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Molecular pharmacology of human NMDA receptors

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Abstract

N-methyl-D-aspartate (NMDA) receptors are ionotropic glutamate receptors that mediate excitatory neurotransmission. NMDA receptors are also important drug targets that are implicated in a number of pathophysiological conditions. To facilitate the transition from lead compounds in pre-clinical animal models to drug candidates for human use, it is important to establish whether NMDA receptor ligands have similar properties at rodent and human NMDA receptors. Here, we compare amino acid sequences for human and rat NMDA receptor subunits and discuss inter-species variation in the context of our current knowledge of the relationship between NMDA receptor structure and function. We summarize studies on the biophysical properties of human NMDA receptors and compare these properties to those of rat orthologs. Finally, we provide a comprehensive pharmacological characterization that allows side-by-side comparison of agonists, un-competitive antagonists, GluN2B-selective non-competitive antagonists, and GluN2C/D-selective modulators at recombinant human and rat NMDA receptors. The evaluation of biophysical properties and pharmacological probes acting at different sites on the receptor suggest that the binding sites and conformational changes leading to channel gating in response to agonist binding are highly conserved between human and rat NMDA receptors. In summary, the results of this study suggest that no major detectable differences exist in the pharmacological and functional properties of human and rat NMDA receptors.

Keywords

ionotropic glutamate receptor; NMDA; pharmacology; electrophysiology; structure-function

1. Introduction

NMDA receptors are involved in neuronal development, synaptic plasticity, learning, and memory in the mammalian central nervous system (Traynelis et al., 2010). NMDA receptors are implicated in a number of pathophysiological conditions, including neuropathic pain, epilepsy, stroke, neurodegenerative diseases (e.g. Parkinson's, Huntington's, and Alzheimer's diseases), and psychiatric disorders (e.g. schizophrenia and major depression)

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(Hardingham and Bading, 2010; Kalia et al., 2008; Lau and Zukin, 2007; Skolnick et al., 2009; Traynelis et al., 2010). NMDA receptors have therefore been intensively studied for the past decades as potential drug targets for a number of neurological and psychiatric indications (Koller and Urwyler, 2010; Waxman and Lynch, 2005). Despite these efforts, only few compounds targeting these receptors have been converted from pre-clinically active compounds into drugs for human use. Lowaffinity un-competitive antagonists (i.e. use-dependent channel blockers) and allosteric modulators of NMDA receptors have demonstrated improved safety profiles compared to competitive antagonists, which have failed in the clinic due to their narrow therapeutic windows. The most recent NMDA receptor ligand to be approved for human use is the low-affinity channel blocker memantine (Alzheimer's disease) (Lipton, 2006). Negative allosteric modulators selective for the GluN2B NMDA receptor subunit could be beneficial in the treatment of traumatic brain injury, neuropathic pain, and treatment-resistant depression and are also associated with few adverse side effects (Traynelis et al., 2010). These compounds and novel ligands in clinical trials have demonstrated that NMDA receptor antagonist can be used for therapeutic gain, and suggest a huge potential of NMDA receptor ligands, both inhibitors and potentiators, in the treatment of several neurological and psychiatric conditions. In addition, increasingly precise anatomical localization of the different NMDA receptor subunits has reinforced the therapeutic rationale for the development of subunit-selective NMDA receptor ligands (e.g. see Hallett and Standaert, 2004).

Seven NMDA receptor subunits have been identified; the GluN1 subunit, four different GluN2 subunits (GluN2A–D), and two GluN3 subunits (GluN3A,B). NMDA receptors are assembled from two GluN1 subunits and two GluN2 subunits and are activated by simultaneous binding of glycine and glutamate to GluN1 and GluN2 subunits, respectively (reviewed in Traynelis et al., 2010). The four GluN2 subunits play different roles during neuronal development and in the adult central nervous system (e.g. see Monyer et al., 1994), and endow NMDA receptors with different biophysical and pharmacological properties (Monyer et al., 1994; Vicini et al., 1998; Yuan et al., 2009). GluN3 subunits are capable of assembling with GluN1–GluN2 to modify functional properties, but many details of expression, assembly, and physiological roles of GluN3 subunits in the central nervous system are still unclear (Low and Wee, 2010). In this study, we focus on the GluN1 and GluN2A–D subunits.

Several unique pharmacological and biophysical features, including the requirement for simultaneous binding of the co-agonists glycine and glutamate for activation, slow deactivation, voltage-dependent Mg^{2+} block, and high permeability to Ca^{2+} , distinguish NMDA receptors from the other ionotropic glutamate receptors. The activities of a wide range of agonists, competitive antagonists, channel blockers and allosteric modulators are well-characterized across the different NMDA receptor subtypes (Dravid et al., 2007; Erreger et al., 2007; Hansen et al., 2008; Hansen et al., 2010a). Considering the therapeutic potential of NMDA receptor ligands, it is surprising that the vast majority of studies on pharmacological and functional properties of NMDA receptor subtypes have been conducted on recombinant rat NMDA receptors. In other words, there is an apparent lack of data from human NMDA receptors. Relatively few studies have described functional properties of heterologously expressed human NMDA receptors (Bednar et al., 2004; Claiborne et al., 2003; Curtis et al., 2003; Daggett et al., 1998; Feuerbach et al., 2010; Grimwood et al., 1996a; Grimwood et al., 1996b; Hess et al., 1996; Hess et al., 1998; Le Bourdelles et al., 1994; Priestley et al., 1996; Priestley et al., 1995; Steinmetz et al., 2002; Steinmetz et al., 2004; Usala et al., 2003; Varney et al., 1996; Wafford et al., 1995). Although the amino acid sequence is highly conserved between rat and human subunits, some variation exist that may nonetheless confer significant pharmacological and functional differences.

1.1 Comparison of amino acid sequences for rat and human NMDA receptor subunits

The overall sequence identities between rat and human orthologs of GluN1, GluN2A, GluN2B, GluN2C, and GluN2D are 99.3%, 95.3%, 98.5%, 87.1%, and 95.6%, respectively (Fig. 1 and Table 1). GluN1 shows high sequence identity between rat and human NMDA receptors with only 7 non-identical amino acids out of 938, whereas GluN2C is remarkably divergent with 162 non-identical amino acids out of 1236. The majority of variation between rat and human orthologs are located in the extracellular amino-terminal domain (ATD) and the intracellular carboxyl-terminal domain (CTD) (Fig. 1D and Table 1). Only few differences are located in the agonist binding and the transmembrane domains, suggesting conservation of key conformational changes that lead to channel gating in response to agonist binding. More detailed analyses of cDNAs and deduced amino acid sequences for human NMDA receptor subunits are described in the original cloning papers (Collins et al., 1993; Daggett et al., 1998; Foldes et al., 1994a; Foldes et al., 1993, 1994b; Hess et al., 1996; Hess et al., 1998; Karp et al., 1993; Le Bourdelles et al., 1994; Lin et al., 1996; Planells-Cases et al., 1993).

The intracellular CTD varies greatly among different ionotropic glutamate receptor subunits. This domain may contain different phosphorylation sites and binding sites for intracellular proteins involved in regulation of membrane trafficking and receptor function (Traynelis et al., 2010). Deletion of the CTD of GluN1 and GluN2A does not abolish function, but does alter regulation (Ehlers et al., 1996; Kohr and Seeburg, 1996; Krupp et al., 1998; Vissel et al., 2001). The variation between CTDs of rat and human NMDA receptor subunits raises the possibility that differences might exist in membrane trafficking and phosphorylation, especially for GluN2C whose CTD is just 71% identical between rat and human orthologs (Fig. 1 and Table 1).

The ATD is a semi-autonomous domain that consists of the first ~400–450 residues starting at the N-terminus. Mutant NMDA receptor subunits, where the ATD has been completely removed, are capable of forming functional receptors (Gielen et al., 2009; Yuan et al., 2009). However, truncations of the ATD have been found to influence several key subunit-specific features of NMDA receptor function such as open probability, deactivation, desensitization (Gielen et al., 2009; Yuan et al., 2009). Thus, the ATD plays a regulatory role by influencing many of the differences in biophysical properties that exist among the NMDA receptor subtypes. Moreover, multiple NMDA receptor modulators bind the ATD (Hansen et al., 2010a), and some of the distinct pharmacological properties of different NMDA receptor subtypes are mediated by the GluN2 ATD (Gielen et al., 2009; Yuan et al., 2009). For example, the GluN2 ATD contributes to the difference in glutamate potency between GluN1/GluN2A and GluN1/GluN2D receptors (Yuan et al., 2009); glutamate potency at GluN1/GluN2A is ~10-fold lower than at GluN1/GluN2D. In addition, non-competitive antagonists, typified by the phenylethanolamines such as ifenprodil, bind to a site at the interface between the ATDs of GluN1 and GluN2B (Karakas et al., 2011). Since variations between ATDs of rat and human NMDA receptor subunits exist, there could be inter-species differences in biophysical and pharmacological properties.

1.2 Biophysical properties of rat and human NMDA receptors

NMDA receptors that contain different GluN2 subunits have different deactivation time course, single-channel conductance, and open probability (Table 2). For example, rat GluN1/GluN2A and GluN1/GluN2B receptors have higher mean single-channel conductance (47–48 pS) than rat GluN1/GluN2C and GluN1/GluN2D receptors (30–32 pS) (Table 2). The weighted time constants of deactivation ($\tau_{\text{deactivation}}$) following removal of glutamate also depends on the GluN2 subunit; $\tau_{\text{deactivation}}$ for rat NMDA receptors are ~50 ms for GluN1/ GluN2A, ~300 ms for GluN1/GluN2B, ~400 ms for GluN1/ GluN2C, >2

seconds for GluN1/ GluN2D. In addition, rat NMDA receptors have different open probabilities depending on the GluN2 subunit; ~0.5 for GluN1/GluN2A, ~0.1 for GluN1/ GluN2B, and <0.05 for GluN1/GluN2C and GluN1/GluN2D (Table 2).

Three studies have described the biophysical properties of human NMDA receptors (Daggett et al., 1998; Hess et al., 1998; Varney et al., 1996) (Table 2). Mean channel open times for human NMDA receptor subtypes are similar to those of the corresponding rat NMDA receptor subtypes. However, mean single-channel conductances for human NMDA receptor subtypes appear lower than those of the corresponding rat NMDA receptor subtypes. This difference in mean single-channel conductances likely reflects differences in experimental conditions. For example, values for mean single-channel conductance of rat NMDA receptors are directly measured using single-channel recordings, whereas these values for human NMDA receptors are estimated using noise analysis. In addition, increasing concentrations of extracellular Ca^{2+} reduces mean single-channel conductance (Dravid et al., 2008; Schorge et al., 2005; Wyllie et al., 1996), and single-channel conductances for human subtypes were obtained at higher extracellular Ca^{2+} (2 mM) than those for rat subtypes (0.85–1.0 mM). Deactivation time constants following removal of glutamate or glycine are similar for rat and human GluN1/GluN2C and GluN1/GluN2D receptors (Table 2). No data exist for deactivation of human GluN1/GluN2A and GluN1/GluN2B receptors. Furthermore, open probabilities of the different human NMDA receptor subtypes have not been determined. In summary, these results do not suggest marked inter-species differences in biophysical properties. However, the data for human NMDA receptors are incomplete and more work is required to rule out that differences exist between rat and human orthologs.

1.3 Pharmacology of human NMDA receptors

A number agonists and competitive antagonists have been characterized on human recombinant NMDA receptor subtypes using electrophysiological methods and fluorescence-based measurements of intracellular Ca^{2+} (Bednar et al., 2004; Daggett et al., 1998; Feuerbach et al., 2010; Hess et al., 1996; Hess et al., 1998; Le Bourdelles et al., 1994; Priestley et al., 1995; Varney et al., 1996; Wafford et al., 1995). The GluN2B-selective non-competitive antagonist ifenprodil has also been evaluated on human NMDA receptors with some variation in the obtained IC_{50} -values, ranging from 110 nM to 2.2 μM at human GluN1/GluN2B (Bednar et al., 2004; Feuerbach et al., 2010; Hess et al., 1998; Varney et al., 1996). No studies have provided IC_{50} values at human NMDA receptors for un-competitive antagonist (i.e. voltage-dependent channel blockers) using electrophysiological methods, which is a prerequisite to control membrane potential. Finally, the pharmacological properties of rat and human NMDA receptors have not yet been compared in the same study. Here, we address the lack of comparative data for rat and human NMDA receptor subtypes by providing quantitative evaluation of the actions of agonists, un-competitive antagonists, GluN2B-selective non-competitive antagonists, and newly described GluN2C/D-selective modulators using two-electrode voltage-clamp recordings.

2. Materials and Methods

2.1 DNA constructs

Wild type rat cDNAs for GluN1-1a (GenBank accession numbers U11418 and U08261; hereafter GluN1), GluN2A (D13211), GluN2B (U11419), GluN2C (M91563), and GluN2D (L31611) were provided by Drs. S. Heinemann (Salk Institute), S. Nakanishi (Kyoto University), and P. Seeburg (University of Heidelberg). The full open reading frames of human NMDA receptor subunits were assembled from cDNA fragments of obtained from the I.M.A.G.E. Consortium (Carlsbad, CA) and Origene (Rockville, MD). High GC-content in the open reading frames of human GluN2C and GluN2D subunits was reduced in order to

increase expression in *Xenopus* oocytes (Hess et al., 1998; Monyer et al., 1994). Briefly, the GC-content of cDNAs encoding the first 229 amino acids of human GluN2C and the first 118 amino acids of human GluN2D were reduced without changing the amino acid sequences by replacing this fragment with a custom-synthesized DNA fragment (Genscript USA Inc, Piscataway, NJ). Similarly, the GC-content was reduced for cDNAs of human GluN2C encoding amino acids 956–1236 and human GluN2D encoding amino acids 997–1336. In addition, both the length and GC-content of 5' untranslated regions of GluN1, GluN2C, and GluN2D were reduced. The final cDNAs encode protein sequences corresponding to wild type human NMDA receptor subunits GluN1-1a (GenBank NP_015566), GluN2A (GenBank NP_000824), GluN2B (GenBank NP_000825), GluN2C (intermediate between GenBank NP_000826.1 [EPP insertion at amino acid position 1048 is present] and GenBank NP_000826.2 [arginine residue at position 1212 is present]), and GluN2D (GenBank NP_000827.1).

2.2 Ligands

L-glutamic acid, glycine, and S-(+)-ketamine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). NMDA, memantine hydrochloride, ifenprodil ((1*R**,2*S**)-erythro-2-(4-benzylpiperidino)-1-(4-hydroxyphenyl)-1-propanol hemitartrate), eliprodil (α -(4-chlorophenyl)-4-[(4-fluorophenyl)methyl]-1-piperidineethanol), Ro 25-6981 ((α , β , γ)- α -(4-hydroxyphenyl)- β -methyl-4-(phenylmethyl)-1-piperidinepropanol maleate), and clobenpropit (*N*-(4-chlorobenzyl)-*S*-[3-(4(5)-imidazolyl)propyl]isothiourea dihydrobromide) were purchased from Tocris Bioscience (Ellisville, MO). CIQ ((3-chlorophenyl) [3,4-dihydro-6,7-dimethoxy-1-[(4-methoxyphenoxy)methyl]-2(1*H*)-isoquinoliny]methanone)) was obtained from Tocris Bioscience and synthesized as previously described (Mullasseril et al., 2010). QNZ46 ((E)-4-(6-methoxy-2-(3-nitrostyryl)-4-oxoquinazolin-3(4H)-yl)-benzoic acid) was synthesized as previously described (Mosley et al., 2010).

2.3 Two-electrode voltage-clamp recordings from *Xenopus* oocytes

For expression in *Xenopus* oocytes, DNA constructs were linearized by restriction enzymes to produce cRNAs using the mMessage mMachine kit (Ambion). Injection of cRNA and two-electrode voltage-clamp recordings from *Xenopus laevis* oocytes were performed as previously described (Traynelis et al., 1998). Injected oocytes were maintained at 15°C in Barth's solution containing (in mM) 88 NaCl, 2.4 NaHCO₃, 1 KCl, 0.33 Ca(NO₃)₂, 0.41 CaCl₂, 0.82 MgSO₄, 5 Tris-HCl (pH 7.4 with NaOH). Recordings were performed 3–4 days post-injection at room temperature (23°C). The extracellular oocyte recording solution contained (in mM) 90 NaCl, 1 KCl, 10 HEPES, 0.5 BaCl₂, 0.01 EDTA (pH 7.4 with NaOH). Voltage and current electrodes were filled with 0.3 and 3.0 M KCl, respectively, and current responses were recorded at a holding potential of –40 mV. Data acquisition and voltage control were accomplished with a two-electrode voltage-clamp amplifier (OC725, Warner Instruments, Hamden, CT).

2.4 Data Analysis

Multiple alignments of peptide sequences were performed using the ClustalW algorithm (AlignX, Vector NTI Advance 11, Invitrogen, Carlsbad, CA). Concentration–response data were analyzed using GraphPad Prism (GraphPad Software, La Jolla, CA). Data for individual oocytes were fitted to the Hill equation using variable slope. Fitted EC₅₀ or IC₅₀ values and Hill coefficients (n_H) from individual oocytes were used to calculate the mean and standard error of the mean (SEM). For graphical presentation, data points from individual oocytes were normalized to the maximum current response in the same recording and averaged. The averaged data points were then fitted to the Hill equation and plotted together with the resulting curve. Unpaired t-test (two-tailed) was used for statistical

comparison of log EC₅₀ and log IC₅₀ values as indicated ($P < 0.05$ was considered significant). All data are presented as mean $\pm$ SEM.

3. Results

3.1 Characterization of NMDA receptor agonists

We generated concentration-response data for the two endogenous agonists glutamate and glycine at human and rat NMDA receptor subtypes expressed in *Xenopus* oocytes using two-electrode voltage-clamp electrophysiology. In addition, concentration-response data was generated for the partial GluN2 agonist NMDA. Data for agonists are summarized in Figure 2 and Table 3. EC₅₀ values at human NMDA receptor subtypes were not significantly different than EC₅₀ values at the corresponding rat NMDA receptor subtypes ($P > 0.05$, unpaired t-test). Furthermore, there was significant correlation between EC₅₀ values at human and rat NMDA receptor subtypes for all three agonists (Fig. 2H; Pearson test, $P < 0.05$, $r = 0.99$ for all agonists). The rank order of potencies at the different subtypes was the same for all three agonists, namely GluN1/GluN2D > GluN1/GluN2C > GluN1/GluN2B > GluN1/GluN2A. This rank order of potencies is in agreement with previously published results from recombinant rat NMDA receptors (Chen et al., 2008; Erreger et al., 2007; Hansen et al., 2008). Maximal activation by the partial agonist NMDA was 69–89% compared to maximal activation by the full agonist glutamate and was similar for human and rat subtypes (Fig. 2I). The relative agonist efficacies of NMDA at human subtypes were not significantly correlated with relative efficacies at rat subtypes (Fig. 2I; Pearson test, $P > 0.05$, $r = 0.46$). However, this analysis is complicated by the narrow range of observed efficacies (69–89%). In summary, the results demonstrate that the activities of the GluN1 agonist glycine and the GluN2 agonists glutamate and NMDA are highly similar on human and rat NMDA receptors, suggesting indistinguishable agonist binding affinities and conservation of the conformational changes that lead to channel gating in response to agonist binding.

3.2 Characterization of un-competitive antagonists

Most un-competitive NMDA receptor antagonists are positively charged at physiological pH and function by blocking the cation-permeable pore when the channel opens. Thus, these channel blockers are use-dependent in that they bind to and block agonist-gated open channels more rapidly than closed channels. Furthermore, positively charged channel blockers are also voltage-dependent with increased inhibition at more negative membrane potentials. Uncompetitive NMDA receptor antagonists are neuroprotective in animal models of neurological disorders that involve excessive stimulation of the NMDA receptors, such as traumatic brain injury, epilepsy, and stroke (reviewed by Traynelis et al., 2010). However, clinical trials have not been successful due to dose-limiting side effects and a narrow temporal window for intervention. Interestingly, low affinity channel blockers that show fast blocking and unblocking kinetics are well-tolerated, and one such NMDA receptor antagonist have been approved for clinical use for the treatment of Alzheimer's disease (memantine) (Lipton, 2006). Another channel blocker ketamine is also approved for human use as an anesthetic agent (Annetta et al., 2005). Both ketamine and memantine are known to have antidepressant-like properties, which have motivated the development of NMDA receptor antagonists for treatment of depression (reviewed in Skolnick et al., 2009).

Here, we evaluated inhibition of recombinant human and rat NMDA receptor subtypes by ketamine and memantine (Table 4). Ketamine IC₅₀ values were not significantly different between human and rat GluN1/GluN2A and GluN1/GluN2C ($P > 0.05$, unpaired t-test). However, ketamine IC₅₀ values were 1.6- and 1.7-fold lower at rat GluN1/GluN2B and GluN1/GluN2D, respectively, compared to the corresponding human subtypes ($P < 0.05$,

unpaired t-test). Ketamine IC_{50} values at human subtypes were not significantly correlated with IC_{50} values at rat subtypes (Pearson test, $P > 0.05$, $r = 0.81$). Memantine IC_{50} values were not significantly different between human and rat GluN1/GluN2A, GluN1/GluN2B, and GluN1/GluN2D ($P > 0.05$, unpaired t-test), but were 1.3-fold higher at rat GluN1/GluN2C compared to human GluN1/GluN2C ($P < 0.05$, unpaired t-test). Despite the inter-species difference for GluN1/GluN2C, there was significant correlation for memantine IC_{50} values at human and rat subtypes (Pearson test, $P < 0.05$, $r = 0.99$). The rank order of potencies at both human and rat subtypes was the same for both antagonists (GluN1/GluN2A > GluN1/GluN2B > GluN1/GluN2D > GluN1/GluN2C), and is in agreement with previously published results from recombinant rat NMDA receptors (Dravid et al., 2007). Although some inter-species differences were observed for ketamine and memantine IC_{50} values, the differences were less than 2-fold and were not observed at the same subtypes for the two antagonists. These results suggest that the clinically relevant un-competitive antagonists ketamine and memantine have highly similar activities on human and rat NMDA receptors.

3.3 Characterization of GluN2B-selective non-competitive antagonists

The phenylethanolamine ifenprodil was the first subunit-selective antagonist identified at any NMDA receptor subunit (Williams, 1993), and this compound and analogs have been invaluable pharmacological tools for the dissection of the physiological role of the GluN2B subunit in central nervous system (see (Hansen et al., 2010a). In general, GluN2B-selective antagonists are negative allosteric modulators (i.e. non-competitive antagonists) that bind the interface between GluN1 and GluN2B ATDs (Karakas et al., 2011). These antagonists have shown promising results in clinical trials as therapeutic agents in the treatment of traumatic brain injury, neuropathic pain, and treatment-resistant depression (Preskorn et al., 2008; Skolnick et al., 2009; Traynelis et al., 2010; Yurkewicz et al., 2005).

We evaluated inhibition of recombinant human and rat NMDA receptor subtypes by three GluN2B-selective antagonists, namely ifenprodil, eliprodil, and Ro 25–6981 (Fig. 3 and Table 4). In addition, the histamine H3 receptor antagonist clobenpropit was evaluated, since this compound is also a GluN2B-selective NMDA receptor antagonist with a dual channel-blocking (i.e. un-competitive) and non-competitive mechanism of action (Hansen et al., 2010b). IC_{50} values for all four antagonists were not significantly different between human and rat GluN1/GluN2B ($P > 0.05$, unpaired t-test). There was significant correlation between IC_{50} values at human and rat GluN1/GluN2B (Pearson test, $P < 0.05$, $r = 0.99$). Clobenpropit IC_{50} at rat GluN1/GluN2A was 1.9-fold higher than the IC_{50} at human GluN1/GluN2A ($P < 0.05$, unpaired t-test). The rank order of potencies at rat GluN1/GluN2B was Ro 25–6981 < ifenprodil < clobenpropit < eliprodil, which is in agreement with previously published results from recombinant rat NMDA receptors (Hansen et al., 2010a). The comparison of inhibition by GluN2B-selective antagonists at human and rat NMDA receptors did not identify any marked inter-species differences.

3.4 Characterization of GluN2C/D-selective modulators

Several recent reports have described novel subunit-selective ligands for GluN2C- and GluN2D-containing receptors (Acker et al., 2011; Costa et al., 2010; Hansen and Traynelis, 2011; Mosley et al., 2010; Mullasseril et al., 2010). We evaluated inhibition of recombinant human and rat NMDA receptors by the GluN2C/D-selective non-competitive antagonist QNZ46 (Fig. 4 and Table 4). QNZ46 IC_{50} values at human and rat GluN1/GluN2D were not significantly different ($P > 0.05$, unpaired t-test), whereas QNZ46 IC_{50} at rat GluN1/GluN2C was marginally higher (1.2-fold) than at human GluN1/GluN2C ($P < 0.05$, unpaired t-test). We also evaluated potentiation of recombinant human and rat NMDA receptors by the GluN2C/D-selective positive allosteric modulator CIQ (Fig. 4 and Table 3). Similar to

QNZ46, there were no significant difference between CIQ EC₅₀ values at human and rat GluN1/GluN2D ($P > 0.05$, unpaired t-test), but CIQ EC₅₀ at rat GluN1/GluN2C was 2.0-fold higher than at human GluN1/GluN2C ($P < 0.05$, unpaired t-test). Compared to NMDA receptor currents in the absence of CIQ, the maximal potentiation by CIQ was 270 ± 20 ($N = 8$) and 260 ± 30 ($N = 6$) at human and rat GluN1/GluN2C, respectively, and 250 ± 10 ($N = 8$) and 240 ± 20 ($N = 5$) at human and rat GluN1/GluN2D, respectively. Although the activities of QNZ46 and CIQ were highly similar on human and rat NMDA receptors, both modulators appeared slightly less potent at rat GluN1/GluN2C compared to the human ortholog.

4. Discussion

Here, we systematically compared the amino acid sequences for human and rat NMDA receptor subunits and show that inter-species variation is almost exclusively located in the extracellular ATD and the intracellular CTD with very little or no variation in agonist binding and transmembrane domains. Furthermore, we summarize previously published studies that describe biophysical properties of human NMDA receptors. The synthesis of our efforts on these two fronts suggests that inter-species differences in pharmacological and functional properties could exist for NMDA receptors and that more work is needed to substantiate this idea. Using two-electrode voltage-clamp electrophysiology, we compare pharmacological properties of recombinant rat and human NMDA receptors. However, we were not able to identify more than 2-fold differences in ligand potencies between human and rat NMDA receptor subtypes.

In addition to diheteromeric NMDA receptors composed of GluN1 and one type of GluN2 (e.g. GluN1/GluN2B), NMDA receptors can also assemble into triheteromeric receptors that contain more than one type of GluN2 subunit (e.g. GluN1/GluN2B/GluN2D) (e.g. (Jones and Gibb, 2005)). Despite their potential importance in native neurons, little is known about how combinations of subunits with dissimilar properties impact pharmacology and of triheteromeric receptors. The variation between human and rat subunits located in the extracellular ATD, which is thought to mediate receptor assembly and regulate function (Hansen et al., 2010a), may result in differences in assembly of triheteromeric receptors in the human and rodent brain. Moreover, triheteromeric NMDA receptors may also have different functional properties in human versus rat neurons, a possibility that was not addressed in this study.

To improve the likelihood that NMDA receptor ligands with promising results in preclinical animal models can translate into drugs for human use, it is important to establish early in drug development whether these ligands have different pharmacological properties at rodent and human NMDA receptors. Despite this obvious caveat to the success of NMDA receptor ligands as drug candidates, no studies have directly compared human and rat NMDA receptor pharmacology. We addressed this problem and our results suggest that agonist binding affinities and conformational changes that lead to channel gating in response to agonist binding are highly conserved between human and rat NMDA receptors. Furthermore, the actions of NMDA receptor ligands that modulate receptor function through a diverse range of mechanisms and with marked differences in subunit-selectivity also appear to be highly conserved between human and rat receptors. These results suggest that no marked differences exist in the pharmacological and functional properties of human and rat NMDA receptors.

Highlights

1. Variations between human and rat subunits are mainly located in the ATD and CTD.
2. Human and rat NMDA receptors have similar biophysical properties.
3. Agonist binding and channel gating appear conserved between human and rat receptors.
4. Non-competitive antagonists similarly inhibit human and rat NMDA receptors.
5. The molecular pharmacology of NMDA receptors is conserved between human and rat.

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Figure 1.

Comparison of amino acid sequences for rat and human NMDA receptor subunits. (A) Model of the NMDA receptor based on the crystal structure of the membrane-spanning GluA2 AMPA receptor (Sobolevsky et al., 2009), showing the tetrameric arrangement of two GluN1 and two GluN2 subunits. The intracellular carboxyl-terminal domain (CTD) was not part of the crystal structure and is omitted from the model, which was generated as previously described (Acker et al., 2011). (B) NMDA receptor subunits consist of four semi-autonomous domains, namely the extracellular amino-terminal domain (ATD), the bi-lobed agonist binding domain (ABD), the transmembrane domain (composed of the transmembrane helices M1, M3, and M4, as well as the membrane re-entrant loop M2), and

the intracellular carboxyl-terminal domain (CTD). (C) Linear representation of the polypeptide chain of NMDA receptor subunits, illustrating the relative positions of the four semi-autonomous domains in the amino acid sequence. The agonist binding domain (ABD) is formed by two amino acid segments termed S1 and S2. (D) The bar graph shows the percentage of residues that are not conserved between human and rat subunits for the four different domains and for the total protein. Insertion or deletions in human subunits compared to rat subunits are also categorized as non-identical positions. See Table 1 for more detail.

Figure 2.

Concentration-response data for agonists at human and rat NMDA receptors. Determination of EC_{50} values for glutamate, NMDA, and glycine at recombinant human (A–C) and rat (D–F) NMDA receptors expressed *Xenopus* oocytes was performed using two-electrode voltage-clamp electrophysiology. Responses are normalized to maximal currents to the ligand in the same recording. EC_{50} values are listed in Table 3. Glutamate and NMDA were applied in the continuous presence of 30 μM glycine, and glycine was applied in the continuous presence of 100 μM glutamate. (G) Representative two-electrode voltage-clamp recording of responses from human GluN1/GluN2A receptors to increasing concentrations of glutamate in the continuous presence of 30 μM glycine. (H) EC_{50} values at human and rat NMDA receptor subtypes were significantly correlated for all three agonists (Pearson test, $P < 0.05$, $r = 0.99$ for all agonists). The dashed line represents the situation where EC_{50} values at human and rat receptors are equal. (I) Maximal activation by the partial agonist NMDA was 69–89% compared to maximal activation by the full agonist glutamate in the same recording and was similar for human and rat subtypes.

Figure 3.

Concentration-response data for GluN2B-selective non-competitive antagonists at human and rat NMDA receptors. Determination of IC₅₀ values ifenprodil, eliprodil, clobenpropit, and Ro 25-6981 at recombinant human (A) and rat (B) GluN1/GluN2B receptors expressed *Xenopus* oocytes was performed using two-electrode voltage-clamp electrophysiology. IC₅₀ values are listed in Table 4. Antagonists were applied in the presence of 100 μM glutamate plus 30 μM glycine. (C) IC₅₀ values for GluN2B-selective antagonists at human and rat NMDA receptor GluN1/GluN2B receptors were significantly correlated (Pearson test, $P < 0.05$, $r = 0.99$).

Figure 4.

Concentration-response data for novel GluN2C/D-selective ligands at human and rat NMDA receptors. Determination of potencies for QNZ46 and CIQ at recombinant human (A,B) and rat (C,D) NMDA receptors expressed *Xenopus* oocytes was performed using two-electrode voltage-clamp electrophysiology. EC₅₀ values for CIQ and IC₅₀ values for QNZ46 are listed in Tables 3 and 4, respectively. Data points for GluN1/GluN2A and GluN1/GluN2B receptors could not be fitted to the Hill equation and are connected by straight lines. Ligands were applied in the presence of 100 μM glutamate plus 30 μM glycine.

Table 1

Comparison of amino acid sequences for human and rat NMDA receptor subunits.

Signal peptide # of residues	ATD		ABD		TMD		CTD		Total subunit # of residues	
	non- identical	total	non- identical	total	non- identical	total	non- identical	total	non- identical	total
GluN1	3	20	3	376	1	286	0	106	0	150
GluN2A	4	27	14	377	2	275	0	158	49	627
GluN2B	2	28	1	376	2	276	0	158	18	646
GluN2C	14	19	28	395	6	276	3	158	111	388
GluN2D	0	25	15	402	0	277	0	158	44	474
									59	1336

NMDA receptor subunits consist of the extracellular amino-terminal domain (ATD), the agonist binding domain (ABD), the transmembrane domain (TMD), and the intracellular carboxyl-terminal domain (CTD). Insertion or deletions in human subunits compared to rat subunits are also categorized as non-identical positions. ATD includes the ATD-S1 linker, and TMD is M1M2M3 including pre-M1 and intracellular loops. The ABD is S1 and S2 as well as S1-M1, M3-S2, and S2-M4 linkers. The amino acid sequences of all rat ionotropic glutamate receptor subunits were aligned using the ClustalW algorithm and the regions for each of the NMDA receptor subunits were determined based on GluA2 in this global alignment. In GluA2, the regions were defined as amino acids 1–397 (signal peptide and ATD), 398–414 (ATD-S1 linker), 415–527 (S1), 528–534 (S1-M1 linker), 535–647 (M1M2M3), 648–652 (M3-S2 linker), 653–794 (S2), 795–809 (S2-M4 linker), 810–833 (M4) and 834–884 (CTD). Signal peptides were predicted using the SignalP 3.0 server (Bendtsen et al., 2004). The GenBank IDs for amino acid sequences of human subunits used in the alignment are listed in Materials and Methods. NP_015566.1, NP_000824.1, NP_000825.2, NP_000826.1 (and NP_000826.2), and NP_000827.1 were used in the alignment for rat GluN1, GluN2A, GluN2B, GluN2C, and GluN2D, respectively.

Table 2

Biophysical properties of human and rat NMDA receptor subtypes.

NMDA receptor subtype	$\tau_{\text{deactivation glutamate}}$ (ms)	$\tau_{\text{deactivation glycine}}$ (ms)	Mean open time (ms)	Mean single-channel conductance (pS)	Open probability
GluN1/GluN2A	human	-	2.2 ^b	40 ^b	-
	rat	54 ^k	2.6 ^d	47 ^d	0.50 ^l
GluN1/GluN2B	human	-	2.9 ^b	26 ^b	-
	rat	287 ^k	2.8 ^e	48 ^d	0.12 ^l
GluN1/GluN2C	human	440 ^c	0.7 ^c	21 ^c	-
	rat	420 ^g	0.6 ^d	32 ^d	<0.05 ^g
GluN1/GluN2D	human	6300 ^a	1.0 ^a	25 ^a	-
	rat	3800 ^j	1.1 ^f	30 ^f	<0.05 ^m

NMDA receptors are activated by saturating concentrations of glutamate and glycine. Mean open time and conductance for rat NMDA receptors are obtained using single-channel recordings, whereas these values for human NMDA receptors are obtained using noise analysis. All deactivation time constants are weighted time constants. Unless otherwise stated, data are from recombinant NMDA receptors expressed in HEK293 cells. - indicates data not available.

^aHess et al., 1998; 2 mM extracellular Ca²⁺.

^bVarney et al., 1996; L⁺ cells, 2 mM extracellular Ca²⁺.

^cDaggett et al., 1998; 2 mM extracellular Ca²⁺.

^dStem et al., 1992; *Xenopus* oocytes, 1mM extracellular Ca²⁺.

^eBanke and Traynelis, 2003; 0.7 mM extracellular Ca²⁺.

^fWyllie et al., 1996; *Xenopus* oocytes, 0.85 mM extracellular Ca²⁺.

^gDravid et al., 2008; 0.5 mM extracellular Ca²⁺.

^hMonyer et al., 1992; 1.8 mM extracellular Ca²⁺.

^jHansen and Traynelis, 2011; 0.5 mM extracellular Ca²⁺.

^kVicini et al., 1998; 2 mM extracellular Ca²⁺.

^lErreger et al., 2005; 0.5 mM extracellular Ca^{2+} .
^mWyllie et al., 1998; *Xenopus* oocytes, 0.85 mM extracellular Ca^{2+} .

Table 3

Agonist and positive modulator data for human and rat NMDA receptor subtypes.

Agonists	GluN1/GluN2A				GluN1/GluN2B				GluN1/GluN2C				GluN1/GluN2D			
	EC ₅₀ (μM)	n _H	N		EC ₅₀ (μM)	n _H	N		EC ₅₀ (μM)	n _H	N		EC ₅₀ (μM)	n _H	N	
glutamate	human	4.5 ± 0.3	1.3	5	1.5 ± 0.1	1.8	7		0.87 ± 0.05	1.6	6		0.31 ± 0.01	1.5	4	
	rat	4.8 ± 0.3	1.4	6	1.6 ± 0.1	1.5	7		0.83 ± 0.10	1.4	6		0.32 ± 0.02	1.5	3	
NMDA	human	68 ± 3	1.3	4	23 ± 2	1.4	4		19 ± 1	1.2	4		6.7 ± 0.2	2.1	4	
	rat	71 ± 9	1.5	7	21 ± 4	1.7	7		19 ± 2	1.4	10		7.9 ± 0.9	1.7	9	
glycine	human	1.3 ± 0.1	1.3	6	0.29 ± 0.04	1.7	5		0.25 ± 0.01	1.6	6		0.12 ± 0.01	1.8	7	
	rat	1.2 ± 0.1	1.2	8	0.30 ± 0.01	1.8	5		0.26 ± 0.01	1.5	7		0.11 ± 0.01	1.6	5	
Positive allosteric modulator																
CIQ	human	N.E.	-	4	N.E.	-	4		3.1 ± 0.2	1.6	8		3.5 ± 0.3	1.3	8	
	rat	N.E.	-	4	N.E.	-	4		6.2 ± 1.4 [*]	1.1	6		4.3 ± 0.4	1.3	5	

EC₅₀ ± SEM for agonists and the positive allosteric modulator CIQ at recombinant NMDA receptors expressed in *Xenopus* oocytes using two-electrode voltage-clamp electrophysiology. n_H is the Hill slope and N is the number of oocytes. Data for glutamate and NMDA were generated in the continuous presence of 30 μM glycine, and data for glycine was generated in the continuous presence of 100 μM glutamate. Data for CIQ was generated in the presence of 100 μM glutamate plus 30 μM glycine. N.E. indicates no effect at 30 μM CIQ.

* indicates significantly different from EC₅₀ at the corresponding human NMDA receptor subtype (P < 0.05, two-tailed, un-paired t test: logarithmic EC₅₀ values were used for the test).

Table 4

Antagonist data for human and rat NMDA receptor subtypes.

Un-competitive antagonists		GluN1/GluN2A			GluN1/GluN2B			GluN1/GluN2C			GluN1/GluN2D		
		IC ₅₀ (μM)	n _H	N	IC ₅₀ (μM)	n _H	N	IC ₅₀ (μM)	n _H	N	IC ₅₀ (μM)	n _H	N
(+)ketamine	human	3.2 ± 0.2	1.0	6	2.9 ± 0.3	1.0	4	0.79 ± 0.04	0.9	6	2.4 ± 0.1	0.9	4
	rat	5.0 ± 0.9	1.0	6	1.8 ± 0.2*	0.9	4	0.78 ± 0.13	1.0	6	1.4 ± 0.3*	1.0	4
mefenazine	human	4.1 ± 0.7	1.1	6	0.98 ± 0.06	1.0	5	0.43 ± 0.02	1.0	6	0.56 ± 0.04	0.9	6
	rat	5.2 ± 0.7	1.1	4	0.95 ± 0.02	1.4	4	0.56 ± 0.05*	0.9	5	0.56 ± 0.03	1.0	4
Subunit-selective antagonists													
ifenprodil	human	>100	-	4	0.13 ± 0.01	1.1	6	N.E.	-	4	N.E.	-	5
	rat	52 ± 11	1.2	4	0.11 ± 0.01	1.2	5	>100	-	4	>100	-	5
eliprodil	human	N.E.	-	5	0.93 ± 0.14	1.3	4	N.E.	-	4	N.E.	-	4
	rat	N.E.	-	5	0.78 ± 0.09	1.2	6	N.E.	-	4	N.E.	-	6
Ro 25-6981	human	N.E.	-	4	0.049 ± 0.008	1.3	8	N.E.	-	5	N.E.	-	4
	rat	N.E.	-	4	0.042 ± 0.006	1.3	4	N.E.	-	4	N.E.	-	4
clobenpropit	human	8.1 ± 0.6	1.0	4	0.56 ± 0.05	1.0	5	9.8 ± 1.5	0.9	6	11 ± 1	1.0	8
	rat	15 ± 1	1.6*	4	0.56 ± 0.06	1.3	4	6.4 ± 0.6	0.8	4	9.7 ± 1.2	0.9	6
QNZ46	human	>100	-	4	>100	-	4	5.2 ± 0.2	1.0	7	3.6 ± 0.3	1.0	6
	rat	N.E.	-	4	>100	-	5	6.3 ± 0.3*	1.2	6	3.8 ± 0.2	1.0	4

IC₅₀ ± SEM for antagonists at recombinant NMDA receptors expressed in *Xenopus* oocytes using two-electrode voltage-clamp electrophysiology. The membrane potential was clamped at -40 mV. n_H is the Hill slope and N is the number of oocytes. Data for antagonists were generated in the presence of 100 μM glutamate plus 30 μM glycine. N.E. indicates less than 20% inhibition at 100 μM of the antagonist.

* indicates significantly different from IC₅₀ at the corresponding human NMDA receptor subtype (P < 0.05, two-tailed, un-paired t test; logarithmic IC₅₀ values were used for the test).