Opinion

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Voltage-gated calcium channels function as Ca^{2+}-activated signaling receptors

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Voltage-gated calcium channels (VGCCs) are transmembrane cell surface proteins responsible for multifunctional signals. In response to voltage, VGCCs trigger synaptic transmission, drive muscle contraction, and regulate gene expression. Voltage perturbations open VGCCs enabling Ca^{2+} binding to the low affinity Ca^{2+} binding site of the channel pore. Subsequent to permeation, Ca^{2+} targets selective proteins to activate diverse signaling pathways. It is becoming apparent that the Ca^{2+}-bound channel triggers secretion in excitable cells and drives contraction in cardiomyocytes prior to Ca^{2+} permeation. Here, I highlight recent data implicating receptor-like function of the Ca^{2+}-bound channel in converting external Ca^{2+} into an intracellular signal. The two sequential mechanistic perspectives of VGCC function are discussed in the context of the prevailing and long-standing current models of depolarization-evoked secretion and cardiac contraction.

Introduction

When first characterized, VGCCs were viewed mainly as membrane proteins responsible for Ca^{2+} conduction into the cell. The proposal of a signaling role for the channel in driving contraction and triggering synaptic transmission, where it could act as a membrane receptor with Ca^{2+} as the binding ligand, was virtually overlooked.

The prevailing mechanisms of excitation–contraction (EC) coupling and excitation–secretion (ES) coupling hold that intracellular Ca^{2+} ($[Ca^{2+}]_i$) is the trigger that drives cardiac contraction and synchronous transmitter release. VGCCs contribute to these mechanisms solely through elevating the intracellular concentration of calcium ions.

In 1973, Schneider and Chandler suggested that voltage-dependent charge movement at the skeletal muscle L-type calcium channel (Cav1.1) could initiate EC coupling [1]. Cav1.1 was later confirmed to physically interact with the ryanodine channel (RyR1) independently of Ca^{2+} conductance through the channel [2,3].

Similar to skeletal muscle, membrane depolarization triggers cardiac contraction by EC coupling, and synchronous transmitter release by ES coupling. However, because both processes require Ca^{2+} in the extracellular medium they do not seem to fit the skeletal muscle model of direct signaling. It was therefore natural to suggest that Ca^{2+} influx through the channel followed by Ca^{2+} binding to intracellular proteins is responsible for cardiac contraction and transmitter release. During the past two decades, functional and physiological studies have revealed a versatile signaling role of the VGCC [4,5] that is independent of ion flux [6–10]. It has been shown that the VGCC is a dynamic Ca^{2+}-binding cell membrane protein that upon Ca^{2+} binding activates intracellular proteins prior to Ca^{2+} influx. This signaling activity implies receptor-like activity of the channel, with Ca^{2+} serving as the binding ligand.

The Ca^{2+}-bound channel operates as a signal transduction receptor

The process of depolarization-evoked release involves sequential activation of two Ca^{2+} binding proteins. Initially, Ca^{2+} binds to the selectivity filter of the VGCC that is exposed during membrane depolarization. Subsequent to permeation through the channel, Ca^{2+} binds to the vesicular synaptotagmin (syt1) protein.

The conventional model suggests that Ca^{2+} binding to syt1 C2 domains triggers secretion, endorsing syt1 as the Ca^{2+} sensor protein of secretion [11–14]. However, the excitosome model suggests that the Ca^{2+}-bound channel triggers secretion prior to Ca^{2+} influx. In this model, the Ca^{2+}-bound channel that acts from within the excitosome complex (discussed later) serves as the Ca^{2+} sensor protein of synaptic transmission [6,8,9].

The VGCC is a Ca^{2+} binding protein

The Ca^{2+} binding site called the EEEE locus is generated by four glutamate residues located at the p-loop region of each of the four segments of the pore-forming subunit of the channel. Although the 3D structure of the channel is not available, the site has been modeled to denote two different affinities that define open and closed states of the channel [15]. The open state of the Ca^{2+} binding motif represents a multi-Ca^{2+} ion-occupied pore of low affinity (K_d~13 mM) to

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enable ion flow. In the closed state, the channel pore is occupied with a single Ca^{2+} ion exhibiting high affinity (<1 μM), which blocks ion flow [15].

The excitosome signaling complex: physical and functional interactions of VGCCs, Sx1A, SNAP-25, and Syt1

Fundamental to our understanding of the key determinant of synaptic transmission is the coupling between presynaptic Ca^{2+} channels and the exocytotic proteins. A large number of studies have shown that all types of VGCCs are physically coupled to the synaptic fusion machinery (see reviews [6,7,9,16–18]). The network of protein–protein physical and functional interactions between the channel and the exocytotic proteins constitutes an exocytotic signaling unit that assembles independent of Ca^{2+}, called the excitosome complex [6,19]. Consisting of a VGCC, syntaxin 1A (Sx1A), SNAP-25 (synaptosomal-associated-protein 25), and syt1, this macromolecular complex emerged as the minimal set of proteins required for vesicle fusion in a reconstituted system [6,9,20–23]. The excitosome, which tethers the vesicle to the cell membrane, is responsible for a short latency of Ca^{2+} channel opening coupled to the exocytotic machinery, and ensures a precise, reliable, and fast signaling process.

It interacts with additional proteins such as synaptobrevin [20]. Ras-like protein A (RalA)-GTPase that tethers insulin secretory granules to the VGCC, was proposed to assist excitosome assembly [24]. Also Rab3 interacting molecule (RIM) alone, or with other active zone components, such as α-liprins, Munc-13 (mammalian homologues of the *Caenorhabditis elegans* unc-13 gene), or RIM binding proteins, acts primarily as a physical VGCC tether [25–31]. More support for vesicle tethering to the membrane via the excitosome members could be inferred from studies *in vitro* showing synaptic vesicle tethering directly to the VGCC [32]. Tethers that specifically link synaptic vesicles to the plasma membrane at release sites were demonstrated by fast freezing immobilization followed by superficial sublimation, or by electron tomography [33–36]. Ultimately, high-resolution characterizations will be required to explore whether the excitosome proteins correspond to the 50–100 nm scaffolds that link the vesicle to the presynaptic plasma membrane.

The channel and the vesicle fusion machinery join into an exocytotic signaling unit, which implies that conformational changes induced at the channel could signal vesicle fusion directly, prior to Ca^{2+} entry, reminiscent of EC coupling in skeletal muscle [1,37,38]. The excitosome model, however, postulates that unlike EC coupling in skeletal muscle, ES coupling requires the cooperation of voltage perturbation and Ca^{2+} binding at the open pore of the channel [6,39–41].

Testing synchronous release triggered by the Ca^{2+}-bound channel prior to cation influx

A VGCC-impermeable ligand supports evoked secretion

La^{3+} has an ionic radius (1.06 Å) similar to that of Ca^{2+} (0.99 Å). Despite the three positive charges, La^{3+}, like other members of the lanthanide family, displays high affinity for nearly all Ca^{2+} binding proteins, including the EEEE locus at the channel selectivity filter, but it is excluded from entering the cell [15,41,42]. These studies were further confirmed by structural evidence invoking a hydrated Ca^{2+} binding pore of Cav1 [43]. Implying a dynamic exchange of an inner shell of waters of hydration with local hydrogen binding partners, a switch to La^{3+} could impose an energetic barrier that prevents permeation of the trivalent cation. Thus, to monitor synchronous release triggered independently of cation influx, extracellular Ca^{2+} was replaced with La^{3+}.

Lerner *et al.* have shown that La^{3+} supports evoked catecholamine (CA) release in bovine chromaffin cells when substituted for Ca^{2+} [39]. Similarly, La^{3+} supports insulin release triggered by high glucose levels in pancreatic islets and in insulinoma cells (INS-1E) [44]. The cardiac L-type channel (Cav1.2) is largely responsible for evoked secretion in these cells, therefore inhibition of evoked release with the Cav1.2-selective blocker nifedipine established the VGCC as the La^{3+} site of action. Voltage clamp recordings and fura-2 fluorescence confirmed La^{3+} exclusion from entering the cell.

In addition to La^{3+}, the EEEE locus binds cations such as Ba^{2+}, Sr^{2+}, Ce^{3+}, Pr^{3+}, and others [39–42]. The extent of CA release depends on the type of cations that determine the activity of the cation-bound channel [39]. For example, Cd^{2+}, Eu^{3+}, or Gd^{3+} fail to drive secretion, most probably failing to produce the chemical energy (e.g., cation binding) required for initiating the signal [39]. These functional and structural constraints of cation occupation of the selectivity filter underline the channel as a cation receptor that could operate prior to cation entry.

A Ca^{2+}-impermeable VGCC supports evoked secretion

The use of a Ca^{2+}-impermeable channel mutant further supported the signaling activity of cation-bound channels. $Ca_v1.2$ was mutated by the single amino acid substitution L775P at the sixth transmembrane domain (TMD) of segment II of $\alpha_1$1.2, the pore-forming subunit of the channel [45]. The Ca^{2+}-impermeable mutant $\alpha_1$1.2/L775P retained membrane targeting, voltage sensitivity, and Ca^{2+} binding at the selectivity filter [40]. An additional substitution, T1066Y, conferred nifedipine resistance upon the impermeable mutant. This enabled monitoring of secretion elicited in chromaffin cells expressing the channel mutant, in the presence of a concentration of nifedipine that silenced signaling though the endogenous channels. The extent of CA secretion mediated by the Ca^{2+}-impermeable nifedipine-resistant channel $\alpha_1$1.2/L775P/T1066Y was similar to that of cells infected with the nifedipine-resistant wild type $\alpha_1$1.2/T1066Y mutant, confirming that synchronous release was triggered prior to Ca^{2+} influx [40].

Taken together, the published evidence based on ion substitution and knock-in strategies suggests that the Ca^{2+}-bound channel can trigger release, operating like a membrane receptor.

The prevailing model of ES coupling

The long-established model of exocytosis suggests that a minimal set of six proteins might be required for vesicle fusion. A soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)pin, or trans-SNARE complex linking two membranes, is formed between VAMP2 (vesicle-associated membrane protein 2; a vesicle-SNARE or v-SNARE) and its cognate target SNARE (t-SNARE)