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Trends and Perspectives

TRENDS AND PERSPECTIVES

DREADD: A Chemogenetic GPCR Signaling Platform

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Abstract

Recently, we created a family of engineered G protein-coupled receptors (GPCRs) called DREADD (designer receptors exclusively activated by designer drugs) which can precisely control three major GPCR signaling pathways (Gq, Gi, and Gs). DREADD technology has been successfully applied in a variety of in vivo studies to control GPCR signaling, and here we describe recent advances of DREADD technology and discuss its potential application in drug discovery, gene therapy, and tissue engineering.

Keywords: chemogenetics, GPCRs, GsD, hM3Dq, hM4Di

Introduction

G protein-coupled receptors (GPCRs) represent the largest family of membranous signaling molecules and are characterized structurally by a highly conserved seven-transmembrane domain motif (Allen and Roth, 2011; Meltzer and Roth, 2013). GPCRs recognize an incredible variety of ligands, including odorants, photons, neurotransmitters, lipids, hormones, peptides, and other small molecules, and regulate intracellular response to adapt to the ever-changing environment (Allen and Roth, 2011). Upon agonist activation, GPCRs undergo prototypical structural re-arrangements facilitating the interaction with a family of canonical heterotrimeric G-proteins (e.g., Gq, Gs, Gi, and G12/13) and G protein-independent signaling molecules (Allen and Roth, 2011). GPCR signaling modulates virtually every known physiological response (Allen and Roth, 2011)

To control GPCR signaling for neuropsychopharmacological therapeutics, "selective" agonists or antagonists are typically developed (Besnard et al., 2012; Meltzer and Roth, 2013). However, because most neuronal cells express multiple GPCRs and because most GPCRs are expressed in multiple tissues (Regard et al., 2008), it is essentially impossible to regulate a specific GPCR-signaling pathway in a selective cell population by known pharmacological approaches. Additionally, the presence of endogenous receptor ligands and the lack of true specificity of pharmacological probes for GPCRs further confound the neuropsychopharmacological approach. To overcome these inherent problems associated with GPCR therapeutic approaches, the past two decades have seen a number of chemical-genetic approaches designed to control GPCR signaling *in vivo* (see Sternson and Roth, 2014, for recent review). Although the 'first generation' chemogenetic tools were useful from a heuristic perspective, they were not widely adapted (see Sternson and Roth, 2014, for review).

DREADD: A Useful and Robust Chemogenetic Platform

We adopted a novel strategy to discover useful chemogenetic tools utilizing directed molecular evolution in yeast. We were able to eventually evolve human muscarinic receptors to be activated by nM concentrations of the pharmacologically inert clozapine metabolite clozapine-N-oxide (CNO) and to simultaneously be insensitive to the native ligand acetylcholine (Armbruster et al.,

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2007). Importantly, the engineered receptors which we dubbed DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) had minimal constitutive activity *in vitro* (Armbruster et al., 2007) and *in vivo*, even when expressed at high levels in neurons (Alexander et al., 2009; Zhu et al., 2014).

Since the initial description of DREADD technology (Armbruster et al., 2007), three DREADDs have been mostly commonly used in the neurosciences: hM3Dq, which is coupled to Gαq signaling and induces firing of neurons (Alexander et al., 2009); hM4Di, which is coupled to Gαi signaling and mediates neuronal (Armbruster et al., 2007) and synaptic silencing (Stachniak et al., 2014); and rM3Ds, which is coupled to Gαs signaling and which modulates neuronal activity (Farrell et al., 2013).

A schematic diagram for the application of DREADD technology is shown in Figure 1. The typical strategy for using DREADD technology to gain remote control over neuronal signaling is quite simple and is as follows (see Figure 1 for diagram of ways of expressing DREADDs):

- Express the DREADD in the cell-type of interest via either transgenic or viral technology
  - For virally-mediated transduction wait 2–3 weeks for expression
- Administer CNO either via parenteral or oral administration

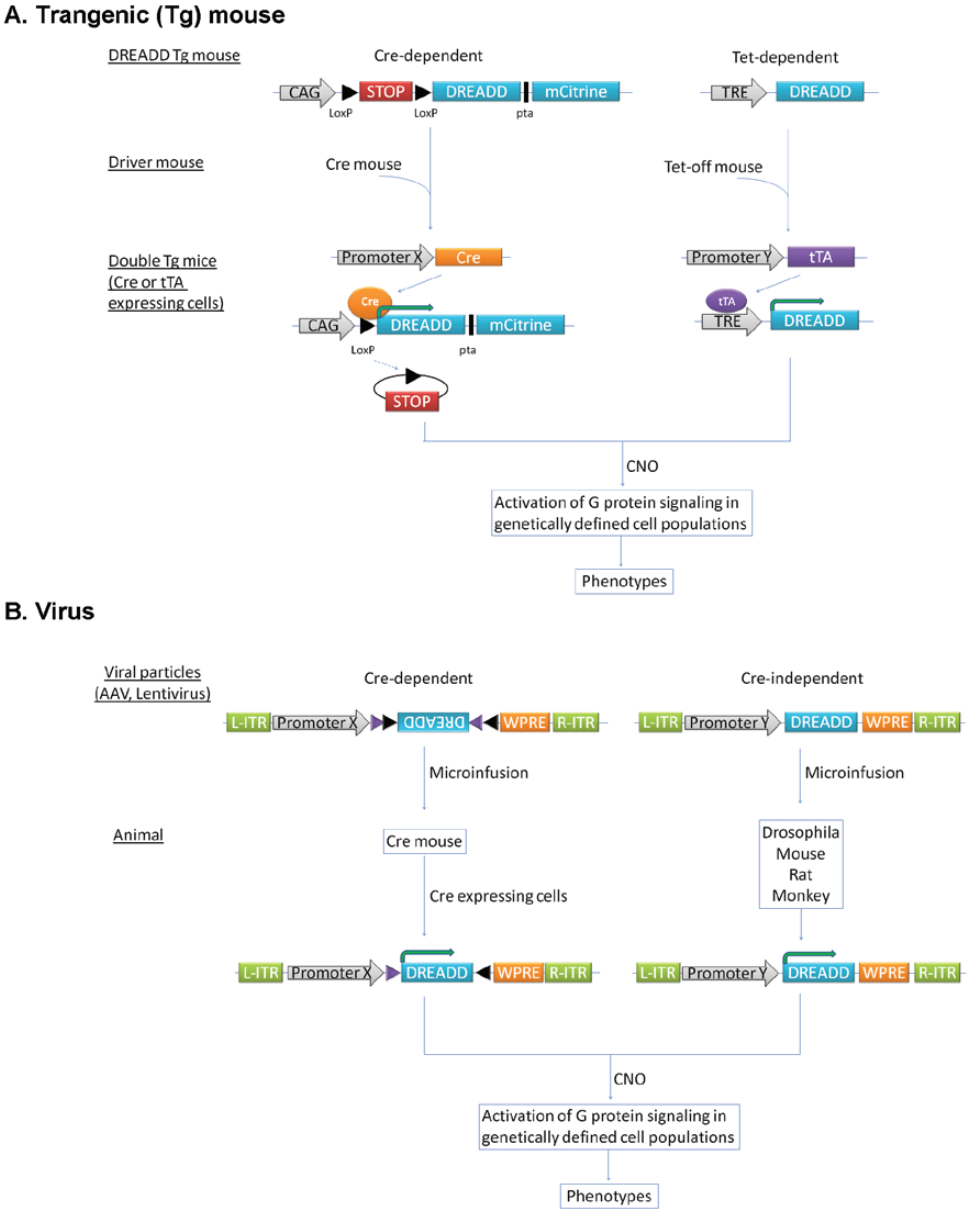

Figure 1. Schematic diagram for the application of DREADD technology in vivo. (A) Two types of DREADD transgenic mouse models have been developed: cre-dependent and tet-dependent. DREADDs can be expressed in genetically defined cell populations after crossing with cre- or tet-off driver mouse lines. (B) Two types of DREADDs viruses were developed: cre-dependent and cre-independent. In the cre-dependent system, virus containing double-floxed inverted DREADDs are microinfused into the appropriate brain region in mice which express cell-type specific Cre drivers. DREADDs are flipped into the correct orientation and expressed after cre-mediated recombination to achieve very precise cell-type specific expression.

- Within a few minutes of CNO administration, the effect due to activation (via hM3Dq), inhibition (via hM4Di), or modulation (via GsD) of neuronal signaling occurs
- Monitor behavior and/or neuronal firing in freely-moving animals
- The effects of CNO will be negligible in animals which do not express DREADDs and profound in animals expressing DREADDs

Widespread Adoption of DREADD Technology

DREADDs have been successfully demonstrated to control individual GPCR signaling in many *in vivo* studies from yeast, drosophila, mice, rats, and—perhaps in the near future—primates (Farrell and Roth, 2012) and, as of August 2014, more than 500 publications have cited DREADD technology. In the

Table 1. Summary of Toolkit of DREADDs Technology

Expression system	Subtype of DREADDs	Vector information	Control system	Source	Publication
Transgenic mice	hM3Dq	TRE-HA-hM3Dq	Tet off	Available at Jackson Labs	Alexander et al. (2009)
		LoxP-STOP-LoxP-HA-hM3Dq-PTA-mCitrine	Cre-LoxP	Roth's lab (In validation)	
		RIPII-hM3Dq	Insulin promoter II	Wess's lab	Guettier et al. (2009)
		C-fos-hM3Dq	C-fos promoter	Mayford's lab	Garner et al. (2012)
	hM4Di	TRE-HA-hM4Di	Tet off	Available at Jackson Labs	Zhu et al. (2014)
		LoxP-STOP-LoxP-HA-hM4Di-PTA-mCitrine	Cre-LoxP	Roth's lab (In validation)	
		CAG-FRT-STOP-FRT-LoxP-mCherry-STOP-loxP-HA-hM4Di	Cre-LoxP and FLP-FRT	Dymecki's lab	Ray et al. (2011)
	rM3Ds	Adora2A-rM3Ds	Adora2A promoter	Available at Jackson Labs	Farrell et al. (2013)
		RIPII-rM3Ds	Insulin promoter II	Wess's lab	Guettier et al. (2009)
AAV virus	hM3Dq	HA-hM3Dq-IRES-mCitrine	hSyn, CaMKIIa, GFAP promoter	UNC Viral Vector Core; plasmids available via ADDGENE	
		HA-hM3Dq-mCherry	hSyn, CaMKIIa, GFAP promoter	UNC Viral Vector Core; plasmids available via ADDGENE	
		hSyn-DIO-hM3Dq-mCherry	Cre-LoxP	UNC Viral Vector Core; plasmids available via ADDGENE	Urban and Roth (in press)
	hM4Di	HA-hM4Di-IRES-mCitrine	hSyn, CaMKIIa, GFAP promoter	UNC Viral Vector Core; plasmids available via ADDGENE	
		HA-hM4Di-mCherry	hSyn, CaMKIIa, GFAP promoter	UNC Viral Vector Core; plasmids available via ADDGENE	Zhu et al. (2014)
		hSyn-DIO-hM4Di-mCherry	Cre-LoxP	UNC Viral Vector Core; plasmids available via ADDGENE	
	rM3Ds	HA-rM3Ds-IRES-mCitrine	CaMKIIa, GFAP promoter	UNC Viral Vector Core; plasmids available via ADDGENE	
		hSyn-DIO-rM3Ds-mCherry	Cre-LoxP	UNC Viral Vector Core; plasmids available via ADDGENE	
Lentivirus	hM3Dq	hM3Dq-mCherry	CMV promoter	Available directly from Roth lab	
		CMV-DIO-hM3Dq-mCherry	Cre-LoxP	Available directly from Roth lab	
	hM4Di	hM3Dq-mCherry	CMV promoter	Available directly from Roth lab	
		CMV-DIO-hM3Dq-mCherry	Cre-LoxP	Available directly from Roth lab	
HSV virus	hM4Di	ENK-hM4Di	Enk promoter	Unknown availability	Ferguson et al. (2011)
		DYN-hM4Di	Dyn promoter	Unknown availability	Ferguson et al. (2011)
CAV2 virus	hM3Dq	hSyn-DIO-rM3Ds-mCherry	Cre-LoxP	Unknown availability	Boender et al. (2014)

central nervous system (CNS), DREADDs have been shown to control neuronal activities in a variety of neurons, including glutamatergic (Alexander et al., 2009; Zhu et al., 2014), serotonergic (Ray et al., 2011), dopaminergic (Dell'Anno et al., 2014), and GABAergic neurons (Kong et al., 2012), to name but a few. DREADDs have also been used to control glial cell activity to modulate the autonomic nervous system (Agulhon et al., 2013). In periphery, DREADDs have been used to control GPCR signaling in pancreatic beta-cells (Guettier et al., 2009), hepatocytes (Li et al., 2013), and breast cancer cells (Yagi et al., 2011). As these applications have been reviewed recently we will not comment on them further here (Urban and Roth, in press).

Recent Advances in the DREADD Toolkit and DREADD Resources

Currently, a substantial collection of DREADD-based tools is available to control GPCR signaling *in vivo* (see Table 1). Three DREADD transgenic mouse lines created by the Roth lab can now be obtained from Jackson Labs (Table 1). Cre-dependent hM3Dq and hM4Di transgenic mice are in the process of validation in the Roth lab and will be made available soon to provide cell-type specific transgenic control of DREADD expression as outlined in Figure 1. Tissue-specific and cell type–specific expression of DREADDs can also be achieved by viral delivery (Krashes et al., 2011; Urban and Roth, in press). For many uses viral delivery is time-saving and yields a more precisely controlled DREADD expression. A variety of viral vectors—Cre-dependent or -independent—are listed in Table 1 and the majority of them can be obtained from UNC viral core facility (http://www.med.unc.edu/genetherapy/vectorcore/research-grade/in-stock-aav-vectors/roth). Current DREADD-based plasmids are available from ADDGENE (http://www.addgene.org/Bryan_Roth/) and as a service to DREADD users a blog is updated frequently (http://chemogenetic.blogspot.com/).

We continue to develop new chemogenetic tools and soon will be describing those which afford bidirectional control of GPCR signaling in same-cell population via different synthetic ligands. In the meantime, new derivatives of DREADDs have been developed by others which are likely to be quite useful. Thus, for example, an arrestin-biased DREADD was generated (Nakajima and Wess, 2012) and an axonal selective hM4D-neurexin variant was developed as a presynaptic silencer (Stachniak et al., 2014).

Future Directions for DREADD Technology

The GPCR superfamily continues to be one of the major targets for drug discovery, although CNS drug discovery remains slow and risky (Meltzer and Roth, 2013). Indeed, less than 5% of CNS drugs entering Phase I clinical trials are eventually approved by the FDA, and many GPCR-targeted drugs fail in Phase II and III clinical trials due to lack of clinical efficiency (Bradley et al., 2014). This raises the questions as to whether current animal models really recapitulate the physiological/pathological condition seen in human disease and whether pharmacological intervention of disease-related GPCR targets can ultimately deliver the desired therapeutic response. We suggest that DREADDs may represent a new "pre-evaluation" approach for GPCR drug discovery. For example, we can pre-evaluate whether pharmacological intervention of a given GPCR-signaling process by DREADD achieves the desired therapeutic response before starting a long and costly drug discovery process (Figure 2).

DREADDs may also represent a novel approach for combining gene therapy, tissue engineering, and synthetic biology. Indeed,

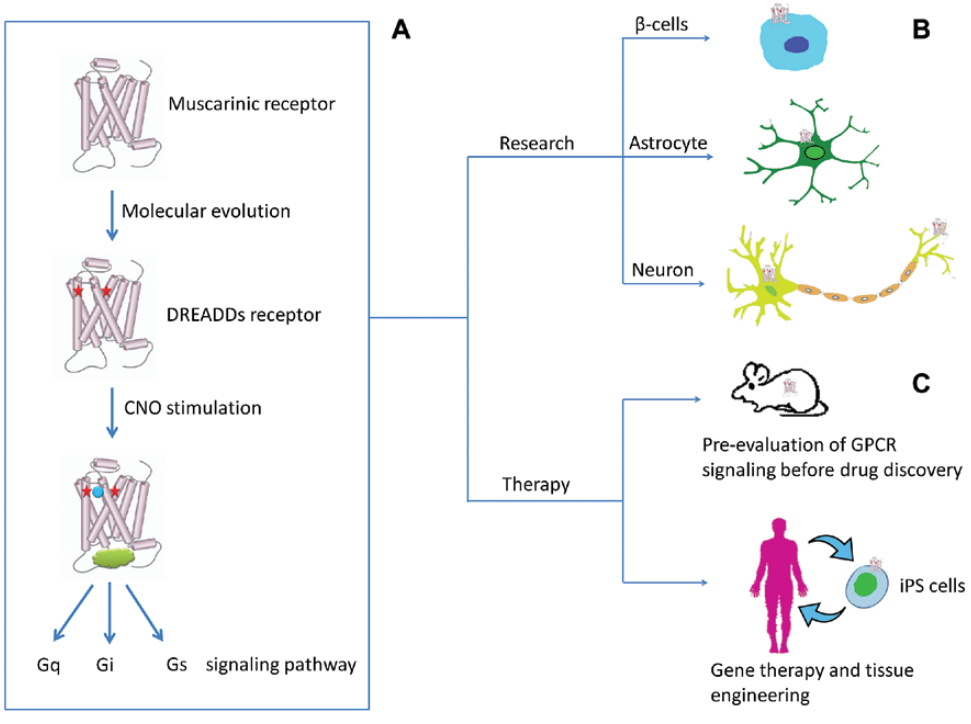

Figure 2. DREADDs represent a chemogenetic tool to modulate GPCR signaling in vivo. (A) Introducing two mutations in transmenbrane III and V of muscarinic receptors which create DREADDs receptors that can precisely control the Gq-, Gi-, or Gs-signaling pathways. (B) DREADDs technology has been successfully applied in β-cells, astrocytes, and a variety of neurons to control GPCR signaling in vivo. (C) DREADDs technology has a great potential to be used in drug discovery, gene therapy, and tissue engineering.

in an important recent study Dell'Anno et al. (2014) demonstrated robust and bidirectional remote control of DREADD-expressing dopaminergic neurons, which induced from iPSCs (Induced pluripotent stem cells), and were able to markedly enhance the beneficial effects in a mouse model of Parkinson's Disease. This represents the first demonstration of combining DREADD technology, gene therapy, and tissue engineering. Theoretically this approach could be potentiated by CRISPR (clustered regularly interspaced short palindromic repeats) technology (Hsu et al., 2014). Thus, for instance, one could envision engineering iPSCs by knocking in DREADDs into the downstream regions of disease-related GPCR targets using CRISPR. DREADDs could then be expressed at physiologically relevant levels in the correct cellular context. Following transplantation, we could then remotely control engineered iPSCs by administrating CNO, a drug-like small molecule, to potentially cure human disease.

Other applications are equally obvious—e.g., by engineering endogenous muscarinic receptors via CRISPR technology one could create mice in which endogenous muscarinic receptors are replaced by DREADDs to achieve precise control over muscarinic receptor subtype signaling. This would allow researchers to deconstruct the roles of various muscarinic receptors in neuropsychiatric disease models. Alternatively, CRISPR-based technology could be used to knock-in DREADDs into essentially any locus in the genome to achieve cell-type specific control of signaling in virtually any tissue and organ. Indeed, with the currently available tissue engineering, gene therapy, and synthetic biology tools we are limited only by our imagination in how we might adapt DREADD technology for interrogating and ultimately treating human neuropsychiatric diseases.

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Statement of Interest

All of the work involving DREADD technology in the Roth lab summarized in this review has been supported by the NIH and by NARSAD. Mice expressing DREADD receptors have been licensed to Takeda Pharmaceuticals; Dr Roth has received a grant from Merck Pharmaceuticals for enhancing DREADD technology. Over the past 18 months Dr Roth has consulted for Pfizer Pharmaceuticals and RuiYi Pharmaceuticals and has received honoraria from Novartis Pharmaceuticals. DREADD technology is an open-source non-patented resource.

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