NMDA receptor NR2 subunit selectivity of a series of novel piperazine-2,3-dicarboxylate derivatives; preferential blockade of extrasynaptic NMDA receptors in the rat hippocampal CA3-CA1 synapse.

Blaise Mathias Costa, Bihua Feng, Timur S. Tsintsadze, Richard M. Morley, Mark W. Irvine, Vera Tsintsadze, Natasha A. Lozovaya, David E. Jane, and Daniel T. Monaghan

Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska 68198-6260 U.S.A. (B.M.C., B.F., D.T.M.), Department of Cellular Membranology, Bogomoletz Institute of Physiology, 01024 Kiev, Ukraine (T.S.T., V. T., N.A.L.) and Department of Physiology & Pharmacology, MRC Centre for Synaptic Plasticity, University of Bristol, Bristol BS8 1TD, U.K. (R.M.M., M.W.I., D.E.J.)
Running title:
NMDA receptor subtype selectivity of UBP141 and UBP145

Corresponding author:
Blaise Mathias Costa, Ph.D.
Department of Pharmacology
985800 Nebraska Medical Center
Omaha, NE 68198-5800
402-559-7132, FAX: 402-559-7495
e-mail: bmathiascosta@unmc.edu

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Abbreviations: AMPA, 2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide; NMDA, N-methyl-D-aspartate; NR2, NMDA receptor subunit 2; NVP-AAM077, [(R)-[(S)-1-(4-bromo-phenyl)-ethylamino]-2,3-dioxo-1,2,3,4-tetrahydroquinoxalin-5-yl]-methyl]-phosphonic acid; (R)-AP5, (R)-2-amino-5-phosphonopentanoate; (R)-CPP, (R)-4-(3-phosphonopropyl)piperazine-2-carboxylic acid; (R)-CPPene,
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(R,E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid; PPDA, (2R*,3S*)-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP125, (2R*,3S*)-1-(4-phenylazobenzoyl)piperazine-2,3-dicarboxylic acid; UBP128, (2R*,3S*)-1-(4-phenylethynylbenzoyl)piperazine-2,3-dicarboxylic acid; UBP129, (2R*,3S*,E)-1-(3-naphthalen-2-ylacryloyl)piperazine-2,3-dicarboxylic acid; UBP133, (2R*,3S*)-1-(9-bromophenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP136, (2R*,3S*)-1-(anthracene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP141, (2R*,3S*)-1-(phenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid; UBP143, (2R*,3R*)-1-(anthracene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP144, (2R*,3R*)-1-(9,10-dioxo-9,10-dihydro-anthracene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP145, (2R*,3S*)-1-(9-bromophenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid; UBP148, (2R*,3S*)-1-(3-oxo-3H-benzo[f]chromene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP150, (-)-cis-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP151, (+)-cis-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid; UBP152, (2R*,3S*)-1-(3-naphthalene-2-ylpropionyl)piperazine-2,3-dicarboxylic acid; UBP160, (2R*,3S*)-1-(9-chlorophenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid; UBP161, (2R*,3S*)-1-(9-iodophenanthrene-3-carbonyl)piperazine-2,3-dicarboxylic acid.

Section: Neuropharmacology
Abstract

NMDA receptor antagonists that are highly-selective for specific NR2 subunits have several potential therapeutic applications, however, to date, only NR2B-selective antagonists have been described. While most glutamate-binding site antagonists display a common pattern of NR2 selectivity - NR2A > NR2B > NR2C > NR2D (high to low affinity), (2S*,3R*)-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid (PPDA) has a low selectivity for NR2C- and NR2D-containing NMDA receptors. A series of PPDA derivatives were synthesized and then tested at recombinant NMDA receptors expressed in *Xenopus* oocytes. In addition, the optical isomers of PPDA were resolved; the (-) isomer displayed a 50- to 80-fold greater potency than the (+) isomer. Replacement of the phenanthrene moiety of PPDA with naphthalene or anthracene did not improve selectivity. However, phenylazobenzoyl (UBP125) or phenylethynylbenzoyl (UBP128) substitution significantly improved selectivity for NR2B-, NR2C-, and NR2D-containing receptors over NR2A-containing NMDA receptors. Phenanthrene attachment at the 3 position (UBP141, UBP145, UBP160, UBP161) displayed improved NR2D selectivity. UBP141 and its 9-brominated homologue (UBP145) both display a 7- to 10-fold selectivity for NR2D-containing receptors over NR2B- or NR2A-containing receptors. Schild analysis indicates that these two compounds are competitive glutamate binding site antagonists. Consistent with a physiological role for NR2D-containing receptors in the hippocampus, UBP141 (5 µM) displayed greater selectivity than PPDA for inhibiting the slow-decaying component of the NMDA receptor-mediated CA3-CA1 synaptic response in rat hippocampal slices. UBP125, UBP128, UBP141 and UBP145 may be useful tools for determining the function of NMDA receptor subtypes.
Introduction:

N-methyl-D-aspartate (NMDA) receptors are a family of glutamate-gated, ion channel receptors that play important roles in synaptic transmission and neuronal plasticity; they are also involved in a wide variety of pathological conditions, such as epilepsy (Meldrum, 2002), neuropathic pain (Childers and Baudy, 2007), and neuronal loss following stroke (Choi, 1998). NMDA receptors are multimeric complexes composed of subunits from at least two families, NR1a-h and NR2A-D (Nakanishi, 1992; Monyer et al., 1994; Mori and Mishina, 1995). In addition, some NMDA receptors may contain an NR3 subunit (Tong et al., 2008). The NR2 subunits contain the glutamate-binding site of the receptor complex (Laube et al., 1997). Accordingly, the NR2 subunit determines the glutamate-site pharmacological properties of the NMDA receptor complex (Ishii et al., 1993; Buller et al., 1994; Laurie and Seeburg, 1994; Buller and Monaghan, 1997).

It is now apparent that the different NR2 subunits underlie differing physiological and cell-signaling properties in NMDA receptor complexes. However, in the absence of antagonists that are highly selective for each of the individual NR2 subunits, progress has been slow in identifying their respective roles in synaptic and brain function. NR2 subtype-selective NMDA receptor antagonists are also likely to have novel therapeutic/adverse effect profiles since these subunits differ significantly in their anatomical and physiological properties (Ishii et al., 1993; Monyer et al., 1994; Vicini et al., 1998). For example, a NR2D-selective antagonist would be expected to be effective in some cases of neuropathic pain (Minami et al., 2001) and NR2C-selective antagonists may be useful in blocking ischemic white matter injury (Karadottir et al., 2005).

A major difficulty in developing subunit-specific glutamate binding site antagonists is the highly conserved structure of the glutamate-binding pocket (Kinarsky et al., 2005). Although there is significant amino acid sequence variation between NR2 subunits within the glutamate binding site domain (segments S1 and S2), receptor-modeling studies have shown that the variable amino acids are located distant to the
glutamate-binding site. Consequently, the commonly used NMDA receptor antagonists that occupy the core of the glutamate binding pocket (e.g. (R)-2-amino-5-phosphonopentaoate, (R)-AP5 and (R)-4-(3-phosphonopropyl)piperazine-2-carboxylic acid, (R)-CPP)) display a similar pattern of NR2 selectivity - high to low affinity in the order of NR2A>NR2B>NR2C > NR2D (Ikeda et al., 1992; Buller et al., 1994; Laurie and Seeburg, 1994; Feng et al., 2005). While small competitive antagonists display some structure-activity relationships that vary the degree of this selectivity pattern (Feng et al., 2005), compounds with bulky aromatic substituents such as NVP-AAM077 [(R)-[(S)-1-(4-bromo-phenyl)-ethylamino]- (2,3-dioxo-1,2,3,4- tetrahydroquinoxalin-5-yl)-methyl]-phosphonic acid) (Auberson et al., 2002), EAB-515 (2-amino-5-(phosphonomethyl)[1,1’-biphenyl]-3-propanoic acid (Urwyler et al., 1996), (2R*,3S*)-1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid, PPDA (Morley et al., 2005), and 4-propyl N-hydroxypyrazol-5-yl glycine (Clausen et al., 2008) have been shown to display an atypical pattern of NMDA receptor subunit selectivity. The altered subunit-specificity of large competitive antagonists may be due to antagonist interactions with subunit-specific amino acid residues at the edge of the glutamate binding pocket (Kinarsky et al., 2005; Clausen et al., 2008).

The compound PPDA is distinct in having a 2-5 fold higher affinity for NR2C- or NR2D-containing receptors than for receptors containing either NR2A or NR2B (Feng et al., 2004; Morley et al., 2005). While PPDA has a low degree of selectivity, studies comparing its action to other competitive antagonists have been able to identify differing physiological actions for NMDA receptors containing different NR2 subunits. By contrasting the actions of PPDA with (R)-(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid ((R)-CPPene) and (R)-AP5, it was possible to first demonstrate that long-term potentiation (LTP) and long-term depression (LTD) are mediated by pharmacologically-distinct NMDA receptor populations (Hrabetova et al., 2000). Likewise, comparing PPDA to (R)-CPP reveals two pharmacologically-distinct, NMDA receptor-mediated components of the CA3-CA1 hippocampal synapse response (Lozovaya et al., 2004). PPDA causes a small, preferential block of the slow-decaying NMDA receptor synaptic current while (R)-CPP selectively blocks the fast-decaying peak response of the
NMDA receptor synaptic current. In initial studies we found that the PPDA derivative UBP141 displayed an improved NR2D and NR2C selectivity over PPDA (Morley et al., 2005). In the present study we extend the structure-activity analysis around the PPDA and UBP141 structures and demonstrate that UBP141 and UBP145 are modestly selective competitive glutamate site antagonists. We also find that UBP125 and UBP128 are modestly selective for NMDA receptors that do not contain NR2A.

Methods

Compounds:

Structures of compounds synthesized and tested for this report are shown in Fig. 1. Compounds were synthesized by reaction of the appropriately substituted acid chloride with (2S*,3R*)-piperazine-2,3-dicarboxylic acid (Morley et al., 2005) under modified Schotten-Bauman conditions (full details will be reported elsewhere). Following synthesis and purification, compound structure was verified by $^1$H NMR and mass spectroscopy. All compounds had elemental analyses where the determined percentage C, H and N were less than 0.4% different from theoretical values.

NR Subunit Expression in Xenopus oocytes

cDNA encoding the NMDAR1a and NMDAR1g (NR1-14b) subunits were a generous gift of Dr. Shigetada Nakanishi (Kyoto, Japan). cDNA encoding the NR2A, NR2C and NR2D were kindly provided by Dr. Peter Seeburg (Heidelberg, Germany) and the NR2B [5' UTR] cDNA was the generous gift of Drs. Dolan Pritchett and David Lynch (Philadelphia, USA). Plasmids were linearized with Not I (NR1a), EcoR I (NR2A, NR2C and NR2D) or Sal I (NR2B) and transcribed in vitro with T3 (NR2A, NR2C), SP6 (NR2B) or T7 (NR1a, NR2D) RNA polymerase using the mMessage mMachine Transcription Kits (Ambion, Austin, TX, USA).

Oocytes were removed from mature female Xenopus laevis (Xenopus One, Ann Arbor, MI, USA) and prepared as previously described (Buller et al., 1994). All animal procedures were performed in accordance with institutional and federal animal care guidelines. NMDA receptor subunit RNAs were dissolved in sterile distilled H$_2$O. NR1 and NR2 RNAs were mixed in a molar ratio of 1:1 to 1:3 and 50 nl of the final RNA mixture was microinjected (15-30 ng total) into the oocyte cytoplasm. Oocytes were incubated in ND-96 solution at 17°C prior to electrophysiological assay (1-5 days).
Electrophysiological responses were measured using a standard two-microelectrode voltage clamp as previously described (Buller et al., 1994) with a Warner Instruments (Hamden, Connecticut) model OC-725B Oocyte Clamp amplifier. The recording buffer contained 116 mM NaCl, 2 mM KCl, 2 mM BaCl2 and 5 mM HEPES, pH 7.4. Ambient Zn2+ levels were estimated to be about 10 nM. Response magnitude was determined by the plateau response elicited by bath application of 10 µM L-glutamate plus 10 µM glycine at a holding potential of –60 mV. Response amplitudes for the NR1/NR2 heteromeric complexes were generally between 50-200 nA. Attempts were made to keep response magnitudes within this range to minimize activation of the endogenous Cl– current. The lack of significant activation of the endogenous Cl– current by Ba2+ in these cells was indicated by the presence of a plateau response.

Antagonist inhibition curves were fit to a single-site with variable slope (GraphPad Prism, ISI Software, San Diego, CA, USA), using a nonlinear regression to calculate IC50. Apparent Ki values were determined by correcting for agonist affinity according to the equation $Ki = IC50/(1 + ([agonist]/EC50))$ (Cheng and Prusoff, 1973). For Ki value calculations from IC50 values and Schild analysis, the L-glutamate Kd values used were averages of those obtained from the literature (Ishii et al., 1993; Frizelle et al., 2006; Erreger et al., 2007; Hansen et al., 2008) and our laboratory (NR1a/NR2A, 2.92 ± 0.27 µM; NR1a/NR2B, 1.93 ± 0.25 µM; NR1a/NR2C, 1.11 ± 0.20 µM; NR1a/NR2D 0.44 ± 0.04 µM). NR1-4b/NR2 L-glutamate affinity values used for Ki determination were: NR2A, 6.8 ± 1.0 µM; NR2B, 5.8 ± 0.4 µM; NR2C, 1.63 ± 0.03 µM; NR2D, 1.60 ± 0.12 µM. Splice form NR1-4b (Hollmann et al., 1993) corresponding to NR1g (Sugihara et al., 1992) and NR1100 (Durand et al., 1993) contains only the 1st of 3 alternatively-spliced cassettes. The predominant NR1 isoform, NR1a (NR1-1a or NR1 011), has only the last two alternatively-spliced cassettes. For Schild analysis, at each of 5 antagonist concentrations NMDA receptors were activated by two concentrations of L-glutamate in combination with 10 µM glycine. L-Glutamate response at each antagonist concentration was fitted by non-linear regression analysis using Prism 5 (GraphPad Software, La Jolla, CA, USA). For each individual experiment, Schild slope was allowed to vary but held to be common for the family of curves. Likewise, Hill slopes were variable and shared within each set of curves. The bottoms of the curves were set to zero response and the maximal responses were set to be a shared value for each set of curves.
Preparation of hippocampal slices

This study was carried out on 21-day-old Wistar rats (WAG/GSto, Moscow, Russia). After decapitation, rat brains were immediately transferred to the chilled (4°C) solution of the following composition: 120 mM NaCl, 5 mM KCl, 26 mM NaHCO₃, 2 mM MgCl₂, 0.5 CaCl₂ and 10 mM glucose. The solution was constantly equilibrated with 95%O₂/5%CO₂ gas mixture to maintain pH = 7.4. During the preincubation, the slices (300-400 mM thick) were kept fully submerged in the extracellular solution: 135 mM NaCl, 5 mM KCl, 26 mM NaHCO₃, 1.5 mM CaCl₂, 1.5 mM MgCl₂, 10 mM glucose (pH = 7.4, equilibrated with 95%O₂/5%CO₂).

Electrophysiological recordings in hippocampal slices

A standard whole-cell patch clamp technique was used to record EPSCs from CA1 pyramidal neurons in situ in response to stimulation of Schaffer collateral/commissural pathway. To prevent the spread of electrical activity from area CA3, mini-slices were prepared by making a cut orthogonal to the stratum pyramidale and extending to the mossy fiber layer. Intracellular solution for patch pipettes contained 100 mM TrisPO₄ or CsF, 40 mM Na₂HPO₄, 10 mM HEPES-CsOH, 10 mM Tris-Cl (pH=7.2). 2-3 mM of N-(2,6-dimethyl-phenylcarbamoylmethyl)-triethylammonium bromide (QX-314) were routinely added to the intracellular solution to block voltage-gated sodium conductance. Patch pipettes were pulled from soft borosilicate glass on a two-stage horizontal puller. When fire-polished and filled with the intracellular solution, they had a resistance of 2-3 MΩ. Currents were digitally sampled at 400 µs intervals by a 12-digit ADC board and filtered at 3kHz. Access resistance was monitored throughout the experiments and ranged typically from 6 to 9 MΩ. When the access resistance was changed by more than 25% during the experiment, the data were discarded. To stimulate Schaffer collateral/commissural pathway, a bipolar Ni/Cr electrode was positioned on the surface of the slice. The current intensity of test stimuli (25-50 μA) was set to produce half-maximal EPSPs. Current pulses were delivered through the isolated stimulator HG 203 (Hi-Med, London, UK) at 0.066-0.2 Hz.
Pharmacologically-isolated EPSP\textsubscript{NMDA} were recorded in a modified extracellular solution containing 135 mM NaCl, 5 mM KCl, 26 mM NaHCO\textsubscript{3}, 0.5 mM Mg\textsuperscript{2+}, 2.5 mM Ca\textsuperscript{2+}, 10 mM glucose (pH = 7.4, equilibrated with 95\%O\textsubscript{2}/5\%CO\textsubscript{2}), in the presence of 10 \( \mu \text{M} \) 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulphonamide (NBQX) to block AMPA receptor currents and 20 \( \mu \text{M} \) bicuculline to suppress inhibitory activity of interneurons.

\textbf{[\textsuperscript{3}H]-AMPA and [\textsuperscript{3}H]-kainate binding assays}

Compound potency at non-NMDA ionotropic glutamate receptors was tested by incubating rat brain tissue sections with radiolabelled \([\textsuperscript{3}H]-\text{AMPA}\) (\([\textsuperscript{3}H]-2\text{-amino-3-hydroxy-5-methylisoxazole-4-propionic acid}\)) or \([\textsuperscript{3}H]-\text{kainate}\) (\([\textsuperscript{3}H]-(2S,3S,4S)-2\text{-carboxy-4-isopropenyl-3-pyrrolidineacetic acid}\)) as previously described (Monaghan, 1993). Following halothane anesthesia, brains were removed from adult male Sprague-Dawley rats (200-250g) and immediately frozen under powdered dry ice. Horizontal sections (8 \( \mu \text{m} \)) were thaw-mounted onto gelatin-subbed slides, and stored at \( -20 \)°C overnight. Slides were preincubated for 20 min at 0°C in assay buffer (\([\textsuperscript{3}H]-\text{AMPA binding assay}: 50 \text{mM Tris-acetate pH 7.2 with 100 mM KSCN}; \text{[\textsuperscript{3}H]-kainate binding assays}: 50 \text{mM Tris-citrate, pH 7.0} \) followed by two additional preincubations for 10 min at 30°C in fresh buffer. Sections were then incubated with 50 nM \([\textsuperscript{3}H]-\text{AMPA}\) (45 Ci/mMol; PerkinElmer, Boston, MA) or with 100 nM \([\textsuperscript{3}H]-\text{kainate}\) (41.2 Ci/mMol; PerkinElmer, Boston, MA) in assay buffer in the presence or absence of 100 \( \mu \text{M} \) of test compound. Non-specific binding was determined with 100 \( \mu \text{M} \) AMPA or 100 \( \mu \text{M} \) kainate, respectively. Sections were washed for 30 s in ice-cold buffer and quickly dried by an airstream at room temperature. Liquid scintillation spectrophotometry was then used to determine radioligand binding.

\textbf{Results}

Compounds (Figure 1) were tested for their ability to block agonist-evoked currents in \textit{Xenopus} oocytes injected with NMDA receptor NR1 and NR2 subunit cRNA. Each of the putative NMDA receptor antagonists were able to block recombinant NMDA receptor responses. Inhibition constants for each of the NR1/NR2 receptors are shown in Table 1. The synthesis and initial biological characterization of UBP129 and UBP141 have been reported elsewhere (Morley et al., 2005); their Ki
values are reported here for comparison to structural homologues. In Figure 2A, an example of a dose-response analysis is shown; increasing concentrations of UBP145 inhibit NR1-4b/NR2D responses to the application of 10 µM L-glutamate and 10 µM glycine. The averaged UBP145 dose-response results are shown in Figure 2B for each of the NR2 subunit-containing receptors in complex with NR1-4b. UBP145 inhibited NR2C- and NR2D-containing receptors with a lower IC$_{50}$ than NR2A- and NR2B-containing receptors, despite the higher potency of L-glutamate at NR2C- and NR2D-containing receptors. Increasing concentrations of antagonist were applied in the presence of agonists, as shown in Fig. 2A. Consistent with the results of Frizelle et al., 2006, pre-application of the antagonist did not change the level of steady-state inhibition by the antagonist.

While the widely-used NMDA receptor antagonists such as (R)-2-amino-5-phosphonopentanoate ((R)-AP5), (R)-2-amino-7-phosphonoheptanoate ((R)-AP7), (2R*,4S*)-4-(phosphonomethyl)piperidine-2-carboxylic acid (CGS-19755) and (R)-CPP display a common selectivity pattern among the different NR1a/NR2-containing receptors of NR2A>NR2B>NR2C>NR2D (high to low affinity) (Feng et al., 2005), none of the PPDA derivatives displayed this pattern of selectivity (Table 1, Figure 3). Of the 15 compounds tested, 12 displayed higher affinity at both NR2C- and NR2D-containing receptors than at either NR2A- or NR2B-containing receptors. Resolution and testing of the optical isomers of PPDA revealed that the (-) isomer (UBP150) is 50- to 80-fold more potent than the (+) isomer (UBP151) and has a subunit selectivity that is similar to that of the racemic mixture (2- to 3-fold selective for NR2C or NR2D over NR2A or NR2B). UBP150 was also tested with the NR1-4b subunit co-expressed with NR2 subunits. L-Glutamate had 2- to 3-fold lower affinity at NMDA receptors containing the NR1-4b splice variant compared to the NR1-1a variant (NR1-4b/NR2A, 6.8 ± 1.0; NR1-4b/NR2B, 5.8 ± 0.4; NR1-4b/NR2C, 1.63 ± 0.03; NR1-4b/NR2D, 1.60 ± 0.12). Using these values for L-glutamate affinity, the estimated Ki values for UBP150 for receptors containing NR1-4b were very similar to that obtained for the NR1a/NR2 receptors (Table 1).

Modeling studies suggest that the phenanthrene ring of PPDA projects directly out of the ligand-binding cavity (Kinarsky et al., 2005), thus longer, linear structures might be tolerated. In two compounds, UBP125 and UBP128, the phenanthrene ring of PPDA was replaced with an extended, linear structure with two benzene rings separated by three covalent bonds (Fig. 1). In both cases, this
modification was tolerated, but was associated with a large reduction in overall affinity compared to PPDA, especially at NR2A-containing receptors. Of all the compounds tested, these most strongly distinguished between NR2A- and NR2B-containing receptors.

Previously, we found that replacing the first aromatic ring in PPDA with an ethenyl linker (generating UBP129) resulted in a 12- and 15-fold reduction in NR2C and NR2D affinity, respectively, but had little effect on NR2A or NR2B affinity. This results in the selectivity pattern of NR2B>NR2A, NR2C>NR2D (high to low affinity). UBP129’s ethenyl double bond appears important for maintaining high affinity at NR2A- and NR2B-containing receptors; saturation of the ethenyl linker to generate UBP152 further reduced antagonist affinity, especially at NR2A- and NR2B-containing receptors.

In UBP141, attaching PPDA’s carbonyl-piperazine group at the 3-position of phenanthrene, instead of the 2-position, reduced receptor affinity overall while increasing selectivity for NR2C- and NR2D-containing receptors. Furthermore, UBP141, in contrast to PPDA, displays a weak selectivity for NR2D over NR2C-containing receptors. With compound UBP133, bromination at the 9-position of the phenanthrene ring in PPDA also increases selectivity for NR2C- and NR2D-containing receptors over NR2B. In UBP145, we tested if the selectivity gained by bromination at the 9-position in PPDA was additive with the selectivity gained by attaching the phenanthrene ring to the carbonyl-piperazine at the 3-position as in UBP141. From IC₅₀ analysis, this modification increased affinity over UBP141 two-fold but did not significantly alter selectivity. However, Schild analysis (see below) suggests that the bromination of UBP141 to make UBP145 may have led to a small increase in NR2D > NR2B selectivity as hypothesized. Since bromination at this position in PPDA (resulting in UBP133) significantly reduces affinity, whereas bromination of UBP141 (resulting in UBP145) increases affinity, it appears that the phenanthrene group in UBP141 occupies a different space in the receptor than it does in PPDA.

Halogenation at the 9-position of UBP141 was further explored by adding a chloro (UBP160) or iodo (UBP161) group. In general, the addition of a halogen group increased receptor affinity. Furthermore, for NR2B, NR2C, and NR2D receptors, increasing halogen size was associated with increases in receptor affinity. NR2A-containing receptors did not display this pattern; the 9-chloro derivative had a two-fold higher affinity for NR2A-containing receptors than for those with NR2B, the 9-bromo derivative displayed equivalent affinities at these two receptors, and the 9-iodo derivative had a
two-fold higher affinity for NR2B-containing receptors. As found for UBP141, and in contrast to PPDA, all of the UBP141 halogenated derivatives displayed higher affinity for NR2D-containing receptors than for those with NR2C.

Substitution of the PPDA’s phenanthrene ring with 9,10-anthraquinone (UBP144) greatly reduced receptor affinity, with minimal effects on receptor selectivity. Likewise, substitution of phenanthrene with the heterocyclic 3-oxo-3H-benzo[f]chromene structure in UBP148 also greatly reduced receptor affinity.

Presently, UBP141 and UBP145 are the most selective antagonists for preferentially blocking NR2C- and NR2D-containing NMDA receptors. To determine if these antagonists are competitive antagonists at the glutamate binding site, and to provide an alternative measure of receptor affinity, a Schild analysis was performed (see example in Figure 4). As expected for a glutamate binding site antagonist, increasingly higher concentrations of L-glutamate were required to obtain similar receptor responses in the presence of increasing concentrations of antagonist. Non-linear fitting of these results indicate a Schild slope of approximately 1, but statistically different from 1. For the average of all experiments, UBP141 Schild slopes were above 1 (1.14 ± 0.02, n = 29, different from 1 with p < 0.0001 two-tailed t-test) whereas UBP145 Schild slopes were below 1 (0.73 ± 0.03, n = 16, p < 0.0001). As shown in Table 2, Schild slopes for each NR2-containing receptor subtype were above 1 for UBP141 and below 1 for UBP145. Each of these were statistically different from 1 except for UBP141 at NR1/NR2C (p = 0.12).

Schild analysis estimates of affinity (K_B values) are fairly close to the K_i values determined by the IC_{50} / Cheng-Prusoff estimation. Schild analysis suggests that UBP141 may have a two-fold lower affinity for NR2A-containing receptors than estimated by IC_{50}-derived K_i values, a 60% higher affinity for NR2B- and NR2C-containing receptors, and essentially identical affinity at NR2D-containing receptors. Schild K_B values for UBP145 were also fairly similar to the IC_{50} derived estimates; UBP145 K_B values for NR1a/NR2A were 40% larger (lower affinity) than the IC_{50}-derived K_i estimates while K_B values for the other receptors indicated that each were fairly similar to the IC_{50}-derived K_i estimates. Thus, for both UBP141 and UBP145, the largest deviation from the K_i estimates was the lower affinity at NR2A-containing receptors. Thus, these two antagonists may be better in distinguishing NR2C and
NR2D from NR2A subunits than previously estimated by IC\textsubscript{50} determination. Schild analysis suggests that UBP145 may be better at discriminating between NR2D and NR2B than UBP141.

To determine the effects of these antagonists on native AMPA and kainate receptors, 100 µM concentrations of UBP125, UBP128, UBP141, and UBP145 were tested as inhibitors of [\textsuperscript{3}H]AMPA and [\textsuperscript{3}H]kainate binding to their respective receptors in rat brain tissue. As shown in Table 3, these compounds display weak activity at AMPA and kainate receptors. UBP128 inhibited approximately half of the binding at AMPA receptors, but only 20% of binding at kainate receptors. The structurally-related compound UBP125 displayed weaker AMPA receptor activity (37% inhibition) and weaker kainate receptor activity (10% inhibition). UBP141 and UBP145 were relatively weak at both AMPA and kainate receptors with less than 20% inhibition.

Previous studies have shown that the NMDA receptor-mediated synaptic currents of the CA3-CA1 hippocampal synapse has multiple components following a short train of high frequency stimulation (Lozovaya et al., 2004). After a few stimulations, the NMDA receptor-mediated EPSC develops an additional slow-decaying current that is pharmacologically-distinct from the fast, early peak response. (R)-CPP, which is moderately selective for NR2A, was able to inhibit much of the early peak response while having significantly less effect on the slow-decaying current. In contrast, PPDA (10 µM) which has a weak selectivity pattern of NR2D>NR2B>NR2A, caused a small, but proportionally greater inhibition of the slow-decaying NMDA receptor-mediated EPSC than the early peak response. This effect was most apparent after superimposing normalized traces after partial inhibition. These and other observations led to the conclusion that NR2B and NR2D subunits contribute to the slow-decay response and NR2A and NR2B subunits contribute to the fast-decaying response (Lozovaya et al., 2004). Since UBP141 is better than PPDA at distinguishing NR2D from NR2A and NR2B, we tested this compound on the early peak and the slowly-decaying NMDA receptor EPSCs to see if it could better discriminate between these two EPSC components.

As shown in Figure 5, when a single stimulation pulse is used to evoke an NMDA receptor synaptic current, 5 µM UBP141 does not inhibit the peak response but causes a small acceleration of the decay. However, after 3 pulses, when the slow-decay current becomes expressed, 5 µM UBP141 clearly inhibits...
a significant portion of the slow component while having little effect on the fast, peak response. After 7 pulses when the slow current is more fully developed, UBP141 now inhibits approximately 40% of the slow-decaying NMDA receptor current while still having little effect on the early, peak response. While both of these components are fully blocked by NMDA receptor antagonists (Lozovaya et al., 2004), it appears that the early and late components are mediated by pharmacologically-distinct subtypes of NMDA receptors. UBP141, unlike the other NMDA receptor antagonists (R)-CPP, ifenprodil (4-[1-hydroxy-2-[4-(phenylmethyl)piperidin-1-yl]propyl]phenol), and PPDA (Lozovaya et al., 2004) or (R)-α-aminoadipate (Diamond, 2001)(Lozovaya, unpublished observations), has little activity on the peak NMDA receptor current at a concentration that significantly inhibits the slow-decaying NMDA receptor current.

Discussion:

Presently, there are few pharmacological tools available for distinguishing NMDA receptor subtypes (Jane et al., 2000; Neyton and Paoletti, 2006). Ifenprodil-like antagonists are available for blocking NR2B-containing receptors (Jane et al., 2000; Neyton and Paoletti, 2006), but compounds that are highly selective for NRA, NR2C, or NR2D have not been identified. At low nanomolar concentrations, Zn$^{2+}$ selectively inhibits NR2A-containing receptors (Neyton and Paoletti, 2006). Most well-characterized competitive NMDA receptor antagonists display the subunit-selectivity pattern of NR2A>NR2B>NR2C>NR2D (high to low affinity) (Ikeda et al., 1992; Ishii et al., 1993; Buller et al., 1994; Laurie and Seeburg, 1994; Buller and Monaghan, 1997; Feng et al., 2005). The compound PPDA was significant in that it was the first compound reported to have a slightly higher affinity for NMDA receptors containing NR2C or NR2D subunits than for those containing NR2A or NR2B (Feng et al., 2004; Morley et al., 2005). However, it is not a selective compound – after considering the higher affinity that L-glutamate displays for NR2C- and NR2D-containing receptors, the IC$_{50}$ values for PPDA at NMDA receptors with various NR2 subunits are fairly similar. We have generated and tested several new PPDA derivatives and further characterized the selectivity of UBP141. These results indicate that UBP141 and UBP145 have an improved selectivity that may be useful for characterizing NR2C- and NR2D-containing receptors.
In this study, we found that the (-) isomer of PPDA (UBP150) is 50 – 80-fold more potent than the (+) isomer (UBP151). Although of lower affinity, the (+) isomer was slightly more selective than the (-) isomer. All of the new compounds, except UBP129, retained selectivity for NR2C/NR2D over NR2A/NR2B. Compared to the more potent isomer of PPDA (UBP150), 10 of the tested compounds have improved selectivity for receptors containing NR2C or NR2D subunits over those with NR2A subunits and 7 compounds had improved selectivity for NR2C and NR2D over NR2B-containing receptors. No compounds displayed a higher overall affinity for NMDA receptors than UBP150. Of these compounds, UBP125 and UBP128 are potentially useful due to their low affinity for NR2A-containing receptors and UBP141 and UBP145 may be useful for their improved selectivity for NR2C-and NR2D-containing receptors.

Schild analysis was used to determine if UBP141 an UBP145 are competitive glutamate site antagonists. It is clear that in the presence of increasing antagonist concentration, higher concentrations of L-glutamate were required to achieve a similar receptor response. Thus, these antagonists appear to be competitive at the glutamate-binding site. Schild slopes for UBP141 and UBP145 were near one, but not identical to one. This suggests that the assumptions necessary for ideal behavior in a Schild analysis (Colquhoun, 2007) may not all be completely valid for NMDA receptors. In the case of a tetrameric NMDA receptor complex with two NR2 glutamate binding sites, it is possible that 1) the antagonist alters the conformation of the receptor or 2) the binding of the first antagonist does not have an identical affinity to the binding of the second antagonist molecule, or 3) that there is a weak agonist response with just one glutamate bound that is not identical to the response with one glutamate and one antagonist bound.

Receptor molecular modeling studies (Kinarsky et al., 2005) suggest the phenanthrene ring of PPDA binds in the S1/S2 cleft along a groove found in S2 at the base of the “H” helix (Armstrong et al., 1998). Along this groove in NR2D there is a non-conserved arginine residue (R737). The selectivity for NR2D by PPDA, and especially UBP141/UBP145, may be due in part to hydrophobic contact of the phenanthrene ring with the hydrophobic hydrocarbon side-chain of NR2D’s R737 as predicted in our modeling studies (Kinarsky et al., 2005). Modeling studies, together with the results of this study, also suggest that the long, linear antagonists such as UBP125 and UBP128 are allowed due to the projection of the distal benzene ring directly out of the ligand-binding pocket.
Relative to PPDA and its active isomer (UBP150), UBP141 displays an increase in selectivity for NR2C- and NR2D-containing receptors. This magnitude of selectivity is similar to that seen for the NR2A/NR2C-preferring antagonist NVP-AAV077 (Feng et al., 2004). To test the usefulness of UBP141 at native NMDA receptors, we evaluated the ability of 5 µM UBP141 to block the NMDA receptor mediated EPSC in the hippocampal CA3-CA1 synapse after burst stimulation. The concentration of UBP141 used, 5 µM, is about twice the Ki value for NR1/NR2D receptors but only 25% of the Ki value for NR1/NR2B receptors. Thus, at this concentration, UBP141 would be expected to block more of NR2D-mediated responses than those due to NR2B-containing receptors. UBP141 was clearly able to block a significant portion (~40%) of the slow-decaying NMDA current while having very little effect (~5%, figure 5) on the peak response that appears to represent NR2A and NR2B-containing NMDA synaptic current (Lozovaya et al., 2004). In contrast to UBP141 (5 µM), ifenprodil inhibited the fast early peak response in addition to inhibiting the very slow-decaying NMDA receptor response. For example, in the representative experiments in figures 4 and 5, Lozovaya et al., 2004, ifenprodil inhibited ~30% of the after-burst peak response and ~40% of the slow-decaying current. The greater selectivity of UBP141 for inhibition of the slow component than that displayed by ifenprodil (or PPDA), supports our previous conclusion that the NR2D subunit may be contributing to this slow-decaying current in the hippocampal CA3-CA1 synapse (Lozovaya et al., 2004).

The fast early peak NMDA receptor-mediated synaptic response appears to be largely mediated by NR2A-containing NMDA receptors since it is fast decaying, preferentially inhibited by (R)-CPP, and, relative to the slow component, more weakly inhibited by ifenprodil and PPDA (Lozovaya et al., 2004). A fast-decaying peak response mediated in part by NR2A, and a very slow-decaying current mediated in part by NR2D, are consistent with the known deactivation kinetics of recombinant NR2A-, NR2B-, and NR2D-containing receptors (Monyer et al., 1994; Vicini et al., 1998). The presence of a functionally-active NR2D subunit in the CA3-CA1 synapse supports our previous suggestion that NR2D-containing NMDA receptors participate in the generation of long term depression (LTD) (Hrabetova et al., 2000). For the interpretation of physiological experiments in native preparations, it will be important to determine the effects of subtype-selective antagonists on triheteromeric (e.g. NR1/NR2A/NR2B or NR1/NR2B/NR2D) NMDA receptors.
The observation that NR2D subunits might primarily contribute to just the slow-decaying NMDA receptor response may be important to the development of neuroprotective agents. The slow response is thought to be due to the activation of extrasynaptic NMDA receptors that are activated by L-glutamate spillover following a train of stimuli (Lozovaya et al., 2004). Various studies have shown that the synaptic and extrasynaptic populations of NMDA receptors activate different intracellular signaling pathways and that the extrasynaptic population plays a key role in causing cell death while the synaptic population may be neuroprotective (Hardingham and Bading, 2003). Thus, it is possible that an NR2D-selective antagonist would provide a more selective neuroprotective action than would an NR2A- or NR2B-selective antagonist or a non-selective NMDA receptor antagonist.

The selectivity of UBP141 (or UBP145) for extrasynaptic currents may be enhanced due to the low μM affinity of UBP141. A low affinity antagonist is likely to display rapid dissociation. Such a competitive antagonist may more readily inhibit extrasynaptic NMDA receptors in the presence of low L-glutamate concentrations than inhibit synaptic NMDA receptors with higher concentrations of L-glutamate (Diamond, 2001). Hence the low affinity of UBP141 may contribute to the apparent selectivity of this compound for extrasynaptic NMDA receptors, in addition to its low selectivity for NR2D-containing NMDA receptors.

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Cheng Y and Prusoff WH (1973) Relationship between the inhibition constant (K1) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction. Biochem Pharmacol 22:3099-3108.


Footnotes:

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Reprint requests may be addressed to Dr. Blaise Mathias Costa, 985800 Nebraska Medical Center, Omaha, NE 68198-5800. Email: bmathiascosta@unmc.edu

1Present addresses: Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115;
3INMED-INSERM U29, Université de la Méditerranée, 163 route de Luminy – BP3, 13273 Marseille, CEDEX 09, France (NAL).
Legend for Figures

Figure 1. Structures of PPDA derivatives. *UBP143 and UBP144 have the trans \((2R^*,3R^*)\) configuration, all other compounds have a cis \((2R^*,3S^*)\) structure.

Figure 2. (A) A representative recording of antagonist blockade of NMDA receptor mediated responses in *Xenopus* oocytes. NR1-4b/NR2D RNA-injected oocytes were voltage clamped at \(-60\) mV and inward currents were evoked by bath application of 10 \(\mu\)M L-glutamate plus 10 \(\mu\)M glycine (heavy bar). Increasing concentrations of the bath-applied antagonist UBP145 reduced the inward currents. (B) Averaged dose-response curves for UBP145 inhibition of NR1-4b/NR2A, NR1-4b/NR2B, NR1-4b/NR2C, and NR1-4b/NR2D receptor complexes.

Figure 3. NMDA receptor subunit selectivity of PPDA derivatives. To compare subunit selectivity profiles, the inhibition constants \((K_i)\) at NR1a/NR2 receptors for each compound were normalized by dividing by the drug’s corresponding \(K_i\) value for its inhibition of NR1a/NR2D receptors. Normalized results for \((R)\)-CPP are from Feng et al., (2005) and are presented for comparison.

Figure 4. Schild analysis of UBP141 inhibition of NMDA receptor responses. A. NR1a/NR2B receptors were activated by bath applied L-glutamate in the presence of different concentrations of UBP141 as indicated. A family of two-point dose-response curves was fitted by non-linear regression analysis (GraphPad Prism) to determine the Schild slope value \((1.14)\) and \(K_B\) \((5.9 \, \mu\)M). B. Schild plot of the same data yields a Schild slope of 1.14 and pKB of -5.24 \((K_B = 5.8 \, \mu\)M).
Figure 5. (A) Pharmacologically-isolated EPSC$_{\text{NMDA}}$ evoked by a single pulse (EPSC$_{\text{NMDA}}^{\text{single}}$) (a), a 3 pulses long train (EPSC$_{\text{NMDA}}^{\text{train}3}$) (b, 200Hz) and a 7 pulses long train (EPSC$_{\text{NMDA}}^{\text{train}7}$) (c) in the control and with UBP 141 (5 μM); stimulation protocols are schematically represented over the traces. Holding voltage was -100mV. (B) The charge transfer of the EPSC$_{\text{NMDA}}^{\text{single}}$ (n1), EPSC$_{\text{NMDA}}^{\text{train}3}$ (n3), and EPSC$_{\text{NMDA}}^{\text{train}7}$ (n7) normalized to the corresponding peak current amplitude (defined here as Q); recordings in control solution and in the presence of UBP 141 (5 μM). The Q value for the control EPSC$_{\text{NMDA}}^{\text{single}}$ was taken as 100%. (C) The contribution of the UBP141-sensitive component ($Q_{\text{CONTROL}} - Q_{\text{UBP141}}$) to the EPSC$_{\text{NMDA}}^{\text{single}}$ and the EPSC$_{\text{NMDA}}^{\text{train}3}$, calculated as: $1 - (Q_{\text{UBP141}} / Q_{\text{CONTROL}})$. (D) The late component of the EPSC$_{\text{NMDA}}^{\text{train}7}$ has greater sensitivity to UBP141 as compared to the early component. The contribution of the UBP 141-sensitive component ($Q_{\text{CONTROL}} - Q_{\text{UBP141}}$) to the early (peak-700 ms) and late (700ms-3000ms) component of the EPSC$_{\text{NMDA}}^{\text{train}7}$ (calculated as $1 - \frac{Q_{\text{UBP141}}}{Q_{\text{CONTROL}}}$).
Table 1  Average antagonist Ki values (µM) ± s.e.m. for inhibiting agonist-evoked responses at recombinant NMDA receptors expressed in *Xenopus* oocytes. Ki values were statistically different from NR1/NR2B (b, B, D), NR1/NR2C (c, C, D), and NR1/NR2D (d, D, D) with p < 0.05 (b, c, d), p < 0.01 (B, C, D), and p < 0.001 (B, C, D). Number of experiments (n) is also indicated. Receptors contained NR1-1a subunits unless indicated otherwise.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>NR1/NR2A</th>
<th>NR1/NR2B</th>
<th>NR1/NR2C</th>
<th>NR1/NR2D</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBP125</td>
<td>99.3 ± 7.0</td>
<td>12.9 ± 1.4</td>
<td>28.0 ± 1.0</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>UBP125</td>
<td>99.3 ± 7.0</td>
<td>12.9 ± 1.4</td>
<td>28.0 ± 1.0</td>
<td>10.8 ± 0.3</td>
</tr>
<tr>
<td>UBP128</td>
<td>138 ± 9</td>
<td>24.1 ± 1.1</td>
<td>21.4 ± 0.4</td>
<td>10.5 ± 1.3</td>
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<tr>
<td>UBP129</td>
<td>0.85 ± 0.08</td>
<td>0.32 ± 0.07</td>
<td>1.14 ± 0.09</td>
<td>1.95 ± 0.14</td>
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<tr>
<td>UBP133</td>
<td>6.00 ± 0.48</td>
<td>8.05 ± 0.93</td>
<td>1.54 ± 0.23</td>
<td>1.59 ± 0.29</td>
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<tr>
<td>UBP136</td>
<td>10.5 ± 2.6</td>
<td>5.15 ± 0.54</td>
<td>3.44 ± 0.37</td>
<td>2.49 ± 0.30</td>
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<tr>
<td>UBP141</td>
<td>22.0 ± 1.4</td>
<td>17.2 ± 1.2</td>
<td>5.24 ± 0.54</td>
<td>2.36 ± 0.12</td>
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<tr>
<td>UBP143</td>
<td>37.8 ± 3.2</td>
<td>19.8 ± 0.4</td>
<td>11.7 ± 1.1</td>
<td>5.7 ± 0.6</td>
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<tr>
<td>UBP144</td>
<td>&gt; 400</td>
<td>227 ± 62</td>
<td>186 ± 39</td>
<td>147 ± 113</td>
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<td>UBP145</td>
<td>11.53 ± 0.79</td>
<td>7.99 ± 0.35</td>
<td>2.79 ± 0.07</td>
<td>1.19 ± 0.06</td>
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<tr>
<td>UBP148</td>
<td>16.47 ± 2.79</td>
<td>10.89 ± 0.73</td>
<td>1.70 ± 0.27</td>
<td>1.53 ± 0.28</td>
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<tr>
<td>UBP148</td>
<td>300 µM</td>
<td>% Inhibition</td>
<td>% Inhibition</td>
<td>% Inhibition</td>
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<tr>
<td></td>
<td>33.9 ± 1.7%</td>
<td>14.8 ± 3.6%</td>
<td>23.0 ± 1.2%</td>
<td>19.2 ± 1.4%</td>
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<tr>
<td>UBP150</td>
<td>0.21 ± 0.02</td>
<td>0.22 ± 0.04</td>
<td>0.068 ± 0.010</td>
<td>0.093 ± 0.008</td>
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<td>UBP150</td>
<td>0.27 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.045 ± 0.007</td>
<td>0.093 ± 0.003</td>
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<td>UBP151</td>
<td>17.6 ± 2.1</td>
<td>13.5 ± 1.0</td>
<td>3.38 ± 0.30</td>
<td>4.64 ± 0.21</td>
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<tr>
<td>UBP152</td>
<td>5.10 ± 0.53</td>
<td>2.27 ± 0.23</td>
<td>4.14 ± 0.29</td>
<td>2.68 ± 0.22</td>
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<td>UBP160</td>
<td>7.16 ± 0.69</td>
<td>15.2 ± 2.6</td>
<td>3.17 ± 0.16</td>
<td>1.68 ± 0.09</td>
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<td>UBP161</td>
<td>7.41 ± 0.92</td>
<td>3.80 ± 0.86</td>
<td>1.86 ± 0.15</td>
<td>1.045 ± 0.11</td>
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Table 2

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<th>KB (µM) ± s.e.m.</th>
<th>n</th>
<th>Schild slope ± s.e.m.</th>
<th>Hill slope ± s.e.m.</th>
<th>R² ± s.e.m.</th>
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<td><strong>UBP141</strong></td>
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<tr>
<td>NR1/NR2A</td>
<td>44.1 ± 1.7</td>
<td>9</td>
<td>1.18 ± 0.03</td>
<td>1.79 ± 0.05</td>
<td>0.998 ± 0.001</td>
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<td>NR1/NR2B</td>
<td>10.4 ± 2.1</td>
<td>9</td>
<td>1.14 ± 0.04</td>
<td>1.66 ± 0.14</td>
<td>0.975 ± 0.005</td>
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<td>NR1/NR2C</td>
<td>3.22 ± 0.31</td>
<td>4</td>
<td>1.05 ± 0.02</td>
<td>1.66 ± 0.24</td>
<td>0.937 ± 0.017</td>
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<tr>
<td>NR1/NR2D</td>
<td>2.57 ± 0.16</td>
<td>4</td>
<td>1.09 ± 0.03</td>
<td>1.53 ± 0.05</td>
<td>0.949 ± 0.022</td>
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<tr>
<td><strong>UBP145</strong></td>
<td></td>
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<tr>
<td>NR1/NR2A</td>
<td>16.1 ± 2.3</td>
<td>4</td>
<td>0.83 ± 0.05</td>
<td>1.65 ± 0.19</td>
<td>0.985 ± 0.005</td>
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<tr>
<td>NR1/NR2B</td>
<td>6.32 ± 0.20</td>
<td>4</td>
<td>0.79 ± 0.04</td>
<td>1.41 ± 0.05</td>
<td>0.995 ± 0.003</td>
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<tr>
<td>NR1/NR2C</td>
<td>2.22 ± 0.55</td>
<td>4</td>
<td>0.66 ± 0.01</td>
<td>1.41 ± 0.08</td>
<td>0.995 ± 0.002</td>
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<tr>
<td>NR1/NR2D</td>
<td>0.94 ± 0.34</td>
<td>4</td>
<td>0.66 ± 0.02</td>
<td>1.25 ± 0.08</td>
<td>0.982 ± 0.004</td>
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Schild analysis of UBP141 and UBP145
Table 3

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<tr>
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<tbody>
<tr>
<td>UBP125</td>
<td>37 ± 2</td>
<td>10 ± 7</td>
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<td>UBP128</td>
<td>53 ± 3</td>
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<tr>
<td>UBP141</td>
<td>15 ± 8</td>
<td>9 ± 9</td>
</tr>
<tr>
<td>UBP145</td>
<td>13 ± 5</td>
<td>15 ± 10</td>
</tr>
</tbody>
</table>

Inhibition of $[^3]$H AMPA and $[^3]$H kainate binding to rat brain AMPA and kainate receptors by select compounds. Values represent mean percent inhibition (± s.e.m., n = 3) by 100 µM of compound.
Figure 1
Figure 2
NR2-Selectivity

Figure 3
Figure 4
Figure 5