Short communication

A mutation in the glycine binding pocket of the \textit{N}-methyl-d-aspartate receptor NR1 subunit alters agonist efficacy

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Abstract

Alanine 714 of the NMDA receptor NR1 subunit resides in the glycine binding pocket. The Ala714Leu mutation substantially shifts glycine affinity, but here no effect on antagonism by DCK is detected. Ala714Leu is also found to limit the efficacy of a partial agonist without altering its apparent affinity. The differential sensitivity of Ala714Leu to glycine agonists suggests that alanine 714 may be an intermediary in transducing the ligand binding signal.

Keywords: NMDA receptor; Pharmacology; Efficacy; Affinity; DCK; HA-966

Glycine and L-glutamate are both required to activate the NMDA receptor [6,7,12]. Although the relationship between these two coagonists is complex [10,17], much progress has been made in defining the binding sites of L-glutamate and glycine at the molecular level. A current image of the NMDA receptor assigns each of the binding sites to separate subunit members. L-glutamate activation is disrupted by mutations in NR2 subunits [9], while numerous studies of mutated residues in the NR1 subunit demonstrate disturbances in the apparent affinity for the coagonist glycine [5,8,20,23]. The extracellular ligand-binding portions of each subunit member are thought to exist in a bilobate conformation [1,16], with the first lobe (S1) represented by portions of the amino terminus, and the second lobe (S2) composed largely of the region connecting the M3 and M4 transmembrane domains. The recent crystallization of a fusion protein constructed from a homologous receptor i.e., iGluR2 offers a high resolution structural image of this model [1].

We previously found that a quadruple mutation (N710R, Y711R, E712A, A714L) in the M3–M4 loop of the NR1 subunit disrupts glycine binding [23]. Dichlorokynurenic acid (DCK) is a high affinity competitive antagonist at the glycine site of the NMDA receptor [4,13]. When measured near their respective EC$_{50}$s for glycine (see Table 1), analysis of DCK affinity revealed no difference between the wild type and quadruple mutant receptors (both IC$_{50}$s = 0.3 \mu M, see Fig. 1 and Table 1). Application of the Cheng–Prussoff equation led to indistinguishable estimates of the affinity constants for DCK as well ($K_s = 0.16 \mu M$, Table 1).

The residue dominant in conferring the shift in glycine affinity in the quadruple mutant was the alteration of alanine 714 to leucine [23]. Although the mutation Ala714Cys did not induce a shift in the apparent affinity for glycine, it was acutely sensitive to alteration by the thiol-modifying reagent, dithionitrobenzoic acid (DTNB). Co-application of glycine, but not L-glutamate, with DTNB could protect A714C from modification by DTNB (see Fig. 7 of Wood, et al. [23]). Unlike glycine, DCK was not able to protect A714C from inhibition by DTNB (Fig. 2). Thus, there is no evidence supporting the involvement of alanine 714 in binding of DCK.

(±)-3-Amino-1-hydroxy-2-pyrrolidone (HA-966) has been used as a partial agonist at the glycine site of the NMDA receptor [17–19]. We performed a comparative analysis of the wild type receptor and the quadruple mutant receptor employing HA-966 as a partial agonist (Fig. 3). Although no significant difference in affinity was seen between the wild type and mutant receptors, the quadruple mutant displayed markedly reduced maximal activation (i.e., efficacy) by HA-966 (Table 2). HA-966 affinity and efficacy were examined for the individual point mutants composing the quadruple mutant. The A714L mutant displayed the most prominent reduction in HA-966 efficacy (Fig. 3C and Table 2).

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Recently, a fusion protein forming a glutamate-receptor ligand-binding core was crystallized. In that structure, the residue analogous to A714 of NR1 is threonine 686 of iGluR2 [1]. T686 resides within the binding pocket for the agonist in the S2 lobe (i.e., kainate in the iGluR2 S1S2 crystal). However, T686 is not a contact point for kainate, but instead forms a hydrogen bond with E402 in the S1 lobe, thereby stabilizing the agonist-bound conformation. In previous work, we also concluded that A714 resided within the glycine-binding pocket of the NMDA receptor, but did not represent a contact point for glycine [23].

The major difficulty in interpreting changes in apparent affinity following mutation of a receptor is distinguishing between a mutation that affects agonist binding (affinity) from a mutation that affects transduction of the binding signal (efficacy). For a careful review of this issue, see Colquhoun [2]. In the case of a ligand-gated ion channel, the simplest model describes three states of the receptor/channel: unoccupied, agonist-occupied/inactive, and agonist-occupied/active. If a mutation affects the binding of agonist, it alters the first equilibrium (i.e., $K_a$), if it affects transduction, it alters the second equilibrium (i.e., $E$). However, for a highly efficacious agonist possessing a large $E$, a mutation that alters the second equilibrium will appear to affect the affinity by causing a shift in affinity following mutation of a receptor.

![Fig. 1. The quadruple NR1 mutation 710:NYEA → RRAL does not alter DCK affinity.](image)

![Fig. 2. DCK does not protect NR1-A714C from DTNB modification.](image)
Fig. 3. Activation by HA-966 is diminished in the quadruple mutation of NR1. Representative traces of whole cell responses of the wild type receptor (A) and quadruple mutation of NR1 (B) are shown. Normalizing concentrations of agonist (100 μM L-Glu + 100 μM Gly) are shown as black bars with test responses to increasing concentrations of HA-966 (10 μM, 100 μM, 2 mM, and 5 mM) + 100 μM L-Glu shown as open bars. Scale bars represent 10 s and 1 μA for the wild type receptor and 2 μA for the quadruple mutant. (C) Dose–response curves for the wild type (open circles), quadruple NR1 mutant (open triangles), and the NR1-A714L mutant (closed circles) are shown. Symbols represent mean responses calculated as in Fig. 1. Error bars represent standard errors.

the EC50 [2]. The A714L mutation did reduce the apparent affinity for the full agonist glycine, as measured by the EC50 [23]. Now, using a less efficacious agonist (i.e., HA-966), we find no change in affinity for a partial agonist and significant reduction in the efficacy as a result of the A714L mutation. Such a phenotype is predicted for a transduction mutant [2]. The lack of effect on antagonism by DCK further supports the idea that this region is not involved in directly binding glycine.

This pharmacological profile described thus far suggests that the role of A714 in NR1 may be the transduction step of NMDA receptor activation. A714 of NR1 is equivalent to residue T686 in iGluR2. T686 is also found within the ligand binding site and forms a hydrogen bond with E402 in the agonist-bound conformation [1]. Thus, it is likely that T686 of iGluR2 stabilizes the agonist-occupied/active conformation of the receptor, and thereby promotes channel opening. So, although residue T686 is not a contact point for glutamate, it may contribute to the molecular determinants for agonist efficacy in iGluR2. Based on the data presented here, we propose that the domain containing A714 in NR1 also contributes to agonist efficacy by stabilizing the agonist-bound conformation.

The necessity for two agonists (L-glutamate and glycine) increases the theoretical complexity of NMDA receptor activation [3,11]. The mechanics that underlie subunit interactions are also unknown. These limitations narrow our ability to interpret mutagenesis data. However, despite these limitations, the data presented here contribute to understanding the pharmacological characteristics of the NMDA receptor. Moreover, this type of analysis in conjunction with the static views generated from crystal structures may assist in developing a dynamic image of a functioning receptor.

Acknowledgements

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Table 2

<table>
<thead>
<tr>
<th>Construct</th>
<th>HA-966 affinity</th>
<th>Glycine affinity (µM)</th>
<th>Alpha (%)</th>
<th>n</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR1</td>
<td>298</td>
<td>1.6</td>
<td>21 ± 2.4</td>
<td>11</td>
<td>200–450</td>
</tr>
<tr>
<td>N710R, Y711R, E712A, A714L</td>
<td>159</td>
<td>530</td>
<td>4.9 ± 0.8</td>
<td>6</td>
<td>110–230</td>
</tr>
<tr>
<td>N710R</td>
<td>149</td>
<td>3.5</td>
<td>24 ± 7</td>
<td>6</td>
<td>40–520</td>
</tr>
<tr>
<td>Y711R</td>
<td>122</td>
<td>3.6</td>
<td>23 ± 6</td>
<td>5</td>
<td>44–340</td>
</tr>
<tr>
<td>E712A</td>
<td>78</td>
<td>4.3</td>
<td>22 ± 6</td>
<td>5</td>
<td>20–310</td>
</tr>
<tr>
<td>A714L</td>
<td>532</td>
<td>100</td>
<td>7 ± 2.5</td>
<td>9</td>
<td>120–2300</td>
</tr>
</tbody>
</table>

*From Wood et al. [23].
References


