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GPCRs of adrenal chromaffin cells & catecholamines: The plot thickens

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ABSTRACT

The circulating catecholamines (CAs) epinephrine (Epi) and norepinephrine (NE) derive from two major sources in the whole organism: the sympathetic nerve endings, which release NE on effector organs, and the chromaffin cells of the adrenal medulla, which are cells that synthesize, store and release Epi (mainly) and NE. All of the Epi in the body and a significant amount of circulating NE derive from the adrenal medulla. The secretion of CAs from adrenal chromaffin cells is regulated in a complex way by a variety of membrane receptors, the vast majority of which are G protein-coupled receptors (GPCRs), including adrenergic receptors (ARs), which act as "presynaptic autoreceptors" in this regard. There is a plethora of CA-secretagogue signals acting on these receptors but some of them, most notably the α_2ARs, inhibit CA secretion. Over the past few years, however, a few new proteins present in chromaffin cells have been uncovered to participate in CA secretion regulation. Most prominent among these are GRK2 and β-arrestin1, which are known to interact with GPCRs regulating receptor signaling and function. The present review will discuss the molecular and signaling mechanisms by which adrenal chromaffin cell-residing GPCRs and their regulatory proteins modulate CA synthesis and secretion. Particular emphasis will be given to the newly discovered roles of GRK2 and β-arrestins in these processes and particular points of focus for future research will be highlighted, as well.

1. Introduction

The circulating catecholamines (CA's) epinephrine (Epi) and norepinephrine (NE) derive from two major sources in the whole organism: the sympathetic nerve endings, which release NE on effector organs on stimulation, and the chromaffin cells of the adrenal medulla, which are cells that synthesize, store and release Epi (mainly) and NE upon acetylcholine (ACh) stimulation of the nicotinic cholinergic receptors (nAChRs) present on their membranes (Eaton and Duplan, 2004; Lymperopoulos et al., 2007b). The chromaffin cells of the adrenal medulla are, in reality, post-ganglionic sympathetic neurons representing the main source of circulating Epi in the body. Precursor cells originating in the neural crest migrate from primitive spinal ganglia to form the primitive sympathetic nervous system located dorsally to the aorta. These primitive sympathetic cells in the human fetal adrenal medulla can give rise to a neural or endocrine–catecholamine-storing phenotype (Hervonen and Kanerva, 1973). The central areas of the primitive elements do not undergo chromaffin differentiation and instead assume characteristics of sympathetic neuroblasts. Splanchnic nerve activity or chemicals that reach the adrenal medulla via the bloodstream may trigger CA release from adrenomedullary chromaffin cells in the human body. CA secretion induced by splanchnic nerve stimulation takes place in situations of fear, anxiety, or organic stress. Allergic reactions or hypotension produce endogenous compounds such as histamine, bradykinin, or angiotensin II that also stimulate catecholamine release. One of the main roles of CA's is to ensure an adequate blood flow and energy supply to vital organs to cope with stressful situations. Several nanograms of CA's per minute are released under basal conditions. However, during a fight-or-flight reaction there is massive Epi (70%) and NE (the rest 30%) input into the circulatory system, increasing their plasma concentrations up to 60 times (Currie, 2010). Normally, Epi is estimated to represent approximately 70–80% of the total adrenal CA secretion and NE to comprise the remaining approximately 20–30% (under physiological, non-stress conditions) (Eaton and Duplan, 2004). However, this ratio varies widely depending on the physiological condition of the adrenal gland and on the general systemic status.

The secretion of CAs from adrenal chromaffin cells in response to ACh released from centrally located sympathetic ganglia is regulated in a complex way by a variety of membrane receptors present

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in these cells (Lymperopoulos et al., 2007b; Currie, 2010). Many of these receptors are GPCRs, including adrenergic receptors (ARs), which thus act as "presynaptic autoreceptors". Of these regulatory ARs, the βARs (mainly of the β_2 subtype but also of the β_1 subtype) enhance CA secretion (facilitatory presynaptic autoreceptors) (Lymperopoulos et al., 2007b; Currie, 2010), whereas the α_2ARs inhibit CA secretion upon agonist stimulation (inhibitory presynaptic autoreceptors) (Brede et al., 2003; Philipp and Hein, 2004). The ARs are the interface between NE and Epi and their target cells and they all belong to the superfamily of G protein-coupled receptors (GPCRs): three α_1AR subtypes, three α_2AR subtypes (α_{2A}, α_{2B}, α_{2C}), and three βAR subtypes (Lymperopoulos et al., 2013). Thus, agonist-induced activation of ARs catalyzes the exchange of guanosine triphosphate (GTP) for guanosine diphosphate (GDP) on the G_α subunit of heterotrimeric G proteins, resulting in the dissociation of the heterotrimer into active G_α and free $G_{\beta\gamma}$ subunits (always associated together, i.e. a heterodimer that functions as a monomer) which can transduce intracellular signals independently of each other (Capote et al., 2015). With specific regards to the adrenal α_2ARs, they appear to be of either the α_{2A}- or the α_{2C} subtype in most species, including mice, rats and humans (Lymperopoulos et al., 2007a,b).

α_2ARs, like most GPCRs, are subject to agonist-promoted (homologous) desensitization and downregulation, a regulatory process that diminishes receptor response to continuous or repeated agonist stimulation (Rengo et al., 2011; Lymperopoulos and Bathgate, 2012). At the molecular level, this process is initiated by receptor phosphorylation by a family of kinases, termed GPCR kinases (GRKs), followed by binding of β-arrestins to the GRK-phosphorylated receptor (Rengo et al., 2009). The β-arrestins then uncouple the receptor from its cognate G proteins, sterically hinder its further binding to them (functional desensitization) and subsequently target the receptor for internalization (Lymperopoulos and Bathgate, 2013). Across all mammalian species, GRK2 and GRK5 are the most physiologically important members of the GRK family because they are expressed ubiquitously and regulate the vast majority of GPCRs. They are particularly abundant in neuronal tissues and in the heart (Lymperopoulos and Negussie, 2013; Capote et al., 2015). The β-arrestin-bound internalized receptor can subsequently either recycle back to the membrane (resensitization) or reach the lysosome for degradation (downregulation). In addition, the β-arrestin-receptor complex is able to elicit new, G protein-independent, signals as it traffics through the intracellular compartments (Lymperopoulos and Bathgate, 2013).

Herein, we review the signaling and regulatory mechanisms of the most important GPCRs expressed and operating in adrenal chromaffin cells to regulate CA secretion, with a specific focus on newly discovered and/or emerging roles of GRKs and β-arrestins in the process of fine-tuning adrenal CA production by these GPCRs.

2. G protein signaling & CA secretion from adrenal chromaffin cells

Chromaffin cells express a wide variety of GPCRs that sense and respond to changes in the local environment and perhaps the overall physiological "status" of the animal through hormones and other blood borne signals. A common theme at chromaffin cells and synapses is feedback modulation, whereby the released transmitters not only convey information to downstream targets but also act in an autocrine manner to modulate subsequent secretory activity. In general, GPCRs that couple to G_i-type G proteins inhibit CA release, whereas G_q-coupled receptors and G_s-coupled receptors potentiate it. Autoreceptors for ATP (P2Y receptors), CA's (α_2ARs, see below), and enkephalin (μ-opioid receptors) all couple to G_i-type G proteins and inhibit Ca^{2+} channels and, consequently, catecholamine release (Fig. 1) (Albillos et al., 1996; Brede et al., 2003; Currie, 2010; Harkins and Fox, 2000; Powell et al., 2000; Ulate et al., 2000). Conversely, elevation of cAMP by G_s-coupled GPCRs (e.g. D_1 dopaminergic, or βARs) can augment electrically evoked catecholamine release by increasing Ca^{2+} influx through L-type Ca^{2+} channels (LTCCs) and/or direct protein kinase A (PKA)-mediated phosphorylation of the exocytotic machinery (Fig. 1) (Carabelli et al., 2003; Nagy et al., 2004; Villanueva and Wightman, 2007). G_q-coupled GPCRs, such as the H_1 histaminergic and the angiotensin II type 1 (AT_1) receptors, release Ca^{2+} from intracellular stores and promote influx of extracellular Ca^{2+} to evoke catecholamine release (Fig. 1). H_1 receptors can also potentiate catecholamine release through generation of diacylglycerol which increases the size of the readily releasable pool of vesicles (Bauer et al., 2007).

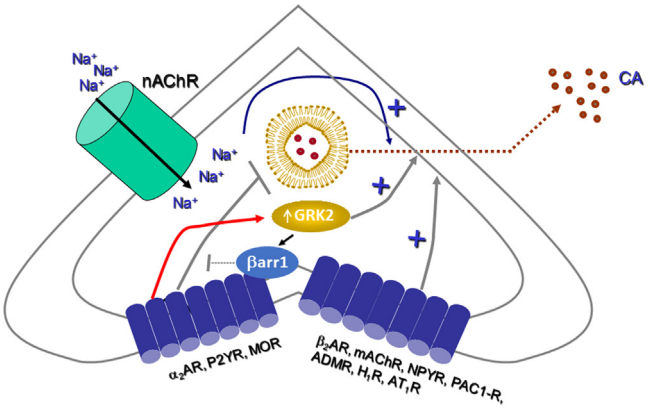

Fig. 1. *Emerging aspects of the regulation of CA secretion by GPCR signaling in the adrenal chromaffin cell.* nAChR: Nicotinic cholinergic receptor; CA: Catecholamine (Epi or NE); GRK2: GPCR-kinase-2; βarr1: β-arrestin1; AR: adrenergic receptor; P2YR: purinergic G protein-coupled receptor; MOR: μ-opioid receptor; mAChR: muscarinic cholinergic receptor; NPYR: neuropeptide Y receptor; PAC1-R: pituitary adenylate cyclase-activating polypeptide 1 receptor; ADMR: adrenomedullin receptor; H_1R: histaminergic type 1 receptor; AT_1R: angiotensin II type 1 receptor. The red arrow indicates the upregulation of GRK2 by chronic activation of the α_2AR in adrenal chromaffin cells. See text for details. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Acute activation of purinergic P2Y or μ-opioid (MORs) receptors or direct application/transfection with $G_{\beta\gamma}$ can inhibit catecholamine release via direct inhibitory effects of the free $G_{\beta\gamma}$ subunits on the LTCCs but also independently of Ca^{2+} channel modulation (Chen et al., 2005; Yoon et al., 2008). It is also interesting to note that concomitant activation of protein kinase C (PKC) seems to prevent the effects of $G_{\beta\gamma}$ on CA secretion (Chen et al., 2005). Therefore, $G_{\beta\gamma}$ and PKC compete with each other at the exocytotic machinery of the chromaffin cell to precisely control fusion pore kinetics, and ultimately, CA release. More specifically, $G_{\beta\gamma}$ can bind directly to several fusion pore machinery components, including syntaxin-1A, synaptobrevin, SNAP25 and the ternary SNARE complex in vitro (Jarvis et al., 2002; Blackmer et al., 2005). Moreover, $G_{\beta\gamma}$ and Ca^{2+}-bound synaptotagmin-1 compete for binding to the SNARE complex in vitro (Yoon et al., 2007). Therefore, $G_{\beta\gamma}$ most likely modulate multiple facets of exocytosis through interactions with SNARE proteins. Of course, $G_{\beta\gamma}$ are known to interact with an increasing number of downstream effectors, as well.

3. Individual GPCRs regulating CA secretion in adrenal chromaffin cells

3.1. NPY receptors

NPY is a 36-amino acid neuropeptide that acts as a co-transmitter, a neuromodulator and a neurohormone, and plays an