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Ion permeation in ionotropic glutamate receptors: Still dynamic after all these years

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Abstract

Ionotropic glutamate receptors (iGluRs) are ligand-gated ion channels that mediate the vast majority of fast synaptic transmission in the nervous system. When the iGluR ion channel is in the open or conducting conformation, it is non-selective for monovalent cations, driving membrane excitation. Often the channel is also permeable to Ca^{2+}. This process of Ca^{2+} permeation and its physiological and pathological consequences depend strongly on the specific iGluR subtype as well as the specific subunits in the oligomeric complex. Recent evidence has highlighted additional levels of diversity to this process including a dependence on specific auxiliary subunits in non-NMDARs and post-translational modifications in NMDARs. Various *de novo* missense mutations associated with neurological disease in NMDAR subunits have been identified in regions critical to Ca^{2+} influx. These features highlight the dynamics of Ca^{2+} influx mediated by iGluRs and its critical role in synaptic physiology and pathology.

Keywords

AMPA receptors; kainate receptors; NMDA receptors; Ca^{2+} permeability; auxiliary subunits; post-translational modification

1. Introduction

We have learned in school, or should have learned, that a fundamental determinant of ion channel function is the ions it allows to pass when in the open or conducting state [1]. Consider ionotropic glutamate receptors (iGluRs), the ligand-gated ion channels that mediate fast excitatory synaptic transmission in the brain. Their ion channel is cation non-selective, being about equally permeable to both K^+ and Na^+ ions [2]. At rest, activation or opening of iGluRs is excitatory because of the strong driving force for Na^+ influx. The iGluR ion channel evolutionarily originated from a two transmembrane K^+ channel [3]. If iGluRs retained their ancient permeability properties of being selectively permeable to K^+

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rather than being cation non-selective, the contribution of iGluRs to membrane physiology would be the exact opposite: K^+-selective iGluRs would be inhibitory rather than excitatory.

Being non-selective to monovalent cations mediates the role of iGluRs in brain excitation. iGluRs also often affect cell-to-cell signaling independent of direct membrane excitation. Some iGluR subtypes can function as metabotropic receptors, acting independent of ion flux [4]. In many instances, the iGluR ion channel is permeable not only to monovalent cations but also Ca^{2+}. Although the charge associated with Ca^{2+} may contribute to excitation, Ca^{2+} predominantly acts as a biochemical signaling molecule. Indeed, this Ca^{2+} signaling often dominates how we think of iGluR function, contributing to their role in synaptic dynamics, neuronal development, and cellular pathology [5,6]. After reviewing some basic features of Ca^{2+} permeation, I will focus on recent efforts that have further highlighted the regulation and dynamic role of Ca^{2+} permeation in iGluRs.

2. The permeation pathway in iGluRs

There are three iGluR subtypes: AMPA (AMPAR), kainate (KAR), and NMDA (NMDARs) receptors, which are composed of various subunits (Figure 1). These receptors share a similar overall structure [7–9], including the topology of the pore-forming or ion permeation pathway.

In iGluRs, the permeation pathway is largely formed by the M3 transmembrane segment and the intracellular M2 pore loop (Figure 1A). This arrangement is like that of a K^+-selective channel, but is inverted in the membrane with the pore loop on the intracellular rather than the extracellular side of the membrane. Within the M2 loop resides a key site for ion permeation, the Q/R/N site (Figures 1A & 1B)(the Q/R site for non-NMDAR subunits and the N site for NMDAR subunits)[10]. The Q/R/N site is located near the tip of the M2 loop and contributes to the narrow constriction in the pore where waters of hydration of permeant ions are stripped for recognition [1]. In non-NMDARs, the Q/R site is edited, at the mRNA level, to encode the positively charged arginine (R) in GluA2 and GluK1 and GluK2, which renders the channel Ca^{2+} impermeable (Figure 1B)[10]. Because of the dominance of the Q/R site in terms of pore properties (single channel current, channel block and Ca^{2+} permeation), AMPARs are distinguished into GluA2-containing (Ca^{2+}-impermeable AMPARs) and GluA2-lacking (GluA1, GluA3, GluA4, or Ca^{2+}-permeable AMPARs) AMPARs. Although KARs (GluK1-K2) are also edited, this editing is less complete [11]. For KARs, the role of Ca^{2+} influx, or the lack of it for edited subunits, in synaptic development and physiology remains poorly defined.

Di-heteromeric assemblies of NMDARs, containing GluN1 and some combination of GluN2 subunits, have a higher selectivity for Ca^{2+} than non-NMDARs (Figure 1B). This difference is due only in part to the Q/R/N site [2]. One determinant of this higher selectivity is the DRPEER motif in the GluN1 M3 segment [9,12]. Although elements that contribute to ion permeation in NMDARs are largely known, the mechanism of this process remains unclear: it follows Goldman-Hodgkin-Katz (GHK) assumptions at physiological Ca^{2+} [13], but deviates at lower and higher concentrations [2]. Structures of iGluRs especially at higher

resolutions and in the open state and molecular dynamics simulations will provide new insights into the mechanistic basis of Ca^{2+} permeation in iGluRs.

One of the hallmarks of GluN2-containing NMDARs is a strong voltage-dependent block of the pore by extracellular Mg^{2+} [10]. One question that has perplexed biophysicists is how the pore of NMDARs can distinguish between Ca^{2+}, which is permeable, and Mg^{2+}, which is largely impermeable. Determinants of both processes, Ca^{2+} permeation and Mg^{2+} block, are shared in the M2 loop. Part of the distinction between Ca^{2+} and Mg^{2+} must involve differences in hydration energy [1]: Mg^{2+} because of its small size and condensed charge holds on to its water of hydration tightly, whereas the larger Ca^{2+} holds onto these waters much less tightly. Recent efforts involving molecular dynamic simulations have started to define how elements in the M2 loop mechanistically distinguish between Ca^{2+} and Mg^{2+} [14*]. In addition, high resolution structures of the M2 loop in the open state, which are now available for AMPARs [15**], will further allow additional mechanistic insights into how the narrow constriction in NMDAR distinguishes between these two critical physiological ions.

An additional element that remains uncertain is how extracellular permeant ions, Na^{+} and Ca^{2+} in particular, access the central permeation pathway defined by the M3 transmembrane segment (Figure 1A). iGluRs are highly modular proteins (upper panel, Figure 1B). The ligand-binding domain (LBD) is positioned on the extracellular end of the ion channel and is connected to the ion channel-forming transmembrane domain (TMD) by a set of short polypeptide linkers, the LBD-TMD linkers. These linkers are highly dynamic during pore opening and contain numerous charged side chains [16]. They presumably represent pathways or portals for permeant ions to access the central permeation pathway. Nevertheless, how permeant ions cross these portals and their impact on selectivity and single channel conductance levels are unknown. This question is of interest since these linkers are promising sites for modulation of receptor function in the clinic [17].

The NMDAR GluN3 subunits, GluN3A & GluN3B, reduce Ca^{2+} influx when part of a di-heteromeric (GluN1 & GluN3) or triheteromeric (GluN1/GluN2/GluN3) complex [18,19]. This difference presumably reflects the composition of the residues occupying the N site (Figure 1B), though this needs to be directly tested. To do so will require approaches where the subunit composition of NMDARs is well-defined [20].

3. Subunit composition: New subunits in the mix

A major determinant of Ca^{2+} permeability in iGluRs is subunit composition (Figure 1B). The AMPAR subunits GluA1-A4 and KAR subunits GluK1–5 are pore-forming. Native AMPARs and KARs are most likely associated with an assortment of auxiliary subunits, non-pore forming subunits that modulate the cell biology and/or functional properties of the pore-forming subunits [21,22]. Stargazin (γ−2) was the first characterized auxiliary subunit, but these subunits are now expansive, encompassing transmembrane AMPA receptor regulatory proteins (TARPs)(γ-2, γ-3, γ-4, γ-5, γ-7, γ-8), cornichons (CNIH-1, -2, -3), GSG1L, CKAMP-44 (CKAMP-44, -39, -52, -59) and Neto (Neto1 & Neto2). These auxiliary subunits show cell-type and developmental specific patterns of expression and add

many layers of diversity to non-NMDARs, altering receptor trafficking and localization, gating, and properties of the permeation pathway including polyamine block, single channel conductance, and, notable here, Ca^{2+} permeation.

Of the tested auxiliary subunits, stargazin (γ−2 and γ-4) and cornichon (CNIH-2) increase Ca^{2+} permeation in AMPARs by about two-fold [23,24]. In contrast, CNIH-1 has no effect and GSG1L decreases permeability by about 50% [24,25]. These properties parallel those for the general effect of these auxiliary subunits on receptor function: γ-2 and CNIH-2 tend to increase receptor function including decreasing the extent of desensitization and polyamine block and increasing single channel conductance, whereas GSG1L has the opposite effects. This raises two interesting points. The first is physiological: why does nature incorporate auxiliary subunits that either increase (γ-2, CNIH-2) or decrease (GSGL1) all receptor functions? Ca^{2+} influx through Ca^{2+}-permeable AMPARs has a strong effect on synaptic physiology, but the parallel effects on receptor function will increase or decrease both the electrical as well as the biochemical function of Ca^{2+}-permeable AMPARs.

The second question is a structure-function one: how do these various auxiliary subunits impact the permeation pathway? High-resolution cryo-EM structures of auxiliary subunits in complex with AMPARs highlight the close contact between membrane spanning domains [26–29] and give guidance but do not reveal mechanistic features. The effect of stargazin on receptor function depends on the residue occupying the Q/R site [30], suggesting that auxiliary subunits might alter the local structure of the narrow constriction, though they do not change pore size [31]. On the other hand, the intracellular C-terminus of CNIH-2 affects pore properties [24], possibly by directly interacting with intracellular determinants of the pore. Nevertheless, how auxiliary subunits mechanistically alter pore properties, especially Ca^{2+} permeation, remains unclear.

The most prominent auxiliary subunits in KARs are Netos (Neto1 & Neto2). Although Netos can alter pore block, they have no apparent effect on Ca^{2+} permeability [32]. Interestingly, Netos apparently reduce polyamine block by enhancing its permeation apparently by changing the structure of the pore loop [33]. What is needed to fully clarify this mechanism of action are high resolution structures of KAR-Neto complexes.

4. Dynamic regulation of Ca^{2+} permeation in NMDARs at synapses

Given the importance of Ca^{2+} influx to synaptic physiology and dynamics, nature finds way to increase functional diversity. Indeed, the magnitude of Ca^{2+} permeation in NMDARs can be regulated in a use-dependent manner at synapses [34,35]. This dynamic action is associated with the GluN2B subunit. At least one of the pathways for it occurs via protein kinase A modulation [34] of Ser1166 in the GluN2B C-terminal domain (CTD) [36*]. This dynamic action has clear biological effects impacting synaptic plasticity, learning and memory, and emotional responses to stress [34–36*].

How phosphorylation of the intracellular CTD modulates pore properties of the receptor is completely unknown. Functional studies of the CTD have been hindered by a lack of

structural information, as the CTD has been excluded from all available iGluR structures [37]. Further complicating a structural understanding is that the GluN2B CTD contains significant regions of intrinsic disorder [38,39**]. The CTD in NMDARs interacts with lipids [40*], which might in some way disrupt the overall structure of the transmembrane domain, where interactions among pore forming elements are important to permeation properties [41]. Alternatively, the CTD is directly connected to the M4 transmembrane segment, which has strong effects on receptor function [42]. Conformational changes in the CTD, induced by post-translation modifications, might alter the orientation of M4 in the membrane, which in turn alters pore properties. In any case, how the conformation of the CTD, driven by post-translational modifications, affects receptor function including pore properties and receptor gating is an area of intense investigation [43]. To define how the CTD impacts receptor function will require integration of various approaches including electrophysiological and optical approaches as well as single molecule techniques that allow details of the conformation of the CTD under various conditions to be ascertained.

5. *De novo* and inherited missense mutations in NMDAR in neurological disorders

The role of iGluRs in neurological diseases has a long history. Recently, an ever expanding list of *de novo* and inherited missense mutations in NMDAR and AMPAR subunits have been identified in patients with neurological disorders including epileptic encephalopathy, intellectual disability, autism, schizophrenia, and motor dysfunction [44–46]. Functionally, most of these mutations have not been characterized in detail, though when characterized, they often have clear effects on receptor function [47–49].

Missense mutations have been identified in the M2 pore loop (Figure 1C) and, when tested, affect Mg^{2+} block [50–54]. In the one instance where tested, which occurred for the positively charged lysine (K) at the N site in GluN2A (Figure 1C), Ca^{2+} permeation was strongly affected [50]. Given the central role of M2 in Ca^{2+} permeation, other M2 loop missense mutations presumably also alter Ca^{2+} though this remains untested. Understanding how these missense mutations might alter Ca^{2+} influx has strong implications for their clinical pathology as well as the mechanism of the permeation process itself. As such, it remains an area of intense interest.

6. Concluding remarks

While the Ca^{2+} permeability of iGluRs and its biological importance was discovered some time ago, it remains an important topic of study. Many mechanistic questions remain such as how the conformational status of the CTD impacts pore properties. Further, Ca^{2+} permeation is not a static property solely dictated by the subunit composition of traditional subunits but depends also on auxiliary subunits, and we do not understand how these changes in Ca^{2+} permeation induced by different auxiliary subunits impact synaptic dynamics including plasticity. Finally, how missense mutations in the M2 loop affect Ca^{2+} permeation and clinical pathologies has not been well established.

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Abbreviations

AMPAR	AMPA receptors
KAR	kainate receptors
NMDAR	NMDA receptors
ATD	amino-terminal domain
LBD	ligand-binding domain
TMD	transmembrane domain
CTD	C-terminal domain

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One star, of special interest

Two stars, of outstanding interest

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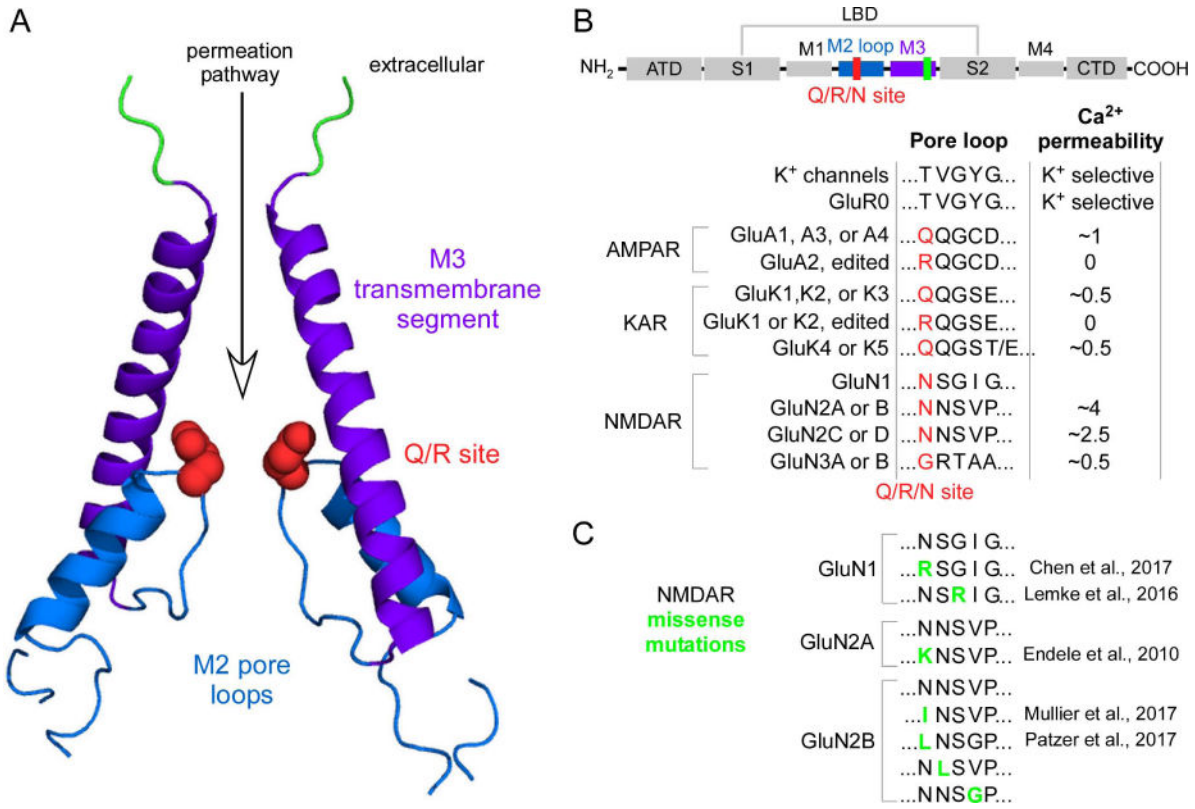

Figure 1. Structure of the permeation pathway and relative Ca^{2+} permeability in iGluRs

(A) Open state structure of an AMPAR (GluA2) (PDB, 5WEO) [15]. The permeation pathway in iGluRs is mainly defined by the M3 transmembrane segment and the M2 pore loop. At the tip of M2, and contributing to the channel's narrow constriction, resides the Q/R/N site (red)(Q/R site, non-NMDARs; N site, NMDARs), a key determinant of ion permeation in iGluRs. In NMDARs, an additional site in the GluN1 M3 segment, the DRPEER motif, also contributes to Ca^{2+} influx [12]. Homologous residues in the AMPAR structure are highlighted in green.

(B) Residues at and around the Q/R/N site in various iGluR subtypes and subunits and associated Ca^{2+} permeability. Top cartoon, domains in iGluR subunits: the extracellular amino-terminal (ATD) and ligand-binding (LBD) domains; the transmembrane domain (TMD), transmembrane segments M1, M3, and M4 and an M2 pore loop; and the intracellular C-terminal (CTD) domain. Single letter amino acid code is shown for the N site (in red) and four adjacent positions located to the C-terminal side.

Ca^{2+} permeabilities (P_{Ca}/Pmonovalent) are approximate and shown just for illustration but are primarily based on fractional Ca^{2+} currents (see Table 18 in Traynelis et al. (2010), where specific values and original references can be found. Additional data was taken from [41]). GluR0 is a prokaryotic iGluR that is K^+ selective [3].

iGluRs are tetrameric complexes composed of the same (monoheteromeric) or two (diheteromeric) or 3 (triheteromeric) different subunits. AMPAR (GluA1-A4) and KAR (GluK1-GluK3) subunits can form monoheteromers but are typically diheteromeric assemblies in native tissue. KAR GluK4 and GluK5 form di- or tri-heteromeric complexes

with the other KAR subunits. NMDAR are obligate di- or tri-heteromers, being composed of GluN1 and some combination of GluN2 (GluN2A-2D) or GluN3 (GluN3A-3B).
(C) *de novo* missense mutations (residues in green) near the tip of the M2 loop. See original publications for clinical phenotype.