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Review

Amino terminal domain regulation of NMDA receptor function

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Abstract

N-Methyl-D-aspartate (NMDA) receptor function is modulated by a wide variety of compounds, several of which appear to bind to globular extracellular amino terminal subunit domains (ATDs). This review focuses on modulators with putative binding sites in ATDs of NMDA receptor subunits, and potential mechanisms by which these compounds exert their effects on receptor function. With an overview that stresses several themes, we explore evidence that the ATDs of NR2 subunits appear to bind modulatory compounds in the cleft of a clamshell-like structure that is analogous to the ligand-binding domain. This modulation influences NMDA receptor function only partially, is dependent on extracellular pH, and affects receptor desensitization. Modulation of the NMDA receptor by the ATD is considered within a framework of functional modularity of multisubunit ion channels. We also consider the potential importance of the ATD in assembly of the receptor.

Keywords: *N*-Methyl-D-aspartate receptor; Amino terminal domain; Modulation; Polyamine; Ifenprodil; Zinc

Contents

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1. Introduction

Ionotropic glutamate receptors mediate excitatory neurotransmission in the central nervous system through the ligand-induced opening of ion channels (reviewed in Dingledine et al., 1999; McBain and Mayer, 1994). Activation of the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors has been linked to long-term potentiation and depression, as well as neuronal development (Dingledine et al., 1999). Additionally, overactivation of the NMDA receptor is a well-documented causative factor in pathological processes such as ischemia-induced excitotoxicity (Lee et al., 1999) and seizures (Loscher, 1998). Therefore, there is great scientific and clinical interest in elucidating NMDA receptor structure–function relationships in order to develop novel pharmaceutical strategies aimed at modifying its activity.

Despite intense investigation, many questions remain unanswered regarding NMDA receptor composition, structure, and the molecular mechanisms of gating and modulation. NMDA receptors are likely to be tetrameric assemblies of the subunits NR1, NR2, and NR3 (Horning and Mayer, 2004; Schorge and Colquhoun, 2003; Sobolevsky et al., 2004), the best studied of which are those composed of NR1 and NR2 subunits. Cloning of NMDA receptor subunits has revealed that the NR1 subunit is a single gene product with eight splice variants, a–h, while the NR2 subunits are the products of four different genes NR2A–NR2D (McBain and Mayer, 1994). Differential expression of NR1 splice variants and NR2 subunits occurs spatially and developmentally, conferring temporal and regional specificity to NMDA receptor composition (Monyer et al., 1994; Paupard et al., 1997; Prybylowski et al., 2001). In addition to NR1 and NR2 subunits, the NR3A and B subunits have been cloned and studied in heterologous expression systems, and may form receptors with unique properties, albeit yet to be extensively characterized (Al-Hallaq et al., 2002; Matsuda et al., 2002; Perez-Otano et al., 2001).

NMDA receptor subunit and splice variant composition determines the sensitivity of receptors to modulation by a wide variety of compounds. Chemicals such as ifenprodil (Masuko et al., 1999; Williams, 1993), and cyanide (Arden et al., 1998) have subunit-specific actions on NMDA receptors. More importantly, endogenous agents modulate NMDA receptors within physiological ranges and in a subunit-specific manner (McBain and Mayer, 1994). These modulatory agents include polyamines (Williams, 1997b), zinc (Paoletti et al., 1997; Zheng et al., 2001), sulfhydryl reducing and oxidizing agents (Aizenman, 1994; Brimecombe et al., 1997), and protons (Traynelis et al., 1995), among others. This review focuses on the modulation of the NMDA receptor by agents that putatively interact with the amino-terminal domain (ATD) of NMDA receptor subunits. We discuss: (1) modulation of NMDA receptors by binding of zinc and ifenprodil to the ATD of NR2 subunits, (2) modulation of NMDA receptors by putative ATD interactors polyamines and redox agents, (3) potential downstream elements such as a pH-sensor and ligand-binding redox sites, and (4) the putative role of the ATD on subunit assembly.

2. Modular structure of NMDA receptors

Attempts to solve the structure of ionotropic glutamate receptors have shown these proteins to be modular with respect to both structure and function. Homology modeling (Fayyazuddin et al., 2000; Masuko et al., 1999; Perin-Dureau et al., 2002) and structural studies (Armstrong et al., 1998; Furukawa and Gouaux, 2003; Horning and Mayer, 2004; Jin et al., 2002, 2003) reveal ionotropic glutamate receptor subunits to be composed of four major domains: the ligand-binding domain, the pore-forming domain, the intracellular domain and the amino terminal domain (Fig. 1).

The two extracellular domains, namely the ligand-binding site and the ATD, have many similarities. Although this

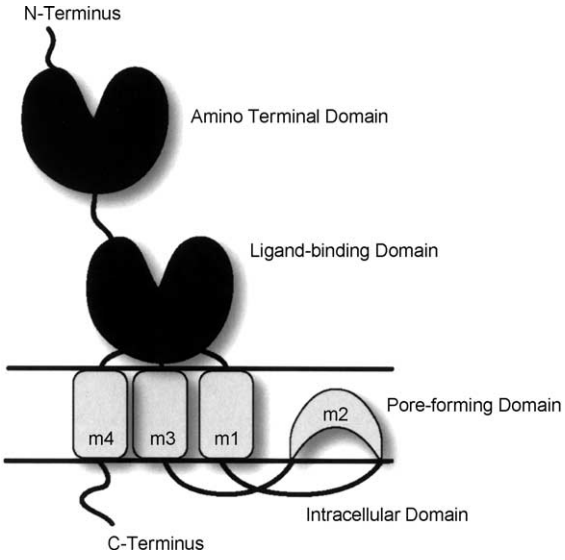

Fig. 1. Modular domains of an NMDA receptor subunit. Cartoon illustrating the main features of NMDA receptor subunit structure, including the clamshell-like domains (amino terminal domain and ligand-binding domain), the pore-forming domain, and the intracellular domain.

review will focus on the function of the ATD, it will rely in part on comparisons with the better-studied ligand-binding domain. As excellent recent reviews exist that outline the structure of the ligand-binding domain of ionotropic glutamate receptors (Madden, 2002; McFeeters and Oswald, 2004), we will only briefly discuss it here in order to highlight its similarities with the ATD.

2.1. The ligand-binding domain

The best-characterized structural domain of the NMDA receptor is the ligand-binding domain, primarily due to the relative ease of determination of changes in the pharmacological properties of glutamate receptors upon perturbation of this functional unit. For NMDA receptors, the binding site for the co-agonist glycine resides within the ligand-binding domain of NR1 (Kuryatov et al., 1994), while the analogous domain of NR2 binds glutamate (Laube et al., 1997). The X-ray crystal structure of the ligand-binding domain of NR1 was recently solved (Furukawa and Gouaux, 2003) and validates predictions from site-directed mutagenesis studies and homology modeling, which suggest that the ligand-binding domain is contained in a globular clamshell-like feature. These ligand-binding domains are conserved among all glutamate receptors (Dingledine et al., 1999; Nakanishi et al., 1990; Nakanishi and Masu, 1994). Ligands bind in the cleft of the clamshell, stabilizing a closed conformation of the two lobes. In a simple model, this movement has been suggested to directly or indirectly gate the channel (Madden, 2002; McFeeters and Oswald, 2004; Qian and Johnson, 2002). Factors that influence the degree of clamshell domain closure affect, in parallel, the degree of activation and desensitization of receptor function (Armstrong and Gouaux, 2000).

Interestingly, this domain appears to maintain function independently from the rest of the protein structure, as ligand-binding domain-only constructs retain binding activity when expressed in isolation. For example, constructs of the ligand-binding domain of NR1 have been expressed in *E. coli* and can bind the glycine site competitive antagonist [^{3}H]MDL105,519 with high affinity (Ivanovic et al., 1998; Neugebauer et al., 2003). Similarly, a ligand-binding domain construct of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor subunit GluR2 (Armstrong and Gouaux, 2000; Armstrong et al., 1998; Jin and Gouaux, 2003) and mutants thereof (Armstrong et al., 2003) have been crystallized, and bind agonists in isolation from the full-length receptor. Furthermore, domain-swapping studies suggest that the ligand-binding domain is a functional unit. For example, ligand-binding domains of kainate receptors can be exchanged with those of non-functional glutamate receptors to produce active receptors with an agonist EC_{50} resembling that of the native kainate receptor (Strutz et al., 2002; Villmann et al., 1997). The ability of the ligand-binding domain to bind ligand in relative isolation suggests that NMDA receptors may have functional modularity throughout their structure (Madden, 2002), as will be discussed more thoroughly below with regard to functional modularity of the amino terminal domain.

2.2. The amino terminal domain

The ATD of eukaryotic glutamate receptor subunits bears a weak homology to the bacterial amino-acid binding protein, leucine isoleucine valine binding protein (LIVBP), which itself has a clamshell-like structure analogous to the ligand-binding domain (Sutcliffe et al., 1996; Wo and Oswald, 1995). The ATD has been referred to as the "LIVBP-like domain" by some groups in reference to this homology (Fayyazuddin et al., 2000; Paoletti et al., 1997, 2000; Perin-Dureau et al., 2002), and "regulatory domain" by others (Masuko et al., 1999), as this domain appears to be an important binding site for various allosteric modulators. It is composed of approximately the first 400 amino acids of the protein, and as of the time of this writing, the structure of this domain had not been solved. Homology modeling with unliganded LIVBP has suggested that the ATD of NR2A is composed of two globular domains with a central cleft (Paoletti et al., 2000). Both globular domains are composed of a beta sheet that is surrounded by alpha helices, and the two lobes of this domain are linked by a three-segment hinge (Paoletti et al., 2000). This domain appears to be globally comparable with the molecular architecture of the ligand-binding domain, and has led to the hypothesis that it may function in analogous manner; via the binding of agents within the clamshell-like domain, and allosteric translation of that binding into alteration of NMDA receptor function.

The subunit dependence of NMDA receptor modulatory agents initially led investigators to assume that if, for example, a modulatory agent is specific for NR2A, then its site of binding and/or action must be present on the NR2A subunit. This approach has proven to be fruitful in determining binding sites in the ATD of NR2A subunits for zinc and for ifenprodil and related compounds in NR2B (Fayyazuddin et al., 2000; Malherbe et al., 2003; Paoletti et al., 1997, 2000; Perin-Dureau et al., 2002; Zheng et al., 2001).

3. Modulation of NMDA receptors by zinc

Considerable progress has been made recently in elucidating the molecular nature of inhibition of NMDA receptors by zinc. NMDA responses in neurons and non-neuronal cells expressing NMDA receptor subunits are modulated by zinc both through a voltage-dependent channel block, and a voltage-independent inhibition (see McBain and Mayer, 1994). Voltage-dependent inhibition of NMDA receptors is present at all NMDA receptor subtypes and is dictated by residues in the pore-forming domain of NMDA receptors. However, voltage-independent zinc

inhibition of NMDA receptor function, while apparent at both NR1/NR2A and NR1/NR2B receptors, is present with a 50-fold higher affinity in NR1/NR2A receptors when compared with NR2B-containing receptors (Chen et al., 1997; Paoletti et al., 1997). NR1/NR2A receptors are thus inhibited by very low concentrations of the metal; that is, concentrations low enough that contaminating levels of Zn^{2+} in recording solutions are sufficient to manifest some degree of block (Paoletti et al., 1997; Zheng et al., 2001).

High-affinity zinc inhibition of NR1/NR2A receptors, hereafter referred to as zinc inhibition, is incomplete, blocking at most 40–80% of the total NMDA-induced current (Chen et al., 1997; Low et al., 2000; Paoletti et al., 1997). Studies revealed zinc inhibition to be involved in the fast desensitization of NR1/NR2A recombinant receptors (Chen et al., 1997; Krupp et al., 1998; Zheng et al., 2001), the molecular basis of which was linked to a high-affinity zinc binding site in the ATD in the NR2A subunits (Krupp et al., 1998; Zheng et al., 2001). An important study also revealed an allosteric interaction between the zinc binding site and the glutamate binding site of NR2A (Zheng et al., 2001). Zheng et al. (2001) characterized the zinc-dependency of fast desensitization of NR1/NR2A receptors, including the observation that mutation of putative zinc-binding residues in the ATD of NR2A eliminates this process. The link between fast desensitization and zinc inhibition suggests that zinc binding to the ATD could allosterically affect the ligand-binding domain in the same subunit. This implies that zinc allosterically increases the affinity of NR2A-containing receptors for glutamate and that the binding of glutamate increases the affinity of the receptor for the inhibitor zinc. Indeed, the sensitivity of NR1/NR2A receptors to zinc is 2.5-fold higher for steady-state currents (presumably desensitized) than for peak currents (Zheng et al., 2001). This study supports predictions from other groups regarding potential mechanisms of desensitization (Paoletti et al., 2000).

What is the molecular basis for this allosteric interaction? This still remains to be fully established; however, progress has been made on the molecular nature of zinc-binding elements in the ATD of NR2A. Mutations of histidine residues (H44G, H128A) in the ATD of NR2A that decrease the affinity of NR1/NR2A receptors for zinc, also significantly decrease the degree of fast desensitization observed (Zheng et al., 2001). According to homology modeling based on LIVBP, these residues are arranged facing each other across a central cleft, and cysteines engineered into the inside of the cleft are inaccessible to modification by the cysteine-modifying agent 2-trimethylammonioethylmethane thiosulfonate (MTSET) in the presence of zinc (Paoletti et al., 2000). Further, trypsin cleavage of isolated NR2A ATD domains is significantly slowed in the presence of zinc (Perin-Dureau et al., 2002). This suggests that zinc binding in the cleft of this domain induces a change in the molecular structure, which then may transduce its functional effects. This allosteric effect appears to be use-dependent and requires the occupancy of the ligand-binding site on the same subunit (Zheng et al., 2001). Paoletti et al. (2000) have hypothesized that the 20-amino acid linker between the LIVBP domain and the ligand-binding domain could transduce zinc-induced LIVBP domain closure to the ligand-binding domain, although this remains to be experimentally evaluated.

Zinc inhibition of NR1/NR2A receptors is proton dependent (Choi and Lipton, 1999; Low et al., 2000; Paoletti et al., 2000; Traynelis et al., 1995; Zheng et al., 2001). In addition, some, but not all, amino acid substitutions in the cleft of the ATD of NR2A that most strongly affect zinc binding are the same residues critical to pH dependency of zinc inhibition (Low et al., 2000). This implies that protonation of a zinc-binding residue may be critical for zinc inhibition, and/or that the zinc binding domains may interact functionally and/or structurally with proton sensitive elements. The interdependency of ATD modulators and pH will be discussed later in more detail.

4. Modulation of NMDA receptors by ifenprodil and derivatives

Ifenprodil and other phenylethanolamines inhibit NMDA responses in an NR2B-specific manner (Dingledine et al., 1999), and produce desensitization of NR1/NR2B receptors via a mechanism that is analogous to zinc-induced desensitization of NR1/NR2A receptors (Zheng et al., 2001). Ifenprodil inhibition is also incomplete and non-competitive (Kew et al., 1998; Legendre and Westbrook, 1991; Masuko et al., 1999; Mott et al., 1998; Williams, 1993). Comparable with the effects of zinc, ifenprodil-like drugs have a slightly higher affinity for presumed desensitized states of NMDA receptors (Kew et al., 1996). In addition, the potency of ifenprodil on NR1/NR2B receptors is highly dependent on pH (Mott et al., 1998).

Several mutagenesis studies (Brimecombe et al., 1998; Masuko et al., 1999; Paoletti et al., 2000; Perin-Dureau et al., 2002) have revealed that the binding site of ifenprodil and related drugs is within the ATD of NR2B. Interestingly, mutant NR1/NR2A receptors, in which the ATD of NR2B has replaced the NR2A ATD, show characteristics of ifenprodil inhibition that are remarkably similar to wild type NR1/NR2B receptors (Perin-Dureau et al., 2002). Trypsin cleavage of isolated NR2B ATDs is significantly slowed by the presence of ifenprodil, and alanine-scanning mutagenesis shows that alteration of four residues, located in the putative cleft of the NR2 ATD, can abolish ifenprodil sensitivity to nearly the same level as replacing the entire amino terminal domain with the NR2A ATD (Perin-Dureau et al., 2002).

However, several other studies have found residues that affect ifenprodil sensitivity in addition to those found within the putative cleft of the ATD. For instance, E201 of NR2B, when mutated, induces a 10-fold reduction in sensitivity to the phenylethanolamines haloperidol and CP101,606, as

measured by single channel responses to NMDA (Brimecombe et al., 1998). In addition, Masuko et al. (1999) showed that mutation of several residues in the ATD of NR1, not NR2B, had a 50-fold effect on the sensitivity of NR1/NR2B receptors to ifenprodil. These findings do not support the hypothesis that all molecular determinants of ifenprodil inhibition are located solely in the ATD of NR2B, as it is possible that all these residues may contribute directly to an ifenprodil-binding site. However, the residues on NR1 may be important for stabilization of the binding site through stearic interactions, or perhaps even subunit–subunit interactions.

While it is unknown what, if any, endogenous ligands may interact with the putative ifenprodil site, recent evidence of the clinical efficacy of NR2B-specific compounds against stroke in animal models and humans has brought to the forefront the consideration ifenprodil modulation of NMDA receptor function (Kemp and McKernan, 2002).

Zinc and ifenprodil modulation of NMDA receptors share important characteristics. First, the block by these compounds is highly subunit-specific, and residues in the ATD of NMDA receptor subunits are at least necessary, if not sufficient, for their effects. Second, the effects of both these compounds are partial, pH-dependent, and affect desensitization of the ligand that binds in the same subunit. This suggests that both zinc and ifenprodil derivatives may alter NMDA receptor function via similar allosteric mechanisms. This begs the question as to whether all agents that allosterically modulate the NMDA receptor through binding sites in the amino terminal domain have similar mechanisms and requirements. We will thus briefly outline results describing modulation of NMDA receptors by agents for which the mechanism of action is not completely understood. This list of potential ATD modulators is by no means complete, especially given the continual stream of newly designed and synthesized compounds aimed at modulating NMDA receptor function, as well as the variety of endogenous agents that have been found to alter the receptor via yet undescribed processes.

5. Other NMDA receptor modulators that potentially interact with the ATD

5.1. Polyamines

Although “ligands” for ATD in NR2 subunits have been well characterized, there is less information regarding potential modulatory agents that bind to the analogous region of NR1. One potential ligand of the ATD of NR1 is spermine, as well as related polyamines. Spermine has several effects on NMDA receptor function (reviewed in Williams, 1997b). These include voltage-dependent channel block at high concentrations, as well as current enhancement due to an increase in the affinity of the receptor for glycine. At low concentrations of spermine and saturating concentrations of glycine, an additional enhancement of NMDA-induced currents is observed in NR1/NR2B receptors, hereafter referred to as spermine potentiation (McGurk et al., 1990; Williams, 1997a,b).

Splice variants of the NR1 subunit that contain the 21-amino acid insert exon 5 (NR1b) are not potentiated as strongly by spermine (Traynelis et al., 1995; Williams, 1997a). In addition, current magnitudes of exon 5-containing receptors resemble those of the spermine-potentiated NR1a receptors, implying that exon 5 mimics the actions of spermine (Traynelis et al., 1995). As such, exon 5 contains several positively charged residues that may structurally function like a polyamine (Traynelis et al., 1995). A model of the predicted secondary structure of NR1’s ATD, based on the LIVBP, places two residues critical for spermine potentiation (E181, E185) very close to the site of the exon 5 insert, supporting the hypothesis that exon 5 masks or constitutively occupies a spermine binding site within the ATD of NR1 (Masuko et al., 1999).

Interestingly, mutation of residues in the N-terminus of NR1 that block spermine potentiation, concomitantly decrease proton inhibition (Masuko et al., 1999; Williams, 1997a). This is in agreement with earlier work demonstrating pharmacologically that spermine potentiation of NR1/NR2B receptors is via relief of proton inhibition (Traynelis et al., 1995).

Additionally, early reports showing that spermine decreased NR1/NR2B receptor desensitization (Lerma, 1992) were later explored further, revealing that both spermine and exon 5 can accelerate deactivation of this subunit configuration (Rumbaugh et al., 2000).

5.2. Redox agents

Physiological responses in neurons mediated by NMDA receptors are potentiated by disulfide reducing agents such as dithiothreitol (Aizenman et al., 1989; Tang and Aizenman, 1993b). Conversely, sulfhydryl oxidants such as 5,5′ -dithiobis(2-nitrobenzoic acid) (Aizenman et al., 1989; Brimecombe et al., 1997), lipoic acid (Tang and Aizenman, 1993a), and pyrroloquinoline quinone (Aizenman et al., 1992) are able to reverse dithiothreitol potentiation. In heterologous systems, NMDA receptors containing the NR1 subunit and either the NR2B, NR2C, or NR2D subunits are rendered relatively insensitive to potentiation by reducing agents when NR1a cysteines 744 and 798 are mutated (Sullivan et al., 1994). A potential mechanism for redox modulation of NR1/NR2B, NR1/NR2C, and NR1/NR2D receptors lies in the structure of the ligand-binding domain. Cysteines 744 and 798 of NR1 are located at the hinge of the cleft of NR1, and therefore, it is likely that oxidation state of the disulfide bond in the NMDA receptor determines the flexibility of the hinge (Furukawa and Gouaux, 2003). As the degree of closure of the ligand-binding clamshell correlates with the activity of the channel (Jin et al., 2003), when the NMDA receptor disulfide bond C744–C798 is oxidized, the flexibility of the

ligand-binding hinge is decreased, and closure of the clamshell domain is inhibited. Conversely, in the absence of the disulfide bond, the constraint is relieved, and the clamshell closes more fully. Although redox sensitivity is nearly abolished when NR1a(C744A,C798A) is co-expressed with NR2B, NR2C, and NR2D receptors, NR1a(C744A,C798A)/NR2A receptors remain sensitive to reducing agents to the same extent as that of wild-type NR1a/NR2A receptors (Brimecombe et al., 1999; Kohr et al., 1994). Choi et al. (2001) demonstrated that the remaining dithiothreitol sensitivity of NR1a(C744A,C798A)/NR2A NMDA receptors may be attributable to cysteines in the N-terminal domains of both NR1a(C79,C308) and NR2A(C87,C320). Mutation of all six of these cysteines renders NR1/NR2A receptors insensitive to modulation by dithiothreitol and the oxidizing agent DTNB (Choi et al., 2001).

In addition, Choi et al. (2001) observed that each pair of cysteines appears to have different kinetics of redox modulation, and that this redox modulation cannot be considered independently from zinc modulation of NR1/NR2A receptors. As such, NR1a(C79S,C308S,C744A,C798A)/NR2A(C87A,C320A) receptors also lack high-affinity zinc block. Mutation of each of the putative cysteine pairs has a cumulative effect on zinc inhibition (Choi et al., 2001). It thus appears that the redox state of these three domains, the ligand-binding domain of NR1, and the ATDs of NR2A and NR2B, allosterically determine the modulation of each domain by other types of substances. Perhaps, in analogy with proposed mechanisms of redox modulation of the ligand-binding domain, the ATD "redox sites" determine the configuration, or flexibility, of ATD clamshell-like lobes.

Homology modeling based on LIVBP suggests that ATD redox sites in NR1 and NR2A are homologous to one another, but not spatially comparable to those in the ligand-binding domain (Lipton et al., 2002). They do not appear to be located at the hinge of the proposed clamshell domain of either NR1 or NR2A. In addition, it is not clear from the model as to whether or not these cysteines form disulfide bonds with each other or whether they do so in an *inter*subunit or *intra*subunit manner.

6. Interactions between NMDA ATD modulators and potential downstream elements

6.1. Protons

NMDA-mediated currents are inhibited by protons with an IC_{50} that corresponds to a pH very near the physiological normal range (Traynelis and Cull-Candy, 1990; 1991). This implies, under normal conditions, small changes in extracellular pH can have a profound effect on NMDA receptor function per se. Importantly, the presence of other modulatory substances can dramatically alter the pK_a of the receptor itself. Indeed, alterations in proton inhibition appear to be a common mechanism among polyamine, zinc, and ifenprodil modulation of NMDA receptors. For example, zinc inhibits NR1/NR2A receptors at very low concentrations by enhancing proton inhibition (Choi and Lipton, 1999; Low et al., 2000; Paoletti et al., 2000; Zheng et al., 2001). Amino acid substitutions in the ATD of NR2A are also critical to pH dependency of zinc inhibition, despite a potential proton sensor located in the regions between the ligand-binding domain and transmembrane regions (Low et al., 2003). Likewise, the elements important in ifenprodil inhibition appear to be linked inextricably to proton modulation (Kashiwagi et al., 1996; Masuko et al., 1999; Mott et al., 1998; Williams et al., 1995). It has been suggested that the reason both zinc and ifenprodil inhibition is partial at physiological pH is due to submaximal proton inhibition; the pH sensitivity of residual currents after maximal zinc inhibition seems to confirm this prediction (Low et al., 2000).

A recent study has suggested that the structural components of pH sensitivity of NR1/NR2A are localized very close to the putative gate in NR1 and NR2 subunits (Low et al., 2003). Agents that allosterically affect the gating elements by definition are likely to be affected themselves by the gating elements (Colquhoun, 1998; Monod et al., 1965). This might explain the common pH dependence of many agents that act at the ATD of NMDA receptor subunits. Potentially, conformational changes in the ATD may be allosterically linked to gating through a pH-sensitive structural element. Interestingly, mutations in the ATD that affect the proton sensitivity of ATD modulation by zinc and ifenprodil only modestly affect the sensitivity of the receptor to protons per se, except for those located very close to the site of insert of exon 5 in NR1 (Low et al., 2003).

6.2. Ligand-binding domain redox sites

Mutation of the ligand-binding domain cysteines, NR1(C744,C798) disrupt several forms of ATD modulation. As mentioned above, zinc and redox modulation appear to be intimately related to one another since NR1(C744A,C798A)/NR2A receptors have diminished high affinity zinc inhibition. NR1(C744A,C798A)/NR2B receptors are similarly insensitive to spermine potentiation (Sullivan et al., 1994). Surprisingly, we also have found that this mutation confers spermine sensitivity to otherwise insensitive NR1/NR2A receptors (Fig. 2). Whether mutation of these cysteines also affects ifenprodil inhibition remains to be determined.

6.3. Ifenprodil and spermine interactions

There appears to be a negative allosteric interaction between ifenprodil and spermine, such that binding of spermine to the NMDA receptor results in a reduction of affinity for ifenprodil (Kew and Kemp, 1998). It is worthy to note that concentrations of spermine (1 and 3 mM) used in the Kew and Kemp (1998) study have been shown to

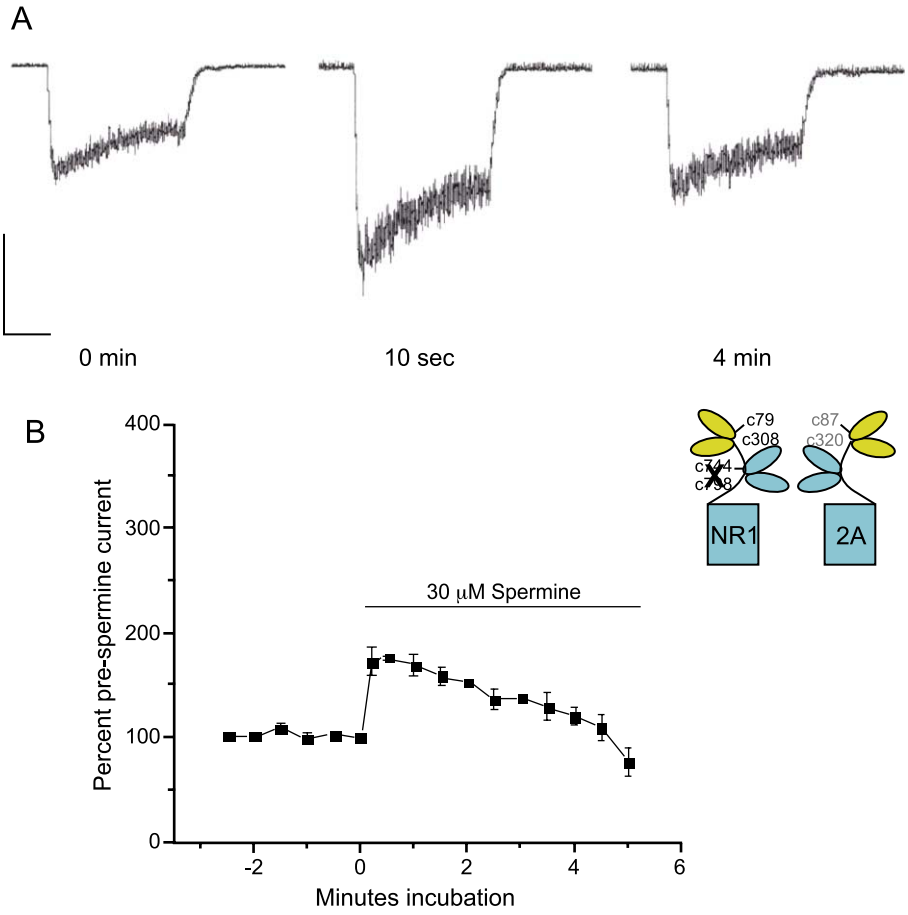

Fig. 2. Spermine transiently potentiates NR1a(C744A,C798A)/NR2A currents. (A) Representative whole cell currents obtained from a Chinese hamster ovary cell transiently expressing recombinant NR1a(C744A,C798A)/NR2A. Scale bar represents 500 pA and 1 s. Currents were measured for 2–3 min in external solution prior to application of 30 μM spermine. Diagram is meant to illustrate relative positions of potential redox sites (see Fig. 1). (B) Average peak current magnitude represented as a percentage of the time 0 magnitude. Bars represent average±S.E.M. (n=5). A one-way analysis of variance between groups (ANOVA) reveals that the difference between the pre-spermine value (−0.5 min) and 0.5 min are highly significant (P<0.001). The value at 4.5 min is not significantly different from −0.5 min (P>0.05) and significantly different from the point at 0.5 min (P<0.001).

inhibit NMDA receptors in a voltage-dependent manner (Williams, 1997b). Therefore, it is unclear whether the actions of spermine in that study are due to the binding of spermine to a site in the ATD, in the channel pore itself, or due to an unspecified mechanism. However, Mott et al. (1998) showed that the presence of 100 μM spermine reduced inhibition of NR1/NR2B receptors by the ifenprodil analogue CP101,606 by 44-fold. It is not known, however, by what mechanism ATD modulators may interact with each other. Potential scenarios include, but are not limited to, subunit–subunit interaction at the ATD and additive interactions through downstream elements.

7. Functional modularity of the ATD

Structurally, ionotropic glutamate receptors appear to be constructed from modular units (Wo and Oswald, 1995). In addition, properties of the ligand-binding and gating suggest that glutamate receptors have “functional modularity.” This means that the conformation change in each subunit and/or domain may not be concerted or “all-or-nothing.” For example, a study by Rosenmund et al. (1998) suggests that ligand binding and “gating” may be linked in a subunit-specific manner. The authors observed subconductance levels that progressed through various steps to reach a full conductance level. Models of waiting times predicted that occupation of the ligand-binding site in each subunit results in a subunit-specific gating event and that the occupation of each binding site is independent of the others. This suggests that each subunit of the receptor has an additive role in the full gating of the channel.

A detailed kinetics study of NR1/NR2B by Banke and Traynelis (2003) also supports some degree of functional modularity for NMDA receptors. The authors demonstrate fast and slow kinetic components associated with gating: the fast component is related to the efficacy of glycine site (NR1) agonists, and the slower component is associated with glutamate site (NR2) agonists (Banke and Traynelis, 2003). While no independent gating events were observed such as those for non-NMDA glutamate receptors, this suggests that the conformational change required for gating

may be directly correlated with the binding of ligand to its own domain.

By comparison, is it true that the NMDA receptor ATD may function in a modular manner? Functional modularity on the domain level would predict that the ATD could bind its "ligand" in relative isolation. Indeed, the studies by Perin-Dureau et al. (2002) have demonstrated that the NR2B subunit-specific ifenprodil inhibition of NMDA receptors can be transferred to NR2A-containing receptors by swapping the ATDs of the respective NR2 subunits. In addition, ifenprodil protects isolated NR2B domains from trypsin digestion, which is suggestive—but not direct—evidence that ifenprodil can bind this domain independently of the rest of the NR2B subunit and/or the entire receptor complex (Perin-Dureau et al., 2002).

Functional modularity of the NMDA receptor ATD at a subunit level would predict that the conformational change induced by binding of agents to the ATD of NR2 would be more likely to affect ligand-binding in same subunit. In support of this idea, zinc and ifenprodil binding to the ATD of NR2A and NR2B, respectively, appear to cause an allosteric intradomain interaction with the glutamate binding site (Zheng et al., 2001).

However, "functional modularity" fails to account for several observations. There appear to be intersubunit interactions, suggesting some degree of cooperativity is required for function of the NMDA receptor. For example, structural elements in the ligand-binding domain are predicted to be responsible for an allosteric interaction between the ligand-binding sites for glutamate and glycine, putatively located in different subunits (Regalado et al., 2001). For the ATD, there are also examples of intersubunit interactions. While both ifenprodil and zinc inhibit NMDA receptor function in a subunit specific manner via binding sites in the NR2 subunit (Fayyazuddin et al., 2000; Low et al., 2000; Paoletti et al., 2000), inhibition by both of these agents is affected by structural elements located in the NR1 subunit, as outlined above. In addition, while more speculative, spermine potentiation is NR2B-specific despite evidence that a binding site may reside in NR1 (reviewed in Williams, 1997b). This does not exclude the possibility that a spermine-binding site may also reside, at least in part, in NR2B.

8. The amino terminal domain as an oligomerization domain

8.1. The role of X-domains in assembly of AMPA receptors

It would be incomplete to discuss the role of the ATD in modulation of fully functional NMDA receptors without consideration of the potential role of this domain in the assembly of the receptor itself. Non-NMDA receptors have been the subject of many studies regarding receptor assembly, targeting, and trafficking. For a recent review of glutamate receptor function including assembly, the reader is referred to Madden (2002). Briefly, the amino terminal domain of non-NMDA receptors, so-called the X-domain, appears to be important in their oligomerization and assembly. A current model of glutamate receptor assembly suggests a preliminary step of family-specific subunit dimerization, followed by dimerization of these dimers to form a tetrameric receptor. It appears that the X-domain is important in family specificity and initial dimerization, but that other molecular determinants are important for tetramer formation, although this remains under debate (for examples, see Ayalon and Stern-Bach, 2001; Greger et al., 2003; Leuschner and Hoch, 1999). Evidence also exists that the X-domain is important for trafficking of AMPA receptors to the cell surface (Xia et al., 2002).

8.2. The role of NMDA ATD in receptor assembly

Though non-NMDA glutamate receptors appear to require the X-domain for preliminary steps of subunit dimerization, less is known regarding the role of the ATD in NMDA receptor assembly. Biochemical (McIlhinney et al., 2003; Meddows et al., 2001) and functional (Schorge and Colquhoun, 2003) studies suggest that NMDA receptors form as a dimer-of-dimers. Additionally, perturbation of the ATD of NR1 compromises formation of functional cell-surface NMDA receptors. Truncation of the first 400 amino acids of NR1 results in a loss of cell-surface expression of NR1/NR2A, as measured functionally in *Xenopus* oocytes and biochemically in human embryonic kidney (HEK-293) cells (Meddows et al., 2001). A loss of immunoprecipitation of NR1 and NR2A subunits accompanied a progressive truncation of the ATD of NR1, suggesting that ATD loss disrupted subunit assembly. Further, it has been observed that the insertion of a flag-tag in the ATD of NR1 prevents functional expression of NR1/NR2A and NR1/NR2B receptors at the cell surface (Papadakis et al., 2004). Although Papadakis et al. (2004) showed that mutation of cysteines in the ATD of NR1 hindered cell-surface expression, functional tests of NMDA receptor function were inconsistent with cell-surface expression results. Interestingly, the cysteines implicated in cell-surface expression of NMDA receptors in this study are also important in the redox modulation of NR1/NR2A receptors (Choi et al., 2001).

9. Remaining questions

In spite of the significant progress in defining the mechanisms of NMDA receptor modulation by agents that bind to the amino terminal domain, there are many questions that still remain unanswered. First and foremost, a structural description of the ATD is necessary. This information would fill several important gaps. For example: What is the relative orientation of the ATD with respect to the ligand-binding domain, and with the ATDs of other

subunits? What type of symmetry does it possess? Homologous domains in other proteins are found both as dimers, such as mGluRs, and tetramers, as in the *lac* repressor (reviewed in Felder et al., 1999). Are the points of contact (if any) between the subunit ATDs important for the mechanism of ATD modulation of NMDA receptors? Mechanistic questions remaining include a fuller description of how "ligand" binding in the ATD affects desensitization in the cases of zinc and Ifenprodil derivatives. Does the degree of clamshell closure affect the mechanism of allosteric modulation, analogous to the ligand-binding domain (Jin and Gouaux, 2003)? How exactly does exon 5 profoundly affect the mechanism of inhibition or potentiation? By what mechanisms are putative downstream elements important in ATD modulation, including the redox site in the ligand-binding domain, as well as the putative proton sensor? New technologies, the drive for clinically tolerable agents that modulate NMDA receptor function in vivo, and emerging data regarding structure–function relationships in related proteins such as non-NMDA receptors will all certainly contribute to the elucidation of plausible explanations to many of these and other questions regarding ATD modulation of the NMDA receptor.

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