Voltage-gated Potassium Channel as a Facilitator of Exocytosis

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Voltage-gated ion channels are well characterized for their function in excitability signals. Accumulating studies, however, have established an ion-independent function for the major classes of ion channels in cellular signaling. During the last few years we established a novel role for Kv2.1, a voltage-gated potassium (Kv) channel, classically known for its role of repolarizing the membrane potential, in facilitation of exocytosis. Kv2.1 induces facilitation of depolarization-induced release through its direct interaction with syntaxin, a protein component of the exocytotic machinery, independently of the potassium ion flow through the channel's pore. Here, we review our recent studies, further characterize the phenomena (using chromaffin cells and carbon fiber amperometry), and suggest plausible mechanisms that can underlie this facilitation of release.

Key words: **exocytosis; Kv2.1 channel; chromaffin cells; PC12 cells; syntaxin; amperometry; large dense-core vesicles release; pore-independent function**

Nonconducting Functions of Voltage-gated Ion Channel

The primary function of voltage-gated ion channels in the nervous system is to generate the wide variety of different firing patterns that are required for processing sensory information and generating motor outputs.[1]

However, recent works have demonstrated that many ion channels can themselves directly influence biochemical events in ways that do not directly depend on their function as ion channels. Such control over cellular signaling has been reported for each of the major classes of ion channels that influence excitability, including the sodium, calcium, and potassium channels (as reviewed in Ref. 1).

Voltage-gated potassium (Kv) channels have classically been viewed to participate in exocytosis solely as inhibitors of neurotransmitter, neuropeptide, and hormone secretion through their ion-conducting function to repolarize membrane potential[2] in presynaptic terminals[3,4] and endocrine cells.[5] Among the cellular behaviors that Kv channels regulate independently of their conducting functions are mitogen-activated protein (MAP) kinase signaling [EAG, (Kv10.1) channels], cell proliferations (EAG, Kv11.1, Kv1.3 channels), and tyrosine phosphorylation (Kv1.3) (as reviewed in Ref. 1). Recently, a novel role for a Kv channel, Kv2.1, as facilitator of dense-core vesicle (DCV)-mediated secretion through a pore-independent function has been demonstrated (see in the following and Refs. 6, 7).

Kv Channels and SNARE Proteins

In secretory cells, such as neurons and neuroendocrine cells, Ca^{2+} influx triggers secretory vesicle exocytosis, releasing neurotransmitters or hormones.[8] Part of the exocytocic

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Mechanisms of Exocytosis: Ann. N.Y. Acad. Sci. 1152: 87–92 (2009).
doi: 10.1111/j.1749-6632.2008.03997.x

machinery are the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which share a cytosolic SNARE motif domain essential for their interaction. Assembly of vesicle SNARE, VAMP, with the plasma membrane t-SNAREs, SNAP25 (synaptosomal-associated protein of 25 kDa), and syntaxin-1A, forms the SNARE complex, which initiates the fusion of the vesicle into the plasma membrane.

Physical interaction between the SNARE proteins, mainly syntaxin, and Kv channels (Kv1.1, Kv2.1) have been shown to modulate the gating function of the channels,[9–12] suggesting that SNARE proteins do not only participate in exocytosis *per se* but also regulate excitability by modulating the ion channels involved in secretion. Accumulating data from different cell types, including rat brain synaptosome,[9] *Xenopus* oocytes,[10] human embryonic kidney (HEK) cells,[13] β-cells,[13] cardiac mycocytes,[14] and esophageal smooth muscles,[15] have established a new paradigm that SNARE proteins can directly modulate the Kv channel superfamily.[8]

Here, however, we will focus on the physical aspect of the syntaxin–Kv channel interaction with regard to exocytosis rather than on the implications of the gating modulations caused by this interaction.

Kv2.1 Facilitation of Exocytosis by Interaction with Syntaxin

The Kv2.1 channel is abundantly expressed in the soma and dendrites of neurons[16–21] where it underlies most of the delayed rectifier current and could influence the release of neuropeptides and neurotrophins. Additionally, Kv2.1 channel is also expressed in neuroendocrine cells[22–24] where it is well positioned to regulate hormone release.

Earlier works have shown that Kv2.1 physically and dynamically interacts with syntaxin and with SNAP25 in PC12 cells, oocytes, β-cells, and *in vitro*.[13,25,26] The Kv2.1 syntaxin-interacting site is located at the cytoplasmatic C terminus between aa 411–522 (termed C1a domain).

Our main interest was to examine the significance of this interaction on modulation of secretion. First, we tested the role of endogenous Kv2.1 in modulation of secretion in cracked PC12 cells, which allowed penetration of peptides and can undergo MgATP priming and Ca^{2+} triggering to release norepinephrine (NE) stored in DCVs. Impairment of the Kv2.1–syntaxin interaction by introducing Kv2.1 syntaxin-binding peptide, Kv2.1-C1, to the cells significantly reduced NE release. Since secretion was measured from cells with permeable plasma membranes, the K^+ ion efflux and membrane potential changes were irrelevant. Hence, it was suggested that Kv2.1 enhances secretion under physiological condition in neuroendocrine cells, independently of its conducting function, through physical interaction with syntaxin (Table 1).[6]

Next, we examined the effect of overexpressed Kv2.1 on secretion from live PC12 cells by measuring secretion of GFP-tagged atrial natriuretic factor (ANF) after Ca^{2+} elevation. Overexpression of Kv2.1 enhanced the release of ANF. A similar enhancement effect was shown for overexpressed mutant nonconducting channel ($Kv2.1^{W365C/Y380T}$),[27] while mutant channel that lacks the syntaxin-binding site (Kv2.1ΔC1a) not only failed to enhance release but also slightly inhibited it. These findings have strengthened the notion that Kv2.1 channels enhance secretion from PC12 by binding a key component of the fusion machinery, syntaxin, through cytoplasmic domain-mediated protein–protein interaction and have led to the suggestion of a novel nonconduction role for the channel (Table 1).[7]

Enhancement of Release by Kv2.1 in Bovine Chromaffin Cells

Here, in an attempt to further characterize this phenomenon and establish Kv2.1 as

TABLE 1. Kv2.1 Effect on Release

Cell type	PC12 (Kv2.1 down-regulation)[6]		PC12 (Kv2.1 overexpression)[7]						Chromaffin (Kv2.1 overexpression)			
assay	NE release (%)		ANF release (%)						CA release (%)			
	Control (GST)	GST-Kv2.1C1	Control (GFP)	Kv2.1	Control (GFP)	Kv2.1$^{W365C/Y380T}$	Control (GFP)	Kv2.1ΔC1a	Control (EGFP)	Kv2.1	Control (EGFP)	Kv2.1$^{W365C/Y380T}$
	100	41*	100	123*	100	134*	100	86*	100	186*	100	170*

Abbreviations: ANF, atrial natriuretic factor; EGFP, enhanced green fluorescent protein; GFP, green fluorescent protein; GST, glutathione-S-transferase; NE, norepinephrine.

*$P < 0.05$.

a common protein in the exocytosis machinery, in addition to its role in plasma membrane hyperpolarization, we overexpressed the channel in bovine chromaffin cells and measured large dense-core vesicle release by amperometry, which enables a highly sensitive measurement and high temporal resolution of secretion.[28]

Bovine chromaffin cells were infected with pSFV1-Kv2.1-IRES-EGFP construct (Kv2.1-expressing cells) or with pSFV1-EGFP alone (control cells). Cells were stimulated by 10-s focal application of high-K^+ solution and charge released from those cells was recorded for 100 s. Five seconds of basal secretion was recorded before stimulation (Fig. 1). Release of catecholamines (CA) in Kv2.1-expressing cells was significantly increased compared to control cells. Notably, the initial secretion during the first 5 s of the stimulation was only slightly larger and became significantly larger only at 10 s (Fig. 1A). Further analysis of secretion data in response to four consecutive stimulations in each cell revealed that the presence of the channel accelerated secretion compared to control cells (Fig. 1B). Expression of mutant nonconducting channel Kv2.1$^{W365C/Y380T}$ revealed a similar extent of enhancement (Fig. 1C), suggesting that a functional pore was not required for the Kv2.1-mediated facilitation of depolarization-evoked release.

These results established the nonconducting role of Kv2.1 as a facilitator protein of exocytosis in chromaffin cells and have characterized

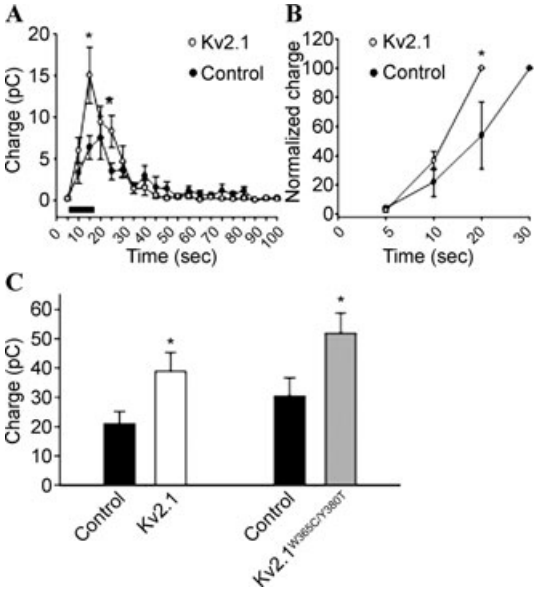

Figure 1. Kv2.1 enhances release evoked by K^+-induced depolarizations in chromaffin cells . (**A**) Average charge released from cells infected with Semliki Forest virus coding for IRES-EGFP (control cell; filled) or with virus coding for Kv2.1-IRES-EGFP (Kv2.1 cell; empty) in response to a 10-s focal application of high-K^+ solution (bar). Accumulated charge during 5 s was plotted. (**B**) Superimposed normalized and averaged charges released from control and Kv2.1-expressing cells. Cells were stimulated by four consecutive 10-s applications of high-K^+ solution with 2-min intervals (first application shown in A). Average values of charge released in control ($n > 9$) and Kv2.1-expressing ($n > 19$) cells were normalized to maximal value in each stimulation. (**C**) Averaged cumulative charge released during 20 s after onset of stimulation in control ($n = 22$; black bars) versus Kv2.1- ($n = 27$; empty bar) expressing cell and in control ($n = 11$) versus Kv2.1$^{W365C/Y380T}$- ($n = 21$; gray bar) expressing cell. Data are shown as mean ± SEM; *$P < 0.05$.

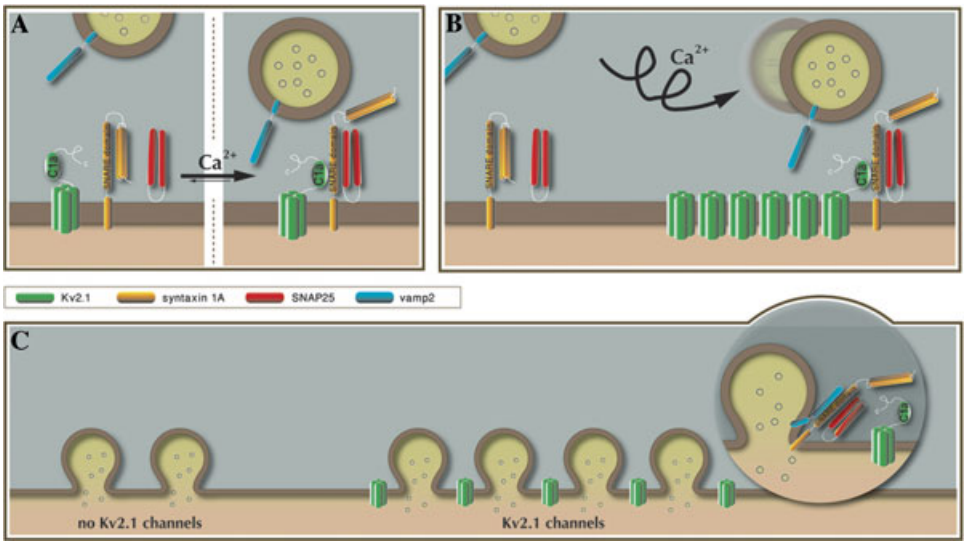

Figure 2. Plausible models for Kv2.1 enhancement of vesicle exocytosis in response to stimulation. (**A**) Ca^{2+}-dependent Kv2.1 binding to syntaxin stabilizes the t-SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) complex. (**B**) Ca^{2+}-dependent relocation of vesicles to a high-density Kv2.1 cluster, which is a favorable site for fusion. (**C**) Both scenarios lead to favorable formation of ternary SNARE complexes at sites with Kv2.1 channels, resulting in an increased charge released. (Note that it has been recently shown that the ternary SNARE complex does not interact with Kv2.1.[45])

the time resolution of this enhancement effect during prolonged stimulation.

Plausible Models for Kv2.1 Facilitation of Release

As summarized in Table 1, Kv2.1 enhances secretion from neuroendocrine cells independently of its ion-conducting function via a physical interaction with syntaxin, a component of the exocytosis machinery. In order to put forward plausible models that can underlie this effect, several considerations were made based on recent data. Interaction of Kv2.1 C1a domain with syntaxin has been shown to underlie the Kv2.1-induced enhancement of neuropeptide release.[7] Kv2.1 has been shown to interact preferentially with the open conformation of syntaxin,[29] thus stabilizing this form of syntaxin, which has been implicated in vesicle docking[30] and priming (the latter via formation of binary t-SNARE complexes).[31,32]

We suggest two scenarios that might lead to increased charge released by Kv2.1 (Fig. 2). In the first scenario (Fig. 2A), the binding of Kv2.1 to open syntaxin is suggested to be Ca^{2+} dependent, based on the previous demonstration of a dynamic association between syntaxin and Kv2.1, which was significantly enhanced following Ca^{2+} triggering of primed cracked PC12 cells.[7] Interestingly, phosphorylation of sites within or near the C1a domain has been suggested to be linked to the Kv2.1-syntaxin interaction.[33] Moreover, dephosphorylation of these sites through a Ca^{2+}/calcineurin-dependent mechanism[21,34] has been shown to result in effects on activation and inactivation gating (hyperpolarizing shifts[35]) that resemble those of syntaxin binding.[26] Thus, Ca^{2+}-dependent dephosphorylation of Kv2.1 could be associated with syntaxin binding. Indeed, Ca^{2+}-dependent phosphorylation of some other proteins has been shown to modulate exocytosis. For example, protein kinase C (PKC) activation was shown to modulate exocytosis by phosphorylation of SNAP-25 at a specific amino acid residue in response to depolarizing stimuli in endocrine cells and neurons,[36–39] and phosphorylation of

Munc18-1 by PKC potentiates vesicle-pool replenishment following a depleting stimulation at a post-docking stage in chromaffin cells.[40] An alternative, but not mutually exclusive, scenario that could account for the Kv2.1 action relates to the recently demonstrated tendency of late-fusing (after 8 s of stimulation) chromaffin LDCVs. It can be suggested that these late-fusing vesicles are not in a favorable location for fusion at the time of stimulation and that they relocate just before fusion to a more favorable site.[41] Similar LDCV motions have been shown to be Ca^{2+} dependent in chromaffin cells[42] and in other tissues.[43,44] Notably, the time scale of relocation, on the order of 8 s, is compatible with the delayed development of the Kv2.1 facilitation of release (Fig. 1A). One possible fusion-favorable site might be an area with high-density Kv2.1 clusters[33] in which docking and/or t-SNARE complex formation are relatively intensified through the interaction of open syntaxin with Kv2.1 channels. Taken together, this scenario suggests relocation of LDCVs from a fusion-unfavorable location to a region of Kv2.1 clusters (Fig. 2B). Both scenarios lead to favorable formation of ternary SNARE complexes at sites with Kv2.1 channels and an increase in charge released (Fig. 2C).

Conflicts of Interest

The authors declare no conflicts of interest.

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