30. Chemogenetics, RASSL, and DREADD

François Noël, August 2022
(reviewed by Dr. Alexandre H. Kihara, UFABC)

RASSLs and DREADDs are artificially engineered receptors designed to be selectively activated by certain ligands. They are the result of **chemogenetics**, a term originally coined to describe the effects of mutations on the substrate specificity of enzymes. Nowadays, this term is used to describe the process by which macromolecules can be engineered to interact only with small molecules not recognized by native proteins (Urban and Roth, 2015).

Historical aspect of chemogenetics and the intrinsic difference between RASSL and DREADD

- Traditional genetic approaches: although genetic manipulation (such as knockout models) is a powerful tool for assessing the function of a protein, it often suffers from issues such as lack of temporal control, compensatory phenomena, or even embryonic lethality. Chemogenetics emerged to overcome these limitations by using a small exogenous ligand to control the activity of an endogenous macromolecule (Keiffer et al., 2020).

- First generation: RASSLs are receptors activated exclusively by a synthetic ligand (RASSL is an acronym for *Receptor Activated Solely by a Synthetic Ligand*). In 1991, Strader and colleagues were the first to design a G-protein–coupled receptor (GPCR), the κ-opioid receptor, that could be activated only by a synthetic compound, thus preventing activation by endogenous agonists. This work was important because it showed that substituting amino acids in the binding site could modify receptor specificity and therefore demonstrated the potential of rational ligand design to act specifically on genetically modified receptors, already foreshadowing the development of DREADDs.

- Second generation: DREADDs, or *Designer Receptors Exclusively Activated by Designer Drugs*. To prevent the synthetic ligands selected to activate RASSLs from also activating endogenous receptors, researchers sought to engineer not only the ligand-binding domain of the receptor but also the small synthetic molecule itself, which led to the creation of DREADDs (Keiffer et al., 2020). These DREADDs were initially developed in the laboratory of Dr. Brian Roth to study 7TM receptor signaling (Armbruster et al., 2007), which is highly complex because agonists can act on more than one receptor subtype and activate different G proteins as well as β-arrestins, as previously discussed in the section on **functional selectivity**. A major advantage of these modified receptors is that they can couple with only one of the heterologous G proteins or one of the arrestin proteins. In addition, DREADDs do not respond to their natural ligands but rather to a pharmacologically inert ligand such as

clozapine-N-oxide (CNO), for example. Another advantage lies in the ability to control the expression of these receptors so that tissue or even cell specificity can be achieved (Thiel, 2015). Several types of these receptors already exist, derived from muscarinic or κ-opioid receptors, and DREADDs are now used ubiquitously to modulate GPCRs non-invasively *in vivo* (Urban and Roth, 2015). Although the initial focus of chemogenetics was GPCRs, emerging research is now targeting ligand-gated ion channels (Keiffer et al., 2020).

Despite the intrinsic differences between RASSLs and DREADDs, these terms are currently often used interchangeably to refer to a rationally designed receptor-ligand system.

Applications

Currently, DREADDs are the most widely used chemogenetic tool, with their primary field of application in neuropsychiatric models, although their use now extends to almost all domains of physiology (Keiffer et al., 2020; Paschon et al., 2020). Importantly, transgenic mice expressing DREADDs already exist, and it has been demonstrated that it is possible to activate or silence specific neurons in the brains of these animals, some of which are already commercially available. This availability is valuable because it enables experiments aimed at unveiling the circuits that control natural behaviors in animals at the cellular level, allowing researchers to elucidate causal relationships between selective neuronal populations and particular types of behavior (Thiel, 2015).

References

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