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Special Issue: Metabolism Through the Lens of GPCRs

Forum

Use of Designer G Protein-Coupled Receptors to Dissect Metabolic Pathways

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G protein-coupled receptors (GPCRs) regulate virtually all metabolic processes, including glucose and energy homeostasis. Recently, the use of designer GPCRs referred to as designer receptors exclusively activated by designer drug (DREADDs) has made it possible to dissect metabolically relevant GPCR signaling pathways in a temporally and spatially controlled fashion *in vivo*.

The DREADD Concept

GPCRs are cell surface receptors that are targeted by an extraordinarily large number of clinically important drugs. Many studies suggest that distinct members of the GPCR superfamily represent potential targets for the treatment of various metabolic disorders including obesity and type 2 diabetes (T2D) [1]. However, research in this area has been hampered by the fact that a particular GPCR is usually expressed in multiple tissues and cell types, making it difficult to predict the *in vivo* metabolic consequences of targeting a specific GPCR with selective ligands.

During the past few years, designer GPCRs referred to as DREADDs have emerged as powerful, novel tools to study the physiological relevance of distinct GPCR signaling pathways in specific tissues *in vivo*. Structurally, DREADDs are mutant muscarinic acetylcholine receptors that can be activated with high potency by clozapine-*N*-oxide (CNO), an otherwise pharmacologically inert agent [2,3]. However, DREADDs cannot be activated by acetylcholine, the endogenous muscarinic receptor agonist [2,3].

Following agonist binding, GPCRs can activate different classes of heterotrimeric G proteins, which can be subdivided into four major functional groups: G_q, G_i, G_s, and G_{12}. Moreover, accumulating evidence suggests that GPCRs can also initiate β-arrestin-dependent (G protein-independent) signaling. Thus, the physiological outcome of activating a specific GPCR (or DREADD) in a particular tissue may also be modulated by β-arrestin-dependent signaling pathways.

At present, the most commonly used DREADDs are mutant muscarinic receptors that stimulate G_q-, G_i-, or G_s-type heterotrimeric G proteins (Figure 1A). More recently, two novel M_3 muscarinic receptor-based DREADDs have been developed (Figure 1B). One of these new designer receptors selectively stimulates β-arrestin-dependent signaling without activating G proteins [4] whereas the other construct selectively stimulates G proteins of the G_q family but lacks the ability to interact with β-arrestins [5]. In the following, I briefly review several studies that have employed DREADD technology to identify metabolically important GPCR signaling pathways *in vivo*. Because of space constraints, I focus on only three cell types that are of central importance for glucose and energy homeostasis: pancreatic β cells, hepatocytes, and AgRP neurons of the hypothalamus (Figure 2).

Modulation of β Cell Function by DREADDs

The function of pancreatic β cells is regulated by a large number of GPCRs [6]. Detailed metabolic studies with transgenic mice expressing M3Dq (G_q DREADD) selectively in pancreatic β cells have led to the identification of novel pathways crucial for the regulation of β cell function [3,7] (Figure 2A). For example, chronic CNO treatment of these mutant mice resulted in pronounced improvements in β cell function, including the upregulation of many genes critical for β cell function [7]. Moreover, chronic stimulation of β cell M3Dq led to a significant increase in pancreatic insulin content due to enhanced β cell proliferation and changes in gene expression promoting insulin synthesis [7]. Chronic activation of β cell M3Dq also greatly ameliorated streptozotocin-induced diabetes and metabolic deficits observed with mice maintained on a high-fat diet [7]. Additional *in vivo* and *in vitro* studies strongly suggested the existence of a novel signaling pathway through which activation of β cell G_q triggers enhanced expression and function of insulin receptor substrate 2 (IRS2) and that IRS2-dependent downstream signaling plays a key role in mediating the improved β cell function observed after chronic activation of M3Dq [7]. In acute CNO administration studies, transgenic mice that expressed the M3Ds designer construct (G_s DREADD) in a β cell-selective fashion showed beneficial metabolic phenotypes similar to those of the β cell M3Dq mutant mice [3]. However, the long-term effects of stimulating M3Ds in mouse β cells have not been investigated so far. These findings strongly suggest that therapeutic strategies aimed at enhancing signaling through β cell G_q and G_s should prove useful for the treatment of diabetes.

Expression of a G_q DREADD in Hepatocytes

The liver plays a central role in regulating whole-body glucose homeostasis. It is well known that glucagon-mediated activation of hepatocyte glucagon receptors, which selectively activate the stimulatory G protein, G_s, strongly promotes hepatic glucose production (HGP) (Figure 2B). Interestingly, CNO treatment of transgenic mice expressing the M3Dq DREADD selectively in hepatocytes led to pronounced increases in blood glucose levels

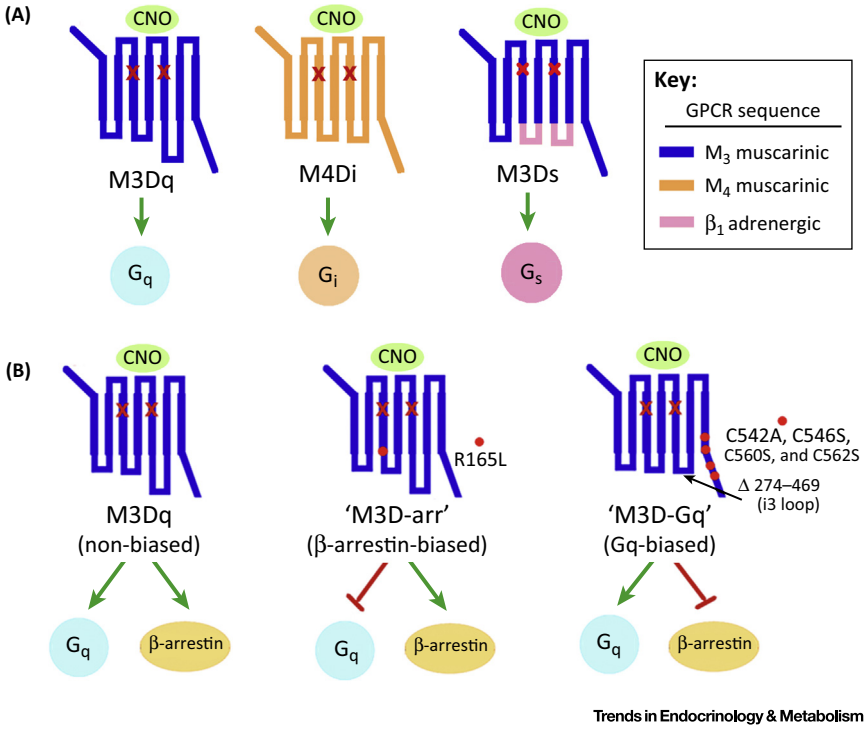

Figure 1. Structure and Coupling Properties of Muscarinic Receptor-Based Designer Receptors Exclusively Activated by Designer Drug (DREADDs). All depicted DREADDs contain the same two point mutations (Y→C and A→G) in transmembrane helices 3 and 5, respectively (red 'x marks') [2–5]. The designer receptors shown are unable to bind acetylcholine, the endogenous muscarinic receptor agonist, but can be activated by clozapine-*N*-oxide (CNO) with high potency and efficacy. (A) Structure and G protein-coupling properties of the M3Dq [2], M4Di [2], and M3Ds [3] DREADDs. (B) Structure of G_q- and β-arrestin-biased DREADDs. M3Dq couples to G_q-type G proteins but can also initiate β-arrestin-dependent signaling [4]. The M3D-arr DREADD (center panel) represents a β-arrestin-biased version of M3Dq [4]. M3D-Gq (right panel) functions as a G_q-biased designer receptor [5]. Amino acid numbers refer to the rat M_3 muscarinic receptor sequence.

due to enhanced hepatic gluconeogenesis and glycogen breakdown [8] (Figure 2B). A recent study employing G_q- and β-arrestin-biased DREADDs (Figure 1B) demonstrated that the M3Dq-mediated increases in HGP are caused by the activation of G_q-type G proteins and do not seem to be modulated by β-arrestins [5]. These observations suggest that inhibitors of G_q-mediated signaling in hepatocytes may prove clinically useful for suppressing HGP and hyperglycemia in T2D, a disease that is characterized by unphysiologically high hepatic glucose output.

Expression of DREADDs in AgRP Neurons

Obese individuals are at high risk of developing T2D. To guide the design of novel appetite-suppressing drugs, it is critical to dissect the neuronal circuits that regulate food intake under physiological and pathophysiological conditions. Recently, several studies have used DREADD technology to study a subpopulation of hypothalamic neurons located in the arcuate nucleus of the hypothalamus that synthesize and release agouti-related peptide (AgRP), a neuropeptide endowed with potent orexigenic activity (Figure 2C). AgRP neurons also store and release two additional appetite-stimulating agents, neuropeptide Y (NPY) and γ-aminobutyric acid (GABA) [9] (Figure 2C).

The activity of AgRP neurons, like that of essentially all other cell types, is predicted to be regulated by GPCRs with different G protein-coupling properties. CNO treatment of mice that selectively expressed the M3Dq DREADD in AgRP neurons triggered neuronal depolarization associated with a pronounced increase in food intake [10] (Figure 2C). By contrast, administration of CNO to mice that selectively expressed the M4Di DREADD in AgRP neurons inhibited the activity of AgRP neurons and caused a reduction in food intake [10] (Figure 2C). In a recent study, Nakajima *et al.* [11] expressed the M3Ds construct (G_s DREADD; Figure 1A) selectively in mouse AgRP neurons. CNO treatment of these animals led to a robust and sustained increase in food intake (Figure 2C). Additional studies suggested that the mechanisms through which the G_q- and G_s-linked DREADDs stimulate appetite are clearly distinct [10,11]. In contrast to M3Dq-induced feeding, the orexigenic effect triggered by M3Ds activation lasted for several days following a single CNO injection and was almost entirely dependent on the release of AgRP [11]. In any case, these findings suggest that drugs capable of blocking signaling through endogenous G_q- and G_s-linked GPCRs expressed by AgRP neurons may prove beneficial as novel appetite-suppressing drugs.

During the past few years, DREADD technology has also been instrumental in mapping several other central pathways that regulate whole-body glucose and energy homeostasis. Because of space constrains, I am unable to discuss these studies in this short Forum article (for a recent review, see [12]).

Comparison of DREADD Technology with Optogenetic Approaches

Like DREADD technology, optogenetic techniques have enabled neuroscientists to inhibit or activate specific sets of neurons to map neural circuitries that regulate feeding behavior and energy expenditure [13,14]. These studies have shown that the activation or inhibition of specific neuronal subpopulations by either DREADD technology or optogenetic approaches