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Engineering Proteins for Custom Inhibition of Ca_V Channels

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Abstract

The influx of Ca^{2+} ions through voltage-dependent calcium (Ca_V) channels links electrical signals to physiological responses in all excitable cells. Not surprisingly, blocking Ca_V channel activity is a powerful method to regulate the function of excitable cells, and this is exploited for both physiological and therapeutic benefit. Nevertheless, the full potential for Ca_V channel inhibition is not being realized by currently available small molecule blockers or second messenger modulators due to limitations in targeting them either to defined groups of cells in an organism or to distinct sub-cellular regions within a single cell. Here, we review early efforts to engineer protein molecule blockers of Ca_V channels to fill this crucial niche. This technology would greatly expand the toolbox available to physiologists studying the biology of excitable cells at the cellular and systems level.

Electrical signals, or action potentials, generated by ionic fluxes through ion channel proteins residing in the plasma membranes of cells, constitute one of the most prevalent and important cell signaling mechanisms in biology. Electrical signals co-ordinate the activity of the millions of cells required to generate the heartbeat; underlie the orchestrated firing of neurons that enable sight, speech, movement, and formation of memories; and regulate the release of hormones that control glucose homeostasis, growth, and development. Although the spectrum of biological responses dependent on electrical signals is impressively diverse, they all utilize a similar signal transduction paradigm: membrane depolarization leads to the opening of voltage-dependent Ca^{2+} (Ca_V) channels, permitting a Ca^{2+} influx that triggers the appropriate cell biological response. The central role Ca_V channels play in transducing electrical signals into biological responses position them as attractive targets for potentially regulating a wide range of physiological processes. Indeed, modulation of Ca_V channels by a variety of second messenger pathways and small molecules is widely exploited as a means to regulate physiology and as a therapy for various diseases. In this review, we will discuss nascent efforts to engineer protein molecules for custom inhibition of Ca_V channels. As a prelude to in-depth discussion of this topic, we will first briefly review the structure-function of Ca_V channels, traditional Ca_V channel blockers, and the rationale for developing such novel protein inhibitors of Ca_V channels.

Structure-function of Ca_V channels

Ca_V channels are divided into two main families depending on their threshold for activation. There are three types of low-voltage-activated ($Ca_{V,LVA}$), or T-type, Ca^{2+} channels ($Ca_V3.1$ – $Ca_V3.3$) encoded by distinct genes (*CACNA1G*, *CACNA1H*, and *CACNA1I*), each with multiple splice variants (93). Functionally, $Ca_{V,LVA}$ channels activate at a relatively negative threshold of around −60 mV, have a small conductance, and rapidly inactivate (111). $Ca_{V,LVA}$ channels are found in many excitable cell types including sinoatrial node cells, smooth

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muscle, and neurons, where they contribute to pacemaking (91). $Ca_{V,LVA}$ channels will not be discussed any further in this review.

High-voltage-activated calcium ($Ca_{V,HVA}$) channels currently include seven members ($Ca_V1.1$–$Ca_V1.4$, $Ca_V2.1$–$Ca_V2.3$) encoded by distinct genes each with multiple splice variants (19). $Ca_{V,HVA}$ channels typically have an activation threshold of around −30 mV, the exception being $Ca_V1.3$ which activates at a threshold of around −50 mV (75,123). Structurally, $Ca_{V,HVA}$ channels are hetero-multimeric proteins comprised of a main α_1 subunit assembled with auxiliary β and $\alpha_2\delta$ subunits, calmodulin, and sometimes a γ subunit (Figure 1).

α_1 subunits

$Ca_{V,HVA}$ channel α_1 subunits are the pore-forming proteins and define the identity of the channel complex. All $Ca_{V,HVA}$ channel α_1 subunits have a similar architecture, comprised of four homologous domains (I–IV), each with six membrane spanning segments (S1–S6). The four domains are connected by intracellular loops of varying lengths, along with cytosolic N- and C-termini. The S4 segment of each domain contains positively charged residues that are an integral part of the voltage sensor. The S5–S6 pore loops from each domain collaborate to form the selectivity filter, and the S6 segments line the channel pore (19).

β subunits

There are four auxiliary $Ca_V\beta$ subunits (β1–β4) encoded by different genes, each with multiple splice variants (17,18,31,92,98,101,110). At the primary sequence level the different $Ca_V\beta$s display two conserved domains separated by an alternatively spliced linker region, and variable N- and C-termini. Crystal structures revealed the conserved core of $Ca_V\beta$s contain *src* homology 3 (SH3) and guanylate kinase-like (GK) motifs that interact intramolecularly (23, 88,117). This functional signature suggests a kinship to the membrane-associated guanylate kinase (MAGUK) super-family of scaffold proteins, which all contain an SH3-GK module, and organize intracellular signaling pathways by co-localizing diverse proteins (2,48). Functionally, $Ca_V\beta$s: are necessary for trafficking pore-forming α_1 subunits to the plasma membrane; produce depolarizing shifts in the voltage-dependence of channel activation; elevate single-channel open probability (P_o); and impart characteristic inactivation properties to $Ca_{V,HVA}$ channels (24,29,63,70,86,92,105). $Ca_V\beta$s bind with high affinity to α_1 subunits using an `α binding pocket' (ABP) formed by non-contiguous residues in the GK motif, and a conserved 18-residue sequence, the `α interaction domain' (AID), located in the intracellular loop connecting α_1-subunit domains I and II (23,88,97,117). Binding of $Ca_V\beta$ to α_1 increases the helical propensity of the region from the AID to the end of IS6, suggesting formation of a rigid helix that spans the AID and IS6 (89). This rigid IS6-AID helix is important for the ability of $Ca_V\beta$s to modulate activation and inactivation gating (42,118).

$\alpha_2\delta$ subunits

There are currently four $\alpha_2\delta$ subunit types ($\alpha_2\delta$-1 – $\alpha_2\delta$-4) encoded by different genes (34,49, 68,99). The $\alpha_2\delta$ subunit is generated in cells as a single gene product which is post-translationally cleaved to generate separate α_2 and δ proteins that are held together by disulfide bonds (61). Both the α_2 and δ subunits are heavily glycosylated. Topologically, the α_2 component is entirely extracellular, while the distal part of the δ peptide spans the plasma membrane. Functionally, $\alpha_2\delta$ subunits typically increase current amplitude by increasing the surface density of α_1 subunits, and also influence channel activation and inactivation gating (40,104).

Calmodulin

$Ca_{V,HVA}$ channels are subject to rich positive and negative feedback regulation by Ca^{2+} (37). The Ca^{2+} sensor for the effects of intracellular Ca^{2+} on $Ca_{V,HVA}$ channels is calmodulin (71, 94,126), which associates in the basal state with an IQ motif in the cytoplasmic C-terminus of $Ca_{V,HVA}$ channel α_1 subunits (66,82,116). There is also evidence that calmodulin binding is important for trafficking $Ca_{V,HVA}$ channel α_1 subunits (119).

γ subunits

The first γ subunit identified (γ_1) was originally isolated as one of the component subunits of $Ca_{V,HVA}$ channel purified from skeletal muscle (14). Currently, there are eight members of this protein family, but only a subset has been shown to interact with some $Ca_{V,HVA}$ channel α_1 subunits (64). Topologically, γ subunits are predicted to have four transmembrane with cytoplasmic N- and C-termini. Functionally, they appear to have inhibitory effects on some $Ca_{V,HVA}$ channels (64).

Traditional $Ca_{V,HVA}$ channel blockers

Small molecules that block $Ca_{V,HVA}$ channels have historically played a critical role in advancing understanding of the different $Ca_{V,HVA}$ channel subtypes and their respective biological functions. Furthermore, small molecule $Ca_{V,HVA}$ channel blockers are used pharmacologically as an important therapy for various cardiovascular and neurological diseases (69,114).

Ca_V1 channels

$Ca_V1.1$–$Ca_V1.4$ channels, also referred to as L-type channels, are inhibited by three classes of drugs— dihydropyridines, phenylalkylamines, and benzothiazepines— in a state-dependent manner (108). Pharmacological blockade of L-type channels is an important therapy for cardiovascular diseases such as hypertension, angina, and some cardiac arrhythmias (114). The three drug classes inhibit Ca_V1 channels by binding to partially overlapping residues localized in domains III and IV of the respective pore-forming α_1 subunits (53,57,95,102,108). Drug binding to the Ca_V1 α_1 subunits is believed to couple allosterically to the channel pore and gating machinery (108).

Ca_V2 channels

Unlike Ca_V1 channels, there is a dearth of small organic blockers for $Ca_V2.1$–$Ca_V2.3$ channels. However, Ca_V2 channel family members are potently blocked by various peptide toxins isolated from predatory marine snails or spider venom (115). Specifically, $Ca_V2.1$ (P/Q-type) channels are selectively blocked by ω-agatoxin IVA (80,120); $Ca_V2.2$ channels are inhibited by ω-conotoxin GVIA (3,96); and $Ca_V2.3$ channels repressed by SNX-482 (85,113). These toxins act by binding the respective pore-forming α_1 subunit, and either physically occluding the pore or modifying channel gating. Blockade of Ca_V2 channels is an effective or potential therapy for an assortment of disorders including neuropathic pain, epilepsy, stroke, and neurodegenerative conditions (114).

Gabapentin

Gabapentin is efficacious in the treatment of neuropathic pain and seizures (106). Though this drug was originally designed as a γ-aminobutyric acid (GABA) derivative, its analgesic action stems from a high-affinity interaction with the $\alpha_2\delta$-1 (and $\alpha_2\delta$-2) subunit of $Ca_{V,HVA}$ channels (13,41,51). Under control physiological conditions, gabapentin has only moderate effects on I_{Ca}. However, during nerve injury there is a marked up-regulation of $\alpha_2\delta$-1 subunits in dorsal root ganglion (DRG) neurons and the spinal dorsal horn (73). Under this condition, gabapentin

acutely inhibits I_{Ca} in DRG neurons, and this effect may underlie the analgesic effect (74). Chronic exposure to gabapentin suppresses $Ca_V2.1$ channel trafficking to the plasma membrane and this may also be a contributing mechanism to the therapeutic effects of the drug (54). Overall, gabapentin provides a nice proof-of-concept that it is possible to design efficacious $Ca_{V,HVA}$ channel modulating molecules that target auxiliary subunits rather than the pore-forming α_1 subunit (114).

Rationale for engineering proteins to inactivate Ca_V channels

There are many potential applications of $Ca_{V,HVA}$ channel inhibition that cannot be achieved using the traditional $Ca_{V,HVA}$ channel blockers described above. The limitations arise because it is difficult to specifically target these inhibitors to either a select group of cells in a tissue or organ, or to $Ca_{V,HVA}$ channels localized in spatially distinct regions within a single cell (Figure 2). By contrast, these limitations may be overcome with engineered intracellular proteins that block $Ca_{V,HVA}$ channels because these have the capacity to be deployed in defined cell types, and may also be targeted to spatially distinct sub-cellular sites using appropriate addressing motifs. To illustrate the potential niches that can be uniquely filled by engineered protein blockers of $Ca_{V,HVA}$ channels, we draw on specific examples from neuroscience and cardiac biology, although the potential applications extend to all excitable cells.

Macroscopic neuroscience applications

An important tool for neurophysiologists studying the intricacies of the mammalian brain, or the function of neural circuits in model organisms is the ability to functionally eliminate specific neurons in a living animal and observe the resulting behavioral consequences (Figure 2A) (77). Various tools have been developed to advance this capability, each with its own set of limitations (77). These include: suppressing neuronal excitability by over-expressing potassium (62) or chloride ion channels (72), or a light-gated chloride pump (125); and, inactivating synaptic transmission using small-molecule-mediated cross-linking of synaptic vesicle fusion proteins (65). In principle, blocking Ca_V2 channels should be highly effective in eliminating neurotransmission because synaptic vesicle fusion is steeply dependent on Ca^{2+} influx via these channels (transmitter release $\propto I_{Ca}^{n}$, where $n = 3 – 5$) (12, 30). However, traditional peptide toxin blockers of Ca_V2 channels cannot be easily targeted to specific neurons in living animals, thus limiting their utility for this purpose. Genetically encoded intracellular blockers of $Ca_{V,HVA}$ channels have the advantage that they can be expressed in specified neurons using a number of different approaches that have been developed including utilizing appropriate *cis*-regulating elements (77).

Microscopic neuroscience applications

Neurons have a highly compartmentalized architecture. A single neuron usually has multiple $Ca_{V,HVA}$ channel types that are differentially distributed in different sub-cellular compartments. A single $Ca_{V,HVA}$ channel type expressed in a neuron may mediate different biological responses upon Ca^{2+} influx depending on its sub-cellular localization within the cell (Figure 2B). For example, Ca^{2+} influx through presynaptic terminus-localized Ca_V2 channels is the dominant trigger for neurotransmitter release in most neurons (20, 87). However, these same channels regulate neuronal excitability by coupling to Ca^{2+}-activated K^+ channels in axons and dendrites (39, 79, 122). Having the capacity to selectively inhibit a particular $Ca_{V,HVA}$ channel type located in spatially distinct regions of a neuron would not only advance fundamental understanding of Ca^{2+} signaling mechanisms in neurons but also permit a more fine-tuned regulation of neuronal activity. While such micro-scale targeting of $Ca_{V,HVA}$ channels in single neurons is not possible with the currently available toxin blockers, it may be possible to achieve this objective using engineered protein inhibitors.

Macroscopic cardiac applications

In heart, $Ca_V1.2$ channels are critical for excitation-contraction (EC) coupling, membrane excitability, and conduction velocity through the atrio-ventricular (AV) node. Atrial fibrillation (AF) is a prevalent arrhythmia characterized by rapid and un-coordinated activation of the atria due to re-entrant excitation or abnormal impulse formation from ectopic foci (84). A significant portion of the adverse effects associated with AF are due to ventricular tachycardia produced by abnormal activation of the ventricles as a result of the electrical activity in the atria. A treatment for AF is ablation of the AV node to electrically uncouple the atria and ventricles. This treatment is invasive and irreversible, and it has been proposed that inhibiting $Ca_V1.2$ channels in the AV node may represent a viable alternative that is reversible (Figure 2C) (32). Protein inhibitors of $Ca_V1.2$ channels have the advantage over the small organic blockers because they can be focally expressed, and thus specifically targeted, to the AV node (32,83).

Microscopic cardiac applications

In single ventricular myocytes most $Ca_V1.2$ channels are localized in transverse tubules where they are apposed to nearby clusters of ryanodine receptors (RYR) in the junctional sarcoplasmic reticulum (SR; Figure 2D) (16, 50, 103, 109). This spatial arrangement of $Ca_V1.2$ channels and RYRs is critical for the calcium-induced calcium release that underlies cardiac EC coupling (15, 38, 107, 121). However, a portion of ventricular $Ca_V1.2$ channels are found localized within caveolae (5). It has been hypothesized that caveolae $Ca_V1.2$ channels in heart locally activate Ca^{2+}-sensitive molecules that signal to responses other than contraction, including, potentially, cardiac hypertrophy (52, 81). Testing the function of caveolae-localized $Ca_V1.2$ channels in heart requires the selective inactivation of this channel pool in single ventricular myocytes. This requirement is beyond the capabilities of organic $Ca_V1.2$ channel blockers, but may be achievable with appropriately targeted protein inhibitors.

Engineering proteins for custom inhibition of $Ca_{V,HVA}$ channels

Inducible inhibition of Ca_V1–2 channels is widely exploited as a mechanism to regulate diverse physiological processes in organisms (37,67). For example, Ca_V2 channels are inhibited by many G-protein coupled receptor (GPCR) ligands. In one form of modulation, Gβγ subunits released from heterotrimeric G-proteins upon GPCR activation, bind Ca_V2 channel α_1 subunits and shift them into a reluctant gating mode where they require large depolarizations to open (6,33,35,56,59). Hallmarks of Gβγ-induced inhibition of Ca_V2 channels include a slowing of current activation kinetics and relief of inhibition by either large depolarizations or high frequency action potential waveforms (25,36,76). In another paradigm, binding of GPCR ligands inhibit I_{Ca} by causing the removal of $Ca_{V,HVA}$ channels from the cell surface (1,112). In principle, the myriad physiological mechanisms that exist to inhibit $Ca_{V,HVA}$ channels are potential candidate templates for engineering to create novel derivatives for custom applications. In one approach, the engineering is performed at the level of the GPCR, to evolve forms that can be activated by pharmacologically inert ligands (4). In another approach, membrane-tethered ω-conotoxin MVIIA was used to selectively and potently block co-expressed $Ca_V2.2$ channels in *Xenopus* oocytes (58). We will, however, focus the rest of the review on the Rad/Rem/Gem/Kir (RGK) GTPases which have several features that make them particularly well-suited for this purpose.

RGK GTPases

The RGK protein family currently consists of four members; Rem, Rem2, Rad, and Gem (mouse homolog also referred to as Kir). These proteins belong to the Ras superfamily of monomeric GTP binding proteins that function as GTP-regulated switches to regulate a wide variety of essential biological processes in cells (26). Structurally, RGK proteins have several unique features that distinguish them from other Ras GTPases including non-conservative

substitutions in the GTP binding domain of residues involved in nucleotide binding and hydrolysis, a long N-terminus extension that is variable within the family, and a relatively conserved C-terminus extension (27,43,47,78,100). The C-terminus extension of RGK GTPases lack the CAAX prenylation motif found in many Ras-like GTPases (26). Nevertheless, the distal C-terminus extension effectively targets RGK proteins to the plasma membrane utilizing a combination of electrostatic and hydrophobic interactions involving basic and aromatic (or aliphatic) residues with the membrane (55). The C-terminus of RGK GTPases binds calmodulin and 14-3-3 proteins *in vitro*, and these interactions may regulate the sub-cellular localization of these proteins (8,9). Functionally, Gem and Rad regulate cytoskeleton remodeling via interactions with Rho kinase (27); siRNA knockdown of Rem2 in neurons inhibits synapse development (90); and loss of Rad in heart leads to heart failure (21).

Crosstalk of RGK GTPases with $Ca_{V,HVA}$ channels

A yeast two-hybrid screen using $Ca_V\beta_3$ as bait fished out Gem GTPase as an interaction partner (10). Electrophysiological experiments on recombinant channels reconstituted in *Xenopus* oocytes revealed that Gem effectively eliminated $Ca_V1.2$ and $Ca_V1.3$ channel currents (10). Subsequently, the property of dramatically inhibiting $Ca_{V,HVA}$ channels was found to extend to all members of the RGK GTPase protein family (45). The mechanism of RGK proteins inhibition of $Ca_{V,HVA}$ channels was initially believed to involve disruption of the α_1-β subunit interaction (10). However, there is an emerging consensus that this does not occur and that RGK GTPases rather form a ternary complex with α_1 and β subunits to inhibit I_{Ca} (11,44). RGK GTPases inhibit I_{Ca} by different mechanisms. In some studies, RGK proteins reduce the surface density of $Ca_{V,HVA}$ channels (7–10), although this is not universally found (22,46). A significant portion of inhibition involves channels that remain on the cell surface but are either held in a low open probability mode or else display immobilized voltage sensors.

Engineering RGK proteins for custom applications

RGK proteins are promising candidates for engineering custom $Ca_{V,HVA}$ channel blockers given their extreme potency in inhibiting all $Ca_{V,HVA}$ channel types. Indeed, over-expression of wild-type RGK GTPases in tissues can be used to achieve a constitutive block of native $Ca_{V,HVA}$ channels. In a nice demonstration of this, viral-mediated expression of Gem in adult heart cells ablated native $Ca_V1.2$ channel currents, and its focal delivery to the AV node slowed AV nodal conduction and heart rate in an animal model of atrial fibrillation (83). One limitation of using wild-type RGK GTPases is that the inhibition is constitutive, with no facile way for temporal control of channel block. A second disadvantage is that the magnitude of channel block cannot be easily regulated. Whether these capabilities could be engineered into RGK GTPases provided a crucial initial test of the feasibility of exploiting these proteins for custom applications. An important finding that advanced this possibility is that deleting the distal C-terminus of RGK GTPases eliminates their ability to block I_{Ca} (22,45,124). For both Rem and Rem2, deleting the distal C-terminus results in a redistribution of the GTPase from the plasma membrane to the cytosol. Moreover, constitutively targeting the inactive truncated RGK GTPases to the plasma membrane, using generic membrane-targeting modules, recapitulated their capacity to inhibit I_{Ca} (22,124). This suggested that membrane targeting is essential for the ability of RGK GTPases to inhibit I_{Ca}. We took advantage of this feature of RGK GTPases to develop a chemical genetic hybrid approach where Rem derivatives are cytosolic and inactive in the basal state but can be acutely activated to block I_{Ca} by induced translocation to the plasma membrane using a small molecule (124). These engineered proteins were termed genetically encoded molecules for inactivating Ca_V channels (GEMIICCs). The first generation GEMIICC featured the C1 domain from protein kinase Cγ fused to the N-terminus of a C-terminus-truncated Rem, termed Rem_{265}. When expressed in HEK293 cells, $C1_{PKC\gamma}$-YFP-Rem_{265} was predominantly cytosolic. Application of 1 μM phorbol-12,13-dibutyrate

(PdBu) resulted in a rapid translocation of $C1_{PKC\gamma}$-YFP-Rem_{265} to the plasma membrane. In cells co-expressing recombinant $Ca_V2.2$ channels and $C1_{PKC\gamma}$-YFP-Rem_{265}, I_{Ca} was high in the basal state reflecting the inability of a cytosol-localized truncated Rem derivative to block $Ca_{V,HVA}$ channels. Upon adding PdBu, I_{Ca} decreased concomitantly with translocation of $C1_{PKC\gamma}$-YFP-Rem_{265} to the plasma membrane. Moreover, the magnitude of $Ca_V2.2$ channel inhibition was easily regulated simply by varying the concentration of PdBu (IC_{50} = 56 nM). One surprise with the $C1_{PKC\gamma}$-YFP-Rem_{265} GEMIICC was that it showed selectivity in inhibition. Both $Ca_V2.2$ and $Ca_V1.2$ channels were significantly inhibited by $C1_{PKC\gamma}$-YFP-Rem_{265} in a PdBu-inducible manner. However, $Ca_V2.1$ and $Ca_V2.3$ channels were unresponsive. This contrasts to wild-type Rem, which effectively inhibits all $Ca_{V,HVA}$ channels. The reason for the selectivity of the GEMIICC is unknown, but could reflect different geometric constraints for the distinct channel types. Ultimately, the selectivity of $C1_{PKC\gamma}$-YFP-Rem_{265} may prove fortuitous as it suggests that it may be possible to develop GEMIICCs that are specific for individual $Ca_{V,HVA}$ channels.

Many of the potential applications of GEMIICCs may rely on their inducible targeting to spatially distinct plasma membrane sites within a single cell. This level of spatial precision is not possible with the $C1_{PKC\gamma}$-YFP-Rem_{265} GEMIICC. As a prelude to developing GEMIICCs that can be targeted with specificity to defined regions of the plasma membrane, we evaluated the feasibility of using a rapamycin-mediated heterodimerization strategy to facilitate membrane translocation of a Rem_{265} derivative (28). Rapamycin is an immunosuppressant drug that acts by heterodimerizing two proteins, FKBP and the kinase mTOR. To implement the approach, FKBP was fused to the N-terminus of Rem_{265}, and the rapamycin binding domain of mTOR (FRB) was fused constitutively to the plasma membrane using the membrane-targeting module from Lyn kinase (60) (Figure 3). In the basal state, cells co-expressing YFP-FKBP-Rem_{265} (YFR) and Lyn-FRB (LDR) displayed YFP fluorescence in the cytosol. Addition of 1 μM rapamycin caused a rapid translocation of YFR to the plasma membrane, with a concomitant inhibition of co-expressed $Ca_V2.2$ channels (Figure 3B). The successful implementation of the heterodimerization approach greatly advances the prospect of developing GEMIICCs that target $Ca_{V,HVA}$ channels with sub-cellular precision in single excitable cells.

In summary, we have described recent ongoing efforts to develop technologies that permit spatially restricted and temporally regulated inactivation of Ca_V channels *in vitro* and *in vivo*. It is anticipated that this capability would significantly expand the toolkit available to physiologists to probe and manipulate the biology of excitable cells at the cellular and systems level. Potential uses of the technology include: (1) manipulating neuronal excitability *in vivo* to evaluate the function of specific neural circuits, or as a treatment for neurological disorders such as epilepsy and stroke that are characterized by excessive neural activity and excitotoxicity; (2) selectively inactivating spatially distinct pools of Ca_V channels within single heart and neuronal cells to discover novel functional paradigms of Ca_V channel signaling in these cells; and (3) developing inducible animal models of diseases, such as atrial fibrillation, in which a diminished I_{Ca} is an important factor.

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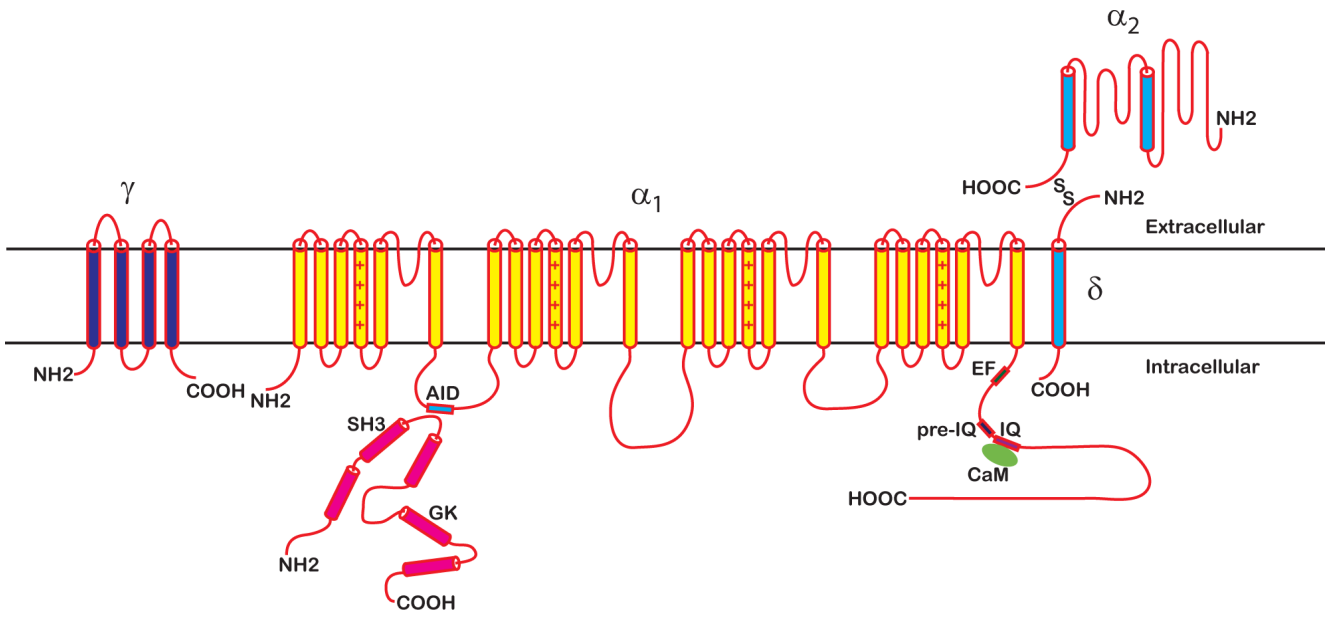

Figure 1. Multi-subunit structure of $Ca_V1–2$ channels

$Ca_V1–2$ channels are comprised of a main pore-forming α_1 subunit together with accessory proteins that include β and $\alpha_2\delta$ subunits, and calmodulin. Some channel complexes also include a γ subunit.

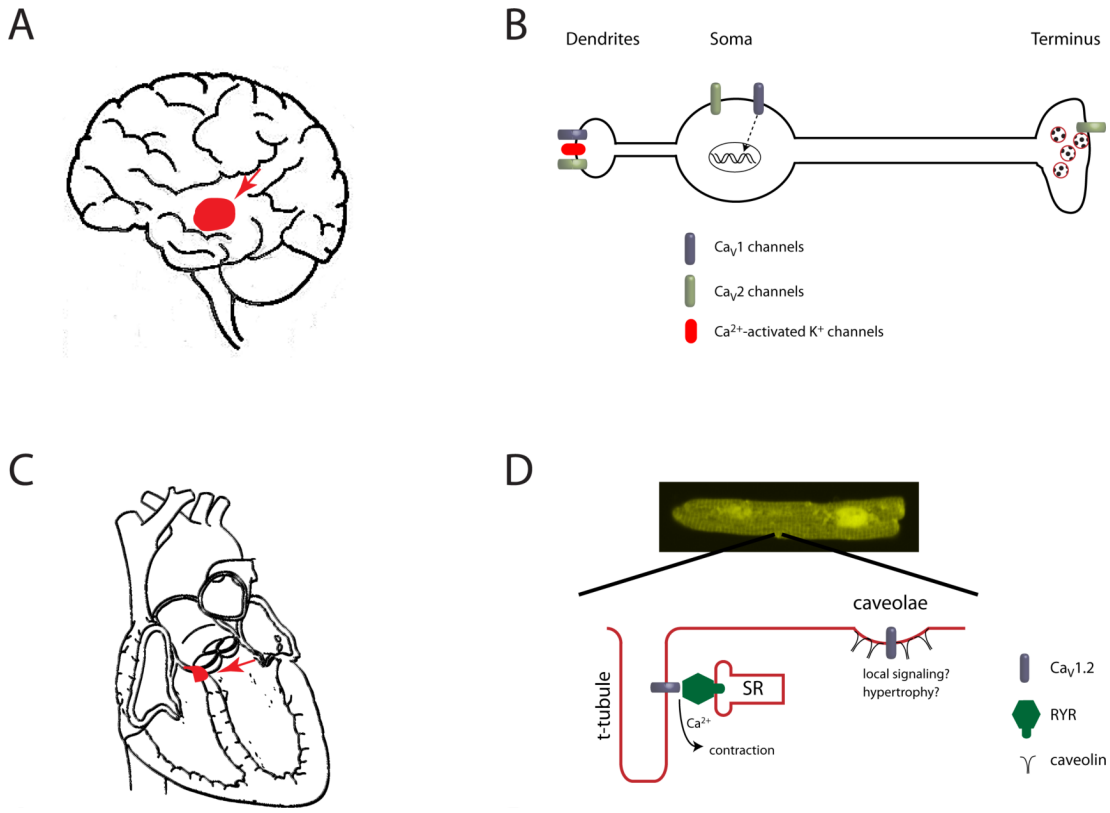

Figure 2. Potential applications for genetically encoded Ca_V channel inhibitors

(A) Disruption of specific neural circuits in a living organism. (B) Targeting functionally distinct $Ca_{V,HVA}$ channels in localized in different compartments within a single neuron. (C) Focal inhibition $Ca_V1.2$ channels in defined region of the heart in living animals. (D) Targeting $Ca_V1.2$ channels in a single myocyte with sub-cellular specificity.

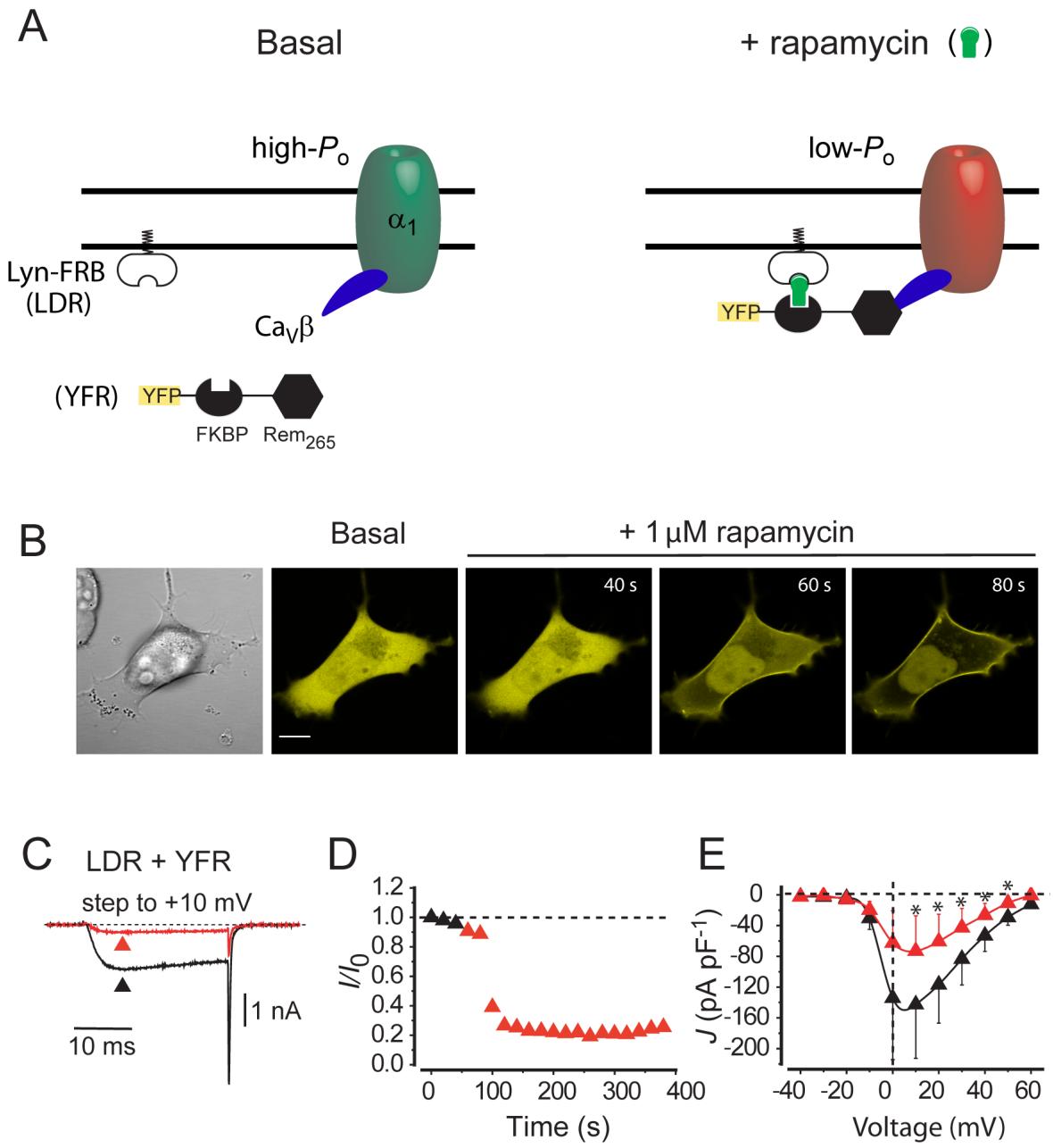

Figure 3. Engineering Rem for acutely inducible inhibition of Ca_V channels

(A) Concept for generating inducible inhibition of $Ca_{V,HVA}$ channels using a small molecule dimerizer-mediated recruitment of a Rem_{265} derivative to the plasma membrane. (B) Confocal images showing rapamycin-induced translocation of YFP-FKBP-Rem_{265} to the plasma membrane in a HEK 293 cell. (C) Whole-cell $Ca_V2.2$ currents from a cell co-expressing LDR and YFR before (▲) and after (▲) exposure to 1 μM rapamycin. (D) Time course of rapamycin-mediated inhibition of I_{Ca}. (E) Population current density (J) versus voltage (V) plots before (▲) and after (▲) rapamycin in cells co-expressing LDR and YFR. (Figure adapted from Yang et al., 2007).