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PERSPECTIVE

Acid Tests of *N*-Methyl-D-aspartate Receptor Gating Basics

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In this issue of *Molecular Pharmacology*, Low et al. (2003) delve into the mechanisms by which pH modulates the glutamate receptor subtype specifically activated by *N*–methyl-D-aspartate (NMDA). Based on a combination of scanning mutagenesis, patch clamp physiology, and molecular modeling, the authors conclude that amino acids involved in NMDA receptor modulation by protons are clustered in regions of the receptor that are likely to link agonist binding with channel opening.

pH plays an exceptionally broad role in mammalian physiology. Ion channels constitute a major class of proteins that are modulated by protons. Ion channels that exhibit sensitivity to pH changes near the physiological range include voltage-gated K^+, Na^+, and Ca^{2+} channels, inward-rectifier K^+ channels, gap junction channels, ClC chloride channels, Ca^{2+}-activated K^+ channels, degenerin/epithelial Na^+ ion channels (including acid-sensing ion channels), vanilloid receptors, muscle and neuronal nicotinic acetylcholine receptors, $GABA_A$ receptors, and NMDA receptors. Proton effects on ion channels have been proposed to be mediated by one or more of a diversity of mechanisms, including protonation of: membrane surface charges (Hille, 1968), carbohydrate moieties added by glycosylation (Freeman et al., 2000), and amino acids involved in agonist binding (Abdrakhmanova et al., 2002), the ion conduction pathway (Woodhull, 1973), "ball-and-chain" style channel gating (Morley et al., 1996), and channel gating caused by generalized structural rearrangements (Schulte and Fakler, 2000). Because of the variety of potential actions of protons, dissecting the mechanisms by which pH modulates the activity of any channel type is challenging. Some of the effects of pH on channel function have been studied for decades yet remain incompletely understood.

Interest in the pH sensitivity of NMDA receptor has been spurred by the diverse and powerful effects of NMDA receptor activation on the mammalian nervous system. NMDA receptors have been implicated in a remarkable range of nervous system physiology (from synapse stabilization during development to synaptic plasticity in adults) and nervous system pathology (from schizophrenia to excitotoxic neuronal death after stroke). Thus, it has been proposed that changes in brain pH may provide, for example, protection of neurons from glutamate excitotoxicity. In addition, determining the location and makeup of the "proton sensor" on NMDA receptors may lead to improved understanding of receptor structure. NMDA receptors are thought to be heterotetramers, composed predominantly of NR1 subunits combined with NR2A, NR2B, NR2C, and/or NR2D subunits. Despite extensive research, such basic questions as the nature of the channel gate and how it functions remain unresolved. The insights into proton modulation of NMDA receptors provided by Low et al. (2003) may have important implications for channel gating.

Numerous excellent studies, many involving the authors of Low et al. (2003), underlie our current understanding of NMDA receptor modulation by protons. At physiological extracellular pH, NMDA receptors are about 50% inhibited by protons. Increasing the extracellular pH potentiates NMDA responses, whereas decreasing the extracellular pH leads to full inhibition of NMDA responses. Fitting of $[H^+]$-NMDA response curves reveal in some instances a Hill coefficient near 1, an observation that seems consistent with the idea that protonation of a single proton sensor in NMDA receptors leads to receptor inactivity. In seeming contrast to this straightforward idea, site-directed mutagenesis studies have revealed that remarkably many amino acids, spread over multiple regions of NR1 and NR2 subunits, influence the proton sensitivity of NMDA receptors. In addition, numerous other modulators of NMDA receptor function, including Zn^{2+}, polyamines, and ifenprodil, act, at least in part, by affecting the proton sensitivity of NMDA receptors.

Low et al. (2003) integrate an extensive volume of data on the diverse locations of amino acids in NMDA receptors involved in proton sensitivity. They first expanded the list of amino acids that affect the proton sensitivity of NMDA receptors by making 88 mutations of amino acids in NR1, along with several mutations in NR2. They combined the results of their mutational analysis with data on 53 other NR1 mu-

ABBREVIATIONS: NMDA, *N*-methyl-D-aspartate; ATD, amino-terminal domain.

tants culled from previous publications. They then investigated the likely spatial relation of important amino acids through homology modeling of specific regions of NMDA receptors. Homology models, although of unknown accuracy, often provide useful guesses of protein structure. The results reveal three regions in which amino acids important to proton sensitivity are clustered.

The first region considered by Low et al. (2003) is the extracellular amino terminal domain (ATD) of NR1 (Fig. 1). Amino acids in this region that strongly affect proton sensitivity were previously described, and the ATD of NR2A and NR2B were previously shown to influence the proton sensitivity of NMDA receptors. The amino acids in the ATD of NR1 that most strongly affect proton sensitivity are scattered in the primary structure of NR1, separated by dozens to hundreds of other amino acids. To rationalize this surprising observation, Low et al. (2003) considered the likely three-dimensional structure of the ATD of NMDA receptor subunits. The structure of NMDA receptors has not been solved, nor has the structure of any of its domains. However, several NMDA receptor domains are thought to be homologous to other proteins or protein domains of known structure. A homology model of the ATD of NR1, which exhibits weak sequence similarity to bacterial leucine/isoleucine/valine binding proteins (LIVBP; O'Hara et al., 1993), was based on the known structure of a related region of metabotropic glutamate receptors. The homology model revealed that the amino acids that most strongly affect proton sensitivity in the NR1 ATD actually lie close together along a section that links the two lobes of this domain.

The authors found two other regions that are more critical to proton sensitivity than the ATDs: the two series of amino acids that join M3 to S2 (the M3-S2 linker) and S2 to M4 (the S2-M4 linker; Fig. 1) of both NR1 and NR2 subunits. The S2 region, which constitutes most of the long extracellular loop that separates transmembrane regions M3 and M4, and the S1 region form the agonist binding site on each NMDA receptor subunit. The amino acids that make up the M3-S2 linker are thought to be critical in transducing agonist binding into channel gating (Qian and Johnson, 2002). Based on the observation that some of the M3-S2 linker amino acids also regulate the proton sensitivity of NMDA receptors, the authors suggest a close connection between the proton sensor and the channel gate. Based on the observation that amino acids in the S2-M4 linker also strongly affect proton sensitivity, the authors suggest that amino acids on both sides of the S2 region may play related roles in channel gating. Note, however, that NMDA receptors can still gate even when the M4 region is physically separated from the S2 region (Schorge and Colquhoun, 2003). To determine the likely spatial proximity of the amino acids in the M3-S2 and S2-M4 linkers that affect proton sensitivity, the authors again used a homology model. This time the model was based on the known structure of a construct of the S1 and S2 regions of a glutamate receptor closely related to NMDA receptors (Armstrong et al., 1998). As was found in the ATD region, the model revealed that the amino acids important for proton sensitivity on opposite sides of the S2 region nevertheless are spatially clustered. The potential association of the proton sensor and the channel gate was further investigated using homology models of NMDA receptors based on the bacterial K^+ channels KcsA and MthK, which are thought to be structurally related to glutamate receptors. The crystal structures of both of these bacterial channels have been solved, probably with KcsA in the closed state and MthK in the open state (Jiang et al., 2002). These homology models showed that amino acids in the M3-S2 linker that affect proton sensitivity are strategically located near a potential channel gating region.

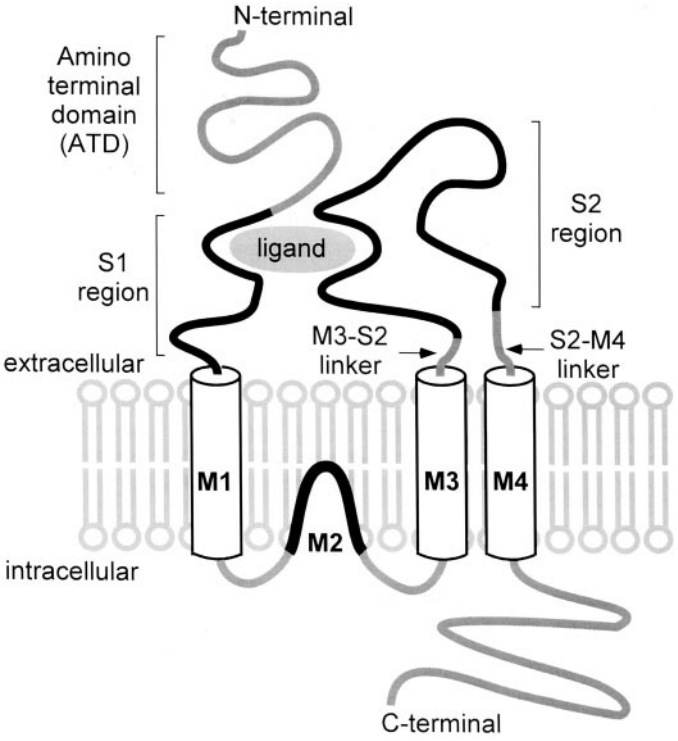

Fig. 1. Schematic of the topology of a single NMDA receptor subunit. Regions important to the conclusions of Low et al. (2003) are identified. The structural features illustrated are shared by all NR1 and NR2 subunits. Changes of shading of the subunit are used only to distinguish different regions.

The findings of Low et al. (2003) should help focus questions for future studies. A fundamental question for future work is: where is the actual proton sensor on NMDA receptors? The authors' results point to the M3-S2 and S2-M4 linkers. It will be particularly challenging to determine how such a large group of amino acids can cooperate to form and influence a proton sensor that acts in some respects as a single entity. Additional intriguing questions involve the ATDs: how do they interact with a distant proton sensor, and more generally, what are their functional roles? Low et al. (2003) do not favor the idea that any of the ATDs form the actual proton sensor. However, the similarities of the glutamate receptor ATDs to amino acid binding proteins suggest that the ATDs are involved in the binding of ligands. Zn^{2+} seems to be the high-affinity endogenous ligand bound by the ATD of NR2A (Choi and Lipton, 1999; Fayyazuddin et al., 2000; Low et al., 2000), but no endogenous ligand of any of the other NMDA receptor ATDs has been identified. Finally, what is the relation between the proton sensor and the channel gate? With luck, further acid tests may reveal the basics of channel gating.

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