Journal Club

Reassessing the molecular mechanism of β-adrenergic stimulation of cardiac L-type Ca²⁺ current

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In the heart, Ca²⁺ influx via the L-type Ca²⁺ channel (CaV₁.2) underlies the plateau phase of the cardiac action potential and gives rise to contracture of the myocardium by gating the cardiac ryanodine receptor (RyR2). During β-adrenergic stimulation (the “fight or flight” response), CaV₁.2 channels become more sensitive to changes in membrane potential and enter a high P⁰ gating mode in order to provide the greater Ca²⁺ influx required to enhance contractile force (for a review of this topic, see ref. 1). Thus, adrenergic stimulation of the cardiac L-type current is initiated by binding of noradrenaline to β-adrenergic receptors on the external face of the sarcolemma, causing dissociation of choline toxin-sensitive G protein heterotrimers on the myoplasmic side of the plasma membrane (Fig. 1). The liberated Gα₁ subunits stimulate cAMP production by adenylyl cyclase, leading to activation of cAMP-dependent protein kinase (PKA). After much initial controversy, the requirement for PKA-mediated phosphorylation in adrenergic upregulation of cardiac L-type current has been firmly established.¹⁻³ Beyond activation of PKA, however, the molecular events that mediate stimulation of CaV₁.2 during sympathetic activity are less clear.

Phosphorylation of residue S1928 within the CaV₁.2 carboxyl-terminus has been proposed to be the critical event underlying adrenergic modulation of CaV₁.2 channels²⁻⁴ (Fig. 1A), largely because S1928 is the only detectable PKA phosphorylation site on the principle α₁ subunit of the CaV₁.2 heteromultimer.³⁻⁵ However, probing the physiological significance of PKA-mediated phosphorylation of S1928 has been somewhat problematic. Such difficulty has been due, at least in part, to the inconsistent reproduction of β-adrenergic stimulation of CaV₁.2 in heterologous expression systems. In this regard, multiple groups have been unable to reconstitute this signaling pathway in HEK293 cells, CHO cells or Xenopus oocytes.⁶⁻⁷ In contrast, other laboratories have demonstrated that direct activation of either adenyl cyclase by forskolin or PKA by 8-bromo cAMP (i.e., pharmacologically bypassing β-receptor activation) produces stimulation of heterologously-expressed CaV₁.2 channels in HEK293 cells.⁴ In particular, the increase in CaV₁.2 current amplitude observed by Gao and colleagues⁴ was modest (<100%) compared to that typically observed in cardiomyocytes (>200%), and required both coexpression of an A-Kinase Anchoring Protein (AKAP) and the presence of phosphatase inhibitors. Within this experimental system, currents mediated by a CaV₁.2 S1928A phosphorylation-deficient mutant were unaffected by application of either forskolin or 8-bromo cAMP.⁴ Interestingly, the same laboratory demonstrated that CaV₁.2 channels truncated at residue 1905 (and therefore lacking S1928) were readily stimulated by purified PKA catalytic subunits introduced via the patch pipette, though the hyperpolarizing shift in the I-V relationship that accompanies the increased current amplitude in native cardiomyocytes was absent.⁸

Needless to say, these conflicting reports have led to a lack of consensus concerning the fidelity of PKA-mediated stimulation of CaV₁.2 in heterologous systems. Even so, phosphorylation of S1928 has persisted as the generally-accepted mechanism for adrenergic upregulation of the cardiac L-type current.

Not so long ago, Ganesan et al.⁹ presented evidence suggesting that adrenergic stimulation of the cardiac L-type current may not be entirely attributable to phosphorylation of S1928. In order to investigate the role of S1928 in a more physiological environment than cloned cell lines, a dihydropyridine (DHP)-resistant CaV₁.2 S1928A mutant subunit was overexpressed in dissociated ventricular myocytes. The DHP-resistant component of the Ca²⁺ current (i.e., the current presumably attributable to the S1928A mutant channel) in successfully transduced myocytes was found to have a similar response (~75% increase) to the β-receptor agonist isoproterenol as DHP-resistant CaV₁.2 channels with serines at position 1928. Thus, Ganesan and colleagues concluded that phosphorylation of S1928 is not critical for PKA-mediated modulation of CaV₁.2 in the myocardium. While this assessment seems
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In a more recent study, Lemke and colleagues\textsuperscript{3} generated an S1928A knock-in mouse as a means to directly test the importance of phosphorylation of S1928 in the adrenergic response. The substitution of alanine for serine at this position eliminated the influence of G\(\alpha\) subunits. Specifically, no compensatory alterations in the expression of CaV\(\alpha_{1,2}\) \(\beta_{2a}\) subunit suggests the involvement of another PKA-phosphorylated protein(s) in the adrenergic signaling pathway (Fig. 1B). If this idea is correct, one candidate to mediate the signal between PKA and CaV\(\alpha_{1,2}\) is the channel \(\beta_{2a}\) subunit. To this effect, \(\beta_{2a}\) may possibly be the direct target of PKA as it contains three residues (S459, S478, S479) that have been identified as PKA substrates. In particular, two of these residues (S478 and S479) were shown to mediate increased CaV\(\alpha_{1,2}\) Ba\textsuperscript{2+} current density in HEK293 cells dialyzed with purified PKA catalytic subunits.\textsuperscript{3} However, such a requirement for direct phosphorylation of \(\beta_{2a}\) is uncertain since L-type currents of ventricular myocytes overexpressing a \(\beta_{2a}\) triple mutant (S459A, S478A, S479A) were found to be as sensitive to forskolin as those of myocytes overexpressing wild-type \(\beta_{2a}\).\textsuperscript{7} Alternatively, \(\beta_{2a}\) has been proposed to be indirectly involved in the adrenergic response, whereby PKA-mediated phosphorylation of the immense protein Ahnak disrupts an inhibitory interaction between Ahnak and \(\beta_{2a}\), leading to an enhancement of L-type current reminiscent of adrenergic upregulation.\textsuperscript{10} Finally, it is not beyond the realm of possibility that the intermediate protein(s) has not yet been identified as a component of the CaV\(\alpha_{1,2}\)-centric signaling apparatus; proteomic approaches may prove useful in the identification of new players in this signaling pathway.

Figure 1. Adrenergic upregulation of cardiac L-type (CaV\(\alpha_{1,2}\)) Ca\textsuperscript{2+} channels. In both (A and B), binding of noradrenaline (NA) to \(\beta\)-adrenergic receptors causes dissociation of cholera toxin-sensitive G protein heterotrimers. G\(\alpha_{i}\)-GTP subunit increases cAMP levels by activating adenyl cyclase. cAMP then binds to the regulatory subunits of the Protein Kinase A (PKA) holoenzyme and engages kinase activity by causing dispersal of the catalytic subunits. For clarity, these latter events are shown collectively as “PKA.” (A) Shows a generally-accepted model of PKA upregulation of CaV\(\alpha_{1,2}\) channels in which AKAP15/18-anchored PKA directly phosphorylates residue S1928 in the proteolytically-cleaved distal carboxyl-terminus of CaV\(\alpha_{1,2}\) (see ref. 1). As shown by the four red lines, this region of CaV\(\alpha_{1,2}\) re-associates with the channel complex via a non-covalent interaction with the proximal CaV\(\alpha_{1,2}\) carboxy-terminus. (B) shows an alternative model based on the findings of Lemke et al.\textsuperscript{3} in which stimulation of cardiac L-type current requires AKAP15/18-anchored PKA-mediated phosphorylation of another yet-to-be determined protein (denoted by “?”).

In the previous work, isoproterenol failed to produce further enhancement of the L-type current in either wild-type or S1928A myocytes following forskolin treatment, arguing against a direct stimulatory influence of G\(\alpha_{i}\) subunits. Furthermore, the kinase inhibitor H-89 blocked the stimulatory effect of isoproterenol in S1928A myocytes, confirming the involvement of PKA in stimulation of the L-type current.

In addition to providing electrophysiological evidence that phosphorylation of S1928 is not essential for \(\beta\)-adrenergic modulation of cardiac L-type current, Lemke and colleagues also examined the effect of the S1928A mutation on cardiac function in vivo. Using ECG telemetry, the authors found no significant difference in heart rate, either before or following infusion of isoproterenol, between S1928A mice and wild-type mice. In experiments that assessed the ability of the mutant L-type channel to support the adrenergic response more directly, both S1928A and wild-type hearts displayed similar isoproterenol-induced augmentation of myocardial contractility (gauged by fractional shortening during systole). Moreover, no major behavioral changes were observed in the S1928A mice.

The persistence of the adrenergic response in S1928A mice despite the loss of all detectable PKA-mediated phosphorylation of the CaV\(\alpha_{1,2}\) \(\alpha_{1}\) subunit suggests the involvement of another PKA-phosphorylated protein(s) in the adrenergic signaling pathway (Fig. 1B). If this idea is correct, one candidate to mediate the signal between PKA and CaV\(\alpha_{1,2}\) is the channel \(\beta_{2a}\) subunit. To this effect, \(\beta_{2a}\) may possibly be the direct target of PKA as it contains three residues (S459, S478, S479) that have been identified as PKA substrates. In particular, two of these residues (S478 and S479) were shown to mediate increased CaV\(\alpha_{1,2}\) Ba\textsuperscript{2+} current density in HEK293 cells dialyzed with purified PKA catalytic subunits.\textsuperscript{3} However, such a requirement for direct phosphorylation of \(\beta_{2a}\) is uncertain since L-type currents of ventricular myocytes overexpressing a \(\beta_{2a}\) triple mutant (S459A, S478A, S479A) were found to be as sensitive to forskolin as those of myocytes overexpressing wild-type \(\beta_{2a}\).\textsuperscript{7} Alternatively, \(\beta_{2a}\) has been proposed to be indirectly involved in the adrenergic response, whereby PKA-mediated phosphorylation of the immense protein Ahnak disrupts an inhibitory interaction between Ahnak and \(\beta_{2a}\), leading to an enhancement of L-type current reminiscent of adrenergic upregulation.\textsuperscript{10} Finally, it is not beyond the realm of possibility that the intermediate protein(s) has not yet been identified as a component of the CaV\(\alpha_{1,2}\)-centric signaling apparatus; proteomic approaches may prove useful in the identification of new players in this signaling pathway.

Building on the initial findings of Ganesan et al.,\textsuperscript{9} Lemke et al.\textsuperscript{3} have provided compelling evidence arguing against an essential role for phosphorylation of S1928 in adrenergic modulation of cardiac L-type Ca\textsuperscript{2+} channels. In doing so, they have exposed a gap in our understanding of PKA-dependent modulation of cardiac L-type channels and have underscored the need for further investigation of this important physiological mechanism.
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