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Inhibition of Ca²⁺ Channels and Adrenal Catecholamine Release by G Protein Coupled Receptors

Kevin P. M. Currie

Departments of Anesthesiology, Pharmacology, and Center for Molecular Neuroscience, Vanderbilt University School of Medicine, 1161 21st Avenue South, Nashville, TN 37232, USA

Kevin P. M. Currie: kevin.currie@vanderbilt.edu

Abstract

Catecholamines and other transmitters released from adrenal chromaffin cells play central roles in the “fight-or-flight” response and exert profound effects on cardiovascular, endocrine, immune, and nervous system function. As such, precise regulation of chromaffin cell exocytosis is key to maintaining normal physiological function and appropriate responsiveness to acute stress. Chromaffin cells express a number of different G protein coupled receptors (GPCRs) that sense the local environment and orchestrate this precise control of transmitter release. The primary trigger for catecholamine release is Ca²⁺ entry through voltage-gated Ca²⁺ channels, so it makes sense that these channels are subject to complex regulation by GPCRs. In particular G protein $\beta\gamma$ heterodimers ($G\beta\gamma$) bind to and inhibit Ca²⁺ channels. Here I review the mechanisms by which GPCRs inhibit Ca²⁺ channels in chromaffin cells and how this might be altered by cellular context. This is related to the potent autocrine inhibition of Ca²⁺ entry and transmitter release seen in chromaffin cells. Recent data that implicate an additional inhibitory target of $G\beta\gamma$ on the exocytotic machinery and how this might fine tune neuroendocrine secretion are also discussed.

Keywords

Chromaffin; Calcium channel; G protein; Exocytosis; Secretion; Catecholamine; GPCR

The Fight-or-Flight Response and Stimulus-Secretion Coupling in Adrenal Chromaffin Cells

Adrenal chromaffin cells play important physiological roles in both homeostatic regulation and the coordinated response to stress or danger—i.e., the “fight-or-flight” response. For example, heart rate and contractility are increased, vascular tone and blood flow to skeletal muscle are increased, insulin release is suppressed, and glucose is mobilized from the liver. These and a myriad of other effects prime the body for a survival response to either fight or flee an enemy or dangerous situation. These diverse physiological effects are mediated by release of catecholamines (epinephrine and norepinephrine) and a number of other biologically active peptides and hormones from chromaffin cells. While beneficial in the short term, inappropriate responsiveness to stressors and/or chronic stress can be harmful. For example, elevated catecholamine levels are closely correlated with hypertension (Takiyyuddin et al. 1993) and contribute to the morbidity and mortality of chronic heart failure (Floras 2003).

Chromaffin cells, like neurons, release transmitters by Ca^{2+} -regulated exocytosis. Unlike most neurons, the cells are ideally suited for patch-clamp electrophysiology to record ion channel currents and changes in membrane capacitance that track vesicle exocytosis and endocytosis with high time resolution (Lindau and Neher 1988; Gillis 2000). Another technique that directly detects catecholamine release with exquisite resolution is carbon fiber amperometry (Wightman et al. 1991). Catecholamines released from the cell are rapidly oxidized generating an electrical current that is directly proportional to the number of catecholamine molecules. With suitable stimulation protocols transient current spikes reflecting catecholamine release from individual vesicular fusion events can be detected and analyzed. These approaches combined with other techniques such as total internal reflection fluorescence (TIRF) microscopy and photorelease of “caged” Ca^{2+} can be applied to cells or adrenal slices from wild-type and transgenic mice to provide detailed insight into the cellular and molecular mechanisms that control neurosecretion.

In situ, chromaffin cells are innervated by splanchnic nerve terminals that release acetylcholine (ACh) and other transmitters including PACAP. Activation of nicotinic ACh receptors on the chromaffin cells causes membrane depolarization/action potential firing, opening of voltage-gated Ca^{2+} channels (Ca^{2+} channels), and Ca^{2+} influx that triggers fusion of the vesicles with the plasma membrane (Boarder et al. 1987). Consequently, Ca^{2+} channels play pivotal roles in stimulus-secretion coupling and are important targets for mechanisms that control transmitter release. Significant advances have also been made toward identifying the protein–protein interactions involved in exocytosis (for recent reviews see Rizo and Rosenmund 2008; Sorensen 2009; Sudhof and Rothman 2009). It is generally accepted that SNARE proteins constitute the core fusion machinery. Syntaxin and SNAP25 on the plasma membrane along with synaptobrevin on the vesicular membrane form a coiled-coil structure (the ternary SNARE complex) that positions the vesicle in close apposition to the plasma membrane. When the SNARE complex “zippers-up” it provides the force required to fuse the two adjacent lipid bilayers. Several members of the synaptotagmin family serve as Ca^{2+} sensors for exocytosis (Chapman 2008), and in particular synaptotagmins 1 and 7 are involved in chromaffin cells (Schonn et al. 2008). Although the precise mechanisms are still being defined, synaptotagmin-1 binds the plasma membrane and the SNARE complex in a Ca^{2+} -dependent manner inducing conformational changes that trigger the final fusion event. The SNAREs are also involved in other aspects of the secretory vesicle cycle and recently it was proposed that docking of vesicles in mouse chromaffin cells is mediated by binding of synaptotagmin-1 to binary t-SNARE complexes (de Wit et al. 2009).

G protein Coupled Receptors (GPCRs) in Chromaffin Cells

GPCRs play central roles in orchestrating the dynamic modulation of transmitter release. Heterotrimeric G proteins act as molecular switches to transduce extracellular ligand binding to the GPCR into intracellular signaling cascades. GPCRs are often characterized according to the family of $G\alpha$ subunit to which they couple: G_s -coupled receptors typically elevate cAMP; G_i -coupled receptors inhibit cAMP; G_q -coupled receptors activate phospholipase C; while G_{12} receptor signaling is less well defined. Agonist binding to the GPCR catalyzes the exchange of GDP to GTP on the G protein α -subunit ($G\alpha$) and both $G\alpha$ and the $G\beta\gamma$ heterodimer signal to downstream effectors. Of note for this review, these $G\beta\gamma$ effectors include voltage-gated Ca^{2+} channels and SNARE proteins (see below).

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 Gi-type G proteins inhibit catecholamine release, whereas Gq-coupled receptors and Gs-coupled receptors potentiate catecholamine release. Autoreceptors for ATP (P2Y receptors), catecholamines (α -adrenergic), and enkephalin (μ -opioid receptors) all couple to Gi-type G proteins and inhibit Ca^{2+} channels and transmitter release (Albillos et al. 1996b; Currie and Fox 1996; Harkins and Fox 2000; Powell et al. 2000; Ulate et al. 2000; Brede et al. 2003). Conversely, elevation of cAMP by Gs-coupled GPCRs (e.g., D1 dopamine, or β -adrenergic) can augment electrically evoked catecholamine release by increasing Ca^{2+} influx through L-type Ca^{2+} channels and/or direct protein kinase-A mediated phosphorylation of the exocytotic machinery (Artalejo et al. 1990; Carabelli et al. 2003; Nagy et al. 2004; Villanueva and Wightman 2007). Gq-coupled GPCRs (e.g., H1 histamine receptors) can release Ca^{2+} from intracellular stores and promote influx of extracellular Ca^{2+} to evoke catecholamine release. H1 receptors can also potentiate catecholamine release through generation of diacylglycerol which binds munc13 to increase the size of the readily releasable pool of vesicles (Bauer et al. 2007). PACAP/PAC1 receptors also play important roles in stimulating catecholamine release, particularly during periods of stress (Hamelink et al. 2002; Kuri et al. 2009).

Voltage-Gated Ca^{2+} Channels

As outlined above Ca^{2+} channels play pivotal roles in stimulus-secretion coupling and a multitude of other cellular functions. In large part this is due to their role in transducing the electrical inputs of excitable cells into biochemical outputs by influx of the ubiquitous second messenger Ca^{2+} . The channels are hetero-oligomeric protein complexes containing a pore forming α_1 subunit, a cytoplasmic β -subunit, a membrane associated $\alpha_2\delta$ -subunit, and perhaps a γ -subunit (Catterall 2000). Ten genes encode three major families of the pore forming α_1 subunit: four Ca_v1 (L-type channels), three Ca_v2 (P/Q-type, N-type, and R-type channels), and three Ca_v3 (T-type channels). The Ca_v3 family is referred to as low-voltage activated channels because they require only modest membrane depolarization to open. Ca_v1 and Ca_v2 channels are referred to as high-voltage activated as they typically require stronger membrane depolarization to open, although $\text{Ca}_v1.3$ and $\text{Ca}_v2.3$ channels activate at relatively hyperpolarized potentials. Indeed $\text{Ca}_v1.3$ channels play an important role in determining the spontaneous electrical activity of mouse chromaffin cells (Marcantoni et al. 2010). The pore forming α_1 -subunit consists of four homologous repeats (domains I–IV) each with six transmembrane spanning α -helical segments (S1–S6) and a ‘P-loop’ between S5 and S6. The intracellular regions of the channel including the N- and C-termini and the cytoplasmic loops connecting the domains I–IV are important for interaction with other proteins including $G\beta\gamma$ and regulatory mechanisms such as phosphorylation. Alternative splicing of the α_1 subunit can increase functional diversity, for example by introducing a site for tyrosine kinase phosphorylation in the C-terminus of $\text{Ca}_v2.2$ (Gray et al. 2007). Four genes encode β -subunits, with multiple splice variants for each, four genes encode $\alpha_2\delta$ subunits, and ten putative γ subunit genes exist. Little is known about the role of γ subunits and it is not clear that they functionally associate with the channels in neurons and neuroendocrine cells. Both the $\alpha_2\delta$ and β subunits increase the expression and modulate activation and inactivation kinetics of Ca_v1 and Ca_v2 channels. The β -subunit might also be targeted by kinases to modulate channel activity (Grueter et al. 2006) and the $\alpha_2\delta$ subunits can bind extracellular ligands and drugs including thrombospondin and gabapentin/pregabalin (Davies et al. 2007; Eroglu et al. 2009).

Chromaffin cells can express channels from all three Ca_v families. Cell-to-cell and species-to-species variability in the relative expression of the Ca^{2+} channels has been reported by several groups (Garcia et al. 2006). Also of note, different channel types can be recruited by physiological or pathophysiological stressors. For example, Ca_v3 channels are typically not

seen in recordings from adult chromaffin cells, but hypoxia or sustained β -adrenergic receptor signaling can increase plasma membrane expression of these channels and thereby shift the voltage-dependence of Ca^{2+} entry and exocytosis to more hyperpolarized potentials (Novara et al. 2004; Carabelli et al. 2007). Some studies have reported preferential coupling of specific channels to secretion or distinct vesicle pools, while others suggest the channels couple to secretion with similar efficiency (Artalejo et al. 1994; Engisch and Nowycky 1996; Lukyanetz and Neher 1999; Alvarez et al. 2008). The contribution of specific channel types can also change in an activity-dependent manner (Villarroya et al. 1999; Chan et al. 2005; Polo-Parada et al. 2006). Of note for this review, the Ca^{2+} -channel subtypes are differentially modulated by G proteins and other second messenger pathways (Fox et al. 2008; Marcantoni et al. 2008).

Modulation of Ca^{2+} Channels by G Protein $\beta\gamma$ Subunits

Inhibition of Ca^{2+} channels by neurotransmitter receptors is now recognized to be a widespread and important mechanism that controls neurotransmitter and hormone release (for reviews see Dolphin 2003; Tedford and Zamponi 2006; Catterall and Few 2008; Stephens 2009). Inhibition by GPCRs predominantly targets $\text{Ca}_v2.1$ (P/Q-type) and $\text{Ca}_v2.2$ (N-type) channels although Ca_v1 and Ca_v3 channels can also be inhibited, albeit by different molecular mechanisms. Several distinct signaling pathways converge on Ca_v2 channels but the most common and best understood mechanism is the so-called *voltage-dependent inhibition* mediated by direct binding of $G\beta\gamma$ to the channel. $G\beta\gamma$ reduces the peak amplitude of the whole cell Ca^{2+} -channel current (I_{Ca}), and produces characteristic shifts in channel gating: the voltage-dependence of activation is shifted to more depolarized potentials and activation kinetics are slowed; inhibition is diminished at depolarized membrane potentials; a conditioning prepulse to depolarized potentials can relieve most of the inhibition and normalize channel kinetics (hence the inhibition is termed *voltage-dependent*). In addition to being a defining biophysical signature, voltage-dependent relief of the inhibition (also termed facilitation) is physiologically relevant and can occur at least to some extent during high frequency trains of action potential-like waveforms (Womack and McCleskey 1995; Brody et al. 1997; Currie and Fox 2002). Inhibition is mediated by direct binding of $G\beta\gamma$ to the α_1 subunit of the channel, primarily the intracellular loop between domains I and II with other regions in domain I, the N-terminal, and C-terminal also implicated (Tedford and Zamponi 2006). These aspects have been incorporated into models in which the channels exhibit two functional gating states: “willing” in absence of bound $G\beta\gamma$ and “reluctant” when $G\beta\gamma$ is bound (Bean 1989; Elmslie et al. 1990; Colecraft et al. 2000; Lee and Elmslie 2000). Voltage-dependent relief of the inhibition by a prepulse or train of action potential-like stimuli is thought to reflect transient dissociation of $G\beta\gamma$ from the channel at depolarized potentials. At the single channel level $G\beta\gamma$ increases the latency to first opening of the channel in response to membrane depolarization with little impact on other parameters (Carabelli et al. 1996; Patil et al. 1996). Brief channel openings (from the “reluctant” state) have been reported to occur in N-type channels but the probability of such events is low (Colecraft et al. 2000; Lee and Elmslie 2000).

Both N-type and P/Q-type channels are subject to $G\beta\gamma$ mediated inhibition. However, the amplitude P/Q-type I_{Ca} is reduced to a lesser extent than N-type I_{Ca} (Currie and Fox 1997) and the inhibition of P/Q-type I_{Ca} can be relieved more easily by trains of action potential-like stimuli (Currie and Fox 2002). These differences are likely due to lower affinity binding of $G\beta\gamma$ at the P/Q-type channel. Protein kinase C can phosphorylate the I-II linker region of $\text{Ca}_v2.2$ (N-type) channels and thereby diminish binding of $G\beta1$ -containing $G\beta\gamma$ heterodimers, but not heterodimers containing other $G\beta$ subunits (Cooper et al. 2000). More recently it has been shown that $G\beta\gamma$ can reduce inactivation of N-type channels (McDavid and Currie 2006; Weiss et al. 2007). The molecular mechanisms that underlie voltage-

dependent inactivation of Ca^{2+} channels are not fully understood but might involve a “hinged lid” type mechanism, with the intracellular loop connecting domains I and II of α_1 -subunit serving as the “inactivation gate” (Stotz and Zamponi 2001). Intriguingly, the I–II loop is also a primary binding site of $\text{G}\beta\gamma$ on the channel (De Waard et al. 1997; Herlitze et al. 1997). Perhaps binding of $\text{G}\beta\gamma$ disrupts movement of this putative inactivation gate, or its interaction with other channel domains, but this remains to be determined.

Voltage-dependent inhibition by $\text{G}\beta\gamma$ demonstrates substantial plasticity and crosstalk with other signaling pathways that control I_{Ca} . For example, the extent of inhibition and the ability for high frequency bursts of action potentials to relieve the inhibition will depend upon the relative expression of N- and P/Q-type channels in a particular cell or synapse. Concomitant activation of PKC might selectively diminish inhibition of N-type channels, but only when the $\text{G}\beta\gamma$ heterodimer contains $\text{G}\beta 1$ -subunits. Due to reduced inactivation $\text{G}\beta\gamma$ alters the temporal profile as well as the absolute amount of Ca^{2+} influx during trains of action potential-like stimuli. Thus, Ca^{2+} channels integrate multiple convergent signals, both electrical and chemical (2nd messengers), to precisely tune Ca^{2+} entry for a given cellular context.

Voltage-dependent inhibition of N- and P/Q-type I_{Ca} is prominent in adrenal chromaffin cells and thought to underlie potent feedback inhibition of secretory activity. Several GPCRs have been shown to inhibit I_{Ca} including α -adrenergic, opioid and P2Y purinergic receptors (Diverse-Pierluissi et al. 1991; Kleppisch et al. 1992; Gandia et al. 1993; Twitchell and Rane 1993; Albillos et al. 1996a, b; Currie and Fox 1996). These receptors are of particular note because large dense core vesicles store and release catecholamines, ATP/ADP, and enkephalins, along with several other peptides and hormones. A variety of experiments have been performed to assess the impact of endogenous transmitters in chromaffin cells, and the consensus from these studies is that autocrine/paracrine inhibition of I_{Ca} is mediated by P2Y purinergic receptors and μ/δ opioid receptors (for review see Garcia et al. 2006). In contrast $\alpha 2$ adrenergic receptor modulation of I_{Ca} is not consistently seen in chromaffin cells. The inhibition produced by ATP is potent ($\text{EC}_{50} \sim 0.5 \mu\text{M}$) (Currie and Fox 1996), shows little desensitization, and is likely mediated by a P2Y₁₂ receptor subtype (Ennion et al. 2004). Given the high concentration of ATP in the large dense core vesicles it is likely that autocrine purinergic modulation will potently and maximally inhibit I_{Ca} in the intact adrenal.

Voltage-independent inhibition of $\text{Ca}_v 2$ channels also exists and likely represents a conglomerate of several distinct mechanisms. In chromaffin cells purinergic and opioid receptors can produce voltage-independent inhibition by a mechanism involving neuronal Ca^{2+} sensor-1 and src kinase (Weiss and Burgoyne 2001) and Gq-coupled H1 histamine receptors produce voltage-independent inhibition of $\text{Ca}_v 2$ (Currie and Fox 2000). Direct interaction of GPCRs and N-type Ca^{2+} channels has been reported to lead to internalization of the channel when the activated GPCR is endocytosed (Altier et al. 2006) but it is not known if a similar mechanism exists in chromaffin cells.

G proteins activated by purinergic and opioid autoreceptors can also directly inhibit L-type I_{Ca} in chromaffin cells. This pathway is rapid, direct/membrane delimited, but voltage-independent (Hernandez-Guijo et al. 1999). Depending on the species, L-type channels contribute from ~10 to 40% of the whole cell I_{Ca} in chromaffin cells so play important roles in stimulus-secretion coupling (Garcia et al. 2006). $\text{Ca}_v 1.3$ channels also play an important role in the basal firing properties of mouse chromaffin cells, so it will be interesting to see if these channels are inhibited by GPCRs and how this might impact electrical firing. Until recently it was not thought that $\text{Ca}_v 3$ channels were modulated by GPCRs, but it has now been shown that $\text{Ca}_v 3.2$ channels are inhibited by D1 dopamine and corticotropin-releasing factor type 1 receptors, the latter in adrenal cortical cells (Wolfe et al. 2003). At least some

of these pathways involve direct effects of $G\beta\gamma$, albeit in a voltage-independent manner. It will be interesting to determine if $Ca_v3.2$ channels upregulated in chromaffin cells by hypoxia or prolonged β -adrenergic stimulation are subject to modulation by endogenous GPCRs.

Inhibition of Catecholamine Release by $G\beta\gamma$

The link between purinergic/opioid receptor-mediated inhibition of I_{Ca} and transmitter release in chromaffin cells has been shown by several groups (Harkins and Fox 2000; Powell et al. 2000; Ulate et al. 2000; Ennion et al. 2004). These studies used changes in membrane capacitance to assay exocytosis and concluded that inhibition of I_{Ca} is the dominant (if not the only) mechanism that inhibits exocytosis (but see Lim et al. 1997). More recently two studies in rat (Chen et al. 2005) or bovine chromaffin cells (Yoon et al. 2008) concluded that acute activation of P2Y or μ -opioid receptors, or direct application/transfection with $G\beta\gamma$ can inhibit catecholamine release independent from Ca^{2+} channel modulation. Catecholamine release was monitored using carbon fiber amperometry and evoked by methods that bypass Ca^{2+} channels (releasing Ca^{2+} from intracellular stores, applying Ca^{2+} directly through a patch pipette, or using the Ca^{2+} ionophore ionomycin). In both studies G proteins reduced the charge of individual amperometric spikes suggesting that, on average, the number of catecholamine molecules released by each vesicular fusion event was reduced. In bovine cells the number of amperometric spikes was also reduced.

The reduction of spike charge could reflect less catecholamine loaded into the vesicles, although the rapidly reversible effect of GPCR activation suggests this is less likely. Alternatively, $G\beta\gamma$ might preferentially inhibit release of a distinct subset of larger vesicles, and there is evidence for two populations of vesicles in mouse chromaffin cells based on estimates of vesicle size and the charge of amperometric spikes (Grabner et al. 2005). Another possibility discussed in both papers is that $G\beta\gamma$ might shift the mode of exocytosis and favor the occurrence of transient (kiss-and-run) rather than full fusion events. In classical, full fusion exocytosis the vesicle collapses into the plasma membrane thereby emptying the entire contents into the extracellular space. Transient events can also occur in which a fusion pore opens and then re-closes, thus maintaining the integrity of the vesicle and potentially resulting in partial release of the vesicle contents (for reviews see Harata et al. 2006; He and Wu 2007). Work from lamprey reticulospinal synapses supports the idea that GPCRs can modulate the mode of exocytosis (Photowala et al. 2006; Gerachshenko et al. 2009). In chromaffin cells, dynamic shifts from transient fusion to full collapse events have been reported to occur with increased stimulation frequency/ Ca^{2+} entry (Elhamdani et al. 2001, 2006; Fulop et al. 2005; Fulop and Smith 2006). This has the potential to impact the identity as well as the amount of transmitter release from chromaffin cells because the narrow fusion pore in transient events might limit release of larger peptide transmitters by a simple size exclusion mechanism (Fulop et al. 2005). It is also interesting to note that concomitant activation of PKC prevented the effects of $G\beta\gamma$ on spike charge ("quantal size") (Chen et al. 2005). Activation of PKC has also been implicated in the shift from transient to full fusion events during stimuli that mimic acute stress (15 Hz trains of action potential-like stimuli) (Fulop and Smith 2006). Therefore, it is possible that there are opposing actions of $G\beta\gamma$ and PKC on the exocytotic machinery to precisely control fusion pore kinetics.

The molecular targets that underlie these novel effects on catecholamine release remain unclear, but one plausible target is the core fusion machinery. $G\beta\gamma$ can bind to 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). In addition to being Ca^{2+} sensors for exocytosis, synaptotagmins have been implicated in fusion pore modulation

(Wang et al. 2001; Bai et al. 2004; Moore et al. 2006), and vesicle docking in mouse chromaffin cells is mediated by interaction of synaptotagmin-1 with binary t-SNARE complexes (de Wit et al. 2009). Therefore, it is conceivable that $G\beta\gamma$ could modulate multiple facets of exocytosis through interactions with SNARE proteins. Of course $G\beta\gamma$ is known to interact with an increasing number of downstream effectors. Development of molecular tools to dissect the various targets of $G\beta\gamma$ will be important to tease apart the precise mechanisms and relative contribution of Ca^{2+} channels and other targets to the overall control of catecholamine release by $G\beta\gamma$.

Concluding Remarks

It is clear that G proteins utilize multiple signaling pathways to control the timing and amount of Ca^{2+} entry in chromaffin cells. This and potentially other mechanisms that directly target the exocytotic machinery precisely regulate transmitter release. Clearly cellular context matters and must be considered as this field moves forward. As outlined above, $G\beta\gamma$ -mediated inhibition of I_{Ca} can be altered depending on the electrical firing of the cell, either through effects on inactivation of the channels or voltage-dependent relief of $G\beta\gamma$ binding. Concomitant activation of PKC by GPCRs and/or Ca^{2+} entry might antagonize inhibition of N-type I_{Ca} and the effects of $G\beta\gamma$ on the quantal size of release events. Another interesting consideration is cellular remodeling under physiological or pathophysiological conditions and how this might impact G protein control of transmitter release. This might involve changes in the Ca^{2+} channel subtype (as shown for hypoxia or sustained adrenergic receptor stimulation), or changes in the number or type of GPCRs expressed on the plasma membrane. Many other proteins can also regulate G protein signaling. Recent reports suggest that expression of G protein coupled receptor kinase 2 (GRK2) is increased in the adrenal gland in rodent models of heart failure (Lympopoulos et al. 2007). GRK2 is involved in desensitization of α_2 -adrenergic and other GPCRs and it was suggested that increased GRK2 expression resulted in diminished feedback inhibition of catecholamine release. Reducing GRK2 expression in the adrenal gland decreased circulating catecholamines and improved heart function in these rodent models. Thus targeting feedback regulatory mechanisms in adrenal chromaffin cells might prove to be a useful therapeutic strategy in future.

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References

- Albillos A, Carbone E, Gandia L, Garcia AG, Pollo A. Opioid inhibition of Ca^{2+} channel subtypes in bovine chromaffin cells: selectivity of action and voltage-dependence. *Eur J Neurosci* 1996a; 8:1561–1570. [PubMed: 8921248]
- Albillos A, Gandia L, Michelena P, Gilabert JA, del Valle M, Carbone E, Garcia AG. The mechanism of calcium channel facilitation in bovine chromaffin cells. *J Physiol* 1996b;494(Pt 3):687–695. [PubMed: 8865066]
- Altier C, Khosravani H, Evans RM, Hameed S, Peloquin JB, Vartian BA, Chen L, Beedle AM, Ferguson SS, Mezghrani A, Dubel SJ, Bourinet E, McRory JE, Zamponi GW. ORL1 receptor-mediated internalization of N-type calcium channels. *Nat Neurosci* 2006;9:31–40. [PubMed: 16311589]
- Alvarez YD, Ibanez LI, Uchitel OD, Marengo FD. P/Q Ca^{2+} channels are functionally coupled to exocytosis of the immediately releasable pool in mouse chromaffin cells. *Cell Calcium* 2008;43:155–164. [PubMed: 17561253]

- Artalejo CR, Ariano MA, Perlman RL, Fox AP. Activation of facilitation calcium channels in chromaffin cells by D1 dopamine receptors through a cAMP/protein kinase A-dependent mechanism. *Nature* 1990;348:239–242. [PubMed: 2172839]
- Artalejo CR, Adams ME, Fox AP. Three types of Ca^{2+} channel trigger secretion with different efficacies in chromaffin cells. *Nature* 1994;367:72–76. [PubMed: 8107778]
- Bai J, Wang CT, Richards DA, Jackson MB, Chapman ER. Fusion pore dynamics are regulated by synaptotagmin* t -SNARE interactions. *Neuron* 2004;41:929–942. [PubMed: 15046725]
- Bauer CS, Woolley RJ, Teschemacher AG, Seward EP. Potentiation of exocytosis by phospholipase C-coupled G-protein-coupled receptors requires the priming protein Munc13-1. *J Neurosci* 2007;27:212–219. [PubMed: 17202488]
- Bean BP. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature* 1989;340:153–156. [PubMed: 2567963]
- Blackmer T, Larsen EC, Bartleson C, Kowalchuk JA, Yoon EJ, Preininger AM, Alford S, Hamm HE, Martin TF. G protein betagamma directly regulates SNARE protein fusion machinery for secretory granule exocytosis. *Nat Neurosci* 2005;8:421–425. [PubMed: 15778713]
- Boarder MR, Marriott D, Adams M. Stimulus secretion coupling in cultured chromaffin cells. Dependency on external sodium and on dihydropyridine-sensitive calcium channels. *Biochem Pharmacol* 1987;36:163–167. [PubMed: 2432901]
- Brede M, Nagy G, Philipp M, Sorensen JB, Lohse MJ, Hein L. Differential control of adrenal and sympathetic catecholamine release by alpha 2-adrenoceptor subtypes. *Mol Endocrinol* 2003;17:1640–1646. [PubMed: 12764077]
- Brody DL, Patil PG, Mulle JG, Snutch TP, Yue DT. Bursts of action potential waveforms relieve G-protein inhibition of recombinant P/Q-type Ca^{2+} channels in HEK 293 cells. *J Physiol* 1997;499(Pt 3):637–644. [PubMed: 9130160]
- Carabelli V, Lovallo M, Magnelli V, Zucker H, Carbone E. Voltage-dependent modulation of single N-type Ca^{2+} channel kinetics by receptor agonists in IMR32 cells. *Biophys J* 1996;70:2144–2154. [PubMed: 9172738]
- Carabelli V, Gancippoli A, Baldelli P, Carbone E, Artalejo AR. Distinct potentiation of L-type currents and secretion by cAMP in rat chromaffin cells. *Biophys J* 2003;85:1326–1337. [PubMed: 12885675]
- Carabelli V, Marcantoni A, Comunanza V, de Luca A, Diaz J, Borges R, Carbone E. Chronic hypoxia up-regulates alpha1H T-type channels and low-threshold catecholamine secretion in rat chromaffin cells. *J Physiol* 2007;584:149–165. [PubMed: 17690152]
- Catterall WA. Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol* 2000;16:521–555. [PubMed: 11031246]
- Catterall WA, Few AP. Calcium channel regulation and presynaptic plasticity. *Neuron* 2008;59:882–901. [PubMed: 18817729]
- Chan SA, Polo-Parada L, Smith C. Action potential stimulation reveals an increased role for P/Q-calcium channel-dependent exocytosis in mouse adrenal tissue slices. *Arch Biochem Biophys* 2005;435:65–73. [PubMed: 15680908]
- Chapman ER. How does synaptotagmin trigger neurotransmitter release? *Annu Rev Biochem* 2008;77:615–641. [PubMed: 18275379]
- Chen XK, Wang LC, Zhou Y, Cai Q, Prakriya M, Duan KL, Sheng ZH, Lingle C, Zhou Z. Activation of GPCRs modulates quantal size in chromaffin cells through G(betagamma) and PKC. *Nat Neurosci* 2005;8:1160–1168. [PubMed: 16116443]
- Colecraft HM, Patil PG, Yue DT. Differential occurrence of reluctant openings in G-protein-inhibited N- and P/Q-type calcium channels. *J Gen Physiol* 2000;115:175–192. [PubMed: 10653895]
- Cooper CB, Arnot MI, Feng ZP, Jarvis SE, Hamid J, Zamponi GW. Cross-talk between G-protein and protein kinase C modulation of N-type calcium channels is dependent on the G-protein beta subunit isoform. *J Biol Chem* 2000;275:40777–40781. [PubMed: 11053424]
- Currie KPM, Fox AP. ATP serves as a negative feedback inhibitor of voltage-gated Ca^{2+} channel currents in cultured bovine adrenal chromaffin cells. *Neuron* 1996;16:1027–1036. [PubMed: 8630241]

- Currie KPM, Fox AP. Comparison of N- and P/Q-type voltage-gated calcium channel current inhibition. *J Neurosci* 1997;17:4570–4579. [PubMed: 9169518]
- Currie KP, Fox AP. Voltage-dependent, pertussis toxin insensitive inhibition of calcium currents by histamine in bovine adrenal chromaffin cells. *J Neurophysiol* 2000;83:1435–1442. [PubMed: 10712470]
- Currie KPM, Fox AP. Differential facilitation of N- and P/Q-type calcium channels during trains of action potential-like waveforms. *J Physiol* 2002;539:419–431. [PubMed: 11882675]
- Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the $\alpha(2)\delta$ subunits of voltage-gated calcium channels. *Trends Pharmacol Sci* 2007;28:220–228. [PubMed: 17403543]
- De Waard M, Liu H, Walker D, Scott VE, Gurnett CA, Campbell KP. Direct binding of G-protein betagamma complex to voltage-dependent calcium channels. *Nature* 1997;385:446–450. [PubMed: 9009193]
- de Wit H, Walter AM, Milosevic I, Gulyas-Kovacs A, Riedel D, Sorensen JB, Verhage M. Synaptotagmin-1 docks secretory vesicles to syntaxin-1/SNAP-25 acceptor complexes. *Cell* 2009;138:935–946. [PubMed: 19716167]
- Diverse-Pierluissi M, Dunlap K, Westhead EW. Multiple actions of extracellular ATP on calcium currents in cultured bovine chromaffin cells. *Proc Natl Acad Sci USA* 1991;88:1261–1265. [PubMed: 1847515]
- Dolphin AC. G protein modulation of voltage-gated calcium channels. *Pharmacol Rev* 2003;55:607–627. [PubMed: 14657419]
- Elhamdani A, Palfrey HC, Artalejo CR. Quantal size is dependent on stimulation frequency and calcium entry in calf chromaffin cells. *Neuron* 2001;31:819–830. [PubMed: 11567619]
- Elhamdani A, Azizi F, Artalejo CR. Double patch clamp reveals that transient fusion (kiss-and-run) is a major mechanism of secretion in calf adrenal chromaffin cells: high calcium shifts the mechanism from kiss-and-run to complete fusion. *J Neurosci* 2006;26:3030–3036. [PubMed: 16540581]
- Elmslie KS, Zhou W, Jones SW. LHRH and GTP- γ -S modify calcium current activation in bullfrog sympathetic neurons. *Neuron* 1990;5:75–80. [PubMed: 2164405]
- Engisch KL, Nowycky MC. Calcium dependence of large dense-cored vesicle exocytosis evoked by calcium influx in bovine adrenal chromaffin cells. *J Neurosci* 1996;16:1359–1369. [PubMed: 8778287]
- Ennion SJ, Powell AD, Seward EP. Identification of the P2Y₁₂ receptor in nucleotide inhibition of exocytosis from bovine chromaffin cells. *Mol Pharmacol* 2004;66:601–611. [PubMed: 15322252]
- Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA. Gabapentin receptor $\alpha 2\delta$ -1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 2009;139:380–392. [PubMed: 19818485]
- Floras JS. Sympathetic activation in human heart failure: diverse mechanisms, therapeutic opportunities. *Acta Physiol Scand* 2003;177:391–398. [PubMed: 12609011]
- Fox AP, Cahill AL, Currie KP, Grabner C, Harkins AB, Herring B, Hurley JH, Xie Z. N- and P/Q-type Ca^{2+} channels in adrenal chromaffin cells. *Acta Physiol* 2008;192:247–261.
- Fulop T, Smith C. Physiological stimulation regulates the exocytic mode through calcium activation of protein kinase C in mouse chromaffin cells. *Biochem J* 2006;399:111–119. [PubMed: 16784416]
- Fulop T, Radabaugh S, Smith C. Activity-dependent differential transmitter release in mouse adrenal chromaffin cells. *J Neurosci* 2005;25:7324–7332. [PubMed: 16093382]
- Gandia L, Garcia AG, Morad M. ATP modulation of calcium channels in chromaffin cells. *J Physiol* 1993;470:55–72. [PubMed: 8308743]
- Garcia AG, Garcia-De-Diego AM, Gandia L, Borges R, Garcia-Sancho J. Calcium signaling and exocytosis in adrenal chromaffin cells. *Physiol Rev* 2006;86:1093–1131. [PubMed: 17015485]
- Gerachshenko T, Schwartz E, Bleckert A, Photowala H, Seymour A, Alford S. Presynaptic G-protein-coupled receptors dynamically modify vesicle fusion, synaptic cleft glutamate concentrations, and motor behavior. *J Neurosci* 2009;29:10221–10233. [PubMed: 19692597]

- Gillis KD. Admittance-based measurement of membrane capacitance using the EPC-9 patch-clamp amplifier. *Pflugers Arch* 2000;439:655–664. [PubMed: 10764227]
- Grabner CP, Price SD, Lysakowski A, Fox AP. Mouse chromaffin cells have two populations of dense core vesicles. *J Neurophysiol* 2005;94:2093–2104. [PubMed: 15944233]
- Gray AC, Raingo J, Lipscombe D. Neuronal calcium channels: splicing for optimal performance. *Cell Calcium* 2007;42:409–417. [PubMed: 17512586]
- Grueter CE, Abiria SA, Dzhura I, Wu Y, Ham AJ, Mohler PJ, Anderson ME, Colbran RJ. L-type Ca^{2+} channel facilitation mediated by phosphorylation of the beta subunit by CaMKII. *Mol cell* 2006;23:641–650. [PubMed: 16949361]
- Hamelink C, Tjurmina O, Damadzic R, Young WS, Weihe E, Lee HW, Eiden LE. Pituitary adenylate cyclase-activating polypeptide is a sympathoadrenal neurotransmitter involved in catecholamine regulation and glucohomeostasis. *Proc Natl Acad Sci USA* 2002;99:461–466. [PubMed: 11756684]
- Harata NC, Aravanis AM, Tsien RW. Kiss-and-run and full-collapse fusion as modes of exo-endocytosis in neurosecretion. *J Neurochem* 2006;97:1546–1570. [PubMed: 16805768]
- Harkins AB, Fox AP. Activation of purinergic receptors by ATP inhibits secretion in bovine adrenal chromaffin cells. *Brain Res* 2000;885:231–239. [PubMed: 11102577]
- He L, Wu LG. The debate on the kiss-and-run fusion at synapses. *Trends Neurosci* 2007;30:447–455. [PubMed: 17765328]
- Herlitz S, Hockerman GH, Scheuer T, Catterall WA. Molecular determinants of inactivation and G protein modulation in the intracellular loop connecting domains I and II of the calcium channel $\alpha 1A$ subunit. *Proc Natl Acad Sci USA* 1997;94:1512–1516. [PubMed: 9037084]
- Hernandez-Guijo JM, Carabelli V, Gandia L, Garcia AG, Carbone E. Voltage-independent autocrine modulation of L-type channels mediated by ATP, opioids and catecholamines in rat chromaffin cells. *Eur J Neurosci* 1999;11:3574–3584. [PubMed: 10564365]
- Jarvis SE, Barr W, Feng ZP, Hamid J, Zamponi GW. Molecular determinants of syntaxin 1 modulation of N-type calcium channels. *J Biol Chem* 2002;277:44399–44407. [PubMed: 12221094]
- Kleppisch T, Ahnert-Hilger G, Gollasch M, Spicher K, Hescheler J, Schultz G, Rosenthal W. Inhibition of voltage-dependent Ca^{2+} channels via α 2-adrenergic and opioid receptors in cultured bovine adrenal chromaffin cells. *Pflugers Arch* 1992;421:131–137. [PubMed: 1326743]
- Kuri BA, Chan SA, Smith CB. PACAP regulates immediate catecholamine release from adrenal chromaffin cells in an activity-dependent manner through a protein kinase C-dependent pathway. *J Neurochem* 2009;110:1214–1225. [PubMed: 19508428]
- Lee HK, Elmslie KS. Reluctant gating of single N-type calcium channels during neurotransmitter-induced inhibition in bullfrog sympathetic neurons. *J Neurosci* 2000;20:3115–3128. [PubMed: 10777775]
- Lim W, Kim SJ, Yan HD, Kim J. Ca^{2+} -channel-dependent and -independent inhibition of exocytosis by extracellular ATP in voltage-clamped rat adrenal chromaffin cells. *Pflugers Arch* 1997;435:34–42. [PubMed: 9359901]
- Lindau M, Neher E. Patch-clamp techniques for time-resolved capacitance measurements in single cells. *Pflugers Arch* 1988;411:137–146. [PubMed: 3357753]
- Lukyanetz EA, Neher E. Different types of calcium channels and secretion from bovine chromaffin cells. *Eur J Neurosci* 1999;11:2865–2873. [PubMed: 10457183]
- Lymperopoulos A, Rengo G, Funakoshi H, Eckhart AD, Koch WJ. Adrenal GRK2 upregulation mediates sympathetic over-drive in heart failure. *Nat Med* 2007;13:315–323. [PubMed: 17322894]
- Marcantoni A, Carabelli V, Comunanza V, Hoddah H, Carbone E. Calcium channels in chromaffin cells: focus on L and T types. *Acta Physiol* 2008;192:233–246.
- Marcantoni A, Vandael DH, Mahapatra S, Carabelli V, Sinnegger-Brauns MJ, Striessnig J, Carbone E. Loss of Cav1.3 channels reveals the critical role of L-type and BK channel coupling in pacemaking mouse adrenal chromaffin cells. *J Neurosci* 2010;30:491–504. [PubMed: 20071512]
- McDavid S, Currie KP. G-proteins modulate cumulative inactivation of N-type (Cav2.2) calcium channels. *J Neurosci* 2006;26:13373–13383. [PubMed: 17182788]
- Moore JM, Papke JB, Cahill AL, Harkins AB. Stable gene silencing of synaptotagmin I in rat PC12 cells inhibits Ca^{2+} -evoked release of catecholamine. *Am J Physiol* 2006;291:C270–C281.

- Nagy G, Reim K, Matti U, Brose N, Binz T, Rettig J, Neher E, Sorensen JB. Regulation of releasable vesicle pool sizes by protein kinase A-dependent phosphorylation of SNAP-25. *Neuron* 2004;41:417–429. [PubMed: 14766180]
- Novara M, Baldelli P, Cavallari D, Carabelli V, Giancippoli A, Carbone E. Exposure to cAMP and beta-adrenergic stimulation recruits Ca(V)3 T-type channels in rat chromaffin cells through Epac cAMP-receptor proteins. *J Physiol* 2004;558:433–449. [PubMed: 15133061]
- Patil PG, de Leon M, Reed RR, Dubel S, Snutch TP, Yue DT. Elementary events underlying voltage-dependent G-protein inhibition of N-type calcium channels. *Biophys J* 1996;71:2509–2521. [PubMed: 8913590]
- Photowala H, Blackmer T, Schwartz E, Hamm HE, Alford S. G protein betagamma-subunits activated by serotonin mediate presynaptic inhibition by regulating vesicle fusion properties. *Proc Natl Acad Sci USA* 2006;103:4281–4286. [PubMed: 16537522]
- Polo-Parada L, Chan SA, Smith C. An activity-dependent increased role for L-type calcium channels in exocytosis is regulated by adrenergic signaling in chromaffin cells. *Neuroscience* 2006;143:445–459. [PubMed: 16962713]
- Powell AD, Teschemacher AG, Seward EP. P2Y purinoceptors inhibit exocytosis in adrenal chromaffin cells via modulation of voltage-operated calcium channels. *J Neurosci* 2000;20:606–616. [PubMed: 10632590]
- Rizo J, Rosenmund C. Synaptic vesicle fusion. *Nat Struct Mol Biol* 2008;15:665–674. [PubMed: 18618940]
- Schohn JS, Maximov A, Lao Y, Sudhof TC, Sorensen JB. Synaptotagmin-1 and -7 are functionally overlapping Ca^{2+} sensors for exocytosis in adrenal chromaffin cells. *Proc Natl Acad Sci USA* 2008;105:3998–4003. [PubMed: 18308932]
- Sorensen JB. Conflicting views on the membrane fusion machinery and the fusion pore. *Annu Rev Cell Dev Biol* 2009;25:513–537. [PubMed: 19575641]
- Stephens GJ. G-protein-coupled-receptor-mediated presynaptic inhibition in the cerebellum. *Trends Pharmacol Sci* 2009;30:421–430. [PubMed: 19632729]
- Stotz SC, Zamponi GW. Structural determinants of fast inactivation of high voltage-activated $\text{Ca}(2+)$ channels. *Trends Neurosci* 2001;24:176–181. [PubMed: 11182458]
- Sudhof TC, Rothman JE. Membrane fusion: grappling with SNARE and SM proteins. *Science* 2009;323:474–477. [PubMed: 19164740]
- Takiyuddin MA, De Nicola L, Gabbai FB, Dinh TQ, Kennedy B, Ziegler MG, Sabban EL, Parmer RJ, O'Connor DT. Catecholamine secretory vesicles. Augmented chromogranins and amines in secondary hypertension. *Hypertension* 1993;21:674–679. [PubMed: 8491501]
- Tedford HW, Zamponi GW. Direct G protein modulation of Cav2 calcium channels. *Pharmacol Rev* 2006;58:837–862. [PubMed: 17132857]
- Twitchell WA, Rane SG. Opioid peptide modulation of $\text{Ca}(2+)$ -dependent K^{+} and voltage-activated Ca^{2+} currents in bovine adrenal chromaffin cells. *Neuron* 1993;10:701–709. [PubMed: 8476614]
- Ulate G, Scott SR, Gonzalez J, Gilabert JA, Artalejo AR. Extracellular ATP regulates exocytosis in inhibiting multiple $\text{Ca}(2+)$ channel types in bovine chromaffin cells. *Pflugers Arch* 2000;439:304–314. [PubMed: 10650982]
- Villanueva M, Wightman RM. Facilitation of quantal release induced by a D1-like receptor on bovine chromaffin cells. *Biochemistry* 2007;46:3881–3887. [PubMed: 17338553]
- Villarroya M, Olivares R, Ruiz A, Cano-Abad MF, de Pascual R, Lomax RB, Lopez MG, Mayorgas I, Gandia L, Garcia AG. Voltage inactivation of Ca^{2+} entry and secretion associated with N- and P/Q-type but not L-type Ca^{2+} channels of bovine chromaffin cells. *J Physiol* 1999;516(Pt 2):421–432. [PubMed: 10087342]
- Wang CT, Grishanin R, Earles CA, Chang PY, Martin TF, Chapman ER, Jackson MB. Synaptotagmin modulation of fusion pore kinetics in regulated exocytosis of dense-core vesicles. *Science* 2001;294:1111–1115. [PubMed: 11691996]
- Weiss JL, Burgoyne RD. Voltage-independent inhibition of P/Q-type Ca^{2+} channels in adrenal chromaffin cells via a neuronal Ca^{2+} sensor-1-dependent pathway involves Src family tyrosine kinase. *J Biol Chem* 2001;276:44804–44811. [PubMed: 11583988]

- Weiss N, Tadmouri A, Mikati M, Ronjat M, De Waard M. Importance of voltage-dependent inactivation in N-type calcium channel regulation by G-proteins. *Pflügers Arch* 2007;454:115–129. [PubMed: 17171365]
- Wightman RM, Jankowski JA, Kennedy RT, Kawagoe KT, Schroeder TJ, Leszczyszyn DJ, Near JA, Diliberto EJ Jr, Viveros OH. Temporally resolved catecholamine spikes correspond to single vesicle release from individual chromaffin cells. *Proc Natl Acad Sci USA* 1991;88:10754–10758. [PubMed: 1961743]
- Wolfe JT, Wang H, Howard J, Garrison JC, Barrett PQ. T-type calcium channel regulation by specific G-protein betagamma subunits. *Nature* 2003;424:209–213. [PubMed: 12853961]
- Womack MD, McCleskey EW. Interaction of opioids and membrane potential to modulate Ca^{2+} channels in rat dorsal root ganglion neurons. *J Neurophysiol* 1995;73:1793–1798. [PubMed: 7623080]
- Yoon EJ, Gerachshenko T, Spiegelberg BD, Alford S, Hamm HE. Gbetagamma interferes with Ca^{2+} -dependent binding of synaptotagmin to the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. *Mol Pharmacol* 2007;72:1210–1219. [PubMed: 17715396]
- Yoon EJ, Hamm HE, Currie KP. G protein betagamma subunits modulate the number and nature of exocytotic fusion events in adrenal chromaffin cells independent of calcium entry. *Neurophysiol* 2008;100:2929–2939.