Abstract

Part I. Design and Synthesis of Tool Compounds for Glutamate Delta Receptors (GluDRs)

Glutamate delta receptors (GluDRs) (subunits GluD1 and GluD2) are members of the ionotropic Glutamate receptors (iGluRs) superfamily on the basis of the sequence similarity. For many years, the GluDRs were considered as orphan receptors, which stands in strong contrast to their wide abundance in the CNS. However, recently *D*-Ser was identified as an endogenous ligand for the GluDRs. Different brain disorders including speech and cognitive development delay, impairment in motor coordination, cerebellar ataxia and eye movement abnormalities have been associated with mutation in GluD2 gene (GRID2). Similarly, mutation in GluD1 gene (GRID1) has been implicated in bipolar disorders, schizophrenia, autism and speech and motor skills development disorders. Further studies on the cellular mechanism and the molecular function of GluDRs in significantly dependent on development of GluD2 selective ligands.

In this PhD thesis, three series of ligands with 3 to 75-fold increase in potency (IC_{50}= 3.2 - 79 µM) and moderate-to-high efficacies (I_{max}= 49 - 120%) relative to the endogenous ligand *D*-Ser with 250 µM potency and 100% efficacy, at the GluD2-*Lurcher* mutant, were developed. This development initiated by structure-based design using the available X-ray crystal structure of the ligand binding domain of the receptor in complex with *D*-Ser. Analogues of *D*-Ser with more lipophilic substituents on the *β*-position were designed with the aim of enhancing the potency. Therefore, the amino acid moiety of *D*-Ser is preserved and modification of the hydroxyl group of *D*-Ser with more lipophilic substiuent was carried out. In addition, the geometry and rotational degree of freedom of the extended ligandswere explored by incorporating three spacers with different three-dimentional topological structures. The three spacers investigated in this study were an amide group (series 1), ether (series 2) and alkyne (series 3) with trigonal, tetrahedral and linear spatial geometry respectively. The Schrödinger software package was applied in the specific design of the chemical structure of the lipophilic component. Both induced fit docking (IFD) and rigid docking were used to evaluate the designed ligands. Based on the *in silico* generated binding modes, the docking scores and with consideration of the desirable interactions to the binding site, compounds were selected to be synthesized in the next step, by adapting reported methods for

similar compounds. Electrophysiology studies of the synthesized ligands were done on the spontaneous active GluD2 *Lurcher* mutant ($GluD2^{LC}$) expressed on Xenopus oocytes. Series 1 (amide group) only improved the potencies 2.5 to 6-fold, whereas, series 2 (ether) and 3 (alkyne) improved the potencies with up to an impressive 75-fold. Interestingly, the 2*S*-cofiguration was equipotent to the 2*R*-configuration. Finally, the synthesized compounds were evaluated for selectivity against NMDA receptors by subjecting them as agoniststo the Gly-binding site of GluN1/2A-D and GluN1(F484A/ T518L)/N3A-B subunits in heteromeric form. Series 2 (ether) and 3 (alkyne) displayed neglectable potencies for the investigated NMDA subunits, while searies 1 (amide) retained activity.

Part II. Fatty Acid Conjugated Fab Fragments of 8D3 Antibody as Potential Brain Drug Delivery Mechanism

Permeation of more than 98% of the small molecule therapeutics and almost all macromolecule biotherapeutics to the brain parenchyma is highly restricted by the blood brain barrier (BBB). Furthermore, many effective drug candidates composed of proteins and genetic materials for some brain disorders exist. However, their application is tightly limited due to the lack of functional brain delivery mechanism. Harnessing the BBB transport system to deliver therapeutics to the brain can be a non-invasive and effective method in the brain drug delivery. Several antibodies including 8D3 anti-transferrin antibody has been developed that depict receptor-mediated transcytosis mechanism. However, studies have demonstrated the low brain uptake for these antibodies, which might be due to reverse endocytosis from the brain to the blood. However, some recent studies suggest that monovalency of the antibody increases the brain uptake.

Therefore, the aim of this project was to study the brain uptake of the monovalent 8D3 Fab fragments. The shorter half-life of the Fab fragments were extended by conjugation to the pre-synthesized maleimide-bearing fatty acids (FA). To perform the conjugation with maleimide functionality, free Cys was inserted on the Fab construct. The single mutations of V205C in C_L, A114C in C_H or insertion of the DKTHTCA peptide chain on the C-terminal domain of the hinge region provided the three engineered Fab fragments. Non-engineered Fab fragment and the three Cys-engineered constructs were prepared through HEK cell transfection. Afterwards, the Fab fragments were purified using the affinity chromatography with Äkta Prime. The purity of the constructs was evaluated by SDS-PAGE and HP-SEC. Afterwards, the FAs were successfully

conjugated to the constructs through site-specific Cys-conjugation. The Lys-conjugation method was further established to conjugate NHS-bearing biotin to the Fab-FA constructs. This was performed to establish a Labeled Streptavidin-Biotin (LSAB) complex method for immunohistochemical staining of brain tissue using the dual-conjugated constructs. However, this method requires further optimization. At the time of submitting this thesis, no precise conclusion can be obtained to illustrate whether the 8D3 Fab-FA constructs possess higher brain uptake, though the studies are continuing.