Assessment of Nicotinic Acetylcholine Receptor Detergent Complex Purity and Stability for Structural Studies

Bianca N. Valdés Fernández, Bryan Cobo Torres, Alejandro Carmona. Orlando González & José A. Lasalde Dominicci

The nAChR belongs to the family of ligand-gated ion channels (LGIC) which include GABA type A, serotonin type 3 and glycine receptor (Figure 1). The LGIC are key components of signal transduction mechanisms and important targets for the treatment of several neurodegenerative diseases such as Alzheimer, schizophrenia, depression, cardiovascular diseases, inflammation and nicotine addiction. The currently structure of the nAChR is at a 4.0 Å (Unwin et al., 2005) and was obtained only with the transmembrane domains in a closed state. These structure doesn't provide the information on the coupling of agonist and/or antagonist to ion channel activation or about lipid-protein interaction in the interface. The mechanism by which detergents affects lipid composition, functionality and stability of the detergent-solubilized membrane proteins is poorly understood. During two decades our laboratory have been developing conditions to understand the mechanisms of how non lipid-analog detergents and lipid-analog detergents affects the functionality, stability, lipid composition and aggregation state of the nAChR (Asmar-Rovira et al., 2008; Padilla et al. 2011: Padilla et al., 2015; Padilla et al, 2016; Quesada et al., 2016 (in revision). The aforementioned studies demonstrated that after several years of screening functional activity, stability, aggregation and purity of >50 nAChR-detergent complexes (nAChR-DCs), several nAChR-detergent complexes with lipid-analogs detergents are the best candidates to obtain a high resolution structure of the nAChR. In order to achieve this structure, we must first improve the purity of the nAChR-LFC16 complex. Herein, we proposed to develop an in depth analysis of how lipid-analog detergents affect the presence of intrinsic contaminants (such as ATPase and 43KD-Rapsyn) of the nAChR preparation. The experiments proposed will provide important new information about the consequences of using lipid-analog detergents in: 1) aggregation state, 2) purity and 3) crystallization potential. These approaches can be applied to different membrane proteins and to the development of different detergents and crystallization methodologies. We are confident that the proposed in depth study will succeed in producing a functional high-resolution structure of the nAChR from a natural source. A high-resolution of the nAChR and its complexes with various ligands is of essential for the design of novel agents and novel allosteric modulators that can treat those nervous system pathologies such as Alzheimer's disease, schizophrenia, depression, attention deficit hyperactivity disorder and tobacco addiction. Ultimately, decoding the mechanism(s) by which detergents affect the lipid composition, purity, stability, and functional state of membrane proteins may lead to the development of novel strategies that could improve the likelihood of successful membrane protein crystallization.