitrogen), 100  $\mu\text{g}/\text{ml}$  gentamycin (Biochrom), and 500  $\mu\text{g}/\text{ml}$  G418 (Calbiochem) at 37 °C in a humidified atmosphere of 5%  $\text{CO}_2/95\%$  air. After transfection, 500  $\mu\text{M}$  D-APV was also added to ensure that glutamate in the medium did not have a toxic effect upon cells expressing functional NMDA receptors whilst they were in culture. The cells were thoroughly washed before experiments were performed to ensure the removal of this antagonist.

## 2.3. FLIPR assays

24 h prior to the FLIPR assay, transfected HEK-293 cells were harvested and reseeded on 96-well plates (Corning Costar) at a density of 80,000 cells/well. Medium composition was changed to DMEM supplemented with 10% dialysed FCS (Sigma), 1 mM Glutamax, and 100  $\mu\text{g}/\text{ml}$  gentamycin. In order to prevent glutamate-induced toxicity of the cells in culture, 500  $\mu\text{M}$  D-APV was included. This competitive antagonist was used here rather than ketamine, as ketamine would probably show intracellular accumulation (Honegger et al., 1993) and was predicted to be more difficult to wash out from the 96-well plate format.

Changes in intracellular  $\text{Ca}^{2+}$  levels were measured as relative fluorescence units (RFU) using the fluorometric imaging plate reader (FLIPR) and the Ca-4 Assay Kit (both Molecular Devices, CA). Prior to addition of agonist or antagonist, the medium was aspirated and cells were loaded for 60 min at 37 °C with 150  $\mu\text{l}$  loading buffer consisting of the  $\text{Ca}^{2+}$ -sensitive dye reconstituted in HBSS,  $\text{CaCl}_2$  (1.3 mM), HEPES (20 mM), pyridoxal 5'-phosphate (1.5 mM), sodium pyruvate (2 mM), and glutamic pyruvic transaminase (3 U/ml), pH 7.4. Subsequently, plates were transferred to the FLIPR and pre-incubated with any antagonist to be tested for 8 min at RT before the addition of the agonist (200  $\mu\text{M}$  NMDA and 10  $\mu\text{M}$  D-serine). The mean of the replicated temporal data ( $n = 4$ ) was calculated and used for graphical representation. The changes in  $\text{Ca}^{2+}$  level were determined at the indicated time point. All responses were measured as a percentage of control (the maximum response after 200  $\mu\text{M}$  NMDA and 10  $\mu\text{M}$  D-serine application).  $\text{EC}_{50}$  and  $\text{IC}_{50}$  were calculated according to the logistic equation using Graft software (Erihacus Software Ltd). Corrections for background activity were made immediately before agonist application.

## 2.4. Patch clamp recordings

For a more detailed description of the methods and equipment used, see Parsons and Gilling (2007). Briefly, voltage clamp recordings were made in the whole-cell patch clamp configuration at a holding potential of  $-70$  mV unless otherwise stated. The substances were applied to cells using a fast superfusion device with a stepper motor-driven (Warner Instruments LLC) application pipette, made from modified double-barrelled theta glass. Patch clamp currents were recorded using an EPC-9 amplifier (HEKA), and the TIDA package system (HEKA) was used for the collection and storage of data. Patch clamp pipettes were pulled from borosilicate glass using a horizontal puller (P-97 Puller, Sutter Instruments) and, when filled with intracellular solution, had resistances of 2–4 M $\Omega$ . The intracellular solution consisted of (in mM): 120 CsCl, 10 EGTA, 1  $\text{MgCl}_2$ , 0.2  $\text{CaCl}_2$ , 10 glucose, 22 TEACl, 2 ATP and 0.2 cAMP. The corresponding extracellular bath solution contained (in mM): 140 NaCl, 3 KCl, 10 glucose, 10 HEPES, 0.2  $\text{CaCl}_2$  and 4.5 sucrose.

Antagonism of agonist-induced NMDA receptor-mediated currents was measured as the magnitude of the steady-state blocked current as a percentage of the control current. For non-kinetic antagonistic protocols with 5 cumulatively increasing concentrations of antagonist, the control current for each antagonist concentration was calculated by a linear projection from the steady-state current before and after antagonist application i.e. for the first antagonist concentration control current =  $0.85 \times$  current before antagonist plus  $0.15 \times$  recovery current. For the second antagonist concentration these same currents were multiplied by 0.7 and 0.3 respectively, and so on for all concentrations. For kinetic protocols, single concentrations of antagonist were applied in the continuing presence of agonists, and the control current was taken as the mean of the steady-state current before and after antagonist application. For pre-incubation i.e. partial untrapping experiments, NMDA-induced currents recorded in the presence of antagonist were compared to the mean of those recorded before and during recovery. Potency of compounds was assessed by plotting the mean percentage current magnitude, calculated with standard error (S.E.), against antagonist concentration and then a curve was fit using

the logistic equation for which the variable parameters  $\text{IC}_{50}$  and Hill coefficient ( $n$ ) were free and the range and background values were normally set to 100 and 0, respectively.

To assess voltage-dependency, single concentrations of antagonist were applied to currents in the plateau phase and the holding potential was steadily ramped from  $-70$  mV to  $+80$  mV over 2 s. The pooled data were then fit by the following equation, where  $\text{IC}_{50}(0)$  is the  $\text{IC}_{50}$  at 0 mV,  $\beta$  is the fraction of voltage-independent sites,  $\delta$  is the fraction of the electric field sensed by the voltage-dependent site, and all other parameters have their normal meaning:

$$\text{Fractional current} = \frac{1 - \beta}{1 + \frac{[\text{antagonist}]}{\text{IC}_{50}(0)e^{\delta V/RT}}}$$

Agonist concentration-dependency of memantine equilibrium block of human GluN1/GluN2A receptors was assessed using the endogenous agonist glutamate (3–30  $\mu\text{M}$ ) in the continuous presence of 10  $\mu\text{M}$  glycine to evoke currents. Block of these currents was assessed using the kinetic protocol and memantine (3–30  $\mu\text{M}$ ).

For partial untrapping experiments NMDA (200  $\mu\text{M}$ ) was applied for 10 s every 35 s in the continuous presence of 10  $\mu\text{M}$  glycine. The first application of NMDA in the absence of antagonist was followed by a 15 s interval. Memantine or ketamine (10  $\mu\text{M}$ ) was then pre-incubated for 20 s (open bar) followed by the co-application of NMDA in the continuing presence of antagonist (10  $\mu\text{M}$ , black bar) for 10 s. NMDA was then applied for a third time in the continuing presence of antagonist to demonstrate that steady-state block had been achieved. Thereafter, partial untrapping of memantine and ketamine in the channel in the absence of agonist was permitted by introducing a delay of 33 s in the continuing presence of uncompetitive antagonist plus L-APV (200  $\mu\text{M}$ ). Both were washed off for 2 s before the fourth 10 s application of NMDA alone. L-APV was used instead of D-APV as it has lower affinity and associated faster offset kinetics and should have completely left the orthosteric binding site in the 2 s following its removal before the fourth application of NMDA. The amplitude of the fast component of the rising phase of this response to NMDA was used to estimate the fraction of channels that released uncompetitive antagonist in the absence of NMDA. A fifth 10 s application of NMDA ensured full recovery from antagonism.

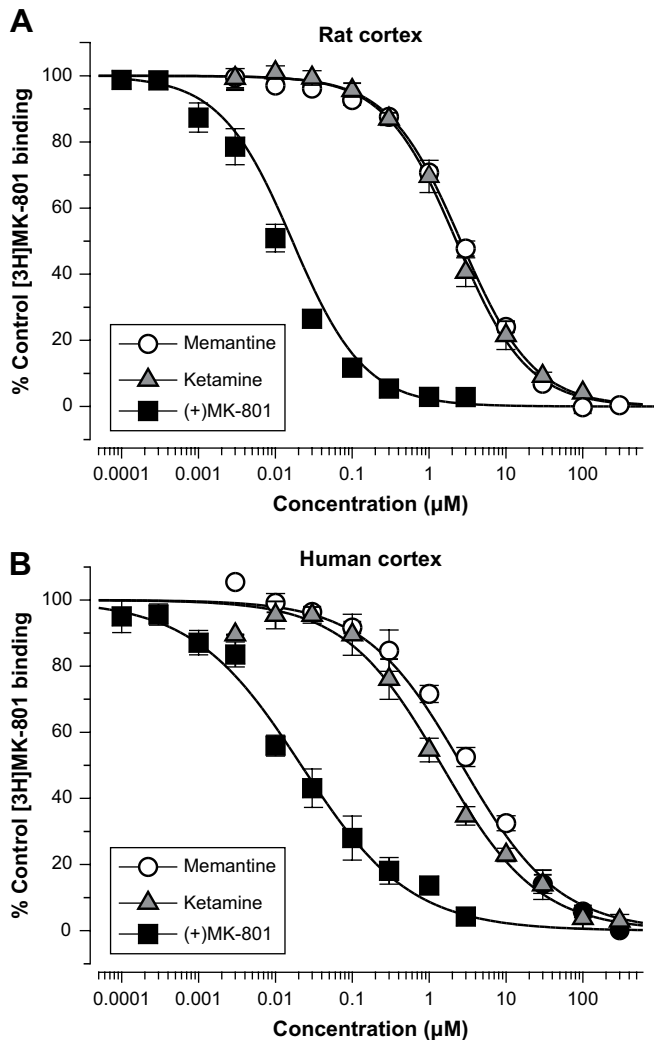
The Student's  $t$  test was applied to determine the significance of differences between two measurements where appropriate (e.g. single versus double exponential fits), calculated using Sigmasat 3.1 software. Statistical significance was taken to be  $p < 0.05$ .

## 3. Results

The results of binding experiments performed using the [ $^3\text{H}$ ](+)-MK-801 assay in rat and human cortical tissues are shown in the graphs in Fig. 1. (+)-MK-801 had a  $K_i$  of  $0.005 \pm 0.001$   $\mu\text{M}$ , and memantine and ketamine displaced the radioligand with similar potency when rat cortical tissue was used ( $K_i$  values of  $1.19 \pm 0.08$   $\mu\text{M}$  and  $1.35 \pm 0.43$   $\mu\text{M}$ , respectively). The absolute values agree well with those previously reviewed by Parsons et al. (1999). When the experiments were performed using human cortical homogenates, ketamine showed somewhat higher affinity than memantine (the  $K_i$  values for (+)-MK-801, memantine and ketamine were:  $0.010 \pm 0.002$   $\mu\text{M}$ ,  $1.11 \pm 0.17$   $\mu\text{M}$ , and  $0.67 \pm 0.15$   $\mu\text{M}$ , respectively).

It may seem fortuitous that the potency of voltage-dependent NMDA receptor channel blockers was similar when examined in this manner to that previously reported in functional assays with membranes clamped at  $-70$  mV as the membrane preparations used would have had no influence of membrane potential. This could be due to issues of binding assay optimization, which adjust assay conditions to give the best possible potency, without much regard for the physiological significance of such conditions. As such, we performed further assays with the FLIPR assay on HEK-293 cells expressing GluN1/GluN2A receptors using more physiological relevant buffers. This assay determines agonist-induced changes in intracellular  $\text{Ca}^{2+}$  without clamping the cells to a particular membrane potential.

The results of  $\text{Ca}^{2+}$ -influx assays performed using the FLIPR are shown in Fig. 2. As would be expected, (+)-MK-801 was still the most potent channel blocker ( $\text{IC}_{50} = 0.025 \pm 0.001$   $\mu\text{M}$ ), and memantine and ketamine were similarly potent, with  $\text{IC}_{50}$  values of  $9.23 \pm 1.03$   $\mu\text{M}$  and  $7.97$   $\mu\text{M}$  respectively. Although these values, at



**Fig. 1.** Graphs showing the binding affinities of memantine, ketamine, and (+) MK-801 upon rat (A) and human (B) cortical homogenate. As expected, (+)MK-801 bound with far greater affinity than memantine and ketamine, and the absolute values agree well with those previously reported (Parsons et al., 1999). For each curve,  $n = 4-6$ . The values for memantine were as follows:  $K_i$  (rat cortex) =  $1.19 \pm 0.08 \mu\text{M}$  and  $K_i$  (human cortex) =  $1.11 \pm 0.07 \mu\text{M}$ ,  $\text{IC}_{50}$  (rat cortex) =  $2.62 \mu\text{M}$  and  $\text{IC}_{50}$  (human cortex) =  $2.43 \mu\text{M}$ . The values for ketamine were as follows:  $K_i$  (rat cortex) =  $1.35 \pm 0.43 \mu\text{M}$  and  $K_i$  (human cortex) =  $0.67 \pm 0.15 \mu\text{M}$ ,  $\text{IC}_{50}$  (rat cortex) =  $2.98 \mu\text{M}$  and  $\text{IC}_{50}$  (human cortex) =  $1.48 \mu\text{M}$ . The values for (+)MK-801 were as follows:  $K_i$  (rat cortex) =  $0.005 \pm 0.001 \mu\text{M}$  and  $K_i$  (human cortex) =  $0.010 \pm 0.002 \mu\text{M}$ ,  $\text{IC}_{50}$  (rat cortex) =  $0.011 \mu\text{M}$  and  $\text{IC}_{50}$  (human cortex) =  $0.022 \mu\text{M}$ .

first glance, appear to be rather high compared to those determined by electrophysiological experiments at  $-70 \text{ mV}$ , it should be noted that the cells may freely depolarize in this assay, thus affecting the apparent potency of voltage-dependent antagonists such as these (see patch clamp experiments later for the voltage-dependency of blockade by e.g. memantine). These values clearly contrast with the binding data in both rat and human tissues, a finding which seems to provide further support for the hypothesis that the optimized conditions used for increasing affinity in binding experiments by adjusting buffers, etc. could give an artificial impression of the real potency of a voltage-dependent antagonist for this site.

For the electrophysiological patch clamp assays, two different protocols were used in order to produce an accurate assessment of potency (see Methods for details). The results derived from these two protocols were very similar, so mean values were pooled from both to produce the concentration–response curves shown in

Fig. 3C. Due to the long incubation and recovery times that would have been required to ensure that (+)MK-801 block is at equilibrium (Huettner and Bean, 1988; Parsons et al., 1993), it was technically impossible to assess the true potency of this antagonist in these patch clamp experiments.

It is clear that both memantine and ketamine were more potent in the electrophysiological assays than would have been expected from the FLIPR data: their respective  $\text{IC}_{50}$  values were  $0.79 \pm 0.02 \mu\text{M}$  and  $0.71 \pm 0.03 \mu\text{M}$ . These measurements agree well with previously published results produced using primary cell cultures and transfected cells expressing rat NMDA receptors (for examples, see Chen et al., 1992; Parsons et al., 1993, 1995; Bresink et al., 1996). For reference,  $\text{Mg}^{2+}$  was also shown to have similar potency at these human receptors ( $\text{IC}_{50} = 19.5 \pm 2.6 \mu\text{M}$ , see Fig. 3C) as has previously been reported for rat GluN1/GluN2A receptors expressed in HEK-293 cells, although recording conditions were slightly different (Tober, 2007).

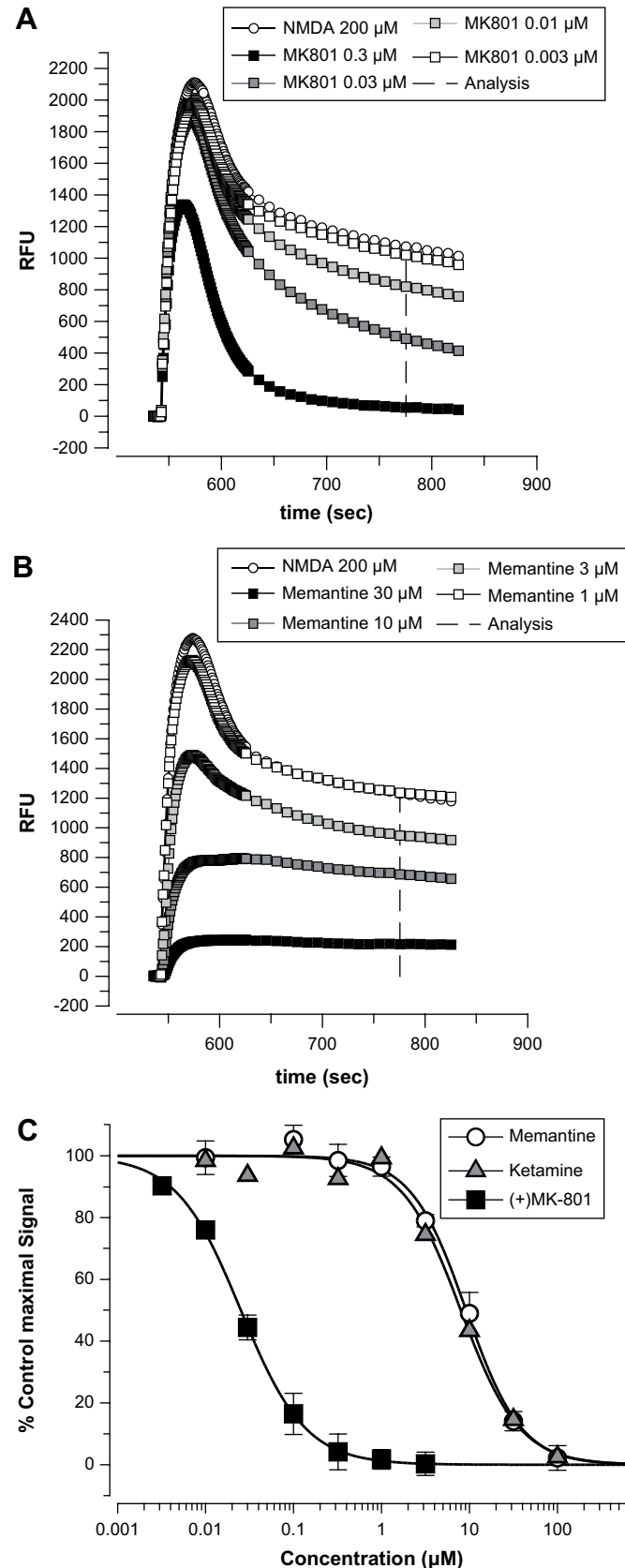
The onset and offset kinetics of the compounds were also measured, from which  $K_d$  can also be estimated. In these kinetic experiments, memantine showed clear double exponential blocking kinetics. For example, at  $10 \mu\text{M}$ , the fast component ( $\tau_{\text{fast}}$ ) of the onset kinetics was  $273 \pm 25 \text{ ms}$  (weight 69%) and the slow component ( $\tau_{\text{slow}}$ ) was  $2756 \pm 296 \text{ ms}$  (weight 31%). The same was true for offset kinetics, where  $\tau_{\text{fast}}$  was  $415 \pm 82 \text{ ms}$  (weight 38%) and  $\tau_{\text{slow}}$  was  $5107 \pm 1204 \text{ ms}$  (weight 62%). In contrast, for ketamine, double exponential fits were not clearly better than single exponential fits. As such, we undertook the following normalizing procedure in order to be able to compare kinetic data. Double exponential fits were integrated to single exponentials according to the following relationship:  $[(\tau_{\text{fast}} \times \text{weight}_{\text{fast}}) + (\tau_{\text{slow}} \times \text{weight}_{\text{slow}})] / (\text{weight}_{\text{fast}} + \text{weight}_{\text{slow}})$ .

As previously reported (Parsons et al., 1995), ketamine was shown to have slower kinetics than memantine. Both the onset and the offset kinetics for memantine were two times faster than those of ketamine. The results for memantine were:  $k_{\text{on}} = 0.32 \pm 0.11 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.53 \pm 0.10 \text{ s}^{-1}$  and the results for ketamine were:  $k_{\text{on}} = 0.15 \pm 0.05 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.22 \pm 0.05 \text{ s}^{-1}$ . The calculated  $K_d$  values confirmed that the two compounds have similar affinity for the receptor despite the difference in kinetics ( $K_d$  values for memantine and ketamine were calculated to be  $1.65 \pm 1.05 \mu\text{M}$  and  $1.47 \pm 0.68 \mu\text{M}$ , respectively) and these values are in moderately good accordance with the measured steady-state  $\text{IC}_{50}$  values.

The voltage-dependence of blockade by the NMDA open-channel blockers was studied on human GluN1/GluN2A receptors, using a voltage ramp protocol (see Methods for details). As can be seen from Fig. 4, antagonism of the NMDA-induced current by memantine showed clear voltage-dependency, and the values of the calculated parameters were similar to those previously reported (Parsons et al., 1995). The fraction of voltage-independent sites,  $\beta$ , was very similar for both memantine and ketamine ( $0.13 \pm 0.06$  and  $0.12 \pm 0.04$  respectively), but the calculated  $\delta$  values indicate that memantine might bind at a deeper site within the channel pore than ketamine ( $\delta$  values are  $0.90 \pm 0.09$  and  $0.79 \pm 0.04$  respectively). From this data, it was also possible to estimate what the  $\text{IC}_{50}$  values of the compounds would have been if measured at a holding potential at  $0 \text{ mV}$  ( $6.91 \pm 1.27 \mu\text{M}$  and  $6.05 \pm 0.66 \mu\text{M}$  for memantine and ketamine, respectively). These values agree well with those obtained in the FLIPR assays where the membrane potential was not clamped to  $-70 \text{ mV}$ .

To address the issue of agonist concentration-dependency of memantine steady-state blockade raised by Chen et al. (1992) the agonist concentration-dependency of memantine equilibrium block of human GluN1/GluN2A receptors in response to the endogenous agonist was tested by applying memantine at

0.3–30  $\mu\text{M}$  to antagonize currents evoked by 3, 10 and 30  $\mu\text{M}$  glutamate (Fig. 5). The  $\text{IC}_{50}$  value of blockade was calculated for each agonist concentration and it is clear from these data that there was not even a trend towards memantine having higher



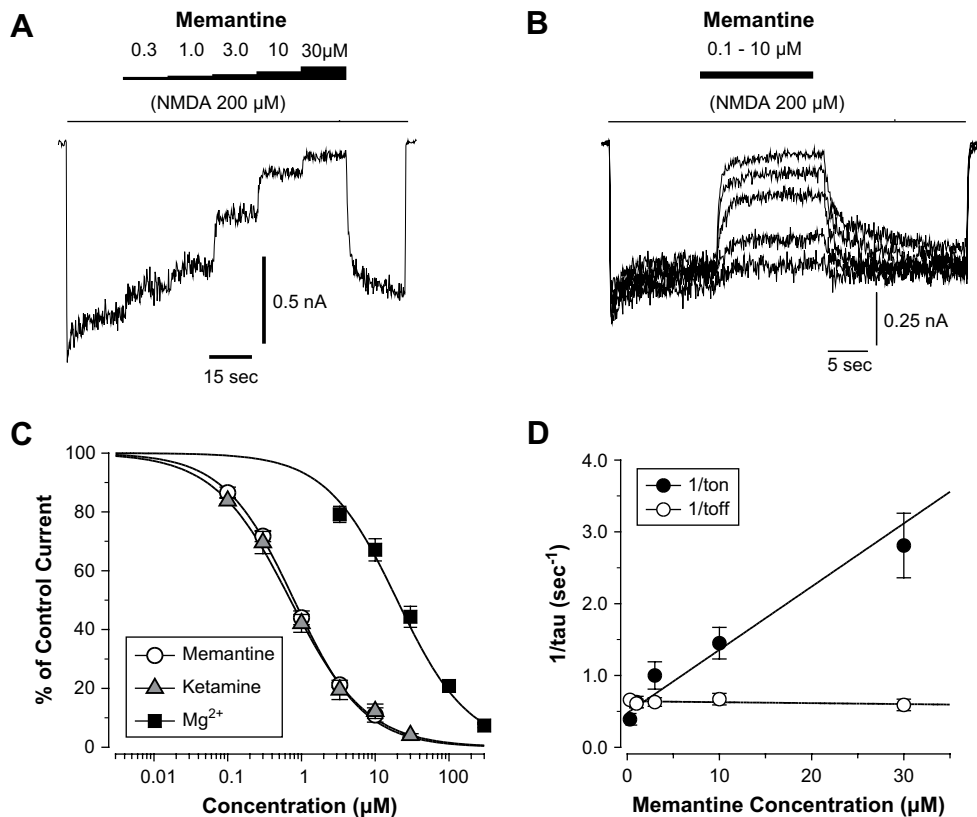
equilibrium potency when higher concentrations of glutamate were used. The  $\text{IC}_{50}$  values for memantine at glutamate concentrations of 3, 10 or 30  $\mu\text{M}$  were  $0.80 \pm 0.05 \mu\text{M}$ ,  $0.63 \pm 0.06 \mu\text{M}$  and  $0.77 \pm 0.09 \mu\text{M}$ , respectively, giving an average  $\text{IC}_{50}$  for all agonist concentrations tested of  $0.73 \pm 0.07 \mu\text{M}$  ( $n = 16$ ). It should be stressed that this concentration of memantine is the therapeutically relevant level achieved in the treatment of Alzheimer's disease. This agonist concentration-independency of memantine equilibrium blocking potency is in complete agreement with a recent study on cultured rat hippocampal neurons (Gilling et al., 2007). In these experiments, the endogenous agonist glutamate was used, but the kinetics of block were very similar to those seen with NMDA. For example, with glutamate (3  $\mu\text{M}$ ) the kinetic parameters were  $k_{\text{on}} = 0.36 \pm 0.01 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.72 \pm 0.01 \text{ s}^{-1}$ ,  $K_d = k_{\text{off}}/k_{\text{on}} = 2.01 \pm 0.45 \mu\text{M}$ . Moreover, the voltage-dependency was very similar -  $\text{IC}_{50} (0 \text{ mv}) = 11.99 \pm 0.36 \mu\text{M}$ ,  $\delta = 0.91 \pm 0.02$  and  $\beta = 0.06 \pm 0.01$  (data not shown).

The final series of experiments investigated the phenomenon of partial untrapping in the absence of NMDA - see legend in Fig. 6 for details. The double exponential offset kinetic time-constants for memantine in classical offset kinetic experiments were very similar to those calculated in these partial untrapping experiments where onset kinetics of the first NMDA application after memantine block were as follows:  $\tau_{\text{fast}}$  was  $310 \pm 40 \text{ ms}$  (weight 64%) and  $\tau_{\text{slow}}$  was  $3190 \pm 1750 \text{ ms}$  (weight 36%). However, the weights of these components were quite different. The fast component of these "NMDA" onset kinetics after the removal of memantine in partial untrapping experiments most likely reflects three processes. First, onset kinetics for NMDA activation of steady-state, non-blocked channels, second, NMDA activation of the population of channels which had released memantine in the absence of agonist and third the fast component of memantine unblock seen in normal kinetic experiments. For both classical kinetic and partial untrapping experiments, this third component should be the same. The weight of this component for classical kinetic experiments was subtracted from that seen in the partial untrapping experiments. The proportion of the fast component due to the activation kinetics of non-blocked NMDA receptors thus obtained was 26%. As memantine 10  $\mu\text{M}$  blocked NMDA receptor currents to 5.3% of control in these experiments, this proportion of receptors was subtracted to reveal the proportion of receptors that showed untrapping, namely 20.7%. This level of partial untrapping from human GluN1/GluN2A receptors was very close to that reported previously for native and cloned rat receptors (Blanpied et al., 1997). In contrast, there was no evidence for partial untrapping by ketamine, as single exponential "offset" kinetics were seen in both experimental paradigms.

#### 4. Discussion

The results from experiments using memantine, ketamine and (+)MK-801 presented here agree very well with most of those previously reported (Parsons et al., 1999). The main difference

**Fig. 2.** Graph showing the results for memantine, ketamine and (+)MK-801 from  $\text{Ca}^{2+}$ -influx assays measured using a FLIPR device. HEK-293 cells were transfected with human GluN1 and GluN2A subunits, and intracellular  $\text{Ca}^{2+}$  levels were measured before and after application of 200  $\mu\text{M}$  NMDA, 10  $\mu\text{M}$  D-serine. A and B show example FLIPR data for (+)MK-801 and memantine, respectively. C shows the dose-response curves for memantine, ketamine and (+)MK-801, and in each case  $n = 2-3$  individual experiments have been performed. The values for memantine were:  $\text{IC}_{50} = 9.23 \pm 1.03 \mu\text{M}$  and Hill coefficient =  $1.04 \pm 0.24$ . The values for ketamine were:  $\text{IC}_{50} = 7.97 \mu\text{M}$  and Hill coefficient = 1.03. The values for (+)MK-801 were:  $\text{IC}_{50} = 0.025 \pm 0.001 \mu\text{M}$  and Hill coefficient =  $1.18 \pm 0.05$ .

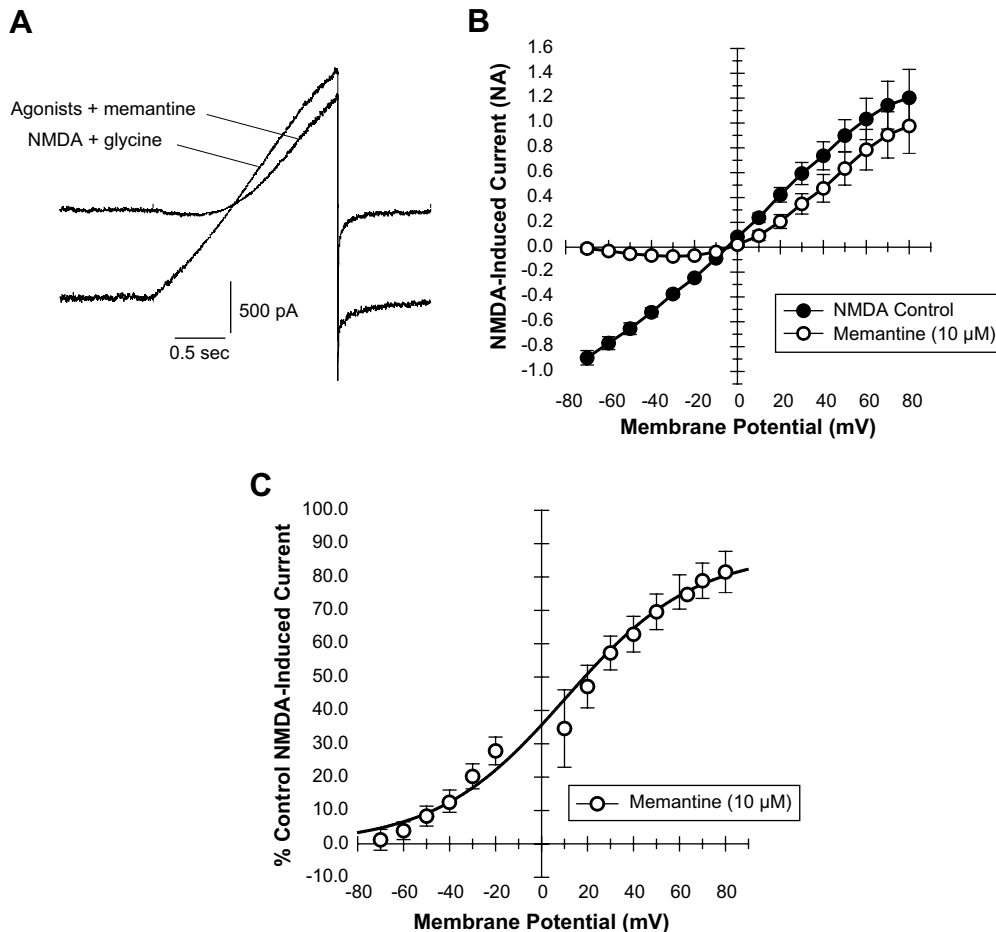


**Fig. 3.** Antagonism of NMDA-evoked currents recorded from HEK-293 cells transfected with human GluN1 and GluN2A subunits measured using electrophysiological patch clamp techniques. (A) Example trace showing the cumulative protocol involving stepwise application of increasing concentrations of antagonist during constant application of agonists (200  $\mu\text{M}$  NMDA and 10  $\mu\text{M}$  glycine in all experiments). For these experiments,  $n = 4$ . (B) Example trace showing the kinetic protocol, where a single concentration of antagonist was applied in the constant presence of agonists. For these experiments,  $n = 6$ . (C) The raw data from the two protocols were pooled to produce the concentration–response curves ( $n = 10$ ). The results were as follows: memantine  $\text{IC}_{50} = 0.79 \pm 0.02 \mu\text{M}$  and Hill coefficient =  $0.92 \pm 0.02$ ; ketamine  $\text{IC}_{50} = 0.71 \pm 0.03 \mu\text{M}$  and Hill coefficient =  $0.84 \pm 0.02$ ;  $\text{Mg}^{2+}$   $\text{IC}_{50} = 19.5 \pm 2.6 \mu\text{M}$  and Hill coefficient =  $0.89 \pm 0.06$ . (D) From the recordings produced using the kinetic protocol, the onset and offset rates of the compounds were measured. Memantine showed double exponential kinetics but ketamine only showed single exponential kinetics. As such, double exponential fits were integrated to single exponentials according to the following relationship:  $[(\tau_{\text{fast}} \times \text{weight}_{\text{fast}}) + (\tau_{\text{slow}} \times \text{weight}_{\text{slow}})] / (\text{weight}_{\text{fast}} + \text{weight}_{\text{slow}})$  to allow comparison of the kinetic data. Mean  $1/\tau$  values were plotted against antagonist concentration and  $k_{\text{on}}$  and  $k_{\text{off}}$  were determined. These values were also used to estimate the  $K_d$ . The results for memantine were:  $k_{\text{on}} = 0.32 \pm 0.11 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.53 \pm 0.10 \text{ s}^{-1}$ , and  $K_d = 1.65 \pm 1.05 \mu\text{M}$ , and the results for ketamine were:  $k_{\text{on}} = 0.15 \pm 0.05 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{\text{off}} = 0.22 \pm 0.05 \text{ s}^{-1}$ , and  $K_d = 1.47 \pm 0.68 \mu\text{M}$ .

between previous studies and the present study was the use of human tissue/receptors in place of rodent. Although electrophysiological experiments have previously been performed to test memantine upon human GluN1/GluN2A receptors expressed in *Xenopus* oocytes (Ferrer-Montiel et al., 1998), the HEK-293 cells used here provide specific advantages – not only that this is a human cell line, but also that faster and more precise voltage- and concentration clamp can be achieved during whole-cell recordings due to their smaller size. The present results clearly contrast to the studies of Ferrer-Montiel et al. (1998) in two major aspects. Firstly, the potency of memantine at human GluN1/GluN2A receptors observed in the present study was very similar to that reported previously for rodent receptors whereas (Ferrer-Montiel et al., 1998) reported an  $\text{IC}_{50}$  for memantine of 220 nM at “resting” membrane potentials. Secondly, memantine was clearly a voltage-dependent blocker of human GluN1/GluN2A receptors in the present study. This agrees with rodent studies, but again, clearly contrasts with the studies of Ferrer-Montiel et al. (1998) where the blockade by memantine was apparently voltage-independent. As such, we feel that the present data provide the first functional evidence that memantine acts in a similar manner on both rodent and human NMDA receptors. It is an uncompetitive, strongly voltage-dependent NMDA receptor antagonist with fast, double exponential blocking kinetics. In addition, partial

trapping of memantine was demonstrated in the present experiments and, as in other recently published results (Gilling et al., 2007), equilibrium blockade of NMDA receptors by memantine was shown to be agonist concentration-independent when using human receptors and the endogenous agonist glutamate.

Taken together, the present results indicate that ketamine had a somewhat higher affinity than memantine for NMDA receptors in binding experiments using post-mortem human tissue, but this finding is probably an artefact. Ketamine and memantine were in fact equipotent when membrane potential was clamped to near resting levels in patch clamp experiments on human GluN1/GluN2A receptors, and memantine was somewhat more voltage-dependent than ketamine. As such, the apparent greater affinity of ketamine in these binding experiments might be explained by the fact that the receptors in such membrane fractions experience no membrane potential, and so the potency of a less voltage-dependent blocker would be influenced to a smaller degree by this factor than the potency of a more voltage-dependent antagonist. This assumption is supported by the fact that ketamine was also slightly more potent than memantine in FLIPR assays. In these assays, cells were again not clamped to a particular membrane potential so were free to depolarize to indefinable levels, and memantine and ketamine were accordingly less potent under these conditions than in patch clamp experiments.

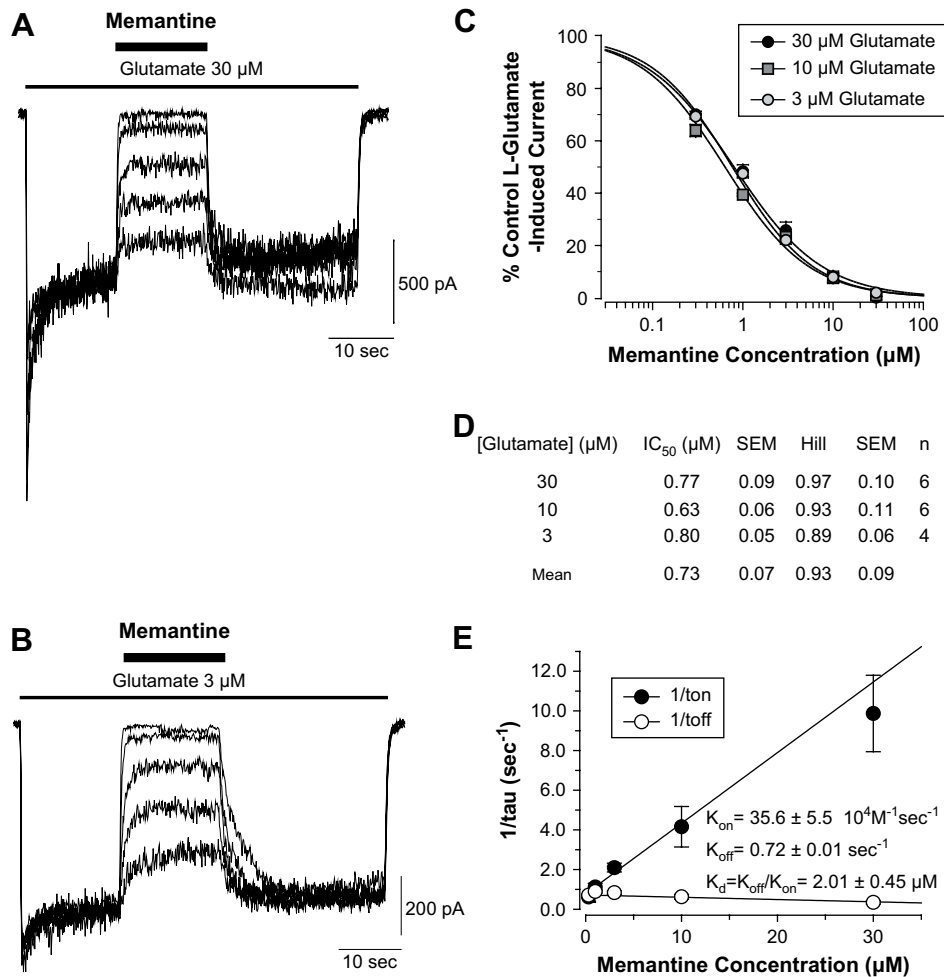


**Fig. 4.** The voltage-dependence of antagonism of currents by a single concentration of antagonist. The agonists used were 200  $\mu$ M NMDA and 10  $\mu$ M glycine and  $n = 4$  for both memantine and ketamine. (A) An example trace showing the voltage ramp protocol, with the currents being blocked by memantine (10  $\mu$ M) in a voltage-dependent manner. (B) The mean current was plotted against membrane potential to highlight the voltage-dependence of the antagonist. (C) The mean percentage blockade of control currents was plotted against holding potential. The values for memantine were:  $IC_{50}$  (0 mV) =  $6.91 \pm 1.27 \mu$ M,  $\delta = 0.90 \pm 0.09$  and  $\beta = 0.13 \pm 0.06$ . The values for ketamine were:  $IC_{50}$  (0 mV) =  $6.05 \pm 0.66 \mu$ M,  $\delta = 0.79 \pm 0.04$  and  $\beta = 0.12 \pm 0.04$ .

Although memantine and ketamine were similarly potent at human GluN1/GluN2A receptors in the electrophysiological assays, there were small but clear differences in the biophysical properties of these two uncompetitive NMDA receptor antagonists. Firstly, as discussed above, memantine was slightly more voltage-dependent than ketamine. Secondly, memantine showed clear double exponential binding kinetics whereas ketamine showed simple single exponential binding kinetics in all experiments. The double exponential kinetics of memantine had to be normalized to single exponentials in order to facilitate quantitative comparison with ketamine. Memantine showed twofold faster onset and offset kinetics than ketamine following this procedure, a difference which could be very important under physiological conditions *in vivo*. In addition, the 20% partial untrapping of memantine previously reported for rodent receptors (Blanpied et al., 1997) was confirmed for human receptors GluN1/GluN2A, whereas ketamine was also confirmed to show little or no partial untrapping. This difference might be related to the lack of a very fast component of unblock by ketamine and lack of binding to a superficial binding site in the NMDA receptor channel (Sobolevsky et al., 1998, 2002; Sobolevsky and Koshelev, 1998).

It should here be stressed that the observed differences in the biophysical properties of memantine and ketamine on human GluN1/GluN2A receptors and previously reported for native rat NMDA receptors were only moderate – i.e. maximal twofold

differences. It seems unlikely that these factors alone can account for the clear therapeutic superiority in the tolerability of memantine over ketamine in humans. Other factors are almost certainly very influential. There is a very clear difference between the pharmacokinetics of memantine and ketamine, both in humans and in rats. Following oral administration, memantine has a half life in humans of around 100 h (for example, see Periclou et al., 2004, 2006) whereas ketamine is extremely rapidly eliminated, with a redistribution half life of around 15 min when administered intravenously or intramuscularly – the only possible routes of administration of ketamine (Clements and Nimmo, 1981). As such, it would probably be nearly impossible to achieve steady-state equilibrium block of human NMDA receptors with ketamine even if one was able to administer this compound orally (Clements et al., 1982). Due to the fundamental role of NMDA receptors in physiological processes such as learning and memory, a stable level of NMDA receptor occupancy would, theoretically, be essential. Even a strongly voltage-dependent antagonist with fast unblocking kinetics could only effectively “modulate” receptor activity if present at relevant concentrations in a stable manner. Such an antagonist would probably impair normal NMDA receptor function if present at excessive concentrations, even if it possessed the desired *in vitro* biophysical properties (see, for example Zajackowski et al., 1997; Zoladz et al., 2006). Other factors, such as the block of human 5-HT<sub>3</sub> receptors by memantine but not ketamine,



**Fig. 5.** Agonist concentration-independency of memantine equilibrium block of human GluN1/GluN2A receptors using the kinetic protocol. Glutamate (3–30 μM) was applied in the continuous presence of 10 μM glycine to evoke currents which were then blocked by memantine (3–30 μM). Memantine showed concentration-dependent double exponential kinetics. Panels A and B show example traces recorded using 30 μM and 3 μM glutamate respectively. From the pooled data presented in panels C and D, it is clear that the concentration of the endogenous agonist also did not affect the potency of blockade by memantine. In panel E, double exponential blocking kinetics of memantine with glutamate 3 μM were integrated to single exponentials as in Fig. 3. Mean  $1/\tau$  values were plotted against antagonist concentration to determine  $k_{on}$  and  $k_{off}$ . The fitted kinetic values were  $k_{on} = 0.36 \pm 0.01 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ ,  $k_{off} = 0.72 \pm 0.01 \text{ s}^{-1}$ ,  $K_d = k_{off}/k_{on} = 2.01 \pm 0.45 \text{ μM}$ .

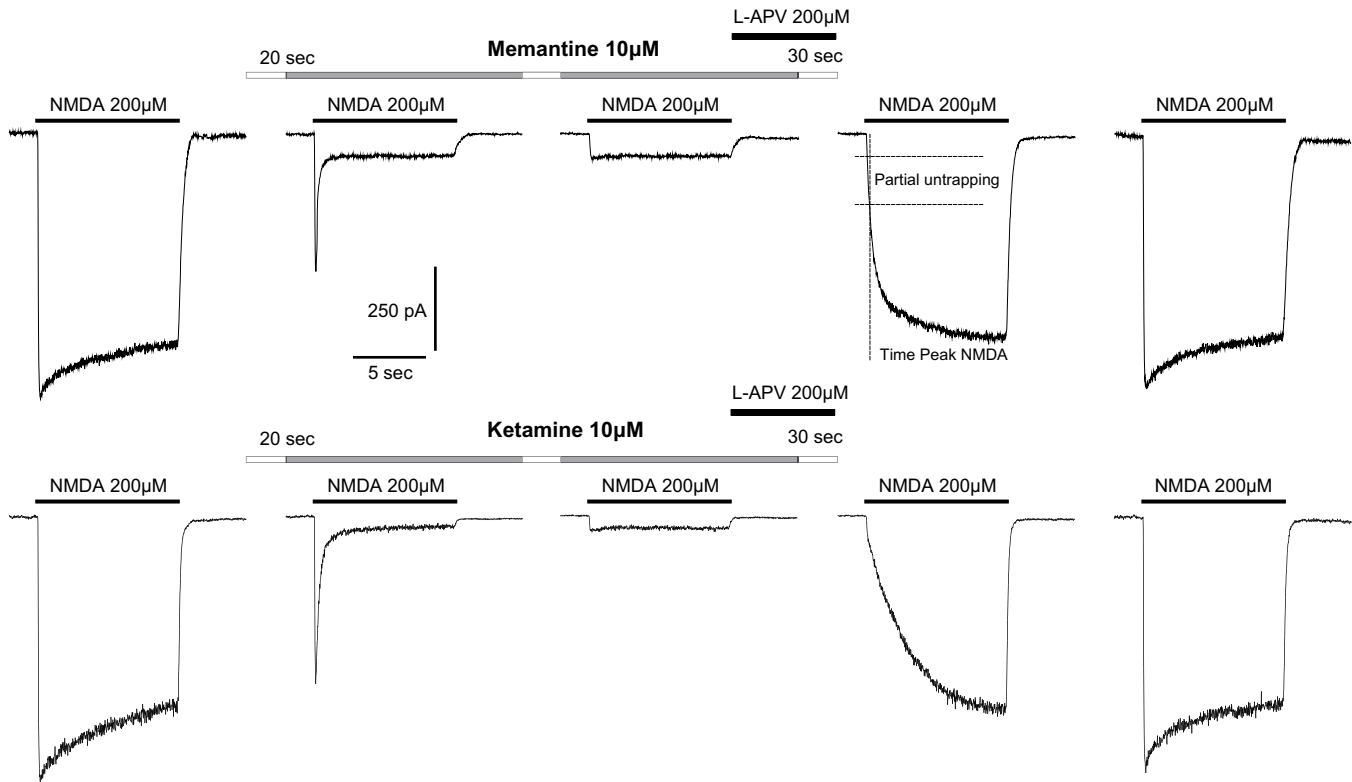
might also be important in the differentiation of the therapeutic tolerability of memantine versus ketamine (Wenk, 1996) but these aspects were not addressed in the current study.

Below, we summarize how we believe certain biophysical properties of memantine, all of which were confirmed in the present studies on human NMDA receptors, are important for its mechanism of action in the treatment of Alzheimer's disease.

Memantine and other well tolerated open-channel blockers show much faster open-channel blocking/unblocking kinetics than compounds burdened with negative psychotropic effects such as (+)MK-801 or phencyclidine (Rogawski et al., 1991; Chen et al., 1992; Rogawski, 1993; Parsons et al., 1993, 1999; Black et al., 1996). The kinetics of (+)MK-801 and phencyclidine are too slow to allow them to leave the channel upon depolarization, which is often reflected in apparently weaker functional voltage-dependency. The unblocking rate of memantine following depolarizing voltage-steps is very rapid and well within the time course of a NMDA receptor-mediated excitatory postsynaptic potential. Physiologically, NMDA receptors are transiently activated by mM concentrations of glutamate (Clements et al., 1992) following strong depolarization of the postsynaptic membrane which rapidly relieves their voltage-dependent blockade by  $\text{Mg}^{2+}$  (Nowak et al., 1984). During pathological activation, however, NMDA receptors are activated by lower

concentrations of glutamate but for much a longer time (Benveniste et al., 1984; Andine et al., 1991; Globus et al., 1991a,b; Mitani et al., 1992; Buisson et al., 1992). The voltage-dependency of the divalent cation  $\text{Mg}^{2+}$  is so pronounced that it leaves the NMDA channel not only during normal physiological signalling, but also under pathological conditions i.e. in the presence of tonically mildly elevated glutamate levels and moderate membrane depolarization due to energy deficits. Uncompetitive antagonists also block the NMDA receptor channel, but high affinity compounds such as (+)MK-801 have much slower unblocking kinetics than  $\text{Mg}^{2+}$  and less pronounced functional voltage-dependency, and are therefore unable to leave the channel within the time course of a normal NMDA receptor-mediated excitatory postsynaptic potential. As a result, (+)MK-801 blocks both the pathological and the physiological activation of NMDA receptors (Parsons et al., 1999).

It was first proposed by this group that the combination of fast offset kinetics and strong voltage-dependency allow memantine to rapidly leave the NMDA channel upon transient activation by mM concentrations of synaptic glutamate but block the sustained activation by μM concentrations of glutamate under moderate pathological conditions (Parsons et al., 1993, 1995, 1996). Although the slower component of the offset kinetic at near resting membrane potentials and room temperature is still too slow to allow synaptic



**Fig. 6.** Partial untrapping of memantine but not ketamine at human GluN1/GluN2A receptor channels. NMDA (200  $\mu$ M) was applied for 10 s every 35 s in the continuous presence of 10  $\mu$ M glycine. In each series (left to right), the first application of NMDA in the absence of antagonist was followed by a 15 s interval. Memantine or ketamine (10  $\mu$ M) was then pre-incubated for 20 s (open bar) followed by the co-application of NMDA in the continuing presence of antagonist (10  $\mu$ M, black bar) for 10 s. Both memantine and ketamine showed clear open-channel block of responses as evidenced by the remaining peak response to NMDA. NMDA was then applied for a third time to demonstrate that steady-state block (to  $5.32 \pm 1.34\%$  and  $3.50 \pm 0.32\%$  of control plateau currents respectively) had been achieved. Thereafter, partial untrapping of memantine and ketamine in the channel in the absence of agonist was permitted by introducing a delay of 33 s in the continuing presence of uncompetitive antagonist plus L-APV (200  $\mu$ M). Both were washed off for 2 s before the fourth 10 s application of NMDA alone. For memantine, this fourth response to NMDA showed clear, double exponential onset kinetics and the average fitted values for all 9 cells tested were  $\tau_{\text{fast}} = 310 \pm 40$  ms (weight 64%) and  $\tau_{\text{slow}} = 3190 \pm 1750$  ms (weight 36%). The amplitude of this fast component was used to estimate the fraction of channels that released memantine in the absence of NMDA (20.7% – see Results text for details). In contrast, for ketamine, this response showed only single exponential kinetics and there was no evidence for partial trapping ( $3500 \pm 350$  ms,  $n = 5$ ). A fifth 10 s application of NMDA was then made ensure full recovery from antagonism.

activation – around 5 s – the relief of blockade in the continuous presence of memantine upon depolarization is much faster due to an increase in the weight of the faster recovery time-constant (Frankiewicz et al., 1996; Bresink et al., 1996; Sobolevsky et al., 1998; Sobolevsky and Koshelev, 1998) and these kinetics are almost certainly even faster *in vivo* due to higher temperatures (Davies et al., 1988). Furthermore, the rate of recovery from memantine blockade is dependent on the open probability of NMDA channels and therefore would be faster in the presence of higher, synaptic concentrations of glutamate (Clements et al., 1992; Gilling et al., 2007).

Given the crucial role of NMDA receptor synaptic activation in neuronal plasticity, simplistic interpretation of the observation that a NMDA receptor antagonist such as memantine can improve cognition and neuronal plasticity under pathological conditions might appear paradoxical. It should, however, be stressed that  $\text{Mg}^{2+}$  is an endogenous NMDA receptor channel blocker and its absence leads both to an impairment in neuronal plasticity (Coan et al., 1989; Frankiewicz and Parsons, 1999) and neuronal death (Furukawa et al., 2000). Any dysfunction of postsynaptic neurons leading to weakened blockade by  $\text{Mg}^{2+}$  may trigger prolonged  $\text{Ca}^{2+}$ -influx and structural deficits (Danysz and Parsons, 2003; Rogawski and Wenk, 2003). It is believed that memantine may serve as a more effective surrogate for  $\text{Mg}^{2+}$  (Parsons et al., 1993). As a result of its somewhat less pronounced functional voltage-dependency, memantine is more effective than  $\text{Mg}^{2+}$  in blocking tonic pathological activation of NMDA receptors, but can leave the NMDA receptor channel with fast, voltage-dependent unblocking kinetics following strong synaptic

activation. Memantine therefore suppresses tonic pathological synaptic “noise” but allows the relevant physiological synaptic signal to be detected, providing both neuroprotection and symptomatic restoration of synaptic plasticity by one and the same mechanism (Parsons et al., 1999). This mechanism therefore seems to be somewhat more relevant for the effects of memantine in Alzheimer’s disease than the partial trapping model (Blanpied et al., 1997) which can only explain the therapeutic tolerability of memantine but not the symptomatic effects on cognition proven in large clinical trials (Danysz and Parsons, 2003).

## 5. Summary

The use of transfected HEK-293 cells has allowed us to further investigate the *in vitro* effects of memantine and ketamine on human GluN1/GluN2A receptors. The present data closely match previously reported data from studies using rodent receptors and confirm that memantine is a fast, voltage-dependent open-channel blocker of human NMDA receptors, in accordance with its proposed mechanism of action in Alzheimer’s disease. However, it should be noted that various factors such as secondary messenger systems may not function in transfected cells as they do in primary cultures of neurons where the receptors are endogenously expressed. This is a technical limitation that cannot be overcome at present – it is not possible to perform similar experiments on cortical/hippocampal neurons from adult human NMDA receptors expressed in their native tissue. Such studies would seem to be highly desirable,

especially in view of the fact that human NMDA receptors expressed in the cortex/hippocampus certainly do not only express the GluN1 and GluN2A subunits.

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