Site within N-Methyl-D-aspartate Receptor Pore Modulates Channel Gating

Nansheng Chen, Bo Li, Timothy H. Murphy, and Lynn A. Raymond

Kinsmen Laboratory, Department of Psychiatry and Brain Research Centre, University of British Columbia, Vancouver, British Columbia, Canada

Received July 10, 2003; accepted October 1, 2003

This article is available online at http://molpharm.aspetjournals.org

ABSTRACT

N-methyl-D-aspartate-type glutamate receptors (NMDARs) are ligand-gated ion channels activated by coagonists glutamate and glycine. NMDARs play a critical role in synaptic plasticity and excitotoxicity, largely because of their high calcium permeability and slow deactivation and desensitization kinetics. NR1 is an obligate subunit in all NMDAR complexes, where it combines with NR2A, 2B, 2C, and/or 2D. NR1 binds glycine, and residue Asn598 in the re-entrant membrane loop M2 largely determines NMDAR calcium permeability. In contrast, NR2 subunits bind glutamate and contain regions that regulate receptor desensitization and deactivation. Here, we report that mutations of NR1(Asn598) in combination with wild-type NR2A, expressed in human embryonic kidney 293 cells, exhibit altered glycine-independent desensitization. In the absence of extracellular calcium, substitution of Arg for Asn598 (NR1R) slowed desensitization by 2- to 3-fold compared with wild-type NR1/NR2A, and glutamate-evoked peak current EC50 and deactivation rate were also affected. Replacement of Asn by Gln (NR1Q) produced two distinct rates of calcium- and glycine-independent desensitization. Moreover, in the presence of extracellular calcium, the voltage-dependent pore block by calcium for the NR1Q mutant mimicked the effects of the positively charged Arg at this site in NR1R on slowing desensitization and deactivation. A kinetic model of the NMDA receptor-channel suggests that these results can be explained by altered gating and not ligand binding. Our data increase understanding of the role that amino acids within the NMDAR pore play in channel gating.
1993; Wollmuth et al., 1996) as well as single channel conductance (Behe et al., 1995). NMDARs have asparagine at this site and relatively high Ca\(^{2+}\) permeability, whereas non-NMDA-type glutamate receptors have either glutamine or arginine and lower or negligible Ca\(^{2+}\) permeability, respectively. Asn598 in NR1 makes the largest contribution to determining the high Ca\(^{2+}\) permeability of NMDARs. Interestingly, one study using single-channel recording showed that substitution of Gln at this site [NR1(N598Q)], combined with NR2A, could alter channel gating under bi-ionic conditions because of differential binding of monovalent ions in the permeation pathway (Schneegugger and Ascher, 1997).

Moreover, recent evidence from studies using the scanning cysteine accessibility method and trapping blockers suggests that Asn598, along with two amino acids in M3—Val626 and Thr630—in NR1 lie very near the channel activation gate, which is postulated to be formed by the M2 loop (Sobolevsky et al., 2002).

To further investigate a role for the Asn/Gln/Arg site deep within the pore in modulating NMDAR channel gating, we re-examined properties of receptors composed of NR2A together with NR1A containing Asn (wild-type), Gln, or Arg at position 598. Recombinant receptors were expressed in HEK293 cells to allow analysis of mutant receptors and facilitate ultra-fast solution exchange during whole-cell patch clamp recording. We report experimental and modeling evidence to support the Asn/Gln/Arg site plays a critical role in channel closing and desensitization of the NMDAR.

**Materials and Methods**

**Cell Culture and Transfection.** Culture and transfection of HEK293 cells (American Type Culture Collection, Manassas, VA) were performed as described previously (Chen et al., 1997). Briefly, cells were passaged every 2 to 4 days. For Ca\(^{2+}\) phosphate transfection, cells were plated at a density of 1 x 10⁶ cells/ml in 10-cm culture dishes (Falcon; Becton Dickinson Labware, Franklin Lakes, NJ.). Cells were transfected with cDNAs encoding NR2A and either wild-type (WT) NR1A, NR1A(N598R), or NR1A(N598Q) at a ratio of 1:1. A total of 10 µg of plasmid cDNA was used for transfection of a 10-cm culture plate. After transfection, 1 mM (±)-2-amino-5-phosphono-pentanoic acid (RBI/Sigma, Natick, MA) was added to the culture media and the cells were transferred onto glass coverslips in 35-mm culture plates (Falcon).

**Electrophysiology.** The whole-cell, patch-clamp recording technique and recording solutions for HEK293 cells were essentially the same as described previously (Chen et al., 1997). Twenty-four to 36 h after the start of transfection, the HEK293 cells were transferred to the recording chamber on the stage of an inverted microscope (Axiovert 100; Carl Zeiss, Thornburg, NY). Agonist-evoked currents were measured at the peak of the response. EC₅₀ was calculated from the equation:

\[
EC₅₀ = \frac{[A]}{(1 + \frac{[A]}{EC₅₀})^H}
\]

where \([A]\) indicates the concentration of agonist, \(EC₅₀\) is the concentration of agonist that causes half-maximal effect, and \(H\) is the Hill coefficient.

**Results**

NMDARs composed of NR2 subunits combined with mutant NR1A containing Gln or Arg instead of Asn at position 598 in the pore exhibit decreased or negligible Ca\(^{2+}\) permeability, respectively (Burnashev et al., 1992; Sakurada et al., 1993). These mutant receptors have been used in previous studies to discern the role of NMDAR-mediated Ca\(^{2+}\) influx in a variety of cellular processes as well as to investigate the stoichiometry of the NMDAR complex (Behe et al., 1995). In this study, we re-examined the properties of NMDARs composed of NR2A and NR1A compared with NR2A and mutant NR1A(N598Q) or NR1A(N598R) (referred to as NR1Q or NR1R, respectively) to determine whether mutations at this site in the pore could affect other receptor channel properties, such as gating.
Wild-type NR1/NR2A (WT)-mediated currents evoked by a 3-s application of 1 mM glutamate (in the presence of saturating glycine of 50 μM) showed significant desensitization when recorded in 1.8 mM extracellular Ca²⁺ (Fig. 1A). Ca²⁺-dependent inactivation (reviewed by McBain and Mayer, 1994) and the acceleration of agonist-induced desensitization by ambient zinc (Chen et al., 1997; Zheng et al., 2001) both may contribute to the rapid rate and large extent of current decay during sustained application of this saturating agonist concentration. When the same cell was superfused with external solution containing no added Ca²⁺ and 1 μM TPEN or 5 mM EGTA to chelate residual Ca²⁺ and zinc, desensitization was characterized by a single exponential decay with a time constant of 1.6 s and high steady state-to-peak current ratio ($I_\text{ss}/I_p$) at the end of the agonist application (Fig. 1A; Table 1), similar to previously published data (Zheng et al., 2001). This process represents agonist-induced Ca²⁺-, Zn²⁺-, and glycine-independent desensitization (Krupp et al., 1998; Zheng et al., 2001).

**N598R Mutation Slows Agonist-Induced Desensitization, Deactivation, and Shifts the Glutamate EC₅₀.** In contrast to wild-type receptors, currents recorded from cells expressing the Ca²⁺-impermeable NR1R showed no Ca²⁺-dependent inactivation (Fig. 1A). Strikingly, these currents also showed minimal agonist-induced desensitization during the 3-s application of 1 mM glutamate in the presence of a zinc chelator and a saturating glycine concentration (Fig. 1A; Table 1). Furthermore, recovery from desensitization for this mutant receptor was also dramatically slowed (Fig. 1, B and C; Table 1; $P < 0.0001$ by two-way analysis of variance). On the other hand, currents recorded in the absence of a zinc chelator exhibited substantial desensitization ($I_\text{ss}/I_p$ of 0.68 ± 0.06, $\tau_d$ of 1.4 ± 0.3 s, $n = 4$; data not shown); this increase in rate and extent of desensitization was qualitatively similar to effects seen for wild-type NR1A/NR2A (Chen et al., 1997; Zheng et al., 2001) caused by contamination of the external solution by nanomolar zinc. This effect could be explained by the allosteric interaction between the zinc and glutamate binding sites (Zheng et al., 2001), independent of desensitization gating. Taken together, these results suggest that the positively charged arginine residue within the pore of the NR1R mutant channels might directly interfere with the desensitization gate.

In addition to the decreased rate and extent of desensitization found for the mutant receptor NR1R, we observed a marked slowing of the 10-to-90% rise time to peak and of current deactivation with offset of the glutamate pulse (Table 1). These results suggested that the NR1 pore mutation might also alter the EC₅₀ for glutamate (measured using 3-s agonist applications). To test this possibility, we constructed concentration-response curves comparing NR1R with wild type. The mutant receptors showed higher sensitivity to glutamate with a peak current EC₅₀ of 1.9 μM compared with 7.7 μM for wild type (Fig. 1D; Table 1). Because the potency of glutamate for activating NMDAR currents reflects the combined processes of receptor binding and channel gating (Colquhoun, 1998), the leftward shift in EC₅₀ indicates that the pore mutation alters glutamate binding affinity and/or influences the gating process.

**Distinct Two-Component Macroscopic Desensitization of NR1Q.** The idea that a mutation at Asn598 of NR1 alters channel gating was further supported by analysis of currents mediated by the partially Ca²⁺-permeable mutant NR1Q. Unlike either wild type or NR1R, these currents showed an apparently rapidly desensitizing component in addition to the slower desensitization when recorded in the presence of a zinc chelator (1–2 μM TPEN) and saturating glycine (Fig. 2A). The rapidly decaying component of the current response to sustained agonist application was consistent with a model for the NR1Q proposed by Schneggensburger and Ascher (1997), in which extracellular sodium favors rapid transition from the main to the subconductance state upon channel opening, leading to apparent macroscopic desensitization (see Discussion). Interestingly, the slowly decaying component of macroscopic desensitization was modulated by extracellular Ca²⁺ in an unexpected manner. In the
absence of extracellular Ca$^{2+}$, this component resembled desensitization of wild-type receptors ($P > 0.05$ by unpaired t test), whereas in the presence of Ca$^{2+}$ (1.8 mM), the rate and extent were strikingly attenuated and resembled those of NR1R ($P > 0.05$ by unpaired t test)(Fig. 2A; Table 1). The rate of current deactivation upon glutamate withdrawal was also influenced by extracellular Ca$^{2+}$—slow, as for NR1R, in 1.8 mM CaCl$_2$ and more rapid, similar to WT, in the absence of Ca$^{2+}$ (Fig. 2B; Table 1). Unlike the NR1R, however, in the presence of external Ca$^{2+}$, the glutamate peak current dose-response curve as well as the 10-to-90% rise-time to peak current for NR1Q were similar to those measured for wild-type receptors (Table 1). On the other hand, recovery from slow desensitization for NR1Q occurred at a rate significantly slower than that measured for WT ($P < 0.0001$, 2-way ANOVA), even in the absence of extracellular Ca$^{2+}$ (Fig. 2, C and D; Table 1).

Pore Block by Ca$^{2+}$ Influences Desensitization and Deactivation Rates for NR1Q. It is known that extracellular Ca$^{2+}$ can bind within the pore and block ion flux through NR1Q in a voltage-dependent manner (Premkumar and Auerbach, 1996). Because onset of slow agonist-induced desensitization, as well as deactivation, in the absence of external Ca$^{2+}$ was similar to that of wild type NR1/NR2A but was not statistically different from that in the presence of Ca$^{2+}$, we hypothesized that Ca$^{2+}$ binding to a site within the pore of NR1Q interfered with channel gating. The effects on gating would only occur after channel opening, which may, in part, explain the lack of effect of external Ca$^{2+}$ on the NR1Q rise time and peak current EC$_{50}$. This hypothesis could be extended to suggest that the slowed desensitization and deactivation of NR1Q currents result from the positively charged arginine residue mimicking the effect of Ca$^{2+}$ binding in the pore of NR1Q.

To test whether Ca$^{2+}$ entry into and/or flux through the pore of NR1Q was necessary to reduce the rate of slow-onset agonist-induced desensitization, we analyzed desensitization of current responses to glutamate at a series of holding potentials ranging from $-100$ to $+100$ mV in the presence of 1.8 mM CaCl$_2$ and 1 to 2 $\mu$M TPEN (Fig. 3A). These channels show voltage-dependent block by external Ca$^{2+}$ (Fig. 3A) and anomalous whole-cell current reversal at holding potentials near 0 mV (not shown), as described previously (Premkumar and Auerbach, 1996; Schneggenburger and Ascher, 1997). As expected, the mean time constant for slow desensitization calculated from current responses at hyperpolarized potentials ($-100$ to $-40$ mV) was similar to the mean time constant measured previously from current responses at a holding potential of $-60$ mV (Fig. 3, A and B). However, the rate of slow, agonist-induced desensitization was significantly accelerated at depolarized holding potentials ($+40$ to $+100$ mV), where Ca$^{2+}$ influx and pore block are dramatically diminished, although it was not as fast as that observed at $-60$ mV for wild-type receptors or NR1Q in the absence of external Ca$^{2+}$ (Fig. 3; A and B; Table 1). These results support the hypothesis that Ca$^{2+}$ binding to a site within the pore of NR1Q (Premkumar and Auerbach, 1996) contributes to the slowing of agonist-induced desensitization.

Results of Modeling Consistent with Effect of Pore Mutations on Channel Gating. We reasoned that the effects of the pore mutations on glutamate-evoked current deactivation and steady-state to peak ratio, as well as that of NR1R on the rise-time and glutamate concentration-response curve, could all be accounted for by altered rates of channel gating without any changes in ligand unbinding rate. Because the kinetic scheme for gating of NR1Q was complicated by asymmetric gating under bi-ionic (external Na$^+$ and internal K$^+$) conditions, which was previously modeled by Schneggenburger and Ascher (1997), we tested this hypothesis only for NR1R, using a standard model to describe NMDAR ligand binding and channel gating (Fig. 4A; see Materials and Methods). For this model, the rates of desensitization and resensitization were set to the values measured experimentally for NR1R (Table 1), and we initially set the glutamate binding and unbinding rates to the values used to model wild-type NR1/NR2A (Chen et al., 2001). We then varied channel opening and closing rates from the rates used for WT (Chen et al., 2001) until the model traces fit our data for the shifted glutamate EC$_{50}$, extent of desensitization at the end of a 3-s pulse of 1 $\mu$M glutamate, and the slowed 10-to-90% rise time to peak and rate of current decay upon withdrawal of glutamate. We found that a $-10$-fold reduction in the channel closing rate, with no change in channel opening rate (Table 2), resulted in a model current response that closely approximated the experimental data (Fig. 4, B and C); the model current showed a rise-time of 13 ms, deactivation

**TABLE 1**

Biophysical properties of NR1R, NR1Q, and WT receptor currents

Results from 3-s applications of 1 mM glutamate/50 $\mu$M glycine with 1–2 $\mu$M TPEN. $n = 6$ to 15 different cells for all kinetic parameters; $n = 4$–7 different cells for EC$_{50}$ measurements.

<table>
<thead>
<tr>
<th></th>
<th>NR1R$^a$</th>
<th>WT$^a$</th>
<th>NR1Q 0 Ca$^{2+}$</th>
<th>NR1Q 1.8 mM Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rise time (ms)</td>
<td>13.4 ± 0.3</td>
<td>6.4 ± 0.2</td>
<td>6.7 ± 0.4</td>
<td>6.8 ± 0.3</td>
</tr>
<tr>
<td>$\tau_{\text{off}}$ (ms)</td>
<td>20.2 ± 0.70</td>
<td>2.42 ± 0.27</td>
<td>14.3 ± 0.54</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>$\tau_{\text{on}}$ (ms)</td>
<td>0.93 ± 0.01</td>
<td>0.74 ± 0.03</td>
<td>0.54 ± 0.04</td>
<td>0.63 ± 0.03</td>
</tr>
<tr>
<td>$\tau_{\text{off}}$ (ms)</td>
<td>170 ± 17 (61%)</td>
<td>56 ± 9 (89%)</td>
<td>89 ± 11 (100%)</td>
<td>122 ± 13 (62%)</td>
</tr>
<tr>
<td>$\tau_{\text{on}}$ (ms)</td>
<td>611 ± 42 (39%)</td>
<td>309 ± 49 (11%)</td>
<td>N.D.</td>
<td>539 ± 45 (38%)</td>
</tr>
<tr>
<td>EC$_{50}$ ($\mu$M; peak current):</td>
<td>1.9 ± 0.2</td>
<td>7.7 ± 0.8</td>
<td>N.D.</td>
<td>8.1 ± 0.5</td>
</tr>
</tbody>
</table>

$^a$N.D. not determined.

$^b$Time course of glutamate-evoked response was not different for individual NR1R-expressing cells switched between external solutions containing no added extracellular Ca$^{2+}$ versus 1.8 mM CaCl$_2$, therefore these data were pooled.

$^c$Results from experiments with no added extracellular Ca$^{2+}$ for WT.

---

time constant of 360 ms (the weighted average time constant from our data was 342 ms), steady-state to peak current ratio of 95%, and EC\textsubscript{50} of 1.75 μM (see Table 1 for comparison with experimental values).

To determine whether a change in ligand unbinding rate, without any alteration in channel opening or closing rates, could also reproduce our data, we reduced the unbinding rate by 5- to 8-fold. Compared with the experimental values for NR1R, a 5-fold rate reduction produced a similar shift in EC\textsubscript{50} but a deactivation rate that was markedly faster (220 ms), whereas an 8-fold reduction reproduced the deactivation rate (350 ms) but shifted the EC\textsubscript{50} to less than 1 μM; both manipulations resulted in a steady state-to-peak current ratio of 72%, indicating markedly more extensive desensitization than that observed experimentally. For comparison, we also modeled WT, using the rates of desensitization and resensitization measured experimentally in the absence of external Ca\textsuperscript{2+} or zinc; the binding/unbinding as well as channel opening/closing rates were the same as we had used previously (Chen et al., 2001) (Fig. 4, B and C; Table 2). Again, the rise-time (7 ms), deactivation time constant (45 ms), steady state-to-peak current ratio (63%), and EC\textsubscript{50} (7 μM) were all similar to our experimental data (see Table 1). In all, these results are consistent with a model in which the positively charged arginine residue near the external mouth of the pore of NR1R interferes with both closing and desensitization gating of the channel. Moreover, the leftward shift in the glutamate concentration-response curve could be explained by these effects on channel gating, without any modification in ligand binding or unbinding rates.

**Discussion**

In this study, we have demonstrated that mutation of NR1 Asn598, a pore residue controlling ion permeation and open channel block, modulates channel gating of NMDA receptors. Furthermore, our data strongly suggest that slowing of desensitization and deactivation gating for the NR1 mutants is
caused by the presence of a positively charged ion at the NR1 Asn598 site. In addition, we have shown a leftward shift of the glutamate concentration-response for NR1R, consistent with previous studies of NR1 pore mutations N598R or A635T expressed with NR2B or NR2A, respectively (Kashima et al., 1997; Kohda et al., 2000). Using a kinetic model, we confirm that this effect results from an alteration in channel gating rather than a more global change in receptor conformation that affects ligand binding.

There is strong evidence indicating that Asn598 in NR1 contributes to controlling the narrow pore constriction involved in determining selective ion permeability and block and single channel conductance (Burnashev et al., 1992; Mori et al., 1992; Sakurada et al., 1993; Behe et al., 1995; Wollmuth et al., 1996); however, the influence of NR1 Asn598 on NMDAR macroscopic desensitization or channel closing has not been explored. On the other hand, a variety of data indicates that residues lining the pore are involved in conformational changes that couple agonist binding to opening of the channel activation gate. For example, the region in and around amino acids within the NR1 C-terminal segment of M3 (M3c), including the highly conserved SYTANLAAF (Lurcher) motif, contributes to lining the channel in the wide, external vestibule (Beck et al., 1999) and largely determines the effect of protons on channel gating (Low et al., 2003). As well, recent data suggest that the walls of this external vestibule constrict in the channel’s closed state but do not form the activation gate (Sobolevsky et al., 2002). Moreover, the Lurcher mutation in the SYTANLAAF motif of NR1 (A635T – NR1Lc) when coexpressed with wild-type NR2A shows marked slowing of deactivation and glutamate-induced desensitization, a 10-fold leftward shift in the glutamate dose-response curve, and a markedly slower channel closing rate (by noise analysis) compared with wild-type NR1/NR2A (Kohda et al., 2000), results that closely match our data for NR1R.

Results of previous studies have also suggested a role for M2 in channel gating. State-dependent accessibility of a substituted cysteine in NR1(N598C) supports a role for this site in gating (Kuner et al., 1996). Another study also used the cysteine accessibility method to compare modification rates of NR1 residues in the external vestibule as well as deeper in the pore (including N598C in M2), in the ligand-bound versus unbound state, with or without the presence of a channel blocking agent; from these data, the authors proposed that the activation gate lies deep within the pore, near the level of Asn598 (Sobolevsky et al., 2002). Consistent with this idea, mutation of the tryptophan five residues toward the N terminus from the Asn/Gln/Arg site (and closer to the internal mouth of the channel) in M2 of both NR1 and NR2A resulted in a change in NMDA channel open probability (Buck et al., 2000). Our data, combined with a kinetic model, demonstrate that mutation of NR1 Asn598 to the positively charged arginine, or Ca²⁺ bound in the pore with a glutamine substitution at this site, significantly slows onset of desensitization as well as channel closing. It may be that the activation gate is formed by the M2 loop in NMDARs, similar to the role of the P loop in gating for cyclic nucleotide-gated channels (Sun et al., 1996).

It has frequently been noted that ionotropic glutamate receptors show significant structural homology with voltage-gated potassium channels in the pore region (MacKinnon, 1995; Wo and Oswald, 1995; Wood et al., 1995); in both families, the pore is formed by a re-entrant loop (M2 for glutamate receptors and the P loop for potassium channels) along with contributions from transmembrane segments (M1, M3, and M4 for NMDARs and S5 and S6 for K⁺ channels). Previous studies have shown that pore mutations in Shaker K⁺ channels can alter rates of channel closing and C-type inactivation (Molina et al., 1998; Rasmussen et al., 1998). Notably, the latter is thought to be mediated by direct constriction (or “collapse”) of the pore itself, and this process can be slowed by occupation of the pore by permeant ions (Kiss and Korn, 1998; Molina et al., 1998; Rasmussen et al., 1998).

Similar to the data for C-type inactivation of potassium channels, previous studies have indicated that occupation of the NMDAR pore by organic cations or other channel-blocking agents alters macroscopic gating. NMDARs composed of NR2A and mutant NR1 in which cysteine was substituted at

![Fig. 4. A kinetic model suggests changes in channel desensitization and closing rate account for differences in macroscopic current kinetics and agonist potency between NR1R and WT. A, State diagram used to model NMDAR channel behavior. B, traces generated using parameters shown in Table 2 to model current response to 3-s application of 1 mM glutamate (+ 50 μM glycine) for NR1R or WT NMDAR. C, concentration-response curve for peak glutamate-evoked current (in 50 μM glycine) based on model simulation and fitted to Hill equation.](image-url)
Ala634 (one residue before the Lurcher site) exhibited markedly slowed deactivation after modification with methanethiosulfonate ethylammonium (Jones et al., 2002). In other experiments using a series of organic ionic species of different sizes that bind to and produce rapid block of the open NMDAR channel, it was shown that the activation/deactivation and desensitization gates are near the external entrance of the narrow constriction of the pore (i.e., at the level of Asn598), because some of these agents prevented desensitization and/or deactivation (Sobolevsky et al., 1999). Similarly, occupation by adamantane derivatives of a binding site in the external vestibule of NMDARs prevented channel closure (Antonov and Johnson, 1996). These studies together with our data are consistent with a model in which the presence of a cation or blocking molecule at or near the narrow pore constriction of NMDARs markedly slows or prevents channel closing and/or desensitization.

An elegant study by Schneggenburger and Ascher (1997) demonstrated that NR1Q shows gating asymmetry under bi-ionic conditions. Specifically, in recordings of agonist-evoked current responses in solutions with Na⁺ as the primary external monovalent cation and Cs⁺ as the internal cation, they observed preferential cycling of channels from closed to main conductance to subconductance and back to closed states. If we assume that internal Cs⁺ and K⁺ act similarly in the model proposed by these authors, then the rapidly decaying macroscopic current we observed in response to sustained agonist application could represent initial opening of channels to the main conductance state followed by a shift toward preferential occupation of the subconductance state as Na⁺ replaces K⁺ at a binding site internal to the narrow constriction within the pore (Schneggenburger and Ascher, 1997). Therefore, the appearance of two distinct components of macroscopic desensitization that we observed probably represents a rapid gating shift from main to subconductance states of the channel, followed by entry into the desensitized state.

It is interesting to note that although glycine-independent desensitization is determined largely by extracellular structural regions in NR2 subunits (Krupp et al., 1998; Villarroel et al., 1998), mutations of a pore residue in NR1 can modify this process as well as deactivation gating. This result is consistent with a model in which extracellular domains of glutamate receptors involved in ligand binding adopt distinct conformations in the closed, open, and desensitized states (Sun et al., 2002), but movement of these extracellular domains is transduced by connector regions to alter the conformation of regions deep within the pore that gate ion flux. The precise nature of the conformational changes associated with gating that occur around the level of Asn598 in the pore remains to be determined.

Acknowledgments

We are grateful to S. Vicini for helpful discussions, J. Ren for technical assistance, and M. Thejomayan for assistance with manuscript preparation.

References

residues in the cyclic nucleotide-gated channel pore: P region structure and function in gating. Neuron 16:141–149.

Address correspondence to: Dr. Lynn A. Raymond, Department of Psychiatry, University of British Columbia, 4N3-2255 Wesbrook Mall, Vancouver, BC, Canada V6T 1Z3. E-mail: lynnr@interchange.ubc.ca