# Abstract

N-methyl-D-aspartate receptors (NMDARs) are heterotetramers containing two obligatory glycine-binding (GluN1) and two glutamate/glycine-binding (GluN2/3) subunits. These receptors mediate excitatory synaptic transmission in the central nervous system and play a key role in high order neuronal processes as a learning and formation of memory. It has been shown that dysregulation of NMDARs is involved in the pathophysiology of neurological and psychiatric disorders.

Each receptor is composed of four protomers exhibiting a conserved domain organization. The most distal part to the cell membrane is the amino-terminal domain that is linked to the ligand binding domain (LBD), which is connected to the pore-forming transmembrane domain (TMD) communicating with the intracellular carboxy-terminal domain. LBD and TMD are connected via three polypeptide chains – linkers. Channel opening is the key step in the NMDAR gating that allows the flux of ions across the membrane. The energy of agonist binding-evoked conformational changes is transferred via linkers to M3 helices forming an ion channel. The rearrangement of M3 helices in activated receptor makes the central cavity of the channel accessible. The details of energy transfer are not yet fully characterized, although accurate knowledge of the receptor gating is a key step for understanding the physiological function of NMDAR. Here, we focused on the structural and pharmacological details of NMDAR channel gating.

To answer the fundamental question, what are the initial steps in NMDAR channel opening, we embarked on functional and molecular biology studies of GluN1/GluN2B receptors and focused on the M3 and initial segments of the M3-S2 linkers. Using deletion/glycine mutations, we found that the initial segments of the M3-S2 linkers in close proximity of TMD (GluN1 (LVL) and GluN2 (MIQ)) affect NMDAR channel gating, specifically GluN1 (L657) and GluN2B (I655) residues.

In addition, we tested the effect of *de novo* mutations associated with mental disabilities in the TMD of human GluN2B. The results showed that mutations had a complete or partial loss-of-function effect. Mutations reduced surface expression, amplitude, and the probability of opening. Moreover, changes in desensitization or agonist-affinity were observed. Pharmacologically, we were able to fully compensate loss-of-function effect observed in hGluN2B(L825V) by androst-5-ene-3β-yl hemisuccinate. This substance has a potential for its use in human medicine.

Last but not least, we report a structure-activity relationship study for perhydrophenanthrenes analogs focusing on the evaluation of the role of the steroidal D-ring in the inhibition of NMDAR. Electrophysiological approach showed that compounds retained an inhibitory character after the D-ring breakdown.

All results revealed new structural and pharmacological details that take place during the NMDAR channel gating.