Classic Fight or Flight Physiology

Ca\textsuperscript{2+} is an essential ion and second messenger that plays a central role in determining membrane excitability and contractility in heart, while also participating in diverse and fundamental activities, such as gene transcription, cell metabolism and survival, vesicle secretion, learning, and memory. Voltage-gated Ca\textsuperscript{2+} channels (Ca\textsubscript{V},x) are the primary entry point for extracellular Ca\textsuperscript{2+} to cross cell membranes and gain access to the intracellular space. In ventricular myocytes, Ca\textsubscript{V}1,2 is the most important pathway for Ca\textsuperscript{2+} entry and provides a trigger for release of intracellular Ca\textsuperscript{2+} from the sarcoplasmic reticulum that activates myofilament crossbridge formation to grade the strength of each heart beat. Thus, improved understanding of Ca\textsuperscript{2+} homeostatic mechanisms, generally, and Ca\textsubscript{V}1,2 regulation, specifically, are important goals for cardiovascular science.

Overview of Ca\textsubscript{V}1,2 Phosphorylation Sites: What Did We Once Think We Knew?

I remember thinking that our field understood, at a molecular level, how Ca\textsubscript{V}1,2 activity increases were driven by \( \beta \)-adrenergic receptor agonist stimulation when Gao et al \(^\textit{1}\) identified the \( \alpha \)-subunit C terminus serine 1928 as a target for \( \beta \)-adrenergic receptor phosphorylation and an indispensable site for increasing \( I_{Ca} \). These studies used heterologous expression systems to build evidence for serine 1928 as an agonist effector. The apparent importance of this site seemed to grow after protein kinase C was also reported to catalyze phosphorylation of serine 1928.\(^\textit{8}\) However, later Ganesan et al \(^\textit{9}\) reported that Ca\textsubscript{V}1,2 mutants expressed in cultured adult ventricular myocytes lacking serine 1928 maintained responsiveness to adrenergic stimulation. Over the years, there have been various reports identifying key regions and individual amino acids as important or essential for fight or flight mediated increases in \( I_{Ca} \). The majority of these reports have relied, at least in part, on heterologous expression systems because of the difficulty, time, and expense required to make individual mutations in Ca\textsubscript{V}1,2 and its auxiliary subunit proteins. In this issue of Circulation Research, Yang et al \(^\textit{10}\) developed a transgenic model of Ca\textsubscript{V}1,2 that harbored a mutation conferring relative insensitivity to dihydropyridine Ca\textsubscript{V}1,2 antagonists driven by a tetracycline on system to operationalize myocardial expression of mutant Ca\textsubscript{V}1,2 channels in mice. The endogenous Ca\textsubscript{V}1,2 could thus be mostly silenced by use of conventional Ca\textsubscript{V}1,2 antagonist drugs, leaving the majority of residual \( I_{Ca} \) via the transgenically expressed, dihydropyridine-resistant Ca\textsubscript{V}1,2. By this approach, Yang et al \(^\textit{10}\) produced new evidence that a recently identified site for proteolytic cleavage of the C terminus (alanine 1800) and a candidate PKA-targeted amino acid (serine 1700) were not by themselves required for isoproterenol-dependent increases in \( I_{Ca} \).

Why Is It So Difficult to Identify the Culprit Site for PKA-Mediated Increases in \( I_{Ca} \)?

Although it is now clear that identifying the key phosphorylation sites and other enabling post-translational modifications on Ca\textsubscript{V}1,2 (and perhaps on any similarly formidable and complex protein) for increasing \( I_{Ca} \) in response to \( \beta \)-adrenergic receptor agonist stimulation is not easy, the nature of the obstacles to successful discovery are less certain. My view is that the entire biological system may be far more complex than originally anticipated and that multiple challenges, yet unsolved, may lie in wait for our field before key post-translational modifications governing
fundamental Ca$_{\alpha}$1.2 α-subunit physiology will be definitively understood. The Figure schematizes various generic scenarios that may exist for a G protein coupled or other cell membrane receptor pathway to influence ion channel behavior. The leftmost scenario (a linear signaling pathway where a single culprit amino acid target for PKA-mediated phosphorylation leads to ion current increases, Figure, A) is the simplest and, therefore, the most likely to be resolved with our current experimental approaches. Unfortunately, the lack of a clear Ca$_{\alpha}$1.2 α-subunit candidate to emerge from experimental work in the postpatch clamp and molecular biology era suggests, at least to me, that this scenario is unlikely to be a valid or sufficiently nuanced reflection of adrenergic agonist stimulation to Ca$_{\alpha}$1.2. Figure, B illustrates another linear model (ie, where agonist activation of a receptor increases ion channel phosphorylation without activating other ion channel-modifying kinases or phosphatases) but where phosphorylation at various sites can lead to increases, decreases, or no net change in ionic current. Although PKA consistently increases I$_{Ca}$, it is a formal possibility that this observed action could be a net result favoring stimulatory over inhibitory actions. It is possible that the history or order of phosphorylation events could be important for net ionic current responses to an upstream stimulus. For example, phosphorylation at site X in advance of site Y could be inhibitory whereas phosphorylation of site Y alone could enhance ionic current. The Figure, B scenario includes 2 potential subcategories: one where the various amino acids are completely redundant and another where the amino acid targets are unique or incompletely redundant for purposes of agonist stimulation. Although simpler than the more rightward scenarios (Figure, C and D), the scenario in Figure, B is already complex and could account for apparent dispensability of various bona fide PKA sites because the key sites are redundant or partially redundant. Figure, C shows a situation where a single receptor leads to activation of multiple signaling cascades, simplified here to show 1 stimulatory and 1 inhibitory pathway working through a single stimulatory phosphorylation site. The model in Figure, C could potentially explain discrepancies between studies in native (primary) cells and cultured cells or in different animal models or in identical animal models from different genetic backgrounds if the potential pathways were different, lacking, or less coupled to the upstream G protein–coupled receptor in one system compared with another. Work by a number of groups does support features of this scenario by suggesting that isoproterenol can recruit kinase pathways (eg, the multifunctional calcium and calmodulin protein kinase II, PKC) in addition to PKA with the potential to enhance I$_{Ca}$. The recruitment of multiple kinase (and phosphatase) pathways targeting multiple redundant or nonredundant amino acids (Figure, D) is further complicated by the potential for distinct phosphorylation targets to produce convergent biophysical phenotypes, such as mode 2 gating. Ultimately, PKA-dependent phosphorylation must modify Ca$_{\alpha}$1.2 channel structure in a manner that enhances I$_{Ca}$. However, detailed structural understanding of these events faces formidable obstacles that will not be resolved when and if a Ca$_{\alpha}$1.2 crystal structure is solved because such a structure will necessarily be static, while the gating consequences of agonist phosphorylation are dynamic.

The Culprit Site Concept May Be Too Simplistic

The increasing complexity of scenarios depicted in Figure, A through D suggest that the likelihood of identifying a culprit phosphorylation site is relatively high for scenario A but steeply declines thereafter (Figure, E). Phosphorylation of various amino acids likely lead to increases in I$_{Ca}$ by lowering the energy barrier (increasing the probability) to assemble activated conformations. Assuming that upstream signaling is conserved in various cell systems, species, and strains, the discovery of a culprit phosphorylation site will depend on the existence of a unique phosphorylation-enabled conformation to achieve increased I$_{Ca}$. However, at this point, we have no evidence to suggest a unique phosphorylation site is in control of this process. If we discover such a site, our field should contemplate this new information with caution because a phosphorylation-resistant mutant protein may produce other unanticipated effects, including allosteric resistance to post-translational modifications at distant sites.

Consider a story where space aliens visit planet Earth and by observation conclude that it will be essential to understand the mechanism of action of automobiles to grasp fundamentals of human society. The alien scientists might first characterize the performance of cars, trucks, and, perhaps, other motorized vehicles. Second, they would disarticulate the machinery to

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Figure. A–E, Schematic diagram of various possible scenarios for receptor signaling to ion channels. **GPCR** indicates G protein coupled receptors.
understand the mechanism of propulsion. One scientist might remove the nonredundant right front tire of a 4-wheeled vehicle and observing that the car was no longer functional conclude that the right front tire was the pathway/mechanism for motorized propulsion. Another scientist might produce conflicting results and could conclude that loss of the nonredundant left rear tire was also essential by its removal. Investigation of complex conveyances, such as a multiwheeled semitrailer, could lead to a conclusion that any individual (nonredundant) tire was dispensable for the mechanism of propulsion because multiple wheels need to be removed before a short-term change could be observed. Thus, it may be with ion channels, such as Ca\textsubscript{v} 1.2, where we vainly seek simple, unique solutions to a complex, nonlinear signaling system with multiple upstream inputs and redundant pathways favoring high-activity conformations.

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None.

**References**


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Why Has It Taken So Long to Learn What We Still Don't Know?
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