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Physiology and pathology of the glutamate-mediated neurotransmission in the brain

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The work in our laboratory focuses on one of the most prevalent means of communication between brain cells which is that mediated by glutamate (Glu). This excitatory neurotransmitter exerts radically opposed properties: On the one hand, its smooth and physiological functioning allows to normally move, feel, perceive, learn and memorize while on the other, its perturbed and pathological function may lead to cognitive, affective or motor deficits. Nowadays, it is well established that Glu exerts a neurotoxicity which plays a critical role in neurological disorders such as epilepsy, stroke, traumatic brain injury and amyotrophic lateral sclerosis.

Our research has two major themes: one focuses on the properties of glutamate receptor channels with the purpose to unravel their molecular structure, elucidate their mechanisms of action and understand their role in the production of short and long term modifications of neuronal function; the other centers on the neuropathological effects of Glu with the aim to contribute in a practical way to the rational development of novel means to achieve a control of Glu actions in neurological disorders.

A. Structure and function of glutamate receptor channels.

In the last three years, we addressed several issues related to the structure of the AMPA receptor subclass of Glu receptor channels:

1) Structure of the transmitter binding domain of a Glu receptor. On the strength of an amino acid sequence similarity of Glu receptors with bacterial periplasmic binding proteins (PBPs), we used computer-assisted homology protein modelling together with extensive site-directed mutagenesis to establish a 3D model structure of the transmitter binding domain of the kainate binding protein, (Paas *at al.* 1996) a member of the Glu receptor family. We found that the transmitter binds in a cleft formed by a bilobated domain. Our 3D model structure turned out to be extremely similar to the experimental X-ray structure elucidated in 1998.

2) The tetrameric structure of AMPA receptors. The number of subunits of the AMPA receptors was investigated. Expressing in Xenopus oocytes defined combinations of the wild type GluR1 subtype of AMPA receptor and of a GluR1 mutant, we observed a binomial distribution much more compatible with a tetrameric than a pentameric structure (Mano and Teichberg, 1998). This finding is in line with the presence in Glu receptor channels of a membranous P loop similar to that of the tetrameric potassium channels. Though debated at first, our suggestion of the tetrameric structure of AMPA receptors has received strong support in the more recent literature.

3) Structure of the active and desensitized states of an AMPA receptor. We have observed that, like the PBPs, the GluR1 receptor channel uses a venus flytrap mechanism to control the interactions with its ligands: activation of the receptor channel takes place upon binding of the agonist to an open-lobe conformation of the ligand binding domain while desensitization of the channel results from the agonist-induced lobe closure (Mano et al. 1996).

We recently confirmed this venus flytrap model in a study devoted to an analysis of the agonist properties of halogenated willardiines at the AMPA subtype of Glu receptors. The interest in the willardiines resides in the fact that, though they differ only by the nature of their halogen substituent, they display very different agonist properties. Thus, we analyzed, with the double-cycle mutant method, the active and desensitized states induced by the willardiines in the GluR1 subtype of AMPA receptors and in GluR1 mutants in which residues located within the agonist binding domain were mutated. We concluded that all willardiines can dock into the active and open-lobe conformation of the ligand binding domain. However, the willardiines with a bulky halogen moiety cannot fit into a closed-lobe conformation and thus, cannot cause receptor desensitization (Kizelsztein et al. 1999).

4) Interaction of the NMDA-R cytoplasmic domain with spectrin. It is well established that dendritic spines undergo morphological changes upon exposure to Glu. This observation suggests that the activation of the dendritic Glu receptors by Glu is transduced within the dendritic spine to a contractile element within the cytoskeleton. Investigating the possible protein partners of the NMDA receptor subtype of Glu receptors, we found

that the actin-binding protein, spectrin, binds selectively to the C-terminal cytoplasmic domains of the NR1, NR2A and NR2B subunits of NMDA-R but not to that of the GluR1 subunit of the AMPA. These interactions are reversible and highly regulated: the spectrin-NR2B interactions are antagonized by Ca2+, fyn-mediated NR2B phosphorylation, but not by Ca2+ /calmodulin (CaM) or CaM/ kinaseII-mediated NR2B phosphorylation. The spectrin-NR1 interactions are unaffected by Ca2+ but inhibited by CaM and by PKA and PKC-mediated phosphorylations of NR1. On the basis of these results, we have proposed that structural rearrangements of the NMDA-R/spectrin/actin scaffold contribute to dendritic morphological changes (Wechsler and Teichberg, 1998).

B. Control of Glu actions in neurological disorders.

The "in vitro evolution" of a Glu scavenging enzyme in the treatment of head trauma and stroke Since Glu neurotoxicity contributes to the process of neuronal death affecting victims of head trauma and stroke, we have started to investigate the use of an enzymatic system as a mean to control Glu levels. Several enzymes, able to scavenge Glu, are being investigated for neuroprotection. As it is necessary to achieve a fast and effective decrease of the neurotoxic Glu levels, we are submitting these enzymes to a process of "in vitro evolution" i.e random mutagenesis and stringent selection, in order to identify a mutant enzyme with optimal Glu scavenging properties. It is our hope that such enzyme which will be effective in the framework of emergency medicine to treat the so far untreatable conditions of head injury and stroke.

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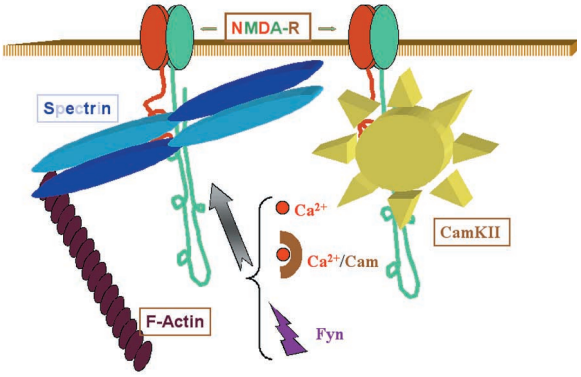

Fig. 1. *Schematic representation of the interactions of the intracellular domains of the N-methyl-D-aspartate receptor (NMDA-R) with spectrin and Ca/Cam kinase and with actin via spectrin. The interactions of spectrin with the NMDA-R subunits are regulated by calcium ions, Ca^{2+}/ calmodulin and by phosphorylation via the fyn kinase.*