Abstract

In the central nervous system, the NMDA receptor subfamily of the ionotropic glutamate receptors plays a crucial role in mechanisms underlying learning and memory. Most abundant are receptors containing GluN1 and GluN2 subunits, activated by the binding of glycine to the GluN1 and glutamate to the GluN2 subunit. This subunit combination is strongly blocked by zinc. The remaining members of the family, the glycine-binding GluN3 subunits, were found a few years later. The GluN3 subunits were soon recognized to modulate all functional characteristics of the conventional GluN1/GluN2-containing receptor when incorporated into the receptor complex: the calcium permeability is lowered significantly, the block by magnesium ions is reduced, and the current responses are decreased. When coexpressed with GluN1 alone, GluN3 gives rise to receptors that are activated by the binding of glycine alone, are barely permeable for calcium and not blocked by magnesium. This subunit combination, for which the name “excitatory glycine receptor” was coined, has been shown to exist in vivo (Piña-Crespo et al., 2010).

Concerning the GluN3A-containing excitatory glycine receptor, it is known that zinc can coactivate and potentiate the glycine-induced current response, and that this potentiation can be further enhanced by the coapplication of a GluN1 glycine binding site antagonist. However, for the GluN3B-containing receptor variant no such information exists.

The aim of this thesis was to find out how the GluN3B-containing excitatory glycine receptor behaves towards zinc, and if this behavior is comparable to that of GluN1/GluN3A-containing or conventional GluN1/GluN2-containing receptors. Also, if there were any modulatory effects of zinc, it would be important to identify the specific sequence elements that mediate these effects.

The GluN3B-containing excitatory glycine receptor combination shows a differential behavior towards zinc as both blocking action and potentiation can be observed, a pattern that is in general independent of the N-terminal splice variant of the GluN1 subunit, GluN1-a or GluN1-b. In addition, the potentiating effect has two components that reside in different parts of the receptor. Though the overall potentiation remains, one of these elements can be influenced by exon 5: when exon 5 is present, the receptor is potentiated to a lesser degree. Mutational analysis suggests a binding site in the vicinity of the amino terminal domain’s (ATD) dimer interface between GluN1 and GluN3B.

With regard to the second element with a weaker potency it is clear that it involves structural elements within the ligand binding domain (LBD), but not directly at the ligand binding site: zinc competes with MDL-29951, a highly efficient GluN1 glycine binding site antagonist that blocks the conventional GluN2-containing receptors, but potentiates current responses in GluN3-containing receptors. Yet when the binding of MDL-29951 is abolished, the zinc potentiation remains.
Abstract

To investigate the comparability of GluN3A- and GluN3B-containing receptors, also GluN3A-containing receptors were tested with the same combinations of concentrations of zinc and MDL-29951. Interestingly, it was found that at the glycine concentrations effective at GluN3B-containing receptors only zinc potentiates the glycine-induced current response, while MDL-29951 has no effect. Furthermore, the proposed “supralinear” potentiation of the glycine-induced current seen upon coapplication of MDL-29951 and zinc (Madry et al., 2008) was not observed. Surprisingly, the removal of the 5’ untranslated region (UTR) of the GluN3A subunit led to the manifestation of such a “supralinear” effect, although MDL-29951 when applied alone still showed no effect. Whereas the GluN3B-containing receptors were found to be sensitive to protons regardless of the GluN1-a or GluN1-b splice variant (Cavara et al., 2009), here the GluN3A-containing receptors showed no sensitivity towards protons.

Additionally, it could be shown that the plasma membrane export of the GluN1-3 variant of the GluN1 subunit is not as pronounced as previously thought. The endoplasmatic reticulum (ER) retention, mediated by an ER retention motif, is supposed to be overridden by a PSD-95/Dig/ZO-1 (PDZ) binding motif, which resides in the additional C2’ sequence in the carboxy-terminal domain (CTD) of this GluN1 variant (Xia et al., 2001; Standley et al., 2000; Scott et al., 2001). However, the small degree of plasma membrane export seen here suggests only a rather weak reduction of ER retention.