doi:10.1016/j.jmb.2008.03.012 *J. Mol. Biol.* (2008) **378**, 673–685

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

NMR Spectroscopy of the Ligand-Binding Core of Ionotropic Glutamate Receptor 2 Bound to 5-Substituted Willardiine Partial Agonists

Michael K. Fenwick and Robert E. Oswald*

Department of Molecular Medicine, Cornell University, Ithaca, NY 14853, USA

Received 5 December 2007; received in revised form 4 March 2008; accepted 6 March 2008
Available online 14 March 2008

Glutamate receptors mediate neuronal intercommunication in the central nervous system by coupling extracellular neurotransmitter–receptor interactions to ion channel conductivity. To gain insight into structural and dynamical factors that underlie this coupling, solution NMR experiments were performed on the bilobed ligand-binding core of glutamate receptor 2 in complexes with a set of willardiine partial agonists. These agonists are valuable for studying structure–function relationships because their 5-position substituent size is correlated with ligand efficacy and extent of receptor desensitization, whereas the substituent electronegativity is correlated with ligand potency. NMR results show that the protein backbone amide chemical shift deviations correlate mainly with efficacy and extent of desensitization. Pronounced deviations occur at specific residues in the ligand-binding site and in the two helical segments that join the lobes by a disulfide bond. Experiments detecting conformational exchange show that micro- to millisecond timescale motions also occur near the disulfide bond and vary largely with efficacy and extent of desensitization. These results thus identify regions displaying structural and dynamical dissimilarity arising from differences in ligand–protein interactions and lobe closure that may play a critical role in receptor response. Furthermore, measures of line broadening and conformational exchange for a portion of the ligand-binding site correlate with ligand EC_{50} data. These results do not have any correlate in the currently available crystal structures and thus provide a novel view of ligand-binding events that may be associated with agonist potency differences.

Keywords: glutamate receptor; AMPA; partial agonists; NMR; chemical exchange

Edited by A. G. Palmer III

Introduction

Glutamate-activated cationic channels constitute one of the three superfamilies of ligand-gated receptors. Within this superfamily, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)-selective receptors are the principal mediators of rapid neuronal excitation at central synapses and are associated with several higher-order neurological functions.[1,2] Structurally, these receptors are tetrameric combinations[3,4] of glutamate receptor (GluR) subunits 1–4 (GluR1–4)[5–8] arranged as dimers of dimers[4,9] on postsynaptic neuronal plasma membranes. Each subunit consists of an extracellular N-terminal domain, an extracellular ligand-binding core, four membrane-associated regions (M1–M4), and an intracellular C-terminal segment.[10–13] Biophysical studies of isolated "S1S2" ligand binding cores (Fig. 1a)[14–19] were initiated by the finding that the artificially linked S1 and S2 segments of GluR4 show pharmacological properties similar to those of the intact GluR4 receptor.[22] Crystal structures of GluR2 S1S2 were subsequently determined in uncomplexed and several complexed states as well as in different dimeric configurations.[21,23–27,20,28–31] These structures, when viewed alongside electrophy-

*Corresponding author. E-mail address: reo1@cornell.edu.

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; GluR, glutamate receptor.

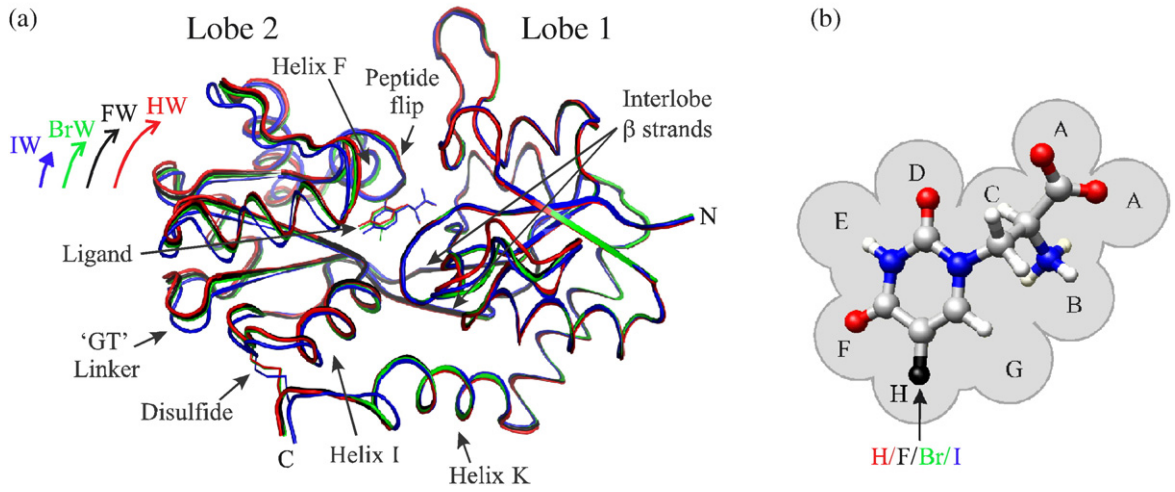

Fig. 1. (a) HW-, FW-, BrW-, and IW-S1S2 crystal structure traces (PDB IDs 1mqj, 1mqi, 1mqh, and 1mqg, respectively).[20] (b) Willardiine compounds unsubstituted or halogenated at the 5-position. The letters (A–H) denote subsites of the ligand-binding pocket as defined by Armstrong and Gouaux.[21] The coloring scheme denoting the 5-position substituent is used throughout the article (HW, red; FW, black; BrW, green; IW, blue).

siological measurements of related receptors, have revealed the structural basis of various aspects of AMPA receptor function.

Electrophysiological studies of the effects of unsubstituted and 5-substituted fluoro-, bromo-, and iodowillardiine partial agonists (HW, FW, BrW, and IW, respectively) on AMPA receptors have demonstrated that distinct current responses can be induced from a single atom change in the ligand, thus pointing to the usefulness of these willardiine derivatives for structure–function studies (Fig. 1b).[20,32–34] Substitution of hydrogen with more strongly electronegative halogens at the 5-position of the ring decreases the ligand pK_a for deprotonation at the 3-position and increases ligand potency and binding affinity for AMPA receptors in the order HW<IW<BrW<FW.[20,32–36] In contrast, the peak current and extent of receptor desensitization vary according to the size of the 5-position substituent as IW<BrW<FW<HW.[20,32,33] Insights into the structural basis of the latter trend were gained from the determination of several crystal structures of S1S2 complexes with willardiine derivatives. A subset of these structures displays degrees of lobe closure that also vary with substituent size according to IW<BrW<FW<HW (Fig. 1a), implying that the extent of lobe closure influences receptor response.[20] However, the crystal structures do not provide an explanation for the correlation between ligand potency and 5-position electronegativity, suggesting that dynamics not evident in these structures may be important.

This is not meant to imply that the crystal structures are completely static. In fact, the structures of several S1S2–ligand complexes display global and/or local conformational variability. For example, IW-S1S2 protomers crystallized in the absence of zinc show differences in their degrees of lobe closure,[20] and BrW- and IW-S1S2 in the absence of zinc show smaller extents of lobe closure relative to forms crystallized in the presence of zinc.[20,28] In addition, several structures of S1S2 exhibit regions of local conformational variability, such as the "peptide flip segment" (Fig. 1a). This region, which comprises residues D651-G653, forms part of the interface between the ligand and the second lobe of S1S2, and in some cases adopts conformations that promote interlobe main-chain hydrogen bonding.[21,25,28] Conformational variability has also been observed in the ligand itself, as can be seen in the crystal structures of IW-S1S2.[20] However, the structure of HW-S1S2, which displays the weakest affinity in the willardiine series, surprisingly shows no indication of conformational variability,[20] although this might possibly be due to differences in crystallization conditions.

Since the extent to which these global and local structural differences depend on crystallization is unclear, we studied the backbone dynamics of the various willardiine-bound S1S2 systems in solution using NMR spectroscopy. Previous NMR backbone studies of GluR2 S1S2 examined the effects of full agonists and identified segments of the protein undergoing motions over a wide range of timescales.[18,37,38] However, as these agonists produce similar degrees of lobe closure in the available crystal structures and similar extents of activation of the corresponding intact receptors, it has been difficult to relate variations in NMR, crystallographic, and electrophysiological observables.

The present article extends previous NMR studies by examining the backbone structural and dynamical differences occurring in S1S2 complexed with HW, FW, BrW, and IW by measurements of main-chain amide chemical shift deviations ($\delta\omega$) and chemical exchange rates (R_{ex}). The $\delta\omega$ between these complexes may identify regions of S1S2 experiencing structural and/or dynamical changes due to differential extents of lobe closure and interaction with ligand. On the other hand, chemical exchange dynamics arising from conformational transitions between two or more chemically unique sites on