Annu. Rev. Pharmacol. Toxicol. 1995. 35:463–82

POLYAMINE REGULATION OF *N*-METHYL-D-ASPARTATE RECEPTOR CHANNELS

David M. Rock
Neuroscience Pharmacology, Parke-Davis Research, Ann Arbor, Michigan 48105, and Neuroscience Program, University of Michigan, Ann Arbor, Michigan 48104

Robert L. Macdonald
Neurology and Physiology Departments, University of Michigan, Ann Arbor, Michigan 48104

KEY WORDS: polyamine pharmacology, spermine, spermidine, NMDA

ABSTRACT

Endogenous polyamines such as spermine and spermidine have multiple effects in the central nervous system and have been suggested to be neurotransmitters or neuromodulators. One effect of the polyamines is to regulate the activity of the *N*-methyl-D-aspartate receptor (NMDAR) channel subtype of glutamate receptor channels. The effects of polyamines on NMDAR currents are complex, suggesting the presence of one or more polyamine-binding sites on the receptor channel. Electrophysiological studies have shown that polyamines enhance NMDAR currents by increasing channel opening frequency and by increasing the affinity of the receptor for glycine. Polyamines have been shown to reduce NMDAR currents by producing voltage-dependent reduction of single-channel amplitudes and/or by producing an open channel block. Recent molecular biological studies have shown that the polyamine effects on NMDAR channels involve interactions with multiple NMDAR subunits and are characterizing the structural basis for the polyamine regulation of NMDAT receptor channels.

Polyamines

Polyamines are aliphatic amines that are polybasic and negatively charged at physiological pH (Figure 1). The charged amine groups are distributed along

0362-1642/95/0415-0463$05.00

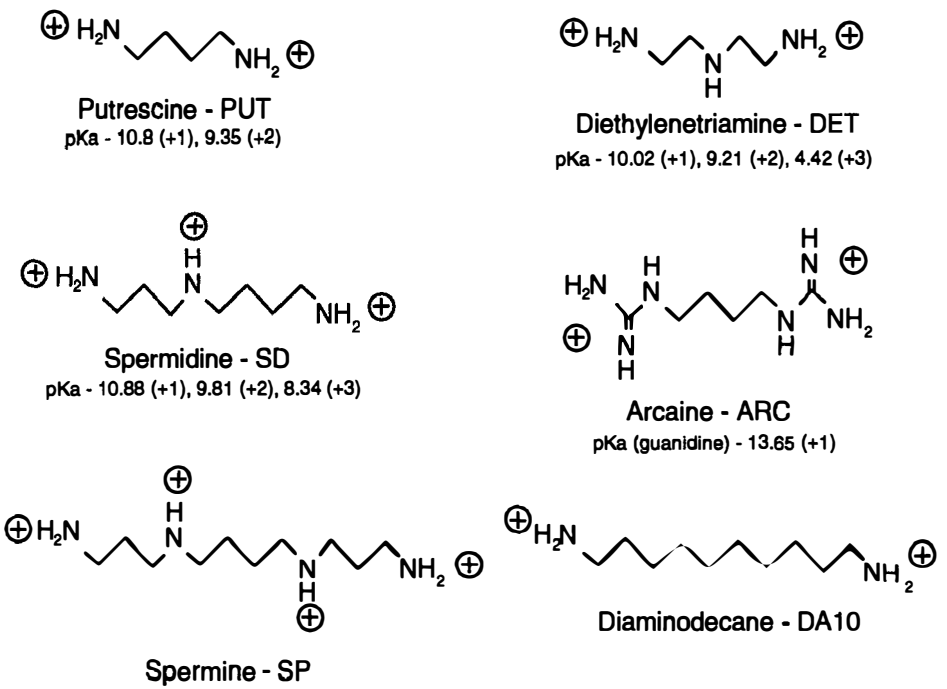

Figure 1 Structure and charge of the endogenous (*left column*) and synthetic (*right column*) polyamines discussed in this review. Net charges (indicated by "+" signs) at pH 7.4 for putrescine (PUT), spermidine (SD), and diethylenetriamine (DET) were estimated from pK_a values for the amine groups, while net charge values for spermine (SP), arcaine (ARC), and DA-10 were estimated from those of structurally similar polyamines. (From Reference 39.)

a carbon-chain backbone, and the orientation and negative charge of these groups are considered important in the electrostatic interaction of polyamines with nucleic acids and proteins (reviewed in 1).

The endogenous polyamines—spermine (SP), spermidine (SD), and putrescine (PUT) (Figure 1)—are the products of ornithine metabolism (Figure 2), which is part of the urea cycle of cellular metabolism (2). In the urea cycle, the enzyme arginase converts the amino acid arginine to ornithine, resulting in the release of urea (2). Ornithine can then be converted to citrulline by adding carbamyl phosphate; this reaction is catalyzed by the enzyme ornithine-carbamyl transferase (2), which is absent in neurons (3). In mammalian cells, ornithine decarboxylase (ODC) decarboxylates ornithine, thereby forming diaminobutane (PUT, putrescine). A propylamine group is transferred from decarboxylated adenosylmethionine to create SD, and a second propylamine grouped is added to SD to form SP (4). Enzymes are

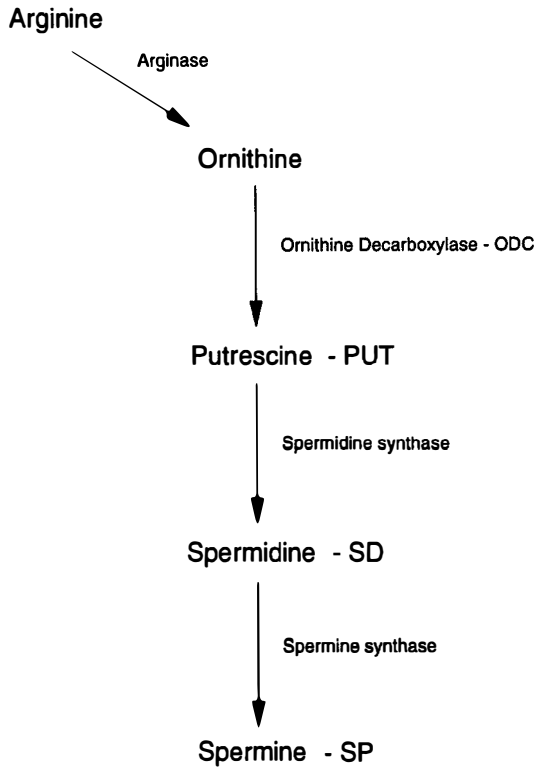

Figure 2 Metabolic pathway for the synthesis of endogenous polyamines from ornithine in mammalian cells.

also present that catalyze the conversion of SP back to SD and of SD back to PUT (5).

The rate-limiting step in the synthesis of polyamines is the conversion of ornithine to PUT by ODC. Polyamine levels are therefore modulated by changes in ODC activity, which is regulated by the rate of synthesis of ODC because of the rapid turnover of this enzyme (turnover time is approximately 10 min).

Polyamines are widely distributed throughout tissue and are found at high intracellular levels, particularly in rapidly dividing cells. Inhibition of polyamine synthesis by inhibition of ornithine decarboxylase stops growth of rapidly replicating cells (4) and affects development (5). Although the exact function of intracellular polyamines is unknown, the interaction of polyamines with the synthesis of DNA, RNA, and proteins indicates a role for these polyamines in the regulation of growth and development (1, 4). Polyamines may also play a

role in the regulation of intracellular and mitochondrial calcium levels (5). Blockers of ODC, which reduce the level of intracellular polyamines, have been tested in clinical trial for the treatment of cancer (4) and have shown efficacy in some models of neurodegeneration (6, 7).

Polyamines are also found at high intracellular concentrations in neurons, suggesting a potential role as neurotransmitters or neuromodulators (1, 4, 8). Evidence from a variety of biochemical and functional studies indicates that the polyamines SP or SD have roles as neurotransmitters or neuromodulators: (*a*) SP and other endogenous polyamines are found at high intracellular concentrations (1, 4); (*b*) SP and SD are released in a Ca^{2+}-dependent manner following either chemical, electrical, or K^+ stimulation (9–11); (*c*) a high-affinity uptake system exists for polyamines for regulation of extracellular polyamine levels and termination of polyamine effects (10, 12); and (*d*) polyamines have been shown to interact with ion channels in the central nervous system (CNS) and to regulate their activity. Although polyamines have been shown to interact with voltage-gated calcium channels (13, 14) (reviewed in 15), most studies have focused on polyamine interactions with a subtype of glutamate receptor channels, *N*-methyl-D-aspartate receptor (NMDAR) channels. This review focuses on the pharmacology and mechanism of polyamine interactions with NMDAR channels.

NMDAR Channels

In the late 1950s Curtis et al (16, 17) found, by measuring increases in firing frequency of these neurons during application, that iontophoretically applied glutamate and aspartate excited spinal cord neurons in vivo. They suggested that glutamate and aspartate were not acting as neurotransmitters but that their actions were nonspecific and, in fact, were possibly involved in convulsive disorders. In work too extensive to review here, biochemical and electrophysiological evidence has been gathered indicating that glutamate acts as a neurotransmitter in the CNS and is currently considered the major excitatory transmitter in the CNS (reviewed in 18–20).

The discovery of selective agonists and antagonists clearly demonstrated that there are several subtypes of glutamate receptors in the CNS (reviewed in 18–20). Subtypes of postsynaptic glutamate receptors have been classified on the basis of selective agonists (18, 20) and can be divided into three broad categories: two ion channel receptor classes and a group of G protein–linked receptors. One of the ion channel subtypes is selectively activated by the NMDA and is selectively blocked by phosphonic acid derivatives (19, 21). The other ion channel subtypes are activated by the selective agonists α-amino-3-hydroxy-4-methyl-5-isoxazolepropionic acid (AMPA) and/or kainic acid (KA) and are selectively blocked by quinoxalinediones (19).

Polyamine Interaction with NMDAR Channels

Ransom & Stec (22) first showed that at low micromolar concentrations the endogenous polyamines SP and SD enhanced the binding of the open channel blocker MK-801 to NMDAR channels. The polyamine-induced enhancement was caused by an increase in affinity for [^{3}H]MK-801 without an increase in the number of sites available for binding. Higher concentrations of SP and SD were not as effective in enhancing the binding of MK-801, leading to a reduction in enhancement at concentrations in the high micromolar–low millimolar range (Figure 3). Several groups showed similar results in preparations from rat forebrain (23–26), in human cortical membranes (27), and in solubilized NMDAR channels (28, 29). Interestingly, SD was not effective in enhancing MK-801 binding in Triton-treated membranes isolated from cerebellum (26, 30), and polyamines showed variable amounts of enhancement of open channel blocker binding in different forebrain regions (31, 32). The polyamine-induced increase in affinity of MK-801 binding was due to an increase in association rate of MK-801 (24, 26, 28), whereas results of polyamine effects on the dissociation rate of MK-801 varied in different preparations (see 24 and 26). The enhancement of open channel blocker binding occurred even in the presence of saturating glutamate and glycine, indicating a site for polyamine interaction that was distinct from the agonist- and coagonist-binding sites (22, 23, 26–28, 30).

Electrophysiological recordings from neurons and oocytes injected with brain mRNA have shown that polyamines affect NMDAR currents. SP and

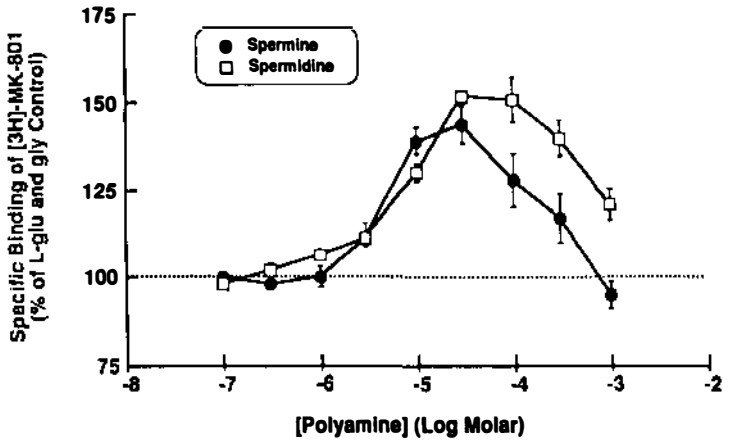

Figure 3 SP- and SD-enhanced [^{3}H]MK-801-receptor binding in membranes isolated from rat brain in a concentration-dependent manner in the presence of saturating glutamate and glycine. The enhancement of MK-801 binding was not as great at higher concentrations of polyamines, leading to a biphasic concentration response curve. (From Reference 23, used with permission.)

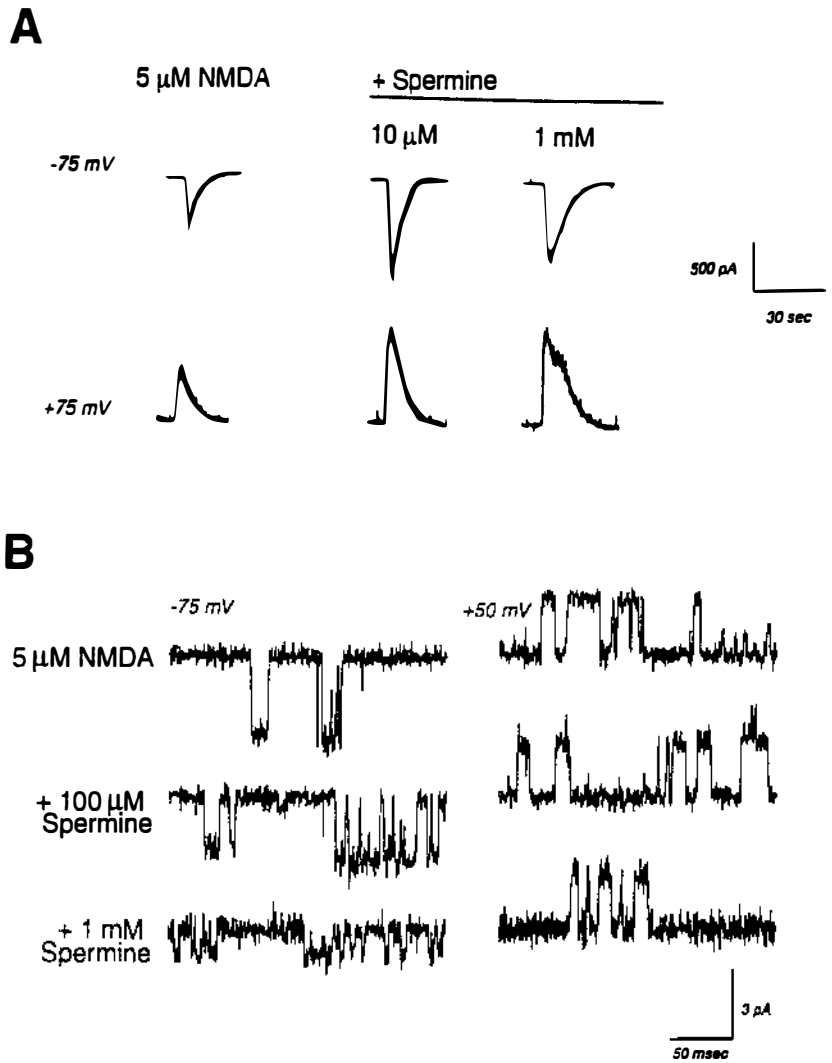

Figure 4 (*A*) Whole-cell voltage-clamp recordings of NMDAR currents were enhanced by SP. Enhancement of 5 μM NMDAR currents by the coapplication of 10 μM and 1 mM SP was voltage dependent; enhancement was limited at higher SP concentrations to a holding potential of −75 mV (*upper trace*). The same neuron shows no difference in enhancement at the same concentrations at +75 mV holding potential (*lower trace*). (*B*) High SP concentrations resulted in a voltage-dependent decrease in NMDA single-channel amplitude. NMDA (5 μM) evoked single-channel openings of around 3.8 pA at −75 mV (*left panel, upper trace*), and single-channel openings were reduced in amplitude by the addition of 100 μM SP (*left panel, middle trace*). A further reduction in amplitude was noted following the addition of 1 mM SP (*left panel, lower trace*). The reduction in amplitude of NMDAR single-channel currents was voltage dependent. At +50 mV holding potential (*right panel, upper trace*), NMDA evoked channels with an amplitude of 2.9 pA. Current amplitude was not changed by the addition of 100 μM or 1 mM SP (*right panel, middle and lower traces*). (From Reference 38.)

SD enhanced voltage-clamped NMDAR current in *Xenopus* oocytes injected with rat brain mRNA (33–35), whereas at higher concentrations of SP (>250 μM) the enhancement was diminished (33). The effect of polyamines on NMDAR currents was partly due to an increase in the affinity of NMDAR channels for the coagonist glycine (33). Whole-cell voltage-clamp recordings from cultured rat neurons have shown that SP and SD enhanced NMDAR currents, but at higher concentrations a voltage-dependent inhibition was noted in some studies (36–42) (Figure 4*A*). Histamine has also been shown to enhance NMDAR currents in acutely isolated hippocampal neurons, possibly through an action at the same site as SP (43). Other electrophysiological experiments in a cortical wedge preparation (44) and extracellular recordings in vivo (45) have shown that polyamines affect NMDA-mediated processes.

Functional Characterization of NMDAR Channels

Although the main component of ionic current generated by activation of postsynaptic glutamate receptors by synaptically released glutamate is mediated by non-NMDARs, the NMDAR subtype is important in a variety of physiological processes as well as under pathophysiological conditions. Activation of NMDAR channels is necessary in several systems to achieve synaptic plasticity (46). For example, in the CA1 region of hippocampus, activation of NMDARs was essential for initiation of increased synaptic efficiency following tetanic stimulation. This increase in synaptic efficacy has been termed long-term potentiation and is thought to be a cellular correlate of learning and memory (46).

Excessive activation of glutamate receptors has been linked to pathophysiological changes in the CNS (47, 48). NMDARs blockers prevented the formation of a seizure focus in an animal model of partial seizures (49), and in vitro studies indicate that NMDAR activation may be important in triggering delayed cell death following ischemia (47, 48). NMDAR activation may also be important in inducing the neuronal damage that is seen following the development of AIDS (50) and may play a role in a variety of psychiatric disorders (51).

Electrophysiological Characterization of NMDAR Channels

The ion channel associated with the NMDAR is a nonselective cation channel permeable to Na^+, K^+, and Ca^{2+} (52–58). Single-channel studies have indicated that under physiological conditions NMDAR channels open to a variety of conductance levels (52, 59–62), with a main conductance level of 50 pS in rat cortical neurons. The 30 pS conductance level was prevalent in cultured rat cerebellar neurons (59, 61).

NMDAR channels were permeable to Ca^{2+} (52, 54, 57) but were blocked by physiological concentrations of Mg^{2+} and other divalent cations (54, 63–66).

Nicoll et al (46) and Choi (47) suggested that many of the effects that occurred after NMDAR activation were partly due to raising the concentration of intracellular calcium (46, 47).

Pharmacology of Regulatory Sites on NMDAR Channels

Because activation of NMDAR channels is an important trigger for physiological changes in neurons and because overactivation can lead to neurotoxic effects, NMDAR channels are highly regulated (Figure 5). At resting membrane potential, NMDAR channels are blocked by physiological concentrations of Mg^{2+} (63, 65, 67). The Mg^{2+} block of NMDAR channels has been attributed to a voltage-dependent fast channel block that is relieved by depolarization so that current flows through NMDAR channels only under depolarized conditions (52, 63, 65). Unlike other ligand-gated ion channels, a second agonist (coagonist), glycine, must be bound in order for gating of the NMDAR channels to occur (68, 69). NMDAR channels are also sensitive to pH, becoming inactive at lower pH (70–72); they are also sensitive to oxidation and reduction (73, 74). The divalent cation Zn^{2+} can act as a fast open channel blocker, similar to Mg^{2+}, but can also produce voltage-independent changes in gating properties of NMDAR single channels, reducing the open duration

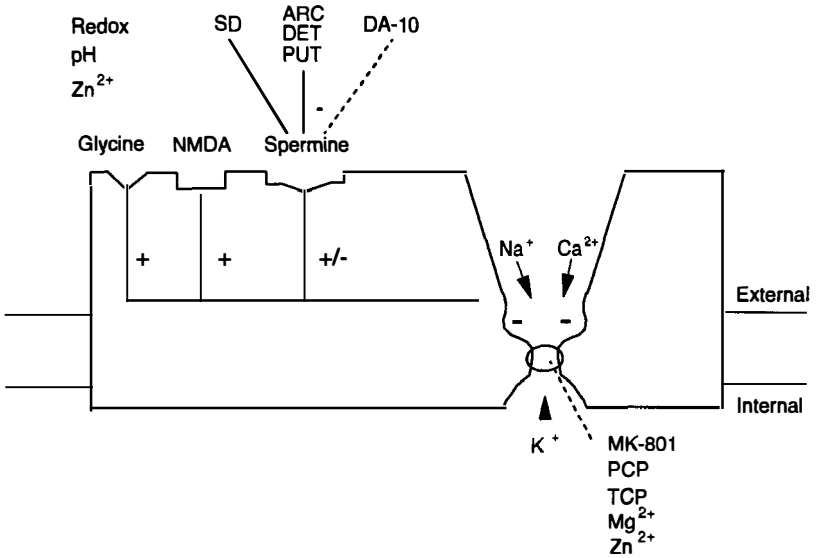

Figure 5 Receptor model outlining regulatory sites on the NMDAR channel. Pharmacology of the polyamine regulatory site is presented as indicated in receptor-binding assays with SP and SD as polyamine agonists; DET, ARC, and PUT as polyamine antagonists; and DA-10 as an inverse agonist acting at the polyamine site. The +/– indicates that polyamine agonists have both enhancing as well as inhibiting effects on NMDAR channel function.

and frequency of channel opening (75, 76). Dissociative anesthetics—such as dizocilpine (MK-801) (77), ketamine, and phencyclidine—interact with NMDAR channels to reduce current by an open channel block mechanism (reviewed in 78). A polyamine regulatory site on the NMDAR channels has also been described (see above).

Pharmacology of the Polyamine-Binding Site on NMDAR Channels

Short-chain diamines (diaminopropane), structurally related triamine polyamines, and aminoglycosides enhance the binding of [^{3}H]MK-801 in a fashion similar to that of SP and SD (23, 24, 26, 79–82). Polyamines that enhanced the binding of open channel blockers to NMDAR channels have been designated agonists at the polyamine site (37).

Structurally related polyamines that blocked the SP- or SD-induced enhancement of open channel blocker binding have been discovered by a number of groups and have been termed polyamine partial agonists or antagonists (23, 24, 26, 37, 79, 80, 83–86). The endogenous polyamine PUT was shown to be a noncompetitive antagonist of polyamine enhancement of open channel blocker binding (23, 24, 26, 29, 80). The synthetic polyamines diethylenetriamine (DET) (23, 37, 80) and arcaine (ARC) (structures in Figure 1) (83, 84, 86), as well as several structurally related polyamines (80, 85, 87), also reduced the polyamine enhancement. These compounds had little or no effect on receptor-binding assays in the absence of polyamines and therefore had the profile of a polyamine antagonist.

Long-chain diamines had a distinct action on the binding of open channel blockers to NMDAR channels. Diamines with eight or more carbons between the primary amines reduced the binding of open channel blockers in the absence of added polyamines (23, 26, 37, 88). The potency of the long-chain diamines increased as the chain length increased (88). The reduction of open channel blocker binding was reversed by the addition of the polyamine antagonist DET (26, 37). On the basis of this evidence, these long-chain diamines have been classified as inverse agonists acting at the polyamine site.

Using the receptor-binding data of various polyamine agonists, antagonists, and inverse agonists, Molinoff's group proposed a model for polyamine interactions with NMDAR channels. In this model polyamines have multi-point interactions with negatively charged or electron-rich aromatic moieties on the surface of NMDAR channels (80). Based on structural data from rigid polyamine analogues, the binding sites appeared to be separated by 5 Å, and interaction with three sites was required for compounds to have an agonist action; antagonists interacted with only two sites (80). To accommodate inverse agonists, a forth interaction site approximately 12 Å from the agonist sites was postulated to be occupied by polyamine inverse agonists (80).

Mechanisms of Polyamine Interactions with NMDAR Channels

Polyamine enhancement of MK-801 binding resembled the effect of divalent and monovalent cations on open channel blocker binding. Many divalent cations (Ca^{2+}, Ba^{2+}, and Mg^{2+}), as well as monovalent cations (Na^{+} and K^{+}), enhanced binding of MK-801 at low concentrations and were less effective at higher concentrations (89–91), which was similar to polyamines. These studies suggested that the effects of polyamines in receptor-binding assays may be partly due to their polyvalent character.

Polyamines had effects on the binding of agonists and antagonists of the glutamate and glycine recognition sites of NMDAR channels. SP has enhanced the binding of competitive antagonists acting at the glutamate agonist site (92, 93), an effect that could be mimicked by the addition of divalent cations (93), but has had the opposite effect on the binding of the agonist [^{3}H]glutamate (92). SP and SD have been shown to enhance the binding of [^{3}H]glycine (94, 95) and decrease the binding of antagonists at the glycine site (96). These allosteric effects of polyamines on binding at the regulatory sites of NMDAR channels may partly explain the enhancement and inhibition of open channel blocker binding by affecting the opening frequency of NMDAR channels.

Electrophysiological studies have shown that polyamines have multiple actions on NMDAR channels (Figure 4). Whole-cell voltage-clamp studies in cultured neurons have shown that a portion of the enhancing effect of SP was caused by an increase in affinity for glycine (41), similar to that in receptor-binding assays, and a decrease in the glycine-sensitive component of desensitization (40, 41). However, polyamines still enhanced NMDAR current in the presence of saturating concentrations of NMDA and glycine, indicating another action of polyamines that is independent of effects at the glycine site (38, 41). Although the increase in glycine affinity occurred in most cells tested (41), the glycine-independent action of polyamines on NMDAR current varied from cell to cell (38, 41). At the single-channel level, the mechanism for the polyamine enhancement of NMDAR current was an increase in the opening frequency of single channels, with no effect on average open duration or average burst duration at low polyamine concentrations (38); there was a report of increased burst duration with polyamine application (42).

Voltage-dependent blocking actions of SP and SD were noted in whole-cell voltage-clamp recordings (38, 39, 41, 42) and in single-channel recordings (38, 39, 42) from cultured neuronal preparations. The voltage-dependent inhibition resulted from a reduction in the amplitude of NMDAR single-channel currents (38, 39, 42) (Figure 4*B*). The data from single-channel recordings indicated that the block was due to either screening of fixed negative charges near the opening of the NMDAR channel pore (38, 39) or an unresolved fast channel block (42). These studies clearly indicate that polyamines have mul-

tiple action on NMDAR channels and these actions may result from interactions with multiple sites on the NMDAR complex.

Polyamine antagonists interacted with NMDAR channels in the absence of added polyamines. The polyamine antagonist DET (37) shared some of the actions of SP and SD; it showed some of the enhancing effects on whole-cell voltage-clamped NMDAR currents (39, 41) and single channel–enhancing and –inhibiting actions (39). Although DET blocked SP or SD enhancement in receptor-binding experiments (37), it was ineffective in blocking the action of SP on whole-cell voltage-clamp NMDAR currents (41) or reversing the voltage-dependent inhibitory actions of SP on single-channel recordings (39). In fact, the inhibitory actions of DET and SP were additive (39). Another polyamine antagonist, ARC, produced a reversible fast open channel block of NMDAR currents (39, 97) and prevented blockade of NMDAR channels by MK-801 (97). ARC, however, did not block the SP-induced enhancement of NMDAR whole-cell voltage-clamped currents (41). These studies indicate that those polyamines that had an antagonist profile in receptor-binding assays interacted with NMDAR channels but did not block the actions of polyamine on NMDAR channels.

The inverse agonist DA-10 (structure in Figure 1) and other long-chain diamines also interact with NMDAR channels. These compounds produce a voltage-dependent block that is consistent with an open channel block mechanism (41, 98–100). The potency of the channel-blocking actions of the diamines increased as the chain length increased (99, 100). The channel-blocking action of DA-10 at the single-channel level could not be reversed by DET (98), and DA-10 could not reverse the action of SP on whole-cell voltage-clamped NMDAR currents (41). These studies indicated that the polyamine inverse agonist had actions on NMDAR channels that were unlike polyamine agonists—actions that could not be blocked by polyamine antagonists.

Variability of polyamine effects on whole-cell voltage-clamped NMDAR currents (41) and on single-channel currents (38) has lead to the suggestion that polyamines act preferentially on subtypes of NMDAR channels (41). Stone (101) has reviewed a variety of receptor-binding and electrophysiological experiments and postulated the existence of subtypes of NMDAR channels. Recent cloning and expression of different subunits of NMDAR channels have provided evidence that polyamines may interact only with certain subtypes of NMDAR channels.

Molecular Biology of NMDAR Channels

NMDAR channels are heteromeric complexes formed by association of multiple subunits. In 1991 Nakanishi's group isolated the first subunit of the NMDAR channel (NR1) by using expression cloning in frog oocytes that had been injected with mRNA isolated from the forebrain of rats (102). NR1

is a 932-amino acid polypeptide with a molecular weight of 105.5 kDa. Based on its hydrophobicity profile, NR1 has large extracellular C and N termini—554 and 110 amino acids, respectively—with four putative membrane-spanning regions. The second membrane-spanning region (TMII) shows homology with the other non-NMDAR clones (e.g. GLUR1-6) and the nicotinic acetylcholine receptor (nAChR) α subunit (102); negatively charged residues flank the TMII region like nAChR channels (103). The TMII region is thought to form a portion of the ion channel pore in non-NMDA and nAChR channels (103–105). Similar clones have been described in rat (106, 107) and mouse (108–110).

NR1 has been shown to have a wide distribution in the CNS (102, 107, 108, 111, 112) and to form functional homomeric channels with a pharmacological profile similar to that of native NMDAR channels (102, 106). Several splice variants of NR1 have been described that showed regional distribution in the CNS (106). These splice variants of NR1 subunits had altered affinity at agonists sites, different pharmacological profiles, and differences in net surface charge (106, 113–115).

A second family of gene products that are subunits of NMDAR channels (NR2) have been identified (107, 108, 110). NR2 subunits are larger than NR1 subunits with an approximate molecular weight of 160 kDa and a similar secondary structure, except for the C-terminal region. The C-terminal region of NR2 subunits, which is greater that 600 amino acids, is much longer than the C-terminal region of NR1 (107, 108). The amino acid sequence of NR2 subunits is only about 20% identical to NR1, but there is between 55 to 70% homology in amino acid sequence between different subtypes of NR2 subunits (107, 108, 110). Expression of NR2 alone did not produce functional NMDAR channels, but coexpression of NR2 and NR1 subunits resulted in NMDA-induced whole-cell currents with larger amplitudes (107, 108, 110).

Four subtypes of the NR2 subunit (NR2A–D) have been described (107, 110–112). These subtypes of NR2 subunits showed differential distribution in the adult CNS (107, 110, 112) and also showed different temporal patterns of expression during development (111). NR2B and NR2D were present prenatally, whereas NR2A and NR2C were first detected by in situ hybridization around the time of birth (111). In adult CNS, NR2A and NR2B were found mainly in the forebrain regions, and NR2C was found primarily in the cerebellum (107, 110). NR2D levels in mature CNS were low and found mainly in midbrain and spinal cord (111, 112).

There are distinct differences in biophysical and pharmacological properties of recombinant NR1 and NR2 channels, depending upon the NR2 subtype present. Single-channel studies have shown that coexpression of NR2 affected the conductance of NMDAR channels (116). Coexpression of NR1 and NR2A or NR2B subunits resulted in single-channel currents with conductances of 38

and 50 pS and an average open duration of 2.8 ms, which were similar to those of NMDAR channels recorded from rat hippocampal or cortical neurons (117). However, coexpression of the NR1 and NR2C subunits resulted in conductance levels of 19 and 36 pS, with average open durations of 0.6 ms (116), resembling results obtained from rat cerebellar NMDAR channel currents (59, 61, 62). There were also differences in the sensitivity to the coagonist glycine; NR1-NR2B and NR1-NR2C receptor channels had a higher affinity for glycine (EC_{50} = 0.3 μM) compared to NR1-NR2A receptor channels (EC_{50} = 2.1–3 μM) (110, 116). By measuring the voltage dependence of the Mg^{2+} block of NMDAR currents, Monyer et al (107, 111) found that coexpression of NR2A or NR2B subunits resulted in greater sensitivity to Mg^{2+} compared to coexpression with NR2C or NR2D subunits. Coexpression of different NR2 subunits also affected the rate of decay of glutamate-induced whole-cell currents (111). The time constant of decay of whole-cell current with coexpression of the NR2A subunit was 120 ms; of the NR2B and NR2C subunits, 380 and 400 ms, respectively; and of the NR2D subunit, 4.8 s (107, 111). These differences in the biophysical and pharmacological properties of the expressions of NR2 subunit subtypes indicate that the changes in the subtype of NR2 subunits could differentially affect the activation of NMDARs.

Point mutations of amino acids in the putative pore region (TMII) of NMDAR channels have identified a region that is important for calcium permeability (104, 105) and divalent cation block of expressed NMDAR channels. However, effects of mutations on the permeability and blocking properties of divalent cations depend upon which subunit is mutated. Replacing asparagine, a negatively charged amino acid, at position 598 of the NR1 subunit, with glutamine, a neutral amino acid, reduced calcium permeability of the mutated NMDAR channels and slightly decreased the Mg^{2+} block, without affecting the blocking action of the open channel blocker MK-801 or the divalent cation Zn^{2+} (109, 118). Replacing glutamate, at position 598, with arginine, a positively charged amino acid, almost completely eliminated calcium permeability and the Mg^{2+} block (118). Interestingly, the effects of replacing asparagine with glutamine at an analogous position, 592, in the NR2A subunit led to a reduction in the Mg^{2+} block without affecting calcium permeability (118), MK-801, or the Zn^{2+} block (109), indicating differential regulation of divalent cation permeability and blockade by subunits of NMDAR channels.

Molecular Sites of Action of Polyamines on NMDAR Channels

Recent studies with NMDAR channels expressed in heterologous expression systems have shown that polyamines have differential effects depending upon the splice variant of NR1 or subtype of NR2 used to express the NMDAR channels. In oocytes expressing NR1 homomeric channels, splice variants of

the NR1 subunit lacking an N-terminal 21–amino acid insert that contained 6 positively charged amino acids ($NR1_a$) showed an enhancement of NMDAR current in response to SP, while NR1 subunit homomeric channels that contained the N-terminal insert did not respond to polyamine agonists (113, 114). Point mutations that replaced the positively charged amino acids in the N-terminal insert restored the sensitivity to the enhancing actions of polyamines (119). The NR2 subunit also played a role in the response of expressed NMDAR channels to polyamines. Coexpression of NR2B with $NR1_a$ resulted in channels that showed both glycine-dependent-enhancing and -independent-enhancing as well as voltage-dependent-inhibiting effects of SP (120), while $NR1_a$-NR2A channels showed only glycine-dependent-enhancing and voltage-dependent-inhibiting effects of SP (120, 121). Coexpression of $NR1_a$ with the NR2C subunit produced channels that showed no effects on NMDAR whole-cell voltage-clamped currents following the additions of polyamines (120). These studies indicated that both NR1 and NR2 subunits play a role in determining the sensitivity of NMDAR channels to polyamines and may explain the variability of polyamine effects in cultured neurons (see 41 and 38). Cultured rat cortical neurons at day 14 in vitro had high levels of mRNA for the NR1 subunit; NR2B and NR2D mRNAs were at a higher levels than NR2A mRNA (122). NR2C mRNA levels were very low in these cultured cortical neurons (122).

Conclusions

In summary, polyamines have multiple actions on NMDAR channels. Receptor-binding experiments have shown that polyamines enhanced the binding of open channel blockers to the NMDAR channel through a site distinct from other regulatory sites on the receptor channel and possibly in a way that is similar to the effects of divalent cations in receptor binding. Electrophysiological experiments, however, indicated that the multiple actions of polyamines were difficult to explain by an action at a single site (Figure 6). Polyamines may in fact interact with multiple sites on the NMDAR complex. Enhancement may occur by actions at the glycine coagonist site to increase activity of NMDAR channels and at a separate site to enhance activity when both glutamate and glycine sites are saturated. Another interaction near or in the opening of the ion channel may lead to the voltage-dependent effect of polyamines on NMDAR currents.

The pharmacology of the polyamine site, as suggested by changes in polyamine-induced effects on open channel blocker binding, was difficult to interpret due to the multi-site interaction, the multiple actions of polyamines, and the indirect nature of the receptor-binding assay. For example, the polyamine antagonist DET did not block the actions of SP; it enhanced and inhibited NMDAR currents in much the same way as did SP. The inverse agonist DA-10

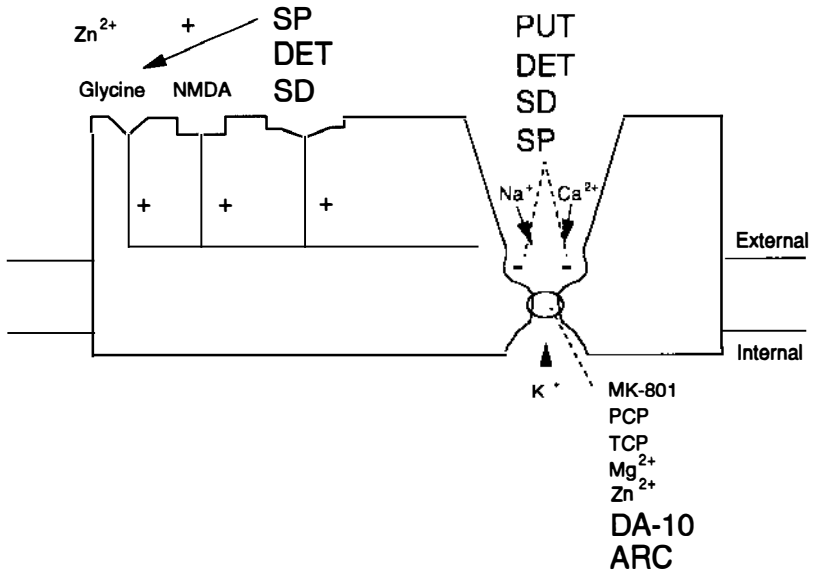

Figure 6 Revised receptor model suggesting potential sites of polyamine interactions with the NMDAR channel. Polyamines act at a site or sites to enhance NMDAR channel function by increasing action at the glycine site (potentially allosteric interaction) as well as by increasing activity through an independent regulatory site. Inhibitory actions of polyamines occur at a site near or in the channel pore, although evidence indicates that DA-10 and ARC act as channel blockers.

blocked the NMDAR current with an open channel block mechanism, an action that was not opposite to the enhancing action of SP, and did not block SP-induced enhancement of NMDAR currents at positive potentials, making the classification of DA-10 as an inverse agonist questionable.

Work on expressed NMDAR channels has provided evidence that polyamines interact with subtypes of NMDAR channels. Polyamine enhancement and inhibition of NMDAR currents occurred with some subunit combinations of expressed NMDAR channels, while other combinations were insensitive to polyamines. Regional variability of polyamine effects in receptor-binding assays as well as cell-to-cell variability of polyamine effects in electrophysiological experiments may be due to polyamines that differentially affect subtypes of NMDAR channels. Future work on the pharmacology and mechanisms of polyamines could focus on expressed NMDAR channels in order to eliminate problems with interpretations that are caused by the interaction of polyamines with multiple sites and subtypes of NMDAR channels.

Literature Cited

1. Seiler N. 1991. Pharmacological properties of the natural polyamines and their depletion by biosynthesis inhibitors as a therapeutic approach. *Prog. Drug Res.* 37:107–60
2. Styrer L. 1981. *Biochemistry,* pp. 411–15. San Francisco: Freeman
3. Bredt DS, Snyder SH. 1992. Nitric oxide, a novel neuronal messenger. *Neuron* 8:3–11
4. Tabor CW, Tabor H. 1984. Polyamines. *Annu. Rev. Biochem.* 53:749–90
5. Paschen W. 1992. Polyamine metabolism in reversible cerebral ischemia. *Cerebrovasc. Brain Metab. Rev.* 4:59–88
6. Porcella A, Fage D, Voltz C, Carter C, Scatton B, et al. 1991. Difluoromethyl ornithine protects against the neurotoxic effects of intrastriatally administered *N*-methyl-D-aspartate in vivo. *Eur. J. Pharmacol.* 199:267–69
7. Kish SJ, Wilson JM, Fletcher PJ. 1991. The polyamine synthesis inhibitor α-difluoromethylorinithine is neuroprotective against *N*-methyl-D-aspartate-induced brain damage in vivo. *Eur. J. Pharmacol.* 209:101–03
8. Williams K, Romano C, Dichter MA, Molinoff PB. 1991. Modulation of the NMDA receptor by polyamines. *Life Sci.* 48:469–98
9. Harman RJ, Shaw GG. 1981. The spontaneous and evoked release of spermine from rat brain in vitro. *Br. J. Pharmacol.* 73:165–74
10. Gilad GM, Gilad VH. 1991. Polyamine uptake, binding and release in rat brain. *Eur. J. Pharmacol.* 193:41–46
11. Fage D, Voltz C, Scatton B, Carter C. 1992. Selective release of spermine and spermidine from the rat striatum by *N*-methyl-D-aspartate receptor activation in vivo. *J. Neurochem.* 58:2170–75
12. Harman RJ, Shaw GG. 1981. High-affinity uptake of spermine by slices of rat cerebral cortex. *J. Neurochem.* 36: 1609–15
13. Herman MD, Reuveny E, Narahashi T. 1993. The effect of polyamines on voltage-activated calcium channels in mouse neuroblastoma cells. *J. Physiol.* 462: 645–60
14. Scott RH, Sweeney MI, Kobrinsky EM, Pearson HA, Timms GH, et al. 1992. Actions of arginine polyamine on voltage and ligand-activated whole cell currents recorded from cultured neurones. *Br. J. Pharmacol.* 106:199–207
15. Scott RH, Sutton KG, Dolphin AC. 1993. Interactions of polyamines with neuronal ion channels. *Trends Neurosci.* 16:153–59
16. Curtis DR, Phillis JW, Watkins JC. 1959. Chemical excitation of spinal neurones. *Nature.* 183:611–12
17. Curtis DR, Phillis JW, Watkins JC. 1960. The chemical excitation of spinal neurones by certain acidic amino acids. *J. Physiol.* 150:656–82
18. Monaghan DT, Bridges RJ, Cotman CW. 1989. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu. Rev. Pharmacol. Toxicol.* 29:345–402
19. Watkins JC, Krogsgaard-Larsen P, Honore T. 1990. Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends Pharmacol. Sci.* 11:25–33
20. Collingridge GL, Lester RAJ. 1989. Excitatory amino acid receptors in the vertebrate central nervous system. *Pharmacol. Rev.* 40:143–210
21. Patneau DK, Mayer ML. 1990. Structure-activity relationships for amino acid transmitter candidates acting at *N*-methyl-D-aspartate and quisqualate receptors. *J. Neurosci.* 10:2385–99
22. Ransom RW, Stec NL. 1988. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor-ion channel complex by L-glutamate, glycine, and polyamines. *J. Neurochem.* 51:830–36
23. Williams K, Romano C, Molinoff PB. 1989. Effects of polyamines on the binding of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor: pharmacological evidence for the existence of a polyamine recognition site. *Mol. Pharmacol.* 36:575–81
24. Sacaan AI, Johnson KM. 1990. Characterization of the stimulatory and inhib-

itory effects of polyamines on [3H]N-(1-[thienyl]cyclohexyl)piperdidine binding to the *N*-methyl-D-aspartate receptor ionophore complex. *Mol. Pharmacol.* 37:572–77

25. Reynolds IJ, Miller RJ. 1989. Ifenprodil is a novel type of NMDA receptor antagonist: interaction with polyamines. *Mol. Pharmacol.* 36:758–65
26. Yoneda Y, Ogita K, Enomoto R. 1991. Characterization of spermidine-dependent [3H](+)-5-methyl-10,11-dihidro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801) binding in brain synaptic membranes treated with triton X-100. *J. Pharmacol. Exp. Ther.* 256:1161–72
27. Steele JE, Bowen DM, Franis PT, Green AR, Cross AJ. 1990. Spermine enhancement of [3H]MK-801 binding to the NMDA receptor complex in human cortical membranes. *Eur. J. Pharmacol.* 189:195–200
28. Romano C, Williams K, Molinoff PB. 1991. Polyamines modulate the binding of [3H]MK-801 to the solubilized *N*-methyl-D-aspartate receptor. *J. Neurochem.* 57:811–18
29. Bakker MHM, McKernan RM, Wong EHF, Foster AC. 1991. [3H]MK-801 binding to *N*-methyl-D-aspartate receptors solubilized from rat brain: effects of glycine site ligands, polyamines, ifenprodil, and desipramine. *J. Neurochem.* 57:39–45
30. Yoneda Y, Ogita K. 1991. Heterogeneity of the *N*-methyl-D-aspartate receptor ionophore complex in rat brain, as revealed by ligand binding techniques. *J. Pharmacol. Exp. Ther.* 259:86–96
31. Subramaniam S, McGonigle P. 1991. Quantitative autoradiographic characterization of the binding of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloh epten-5,10-imine ([3H]MK-801) in rat brain: regional effects of polyamines. *J. Pharmacol. Exp. Ther.* 256:811–19
32. Subramaniam S, McGonigle P. 1993. Regional heterogeneity of polyamine effects on the *N*-methyl-D-aspartate receptor in rat brain. *J. Neurochem.* 60: 2276–84
33. McGurk JF, Bennett MLV, Zukin RS. 1990. Polyamines potentiate the responses of *N*-methyl-D-aspartate receptors expressed in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 87:9971–74
34. Brackely P, Goodnow RJ, Nakanishi K, Sudan HL, Usherwood PNR. 1990. Spermine and philanthotoxin potentiate excitatory amino acid responses of *Xenopus* oocytes injected with rat and chick brain RNA. *Neurosci. Lett.* 114: 51–56
35. Maciver CR, Bednar DL, Karbon W. 1991. Opposite effects of spermine and arcaine on responses of *N*-methyl-D-aspartate receptors expressed in Xenopus oocytes. *Neurosci. Lett.* 132: 146–50
36. Sprosen T, Woodruff GN. 1990. Polyamines potentiate NMDA induced whole-cell currents in cultured striatal neurons. *Eur. J. Pharmacol.* 179:477–78
37. Williams K, Dawson VL, Romano C, Dichter MA, Molinoff PB. 1990. Characterization of polyamines having agonist, antagonist and inverse agonist effects at the polyamine recognition site of the NMDA receptor. *Neuron* 5:199–208
38. Rock DM, Macdonald RL. 1992. The polyamine spermine has multiple actions on *N*-methyl-D-aspartate receptor single-channel currents in cultured cortical neurons. *Mol. Pharmacol.* 41:83–88
39. Rock DM, Macdonald RL. 1992. Spermine and related polyamines produce a voltage-dependent reduction of *N*-methyl-D-aspartate receptor single-channel conductance. *Mol. Pharmacol.* 42: 157–64
40. Lerma J. 1992. Spermine regulates *N*-methyl-D-aspartate receptor desensitization. *Neuron* 8:343–52
41. Benveniste M, Mayer ML. 1993. Multiple effects of spermine on *N*-methyl-D-aspartic acid receptor responses of rat cultured hippocampal neurones. *J. Physiol.* 464:131–63
42. Araneda RC, Zukin RS, Bennett MLV. 1993. Effects of polyamines on NMDA-induced currents in rat hippocampal neurons: a whole-cell and single-channel study. *Neurosci. Lett.* 152:107–12
43. Vorobjev VS, Sharonova IN, Walsh IB, Haas HL. 1993. Histamine potentiates *N*-methyl-D-aspartate responses in acutely isolated hippocampal neurons. *Neuron* 11:837–44
44. Robichaud LJ, Boxer PA. 1993. Polyamine modulation of excitatory amino acid responses in the rat cortical wedge. *Neuropharmacology* 32:1025–35
45. Chida N, Saito H, Abe K. 1992. Spermine facilitates the generation of long-term potentiation of evoked potential in the dentate gyrus of anesthetized rats. *Brain Res.* 593:57–62
46. Nicoll RA, Kauer JA, Malenka RC. 1988. The current excitement about long-term potentiation. *Neuron* 1:97–103
47. Choi DW. 1988. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1:623–34

48. Rothman SM, Olney JW. 1987. Excitotoxicity and the NMDA receptor. *Trends Neurosci.* 10:299–302
49. Rogawski MA. 1992. The NMDA receptor, NMDA antagonists and epilepsy therapy: a status report. *Drugs* 44:279–92
50. Lipton SA. 1992. Models of neuronal injury in AIDS: another role for the NMDA receptor. *Trends Neurosci.* 15: 75–79
51. Olney JW. 1990. Excitotoxic amino acids and neuropsychiatric disorders. *Annu. Rev. Pharmacol. Toxicol.* 30:47–71
52. Ascher P, Nowak L. 1988. The role of divalent cations in the *N*-methyl-D-aspartate responses of mouse central neurons. *J. Physiol.* 399:247–66
53. Ascher P. 1988. Divalent cations and the NMDA channel. *Biomed. Res.* 9 (Suppl. 2):31–37
54. Mayer ML, Westbrook GL. 1987. Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J. Physiol.* 394:501–27
55. Mayer ML, Westbrook GL. 1985. The action of *N*-methyl-D-aspartic acid on mouse spinal cord neurones in culture. *J. Physiol.* 361:65–90
56. Mayer ML, Vyklicky L, Westbrook GL. 1989. Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *J. Physiol.* 415:329–50
57. MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL. 1986. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* 321:519–22
58. Mayer ML, Westbrook GL. 1984. Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. *J. Physiol.* 354:29–53
59. Cull-Candy SG, Usowicz MM. 1987. Multiple-conductance channels activated by excitatory amino acids in cerebellar neurons. *Nature* 325:525–28
60. Jahr CE, Stevens CF. 1987. Glutamate activates multiple single channel conductances in hippocampal neurons. *Nature* 325:522–25
61. Cull-Candy SG, Usowicz MM. 1989. On the multiple-conductance single channels activated by excitatory amino acids in large cerebellar neurones of the rat. *J. Physiol.* 415:555–82
62. Howe JE, Cull-Candy SG, Colquhoun D. 1992. Currents through single glutamate receptor channels in outside-out patches from rat cerebellar granule cells. *J. Physiol.* 432:143–202
63. Nowak L, Bregestovski P, Ascher P, Herbert A, Prochiantz A. 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307: 462–65
64. Westbrook GL, Mayer ML. 1987. Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurons. *Nature* 328:640–43
65. Mayer ML, Westbrook GL, Guthrie PB. 1984. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurones. *Nature* 309:261–63
66. MacDonald JF, Nowak LM. 1990. Mechanism of blockade of excitatory amino acid receptor channels. *Trends Pharmacol. Sci.* 11:167–72
67. Ault B, Evans RH, Francis AA, Oakes DJ, Watkins JC. 1980. Selective depression of excitatory amino acid induced depolarizations by magnesium ions in isolated spinal cord preparations. *J. Physiol.* 307:413–28
68. Johnson JW, Ascher P. 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* 325: 529–31
69. Kleckner N, Dingledine R. 1988. Requirement for glycine for activation of NMDA-receptors in *Xenopus* oocytes. *Science.* 241:835–37
70. Tang C, Dichter M, Morad M. 1990. Modulation of the *N*-methyl-D-aspartate channel by extracellular H^+. *Proc. Natl. Acad. Sci. USA* 87:6445–49
71. Traynelis SF, Cull-Candy SG. 1990. Proton inhibition of *N*-methyl-D-aspartate receptors in cerebellar neurons. *Nature* 345:347–50
72. Vyklicky L, Vlachova V, Krusek J. 1990. The effect of external pH changes on responses to excitatory amino acids in mouse hippocampal neurons. *J. Physiol.* 430:497–517
73. Aizenman E, Lipton SA, Loring RH. 1989. Selective modulation of NMDA responses by reduction and oxidation. *Neuron* 2:1257–63
74. Aizenman E, Hartnett KA, Reynolds IJ. 1992. Oxygen free radicals regulate NMDA receptor function via a redox modulatory site. *Neuron* 5:841–46
75. Christine CW, Choi DW. 1990. Effect of zinc on NMDA receptor-mediated channel currents in cortical neurons. *J. Neurosci.* 10:108–16
76. Legendre P, Westbrook GL. 1990. The inhibition of single *N*-methyl-D-aspartate-activated channels by zinc ions on

cultured rat neurones. *J. Physiol.* 429: 429–49
77. Huettner JE, Bean BP. 1988. Block of *N*-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc. Natl. Acad. Sci. USA* 85:1307–11
78. Lodge D, Johnson KM. 1990. Noncompetitive excitatory amino acid receptor antagonists. *Trends Pharmacol. Sci.* 11: 81–86
79. Williams K, Pullan LM, Romano C, Powel RJ, Salama AI, et al. 1992. An antagonist/partial agonist at the polyamine recognition site of the *N*-methyl-D-aspartate receptor that alters the properties of the glutamate recognition site. *J. Pharmacol. Exp. Ther.* 262:539–44
80. Romano C, Williams K, DePriest S, Seshadri R, Marshall GR, et al. 1992. Effects of mono-, di-, and triamines on the *N*-methyl-D-aspartate receptor complex: a model of the polyamine recognition site. *Mol. Pharmacol.* 41:785–92
81. Reynolds IJ. 1992. 1,5-(diethylamino)piperidine, a novel spermidine analogue that more specifically activates the *N*-methyl-D-aspartate receptor-associated polyamine site. *Mol. Pharmacol.* 41:989–92
82. Pullan LM, Stumpo RJ, Powel RJ, Paschetto KA, Britt M. 1992. Neomycin is an agonist at a polyamine site on the *N*-methyl-D-aspartate receptor. *J. Neurochem.* 59:2087–93
83. Reynolds IJ. 1990. Arcaine is a competitive antagonist of the polyamine site of the NMDA receptor. *Eur. J. Pharmacol.* 177:215–16
84. Reynolds IJ. 1990. Arcaine uncovers dual interactions of polyamines with the *N*-methyl-D-aspartate receptor. *J. Pharmacol. Exp. Ther.* 255:1001–7
85. Reynolds IJ, Baron BM, Edwards ML. 1991. 1,10-bis(guanidino)decane inhibits *N*-methyl-D-aspartate responses in vitro and in vivo. *J. Pharmacol. Exp. Ther.* 259:626–32
86. Sacaan AI, Johnson KM. 1990. Competitive inhibition of magnesium-induced [^{3}H]N-(1-[thienyl]cyclohexyl)-piperidine binding by arcaine: evidence for a shared spermidine-magnesium binding site. *Mol. Pharmacol.* 38:705–10
87. Reynolds IJ. 1992. Interactions between zinc and spermidine on the *N*-methyl-D-aspartate receptor complex: clues to the mechanism of action of 1,10-bis(guanidino)decane and pentamidine. *J. Pharmacol. Exp. Ther.* 263:632–38
88. Berger ML, Seifriz I, Letschnig M, Schodl C, Noe CR. 1992. Interaction of long chain n-alkyl diamines with the NMDA receptor complex. *Neurosci. Lett.* 142:85–88
89. Reynolds IJ, Miller RJ. 1988. [^{3}H]MK-801 binding to the NMDA receptor/ionophore complex is regulated by divalent cations: evidence of multiple regulatory sites. *Eur. J. Pharmacol.* 151: 103–12
90. Rajdev S, Reynolds IJ. 1992. Effects of monovalent and divalent cations on 3-(+)[^{125}I]Iododizocilpine binding to the *N*-methyl-D-aspartate receptor of rat brain membranes. *J. Neurochem.* 58: 1469–76
91. Enomoto R, Ogita K, Han D, Yoneda Y. 1992. Differential modulation by divalent cations of [^{3}H]MK-801 binding in brain synaptic membranes. *J. Neurochem.* 59:473–81
92. Pullan LM, Powel RJ. 1991. Spermine reciprocally changes the affinity of NMDA receptor agonists and antagonists. *Eur. J. Pharmacol.* 207:173–74
93. Reynolds IJ. 1994. [^{3}H]CGP 39653 binding to the agonist site of the *N*-methyl-D-aspartate receptor is modulated by Mg^{2+} and polyamines independently of the arcaine-sensitive polyamine site. *J. Neurochem.* 62:54–62
94. Ransom RW. 1991. Polyamine and ifenprodil interactions with the NMDA receptor's glycine site. *Eur. J. Pharmacol.* 208:67–71
95. Sacaan AI, Johnson KM. 1989. Spermine enhances binding to the glycine site associated with the *N*-methyl-D-aspartate receptor complex. *Mol. Pharmacol.* 36:836–39
96. Yoneda Y, Suzuki T, Ogita K. 1994. Differential profiles of binding of a radiolabeled agonist and antagonist at a glycine recognition domain of the *N*-methyl-D-aspartate receptor ionophore complex in rat brain. *J. Neurochem.* 62:102–12
97. Donevan SD, Jones SM, Rogawski MA. 1992. Arcaine blocks *N*-methyl-D-aspartate receptor responses by an open channel mechanism: whole-cell and single-channel recording studies in cultured hippocampal neurons. *Mol. Pharmacol.* 41:727–35
98. Rock DM, Macdonald RL. 1992. The polyamine diaminodecane (DA-10) produces a voltage-dependent flickery block of single NMDA receptor channels. *Neurosci. Lett.* 144:111–15
99. Subramaniam S, Donevan SD, Rogawski MA. 1992. 1,10-diaminodecane and 1,12-diaminododecane block NMDA receptor currents by an open

channel block mechanism. *Neurosci. Lett.* 147:213–16

100. Subramaniam S, Donevan SD, Rogawski MA. 1994. Hydrophobic interactions of n-alkyl diamines with the *N*-methyl-D-aspartate receptor: voltage-dependent and -independent blocking sites. *Mol. Pharmacol.* 45:117–24
101. Stone TW. 1993. Subtypes of NMDA receptors. *Gen. Pharmacol.* 24:825–32
102. Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, et al. 1991. Molecular cloning and charcterization of the rat NMDA receptor. *Nature* 354: 31–37
103. Imoto K, Busch C, Sakmann B, Mishina M, Konno T, et al. 1988. Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. *Nature* 335:645–48
104. Verdoorn TA, Burnashev N, Monyer H, Seeburg PH, Sakmann B. 1991. Structural determinants of ion flow through recombinant glutamate receptor channels. *Science* 252:1715–18
105. Hume RI, Dingledine R, Heinemann S. 1991. Identification of a site in glutamate receptor subunits that controls calcium permeability. *Science* 253:1028–31
106. Nakanishi N, Axel R, Shneider NA. 1992. Alternative splicing generates functionally distinct *N*-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. USA* 89:8552–56
107. Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, et al. 1992. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* 256:1217–21
108. Meguro H, Mori H, Araki K, Kushlya E, Kutsuwada T, et al. 1992. Functional characterization of a heteromeric NMDA receptor channel expressed from cDNAs. *Nature* 357:70–74
109. Mori H, Masaki H, Yamakura T, Mishina M. 1992. Identification by mutagenisis of a Mg^{2+}-block site of the NMDA receptor channel. *Nature* 358: 673–75
110. Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, et al. 1992. Molecular diversity of the NMDA receptor channel. *Nature* 358:36–41
111. Monyer H, Burnashev N, Laurie DJ, Sakmann B, Seeburg PH. 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron* 12:529–40
112. Tolle TR, Berthele A, Zieglgansberger W, Seeburg PH, Wisden W. 1993. The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. *J. Neurosci.* 13:5009–28
113. Durand GM, Gregor P, Zheng X, Bennett MLV, Uhl GR, et al. 1992. Cloning of an apparent splice variant of the rat *N*-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proc. Natl. Acad. Sci. USA* 89:9359–63
114. Durand GM, Bennett MLV, Zukin RS. 1993. Splice variants of the *N*-methyl-D-aspartate receptor NR1 identify domains involved in regulation by polyamines and protein kinase C. *Proc. Natl. Acad. Sci. USA* 90:6731–35
115. Anantharam V, Panchal RG, Wilson A, Kolchine VV, Treistman SN, et al. 1992. Combinatorial RNA splicing alters the surface charge on the NMDA receptor. *FEBS Lett.* 305:27–30
116. Stern P, Béhé P, Schoepfer R, Colquhoun D. 1992. Single-channel conductances of NMDA receptors expressed from cloned cDNAs: comparison with native receptors. *Proc. R. Soc. London Ser. B* 250:271–77
117. Gibb AJ, Colquhoun D. 1992. Activation of *N*-methyl-D-aspartate receptors by L-glutamate in cells dissociated from adult rat hippocampus. *J. Physiol.* 456: 143–79
118. Burnashev N, Schoepfer R, Monyer H, Ruppersberg JP, Gunther W, et al. 1992. Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science* 257:1415–19
119. Zheng X, Zhang L, Durand GM, Bennett MLV, Zukin RS. 1994. Mutagenesis rescues spermine and Zn^{2+} potentiation of recombinant NMDA receptors. *Neuron* 12:811–18
120. Williams K, Zappia AM, Pritchett DB, Shen YM, Molinoff PB. 1994. Sensitivity of the *N*-methyl-D-aspartate receptor to polyamines is controlled by NR2 subunits. *Mol. Pharmacol.* 45:803–9
121. Lynch DR, Anegawa NJ, Verdoorn TA, Pritchett DB. 1994. *N*-methyl-D-aspartate receptors: different subunit requirements for binding of glutamate antagonist, glycine antagonists and channel-blocking agents. *Mol. Pharmacol.* 45:540–45
122. Zhong J, Russell SL, Pritchett DB, Molinoff PB, Williams K. 1994. Expression of mRNAs encoding subunits of the *N*-methyl-D-aspartate receptor in cultured cortical neurons. *Mol. Pharmacol.* 45:846–53