NMDA RECEPTOR AND THE THERAPEUTIC POTENTIAL OF ITS LIGANDS

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ABSTRACT

NMDA is the excitatory amino acid receptor subtype that is most thoroughly studied. It is an ionotropic receptor with Mg^{2+} blockage in a voltage-dependent manner. Functional NMDA receptor consists of a heterodimer of NMDAR1 and NMDAR2 subunits. Alternative splicing of the products of exons 5, 21 and 22 results in 7 naturally occuring isoforms. Impotant allosteric binding sites on the NMDA receptors are Zn^{2+}, glycine and polyamine binding sites. Since the competitive NMDA antagonists and the noncompetitive antagonists are mostly associated with psychotomimetic effects, attention has recently been paid toward the development of ligands that bind to the glycine and polyamine binding sites. The most promising compounds that have potentials as anticonvulsants are partial and competitive antagonists of the glycine binding site and competitive antagonists of the polyamine bindings sites.

Key words : NMDA receptor, therapeutic potential

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INTRODUCTION

Excitatory amino acids that function as neurotransmitters are chiefly glutamate and aspartate. There are currently 4 subtypes of excitatory amino acid receptors named after the relatively specific agonists[1].

1. NMDA (N-methyl-D-aspartate) receptor
2. AMPA (a-amino-3-hydroxyl-5-methyl-4-isoxazole propionic acid) receptor
3. Kainate receptor
4. Metabotropic receptor

The first 3 subtypes are iono-tropic receptors by having integral cation-specific channel in the receptor molecule. The last subtype couple with G-protein to control the synthesis of intracellular messengers which involves the metabolism of phosphatidylinositol 4,5-bisphosphate.

NMDA receptor contains a single cation channel which is blocked by Mg^{2+} in a voltage-dependent manner. Partial depolarization of the membrane displaces Mg^{2+} from the channel lumen and allow cation entry (Ca^{2+} is preferred over Na^{+}) to depolarize the neuron. Phencyclidine (PCP), ketamine and dizocilpine (MK-801) are noncom-petitive antagonists which block open channel close to the magnesium binding site.

This review will focus on the subunit constitution of NMDA receptor, its important binding sites and the compounds that show some promises in therapeutic uses.

SUBUNIT CONSTITUTION OF NMDA RECEPTOR

Functional NMDA receptor must be at least a heterodimer having 2 subunits, NMDAR1 and NMDAR2[2]. NMDAR1 subunit is the product of a single gene. There are 7 isoforms from alternative splicing of the products of exons 5, 21 and 22[3] . Exon 5 encodes 21 amino acids at the N-terminal while exons 21 and 22 encodes 37 and 38 amino acids, respectively, at the C-terminal[4]. Therefore, there are 8 (2^3) possible splice variants of these exon products. So far, 7 isoforms have been identified (NMDAR1A to NMDAR1G) which contain 938, 959, 901, 922, 885, 922 and 906 amino acid residues, respectively. The splice variant that contains the products of exons 5 and 21 but not the product of exon 22 has not been found in the cDNA library. The characteristics of NMDAR1 subunit are[4]:

1. having a large extracellular N-ter-minal, up to half of the subunit's molecular weight.
2. having three transmembrane dom-ains (TM1, TM3 and TM4)
3. TM2 is bent in the membrane and line the cation channel (pore-forming region or P-region)[5]
4. having S1 and S2 regions that bind glutamate and glycine, respectively.
5. having 4 or 5 residues at the C-terminal that can be phosphorylated by protein kinase C.

NMDAR2 subunit has 4 subtypes from 4 different genes[1,6] (NMDAR2A to NMDAR2D) that contain 1464, 1482, 1250 and 1323 amino acid residues, respectively. Its structure in relation to the cell membrane seems to be similar to NMDAR1 with a large intracellular C-terminal extension from TM4. The

distribution of each subtype in the rat brain is heterogenous. From autoradiographic studies, each subtype is present with high densities in the following brain regions[1]:

NMDAR2A	-	hippocampus and cerebral cortex
NMDAR2B	-	forebrain
NMDAR2C	-	cerebellum
NMDAR2D	-	diencephalon and lower brainstem

ALLOSTERIC SITES ON THE NMDA RECEPTOR

A. Zn^{2+} binding site

Zn^{2+} is a potent noncompetitive NMDA receptor antagonist that blocks the open channel of NMDA receptor with little sensitivity to changes in glycine concentration or membrane voltage[7] . At low concentrations, Zn^{2+} block the response to NMDA in this manner[8,9]. At high concentrations, Zn^{2+} block the open channel in a voltage-dependent manner at the Mg^{2+} binding site. The latter type of blockade is not important because Zn^{2+} concentration has to be very high.

B. Glycine binding site

NMDA receptor is a new type of receptor that requires two different agonists to bind simultaneously to activate the ion channel. Endogenous agonists in this case are glutamate and glycine. Glycine is absolutely required for NMDA receptor activation[10]; without glycine, the receptor could not be activated. Glycine therefore acts as coagonist at this site of NMDA receptor which is strychnine-insensitive. Binding of glycine in the presence of NMDA agonist increases the frequency of cation channel opening with little effect on the mean open time. Extracellular concentration of glycine in most parts of the brain (> 1 mm) is more than enough to saturate the glycine binding sites[11]. In other words, glycine is present in the concentration that is high enough to activate the NMDA receptor together with the NMDA agonist. Although glutamate mediates synaptic transmission, changes in the extracellular glycine levels modulate the NMDA receptor's contribution to the transmission.

Partial agonists for the glycine binding site are HA-966[12] (3-amino-1-hydroxypyrrolidone) and D-cycloserine[13] . HA-966 noncompetitively inhibits glutamate binding and enhances the binding of competitive NMDA antagonists. Competitive antagonists of glycine's action are kynurenic acid derivatives e.g. 7-chlorokynurenic acid[14] and 5,7-dichlorokynurenic acid (MDL 29,814). Kynurenic acid is the product of kynurenine pathway of tryptophan metabolism. It is a nonselective antagonist that binds to the NMDA site, AMPA receptor and kainate receptor. The concentration in human brain tissue is about 1 mm. In the experimental animals it has anticonvulsant effect and protects the animals from global cerebral ischemia. 7-Chlorokynurenic acid antagonizes the action of glycine and HA-966.

In experimental animals R(+)-HA-966 and 5,7-dichlorokynurenic acid have anxiolytic effect[15] and blocks mesolimbic activation and associated hyperactivity by amphetamine[16]. Anxiolysis from these compounds is better than those from NMDA antagonists because there is less muscle relaxation at equivalent anxiolytic doses in the model that measures ultrasonic vocalization of rat pups following the separation from their mothers. 1-Amino-1-carboxycyclopropane and R(+)-cis-4-methyl-HA-966 (L 687414) are partial

agonists with potent anticonvulsant action. The latter compound does not have psychotomimetic effect or behavioral stimulation[17]. D-cycloserine has been shown to produce cognitive enhancing effect in experimental animals[18].

C. Polyamine binding site

Polyamines are highly charged cationic compounds that have their positive charges separated by their chain lengths. They have roles in all enzymatic processes that involve RNA or DNA in cellular growth and development[19]. Spermine and spermidine are polyamines that potentiate the response of NMDA receptor stimulation[20]. NMDA receptor activation is necessary to the potentiating effect of these polyamines. Ifenprodil and its derivative, eliprodil (SL 82.0715) are antagonists at the polyamine binding site[21]. These compounds have the following actions:

1. anticonvulsant action in the maximal electroshock models and in the NMDA-induced[22] and audiogenic seizures[23].
2. cytoprotective effect in focal cerebral ischemia model[24]. Besides the action on NMDA receptor, they have inverse steal action from constriction of pial vessels.
3. neuroprotective effect from the toxicity of NMDA[25]
4. eliprodil blocks voltage-sensitive calcium channel[26] and is a high affinity ligand to the s-receptor with unknown function.

NONCOMPETITIVE NMDA ANTAGONISTS

Although dizocilpine (MK-801) show promising results in many animal models of epilepsy and cerebral ischemia, trials of this compound and related antagonists in patients with epilepsy and stroke are disappointing because of associated psychotomimetic effects. The most promising compound in this group is memantine which is related to the antiviral amantadine. It produces good results in rat ischemic models and is clinically well-tolerated[27].

COMPETITIVE NMDA ANTAGONISTS

D-CPP-ene (SDZ EAA-494), a competitive antagonists at the NMDA binding site on the NMDA receptor, has no anticonvulsant action and many serious adverse effects in human complex partial seizures[28] although promising results have been reported in many animal models of epilepsy[29]. Selfotel (CGS 19755) has been shown to reduce intracranial pressure in patients with severe head injury[30]. Its use in stroke patients has been limited by hallucinations at doses similar to those used in head injury.

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