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# Regulation of Cardiac L-Type Calcium Channels by Protein Kinase A and Protein Kinase C

Timothy J. Kamp, Johannes W. Hell

**Abstract**—Voltage-dependent L-type  $\text{Ca}^{2+}$  channels are multisubunit transmembrane proteins, which allow the influx of  $\text{Ca}^{2+}$  ( $I_{\text{Ca}}$ ) essential for normal excitability and excitation-contraction coupling in cardiac myocytes. A variety of different receptors and signaling pathways provide dynamic regulation of  $I_{\text{Ca}}$  in the intact heart. The present review focuses on recent evidence describing the molecular details of regulation of L-type  $\text{Ca}^{2+}$  channels by protein kinase A (PKA) and protein kinase C (PKC) pathways. Multiple G protein-coupled receptors act through cAMP/PKA pathways to regulate L-type channels.  $\beta$ -Adrenergic receptor stimulation results in a marked increase in  $I_{\text{Ca}}$ , which is mediated by a cAMP/PKA pathway. Growing evidence points to an important role of localized signaling complexes involved in the PKA-mediated regulation of  $I_{\text{Ca}}$ , including A-kinase anchor proteins and binding of phosphatase PP2a to the carboxyl terminus of the  $\alpha_{1\text{C}}$  ( $\text{Ca}_v1.2$ ) subunit. Both  $\alpha_{1\text{C}}$  and  $\beta_{2\text{a}}$  subunits of the channel are substrates for PKA in vivo. The regulation of L-type  $\text{Ca}^{2+}$  channels by Gq-linked receptors and associated PKC activation is complex, with both stimulation and inhibition of  $I_{\text{Ca}}$  being observed. The amino terminus of the  $\alpha_{1\text{C}}$  subunit is critically involved in PKC regulation. Crosstalk between PKA and PKC pathways occurs in the modulation of  $I_{\text{Ca}}$ . Ultimately, precise regulation of  $I_{\text{Ca}}$  is needed for normal cardiac function, and alterations in these regulatory pathways may prove important in heart disease. (*Circ Res.* 2000;87:1095-1102.)

**Key Words:** L-type calcium channel ■ protein kinase C ■ protein kinase A ■ heart ■ regulation ■ phosphorylation

The influx of  $\text{Ca}^{2+}$  ions through voltage-dependent L-type  $\text{Ca}^{2+}$  channels plays an essential role in cardiac excitability and in coupling excitation to contraction. The depolarizing current through L-type  $\text{Ca}^{2+}$  channels ( $I_{\text{Ca}}$ ) contributes to the plateau phase of the cardiac action potential as well as to pacemaker activity in nodal cells. This influx of  $\text{Ca}^{2+}$  triggers the release of intracellular stores of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum, and the ensuing intracellular  $\text{Ca}^{2+}$  transient results in activation of the myofilaments. L-type channels can also impact on other cellular processes modulated by intracellular  $\text{Ca}^{2+}$  such as gene expression and excitation-secretion coupling. Alterations in density or function of L-type  $\text{Ca}^{2+}$  channels have been implicated in a variety of cardiovascular diseases, including atrial fibrillation,<sup>1,2</sup> heart failure,<sup>3-6</sup> and ischemic heart disease.<sup>7</sup>

Cardiac L-type  $\text{Ca}^{2+}$  channels are regulated by a variety of neurotransmitters, hormones, and cytokines. In fact, the first description of currents carried by this channel revealed its regulation by epinephrine.<sup>8</sup> Sperelakis and Schneider<sup>9</sup> and Reuter and Scholz<sup>10</sup> independently hypothesized that  $\beta$ -adrenergic receptor (AR)-mediated stimulation of cardiac L-type  $\text{Ca}^{2+}$  channels was due to phosphorylation of the channel by cAMP-dependent protein kinase A (PKA). Extensive electrophysiology experimentation over the subsequent 2 decades has supported the hypothesis; however, the molecu-

lar details have been slow to follow. The scarcity of this transmembrane protein as well as difficulty in reconstituting regulation in heterologous expression systems has limited progress. Other signaling pathways have also been suggested to regulate the channel by phosphorylation, but the details are even less clear. For example, activation of protein kinase C (PKC) has resulted in widely variable effects on L-type channel activity. The purpose of the present review is to describe recent advances in the understanding of the regulation of L-type  $\text{Ca}^{2+}$  channels by PKA- and PKC-mediated pathways focusing on features that provide specificity and localization to this signaling. Excellent general reviews on the structure and function of L-type  $\text{Ca}^{2+}$  channels are available.<sup>11-14</sup>

## Structure of L-Type $\text{Ca}^{2+}$ Channels

Voltage-dependent  $\text{Ca}^{2+}$  channels are multimeric protein complexes present in many cell types throughout the body. The  $\alpha_1$  subunit is the main functional component of the channel complex. It is composed of 4 homologous domains (I-IV), each containing 6 transmembrane segments (S1-S6) as schematically shown in Figure 1. The  $\alpha_1$  subunit contains the voltage sensor for the channel, which is primarily formed by the positively charged arginine and lysine residues in the S4 segments. The P loops between S5 and S6 line the pore of

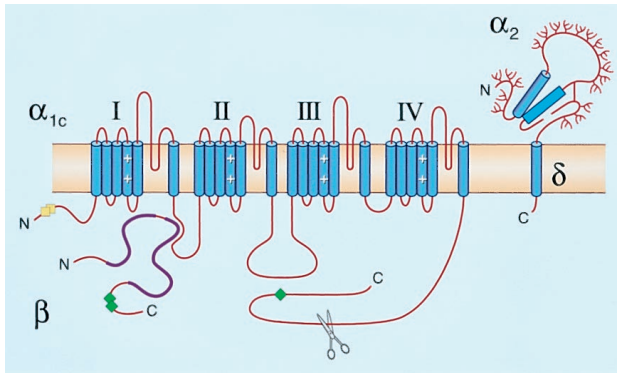
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**Figure 1.** Proposed transmembrane topology and subunit composition of L-type  $\text{Ca}^{2+}$  channel. Shown is the pore-forming  $\alpha_{1c}$  subunit consisting of 4 homologous repeated domains (I–IV), each composed of 6 transmembrane segments as described in text. The cytoplasmic  $\beta$  subunit is formed by 2 highly conserved domains indicated in purple, and the amino-terminal portion of the second conserved domain interacts with the I–II loop of  $\alpha_{1c}$ . The  $\delta$  subunit has a single transmembrane segment with a short cytoplasmic C terminus and is linked by a disulfide bond to the extracellular, glycosylated  $\alpha_2$  subunit. PKA phosphorylation sites of proven functional significance are shown as green diamonds at Ser1928 on  $\alpha_{1c}$  and Ser478 and Ser479 on  $\beta_{2a}$ . PKC phosphorylation sites of proven functional importance at Thr27 and Thr31 on  $\alpha_{1c}$  are indicated by yellow squares.

the channel.<sup>15,16</sup> At least 10 different  $\alpha_1$ -subunit genes have been identified, which provide unique functional properties to  $\text{Ca}^{2+}$  channels present in different cell types.<sup>17</sup> In cardiac muscle, L-type  $\text{Ca}^{2+}$  channels are primarily encoded by the  $\alpha_{1c}$  gene ( $\text{Ca}_v1.2$ ) with possible contribution by  $\alpha_{1D}$  ( $\text{Ca}_v1.3$ ).<sup>18,19</sup> In vivo, a substantial portion of  $\alpha_{1c}$  undergoes proteolytic processing about 400 to 500 residues away from its C terminus, but the C-terminal fragment stays associated with the channel complex.<sup>20–23</sup>

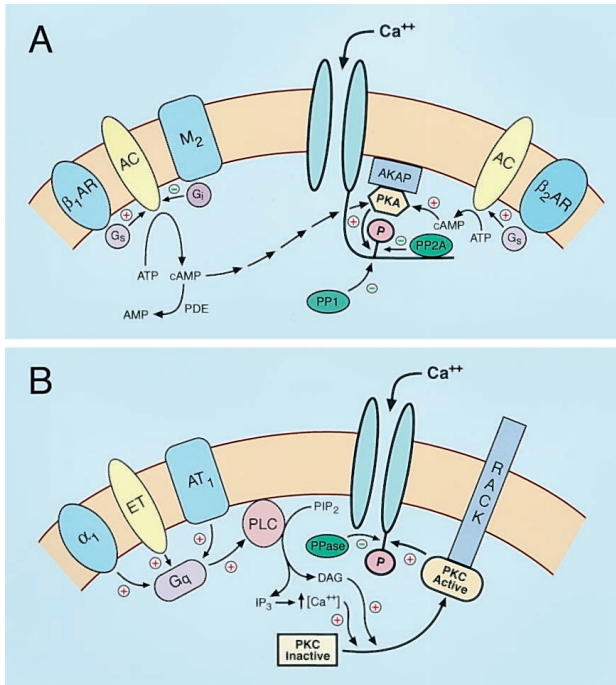
Cardiac L-type  $\text{Ca}^{2+}$  channels are also composed of auxiliary subunits, including  $\beta$  and  $\alpha_2$ - $\delta$ . Additionally, a  $\gamma$  subunit has been found in  $\text{Ca}^{2+}$  channels in skeletal muscle and brain,<sup>24–26</sup> but it remains unclear as to whether cardiac L-type  $\text{Ca}^{2+}$  channels contain a  $\gamma$  subunit.<sup>27</sup> Four distinct genes encode cytoplasmically localized  $\text{Ca}^{2+}$  channel  $\beta$  subunits, each having multiple splice variants.<sup>28</sup> The  $\beta$  subunits are important in trafficking of the channel complex to the surface membrane as well as in modifying its gating properties.<sup>28–31</sup> Although the  $\beta_{2a}$  subunit may be the predominant isoform in heart, there appears to be significant species variation, and multiple isoforms are expressed.<sup>32,33</sup> The  $\alpha_2$ - $\delta$  subunits are created from a precursor protein by proteolytic cleavage.<sup>34</sup> Both fragments remain linked via a disulfide bridge.  $\delta$  is an integral membrane protein with a single transmembrane region, a short intracellular sequence, and a larger extracellular portion, which is differentially glycosylated.<sup>35</sup>  $\alpha_2$  is an extracellular, glycosylated protein.<sup>35</sup> Three  $\alpha_2$ - $\delta$  genes have been identified.<sup>36,37</sup> This subunit has also been implicated in modifying the gating properties of the channel as well as the expression level of the channel complex.<sup>29,37,38</sup> Therefore, a rich variety of different subunit isoforms can combine to produce voltage-dependent  $\text{Ca}^{2+}$  channels in a cell-specific and potentially disease-modulated fashion.

## Regulation by PKA

Multiple G protein-coupled receptors in the heart act through cAMP/PKA pathways to regulate many cellular proteins, including the L-type  $\text{Ca}^{2+}$  channel (Figure 2A). These receptors are coupled to heterotrimeric G proteins, which either stimulate ( $G_s$ ) or inhibit ( $G_i$ ) adenylyl cyclase (AC). An increase in AC activity leads to increased cellular cAMP, which binds to the regulatory subunits of cAMP-dependent protein kinase (PKA), liberating the catalytic subunits to phosphorylate their substrates on specific serine and threonine residues. This cascade is counterbalanced by phosphodiesterases that degrade cAMP into 5'-AMP as well as serine-threonine phosphatases. Multiple laboratories have provided extensive evidence demonstrating robust upregulation of  $I_{Ca}$  by the  $\beta$ AR/cAMP/PKA pathway, and these pioneering electrophysiological studies have been reviewed well elsewhere.<sup>13,14,39</sup> In addition,  $\beta$ -adrenergic activation of  $G_{\alpha_s}$  has been suggested to directly stimulate  $I_{Ca}$  independently of PKA,<sup>40</sup> but the role of this regulation in normal physiology is controversial.<sup>41</sup> The present review will focus on more recent experiments dissecting out the molecular details of PKA-mediated upregulation of channel function.

Most initial studies on the stimulation of cardiac L-type  $\text{Ca}^{2+}$  channel by  $\beta$ AR signaling focused on the  $\beta_1$ AR, the predominant  $\beta$ AR in the normal adult mammalian heart. These studies have clearly demonstrated a cAMP/PKA-dependent stimulation of  $I_{Ca}$ .  $\beta_2$ AR stimulation also increases  $I_{Ca}$  in certain cardiac myocyte preparations depending on the species, developmental stage, and presence of disease.<sup>42,43</sup> Whereas both  $\beta_1$ AR and  $\beta_2$ AR are positively coupled to  $G_s$ , cAMP levels, and L-type  $\text{Ca}^{2+}$  channel activity,  $\beta_2$ AR can in some cases stimulate  $I_{Ca}$  without significantly elevating total cellular cAMP.<sup>44</sup> This finding, as well as the lack of  $\beta_2$ AR effects on PKA-mediated phosphorylation of phospholamban and troponin I, led to the suggestion that regulation of L-type  $\text{Ca}^{2+}$  channels by  $\beta_2$ AR was due to highly localized elevations in cAMP around the channel.<sup>45</sup> In amphibian ventricular myocytes, which contain almost exclusively  $\beta_2$ ARs, regulation of  $I_{Ca}$  is spatially restricted.<sup>46</sup>  $\beta_2$ ARs couple not only to  $G_s$  but also to  $G_i$ . The latter pathway has been suggested to play a role in spatially restricting  $\beta_2$ AR signaling.<sup>47</sup> However, some studies have not been able to demonstrate  $\beta_2$ AR regulation of  $I_{Ca}$ .<sup>48,49</sup> There are multiple other  $G_s$ -coupled receptors in the heart that can upregulate  $I_{Ca}$ , including histamine receptors ( $H_2$ ) and glucagon receptors.<sup>14,39</sup> The specifics of their regulation of  $I_{Ca}$  will likely differ in detail, but less information is available for these receptors.

The muscarinic  $M_2$  receptor represents the best-studied example of a  $G_i$ -coupled receptor that regulates  $I_{Ca}$ .<sup>50</sup> In general, most  $G_i$ -coupled receptors appear not to alter basal  $I_{Ca}$  levels but dramatically inhibit the  $\beta$ AR stimulation of  $I_{Ca}$ . Initial studies suggested that this effect was due to  $G_i$ -mediated inhibition of AC and lowering cAMP levels. However, in the case of muscarinic  $M_2$  receptor-mediated inhibition of  $I_{Ca}$ , other mechanisms are likely in place such as activation of phosphatases<sup>51</sup> and a debatable role of NO and stimulation of cGMP-dependent phosphodiesterase.<sup>52,53</sup> Interestingly,  $\beta_1$ AR- and  $\beta_2$ AR-stimulated responses may exhibit differential sensitivity to muscarinic inhibition.<sup>54</sup> Multiple



**Figure 2.** Signaling cascades regulating L-type  $\text{Ca}^{2+}$  channels. A, Schematic of the cAMP/PKA cascade regulating L-type channels. Stimulation of  $\beta_1\text{AR}$  or  $\beta_2\text{AR}$  leads to  $\text{G}_s$ -mediated activation of AC and increased production of cAMP, which stimulates PKA, as described in text. PKA can then phosphorylate the channel at multiple potential sites indicated schematically by the single P in the diagram. The PKA phosphorylated site(s) is then sensitive to the phosphatases PP1 and PP2A. Whereas  $\beta_1\text{AR}$  regulation causes more global increases in cAMP,  $\beta_2\text{AR}$  stimulation can result in highly localized cAMP level changes and regulation. Regulatory proteins may be localized to the channel by an AKAP for PKA and by binding of PP2A to the C terminus of the channel. Muscarinic  $\text{M}_2$  receptors can oppose the  $\beta\text{AR}$  upregulation of  $I_{\text{Ca}}$  by acting through  $\text{G}_i$  to inhibit AC. B, PLC/PKC signaling cascade regulating L-type  $\text{Ca}^{2+}$  channels. Activation of  $\alpha_1$ -adrenergic, ET, or  $\text{AT}_1$  receptors stimulates  $\text{G}_q$  with resulting activation of PLC, which leads to the production of diacylglycerol and activation of PKC. PKC is proposed to target to the membrane by binding a RACK protein in the vicinity of the L-type  $\text{Ca}^{2+}$  channel, which it then phosphorylates (see text for details). A Ser/Thr phosphatase counterbalances this phosphorylation.  $\text{IP}_3$  indicates inositol trisphosphate;  $\text{PIP}_2$ , phosphatidylinositol 4,5 biphosphate.

other  $\text{G}_i$ -coupled receptors have been implicated in  $I_{\text{Ca}}$  regulation, including adenosine ( $\text{A}_1$ ) receptors, opiate receptors, and atrial natriuretic factor receptors.<sup>14</sup>

An alternative mechanism of PKA-mediated stimulation of L-type  $\text{Ca}^{2+}$  channels occurs as a result of strong depolarization. This process of voltage-dependent facilitation is hypothesized to be caused by a voltage-dependent conformational change in the channel, making it amenable to PKA-dependent phosphorylation.<sup>55</sup> This finding suggested that PKA may be in close proximity to the channel, and in the case of skeletal muscle, an A-kinase anchor protein (AKAP) associating PKA with the channel has been shown to be essential for this regulation.<sup>56</sup> Although state-dependent regulation of the channel has been observed in native ventricular myocytes,<sup>57,58</sup> it has only been variably reproduced in heterologous systems. The neuronal splice variant,  $\alpha_{1\text{C-c}}$ , expressed in

*Xenopus* oocytes has demonstrated pronounced voltage-dependent facilitation that requires PKA and  $\beta$ -subunit coexpression.<sup>59</sup> In contrast, studies in mammalian HEK293 cells expressing cardiac isoforms of  $\alpha_{1\text{C}}$  have demonstrated voltage-dependent facilitation, but it is independent of PKA.<sup>60,61</sup> The reasons for these apparently distinct results, as well as the molecular details of voltage-dependent facilitation of L-type  $\text{Ca}^{2+}$  channel activity, remain largely unknown.

### Biochemical and Functional Characterization of Channel Phosphorylation by PKA

Evidence for a direct phosphorylation of L-type channels by PKA did not become available until it was recognized that the full-length form of  $\alpha_{1\text{C}}$  can be proteolytically truncated at its C terminus.<sup>21</sup> The proteolytic cleavage is mediated in neurons and possibly in the heart by the  $\text{Ca}^{2+}$ -dependent protease calpain.<sup>22</sup> Only the long but not the short form of  $\alpha_{1\text{C}}$  is effectively and stoichiometrically phosphorylated by PKA in vitro.<sup>21,62</sup> Ser1928, which is located in the C-terminal portion that is cleaved off the full-length form (Figure 1), is the only detectable phosphorylation site on  $\alpha_{1\text{C}}$ <sup>20</sup> and is phosphorylated in vivo.<sup>20,62,63</sup> In heart, the prevailing isoform detectable by immunoblotting is the short form.<sup>20</sup> However, the long form is also present, and biochemical and functional evidence indicates that the C-terminal fragment remains tethered to the channel.<sup>23,64</sup> Electrophysiological studies utilizing heterologous expression systems for  $\alpha_{1\text{C}}$  suggested that no other  $\text{Ca}^{2+}$  channel subunit is absolutely required for stimulation of channel activity by PKA.<sup>55,65</sup> Furthermore, mutation of Ser1928 to alanine in  $\alpha_{1\text{C}}$  prevented phosphorylation and upregulation of the channel by PKA.<sup>64</sup>

$\alpha_2$ - $\delta$  is primarily extracellular, and phosphorylation by PKA or PKC is not detectable.<sup>21,63</sup> In contrast,  $\text{Ca}^{2+}$  channel  $\beta$  subunits serve as substrates for multiple kinases in vitro and in intact cells.<sup>13,64</sup> Application of the  $\beta\text{AR}$  agonist isoproterenol in vivo resulted in phosphorylation of 1 or more PKA sites of the cardiac L-type channel  $\beta$  subunits.<sup>66,67</sup> PKA phosphorylates 3 sites of  $\beta_{2a}$  (Ser459, Ser478, and Ser479) in vitro (Figure 1).<sup>68</sup> To test the functional relevance of these phosphorylation sites,  $\beta_{2a}$  was coexpressed with a C-terminally truncated version of  $\alpha_{1\text{C}}$  that lacks Ser1928. Channel activity could be increased by PKA when wild-type  $\beta_{2a}$  was present, indicating that phosphorylation of the  $\beta$  subunit can contribute to the upregulation of channel activity.<sup>69</sup> Mutation of Ser478/Ser479 to alanines but not of Ser459 on  $\beta_{2a}$  prevented upregulation of channel activity.<sup>69</sup> These results indicate that phosphorylation of either Ser478, Ser479, or both contributes to channel regulation by PKA at least in the presence of C-terminally truncated  $\alpha_{1\text{C}}$ .

AKAPs target PKA to various substrates to provide fast and specific signaling.<sup>70–72</sup> When PKA is prevented from binding to AKAPs by a peptide derived from one of the interaction sites, its regulation of skeletal muscle ( $\text{Ca}_v1.1$ ) and cardiac L-type channels is blocked.<sup>56,64</sup> PKA-mediated  $\alpha_{1\text{C}}$  phosphorylation can be reconstituted in HEK293 cells by coexpression of the channel with wild-type AKAP79 but not an AKAP79 mutant deficient in binding of PKA.<sup>64</sup> Recently, association of PKA with  $\alpha_{1\text{C}}$  has been demonstrated in the brain.<sup>63</sup> This interaction may be mediated by microtubule-

associated protein MAP2B,<sup>63</sup> which is the first identified AKAP.<sup>73</sup> Because MAP2B is not expressed in the heart, another AKAP may recruit PKA to cardiac L-type channels. One candidate is mAKAP (AKAP100), which localizes to the region of the transverse tubules and junctional sarcoplasmic reticulum,<sup>74</sup> similar to the predominance of L-type channels in the transverse tubules.<sup>75</sup> Another possibility is AKAP15, which acts as the adaptor protein for PKA association with the skeletal muscle L-type channel<sup>76</sup> and is expressed in the heart.<sup>77</sup>

The functional effects of phosphorylation of cardiac L-type  $\text{Ca}^{2+}$  channels have been examined in single-channel studies. The functional properties of the  $\text{Ca}^{2+}$  channels determine the whole-cell  $I_{\text{Ca}}$  by the equation  $I_{\text{Ca}} = N \times f_{\text{active}} \times p_o \times g \times \Delta V$ , where  $N$  is the total number of L-type  $\text{Ca}^{2+}$  channels,  $f_{\text{active}}$  is the fraction of these channels that are available to open during a depolarization,  $p_o$  is the probability of an active channel to be open,  $g$  is the single-channel conductance, and  $\Delta V$  is the difference between the test potential and the reversal potential for the channel. Single Ca channel currents recorded on consecutive depolarizations have demonstrated a variety of gating patterns that can most simply be divided into blank sweeps (no openings) and active sweeps. The blank sweeps are clustered together in time, as are the active sweeps. One prominent effect of PKA activation is to decrease the number of blank sweeps or increase  $f_{\text{active}}$ . It was hypothesized that phosphorylation of the channel by PKA was necessary for the channels to become active.<sup>78,79</sup> Herzig et al<sup>80</sup> developed a model suggesting that the availability of channels to open could indeed be controlled by a single phosphorylation event. In addition, the activity of the channel during active traces can be markedly increased by PKA stimulation due to increase in  $p_o$  resulting from changed modes of active gating.<sup>81</sup> The relative importance of increased  $f_{\text{active}}$  and  $p_o$  in  $\beta\text{AR}$  stimulation of  $I_{\text{Ca}}$  has been debated and likely varies in different experimental preparations. No changes in single-channel conductance, reversal potential, or the number of channels in the patch have been observed in response to  $\beta\text{AR}$  or PKA stimulation of the channels.

Dynamic regulation of channel activity requires that phosphorylation be readily reversible by phosphatases. The Ser/Thr phosphatases PP1 and PP2A but not PP2B or PP2C have been demonstrated to regulate L-type channels stimulated by PKA.<sup>55,82,83</sup> Experiments with phosphatase inhibitors that differentially inhibit PP1 and PP2A suggest the existence of 2 different phosphorylation sites governing the 2 major changes in gating of L-type  $\text{Ca}^{2+}$  channels observed in response to  $\beta\text{AR}$  stimulation. In rabbit and guinea pig ventricular myocytes, a phosphorylation site sensitive to PP1 regulates the availability of channels ( $f_{\text{active}}$ ), whereas a distinct phosphorylation site sensitive to PP2A controls modal gating during active sweeps.<sup>58,84</sup> However, the case may be different in amphibian ventricular myocytes.<sup>85</sup> Furthermore, rundown of L-type channel activity in inside-out patches obtained from rabbit ventricular myocytes is strongly slowed by an inhibitor of PP1 and PP2A,<sup>83</sup> suggesting that PP1 or PP2A may be linked to the plasma membrane in close proximity to the channel. We recently found that PP2A is directly bound to  $\alpha_{1\text{C}}$  in rat brain and reverses phosphoryla-

tion of Ser1928.<sup>86</sup> Overall, these studies have provided evidence of single L-type  $\text{Ca}^{2+}$  channel complexes being modulated by at least 2 distinct PKA-mediated phosphorylation events and that PKA and PP2A may be highly localized to the channel complex. Investigations have not yet linked the identified PKA phosphorylation sites with specific changes in channel gating in native cells.

### Regulation by PKC

The PKC family of kinases also plays an essential role in the regulation of the L-type  $\text{Ca}^{2+}$  channel in the heart. Multiple  $G_q$  protein-coupled receptors, including endothelin (ET),  $\alpha_1$ -adrenergic, and angiotensin II receptors, trigger the signaling cascade leading to activation of PKC (Figure 2B).<sup>87</sup> Activated  $G_q$  stimulates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate ( $\text{PIP}_2$ ), generating inositol trisphosphate and diacylglycerol (DAG).<sup>88</sup> DAG, phosphatidylserine, and in some cases  $\text{Ca}^{2+}$  collectively activate PKC.

Initial studies of the modulation of  $I_{\text{Ca}}$  by neurohormones linked to PKC have demonstrated a variety of results. For example, ET-1 resulted in clear increases,<sup>89,90</sup> decreases,<sup>91</sup> or no change in basal  $I_{\text{Ca}}$ .<sup>92,93</sup> Some authors have even demonstrated biphasic effect on  $I_{\text{Ca}}$ , ie, a decrease followed by a more sustained increase.<sup>94–96</sup> The range of effects may be due to differences in experimental conditions, species, and methods of studying  $I_{\text{Ca}}$ . Techniques that preserve the cytoplasmic environment, such as the perforated-patch whole-cell approach or cell-attached single-channel method, may be necessary to demonstrate an upregulation of  $I_{\text{Ca}}$  in response to  $\alpha_1$ -adrenergic stimulation, arginine vasopressin, and ET-1.<sup>90,94,95,97,98</sup> In addition, an upregulation of  $I_{\text{Ca}}$  is consistent with the positive inotropic effects and increased intracellular  $\text{Ca}^{2+}$  transients observed in response to many of these neurohormones.<sup>94,99</sup>

Conflicting findings have also resulted from studies of direct activators of PKC, such as dioctanoylglycerol ( $\text{diC}_8$ ) and 1-oleoyl-2-acetyl-*sn*-glycerol, as well as phorbol esters.<sup>100–105</sup> Furthermore, the complexity of the response of  $I_{\text{Ca}}$  to phorbol esters has been demonstrated in studies of neonatal rat ventricular myocytes and adult canine ventricular myocytes showing a biphasic effect on  $I_{\text{Ca}}$  with an initial stimulation followed by an inhibition.<sup>101,103</sup> In some preparations, PKC-independent effects of phorbol esters and DAG analogues on  $I_{\text{Ca}}$  have been observed.<sup>102,106</sup> We recently demonstrated a PKC-independent inhibition of  $I_{\text{Ca}}$  by bath application of  $\text{diC}_8$  but showed in parallel that photorelease of intracellular caged  $\text{diC}_8$  caused a robust PKC-dependent stimulation of  $I_{\text{Ca}}$ .<sup>90</sup> Some PKC inhibitors have also been implicated in directly blocking  $I_{\text{Ca}}$  independently of their effects on PKC.<sup>107</sup> In summary, experiments utilizing direct activators of PKC have demonstrated a range of effects on  $I_{\text{Ca}}$ , not all of which are PKC-dependent.

The ultimate effect of stimulation of PKC on  $I_{\text{Ca}}$  may be closely related to the isoform(s) of PKC activated by a particular signaling pathway or chemical. The PKC isoforms are expressed in developmentally regulated, species-dependent, and disease-specific fashion in the heart.<sup>108–110</sup> Activation of PKC involves translocation of the enzyme to

specific targets, and different isozymes show different patterns of subcellular localization on activation, corresponding to the subcellular localization of the specific substrate proteins. Interestingly, PKC $\epsilon$  translocates to cross-striated regions in ventricular myocytes, which places it near T-tubules where L-type Ca<sup>2+</sup> channels are localized.<sup>111,112</sup> The membrane targeting of PKC isozymes is in part due to interactions with specific anchoring proteins referred to as RACKs (receptors for activated C kinases).<sup>113</sup> The amino-terminal regulatory region of PKC contains the membrane-targeting motifs that interact with RACKs in an isoform-specific manner. Peptides derived from these amino-terminal regions of PKC can be used as isoform-selective translocation inhibitors.<sup>113</sup> A recent study has demonstrated that peptides derived from the corresponding region of PKC $\beta$  specifically block the inhibition of  $I_{Ca}$  by phorbol esters in rat ventricular myocytes, suggesting a role for conventional PKC isoforms in this regulation.<sup>114</sup> It is possible that distinct isoforms of PKC may have opposing effects on L-type Ca<sup>2+</sup> channels, as previously suggested for the effect of phorbol esters on the chronotropic state of neonatal rat ventricular myocytes.<sup>115</sup>

### Molecular Targets for PKC Regulation of L-Type Ca<sup>2+</sup> Channels

PKC-activating pathways can clearly modulate the L-type Ca<sup>2+</sup> channel in cardiac muscle; however, the substrate(s) for PKC and the underlying molecular mechanisms of this regulation remain largely unknown. Biochemical studies in vitro have demonstrated that both the  $\alpha_{1C}$  and  $\beta_{2a}$  subunits of the L-type Ca<sup>2+</sup> channel can be substrates for PKC.<sup>116</sup> When the recombinant rabbit cardiac  $\alpha_{1C}$  was expressed in *Xenopus* oocytes, phorbol 12-myristate 13-acetate (PMA) treatment resulted in an increase followed by a gradual decrease in  $I_{Ca}$ .<sup>117,118</sup> This regulation occurred whether the auxiliary subunits were coexpressed or not.<sup>118</sup> In contrast, channel activity of the human cardiac  $\alpha_{1C}$  subunit expressed in *Xenopus* oocytes was only inhibited by application of PMA, and this inhibition required coexpression of the  $\beta_{1a}$  subunit.<sup>119</sup> It was suggested that the difference in the amino terminus of the rabbit and human clone were responsible for the distinct effects,<sup>119</sup> and recent experiments confirmed that the unique 46 amino acids of the N terminus of the rabbit clone are necessary for PKC-mediated upregulation of  $I_{Ca}$ .<sup>120</sup> It was proposed that PKC stimulates  $I_{Ca}$  by removing the tonic inhibitory effect of the long (rabbit) N terminus on  $I_{Ca}$ . In striking contrast, currents carried by the rabbit heart  $\alpha_{1C}$  expressed in TSA-201 cells are markedly inhibited by PKC.<sup>121</sup> Mutagenesis of threonines at amino acids 27 and 31 in rabbit  $\alpha_{1C}$  demonstrated that these residues are the targets for PKC responsible for the inhibition of  $I_{Ca}$ .<sup>121</sup> Why expressed recombinant L-type channels demonstrate such contrasting regulation in *Xenopus* oocytes compared with mammalian TSA-201 cells is unknown. Important questions remain regarding the regulation of  $I_{Ca}$  in the intact heart by PKC.

### Integrating the Signals/Crosstalk

The regulation of cardiac  $I_{Ca}$  by various signaling pathways has typically been examined by studying each pathway in isolation. In the intact organism, a dynamic mix of cellular

signals regulates the function of the channel. Even in the apparently simple case of a single biologically relevant neurotransmitter, norepinephrine, multiple adrenergic receptor subtypes and their associated signaling cascades are activated in the cardiac myocyte. For example,  $\alpha_1$ ARs activate PLC-/PKC-dependent signaling, whereas  $\beta$ ARs activate cAMP-/PKA-dependent signaling, and both of these pathways have been shown to stimulate  $I_{Ca}$  in most physiological preparations. However, the combination of  $\alpha_1$ AR and  $\beta$ AR activation on  $I_{Ca}$  is not simply additive, as  $\alpha_1$ AR activation strongly blunts the increase in  $I_{Ca}$  by  $\beta$ AR stimulation.<sup>122</sup> Likewise, activation of ET and angiotensin receptors, which are associated with stimulation of PKC, also strongly antagonize the effect of  $\beta$ AR stimulation of  $I_{Ca}$ .<sup>92,123,124</sup> Transgenic overexpression of G $\alpha_q$  and resulting activation of PKC has also been shown to blunt  $\beta$ -adrenergic stimulation of  $I_{Ca}$ .<sup>125</sup> Crosstalk likely occurs at various levels of the signaling cascades to produce these counterregulatory effects, and in some cases it may occur at the level of the channel itself.

There is also evidence for crosstalk with other signaling pathways regulating  $I_{Ca}$ . For example, in human atrial myocytes, tyrosine kinase stimulates  $I_{Ca}$  only after PKC is activated.<sup>126</sup> In guinea pig ventricular myocytes, the tyrosine kinase inhibitor, genistein, increases the sensitivity of  $I_{Ca}$  to  $\beta$ AR stimulation.<sup>127</sup> The status of the cytoskeletal system in the cells can even impact PKA-mediated regulation of  $I_{Ca}$ .<sup>128</sup> Understanding the many interactions between the various signaling cascades and their ultimate impact on channel function is just beginning.

### Conclusions and Future Directions

Given the critical role of the L-type Ca<sup>2+</sup> channel in multiple cellular functions, it is not surprising that this channel is extensively regulated by a variety of signaling pathways. Investigations over the last three decades have defined that the marked upregulation of  $I_{Ca}$  by  $\beta$ AR stimulation results from activation of the cAMP/PKA signaling cascade. However, the molecular details of this regulation have only recently started to be revealed with the discovery of functionally important PKA phosphorylation sites on  $\alpha_{1C}$  and  $\beta_{2a}$ . Many important questions remain, including whether additional phosphorylation sites are involved; how these phosphorylation sites interact; what role the truncated C terminus, including Ser 1928, plays in this regulation; what the functional effects of each site on channel gating are; which sites are important in the intact heart; and how this regulation changes in disease. Additionally, evidence is accumulating for a localized signaling complex that targets regulation to the L-type Ca<sup>2+</sup> channel, including AKAPs to localize PKA and direct binding of PP2a to the C terminus of the  $\alpha_{1C}$  subunit. The composition of these signaling complexes and their functional importance will be exciting areas of future investigation.

PKC regulation of L-type Ca<sup>2+</sup> channels is even more of a mystery. There is clear evidence that activation of PKC can both stimulate and inhibit  $I_{Ca}$  depending on the cells studied and experimental conditions. It seems likely that different PKC isoforms may be activated by different signaling mechanisms, resulting in distinct targeting of the isoforms involved

in this regulation. Likewise, different splice variants of the channel subunits may be critical, especially with regard to the amino terminus of  $\alpha_{1c}$ . Future studies are likely to take advantage of improved tools, including isoform-specific inhibitors, and activators of PKC. Ultimately, understanding the details of these regulatory pathways will provide insights into the role of the L-type  $\text{Ca}^{2+}$  channel in normal physiology and disease.

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### References

- Yue L, Feng J, Gaspo R, Li GR, Wang Z, Nattel S. Ionic remodeling underlying action potential changes in a canine model of atrial fibrillation. *Circ Res*. 1997;81:512–525.
- Van Wagoner DR, Pond AL, Lamorgese M, Rossie SS, McCarthy PM, Nerbonne JM. Atrial L-type  $\text{Ca}^{2+}$  currents and human atrial fibrillation. *Circ Res*. 1999;85:428–436.
- Balke CW, Shorofsky SR. Alterations in calcium handling in cardiac hypertrophy and heart failure. *Cardiovasc Res*. 1998;37:290–299.
- Richard S, Leclercq F, Lemaire S, Piot C, Nargeot J.  $\text{Ca}^{2+}$  currents in compensated hypertrophy and heart failure. *Cardiovasc Res*. 1998;37:300–311.
- Mukherjee R, Spinale FG. L-type calcium channel abundance and function with cardiac hypertrophy and failure: a review. *J Mol Cell Cardiol*. 1998;30:1899–1916.
- He J-Q, Conklin MW, Foell JD, Wolff MR, Haworth RA, Coronado R, Kamp TJ. Reduction in density of transverse tubules and L-type  $\text{Ca}^{2+}$  channels in canine tachycardia-induced heart failure. *Cardiovasc Res*. In press.
- Aggarwal R, Boyden PA. Diminished calcium and barium currents in myocytes surviving in the epicardial border zone of the 5-day infarcted canine heart. *Circ Res*. 1995;77:1180–1191.
- Reuter H. Strom-Spannungsbeziehungen von Purkinje-Fasern bei verschiedenen extracellulären Calcium-Konzentrationen und unter Adrenalinwirkung. *Pflugers Arch*. 1966;287:357–367.
- Sperelakis N, Schneider JA. A metabolic control mechanism for calcium ion influx that may protect the ventricular myocardial cell. *Am J Cardiol*. 1976;37:1079–1085.
- Reuter H, Scholz H. A study of ion selectivity and the kinetic properties of the calcium dependent slow inward current in mammalian cardiac muscle. *J Physiol (Lond)*. 1977;264:17–47.
- Catterall WA. Structure and regulation of voltage-gated  $\text{Ca}^{2+}$  channels. *Annu Rev Cell Dev Biol*. 2000;16:521–555.
- Striessnig J. Pharmacology, structure and function of cardiac L-type  $\text{Ca}^{2+}$  channels. *Cell Physiol Biochem*. 1999;9:242–269.
- Hosey MM, Chien AJ, Puri TS. Structure and regulation of L-type calcium channels: a current assessment of the properties and roles of channel subunits. *Trends Cardiovasc Med*. 1996;6:265–273.
- McDonald TF, Pelzer S, Trautwein W, Pelzer DJ. Regulation and modulation of calcium channels in cardiac, skeletal, and smooth muscle cells. *Physiol Rev*. 1994;74:365–507.
- Yang J, Ellinor PT, Sather WA, Zhang J-F, Tsien RW. Molecular determinants of  $\text{Ca}^{2+}$  selectivity and ion permeation in L-type  $\text{Ca}^{2+}$  channels. *Nature*. 1993;366:158–161.
- Tang S, Mikala G, Bahinski A, Yatani A, Varadi G, Schwartz A. Molecular localization of ion selectivity sites within the pore of a human L-type cardiac calcium channel. *J Biol Chem*. 1993;268:13026–13029.
- Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, Schwartz A, Snutch TP, Tanabe T, Birnbaumer L, Tsien RW, Catterall WA. Nomenclature of voltage-gated calcium channels. *Neuron*. 2000;25:533–535.
- Mikami A, Imoto K, Tanabe T, Niidome T, Mori Y, Takeshima H, Narumiya S, Numa S. Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. *Nature*. 1989;340:230–233.
- Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H, Zheng H, Striessnig J. Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type  $\text{Ca}^{2+}$  channels. *Cell*. 2000;102:89–97.
- De Jongh KS, Murphy BJ, Colvin AA, Hell JW, Takahashi M, Catterall WA. Specific phosphorylation of a site in the full length form of the  $\alpha_1$  subunit of the cardiac L-type calcium channel by adenosine 3',5'-cyclic monophosphate-dependent protein kinase. *Biochemistry*. 1996;35:10392–10402.
- Hell JW, Yokoyama CT, Wong ST, Warner C, Snutch TP, Catterall WA. Differential phosphorylation of two size forms of the neuronal class C L-type calcium channel  $\alpha_1$  subunit. *J Biol Chem*. 1993;268:19451–19457.
- Hell JW, Westenbroek RE, Breeze LJ, Wang KKW, Chavkin C, Catterall WA. N-methyl-D-aspartate receptor-induced proteolytic conversion of postsynaptic class C L-type calcium channels in hippocampal neurons. *Proc Natl Acad Sci U S A*. 1996;93:3362–3367.
- Gerhardstein BL, Gao T, Bünemann M, Puri TS, Adair A, Ma H, Hosey MM. Proteolytic processing of the C terminus of the  $\alpha_{1c}$  subunit of L-type calcium channels and the role of a proline-rich domain in membrane tethering of proteolytic fragments. *J Biol Chem*. 2000;275:8556–8563.
- Jay SD, Ellis SB, McCue AF, Williams ME, Vedvick TS, Harpold MM, Campbell KP. Primary structure of the  $\gamma$  subunit of the DHP-sensitive calcium channel from skeletal muscle. *Science*. 1990;248:490–492.
- Powers PA, Liu S, Hogan K, Gregg RG. Molecular characterization of the gene encoding the  $\gamma$  subunit of the human skeletal muscle 1,4-dihydropyridine-sensitive  $\text{Ca}^{2+}$  channel (CACNLG), cDNA sequence, gene structure, and chromosomal location. *J Biol Chem*. 1993;268:9275–9279.
- Burgess DL, Davis CF, Gefrides LA, Noebels JL. Identification of three novel  $\text{Ca}^{2+}$  channel  $\gamma$  subunit genes reveals molecular diversification by tandem and chromosome duplication. *Genome Res*. 1999;9:1204–1213.
- Klugbauer N, Dai S, Specht V, Lacinova L, Marais E, Bohn G, Hofmann F. A family of  $\gamma$ -like calcium channel subunits. *FEBS Lett*. 2000;470:189–198.
- Birnbaumer L, Qin N, Olcese R, Tareilus E, Platano D, Costantin J, Stefani E. Structures and functions of calcium channel  $\beta$  subunits. *J Bioenerg Biomembr*. 1998;30:357–375.
- Singer D, Biel M, Lotan I, Flockerzi V, Hofmann F, Dascal N. The roles of the subunits in the function of the calcium channel. *Science*. 1991;253:1553–1557.
- Kamp TJ, Perez-Garcia MT, Marbán E. Enhancement of ionic current and charge movement by coexpression of calcium channel  $\beta_{1a}$  with  $\alpha_{1c}$  in a human embryonic kidney cell line. *J Physiol*. 1996;492:89–96.
- Chien AJ, Zhao X, Shirokov RE, Puri TS, Chang CF, Sun D, Rios E, Hosey MM. Roles of a membrane-localized  $\beta$  subunit in the formation and targeting of functional L-type  $\text{Ca}^{2+}$  channels. *J Biol Chem*. 1995;270:30036–30044.
- Biel M, Hullin R, Freundner S, Singer D, Dascal N, Flockerzi V, Hofmann F. Tissue-specific expression of high-voltage-activated dihydropyridine-sensitive L-type calcium channels. *Eur J Biochem*. 1991;200:81–88.
- Collin T, Wang JJ, Nargeot J, Schwartz A. Molecular cloning of three isoforms of the L-type voltage-dependent calcium channel  $\beta$  subunit from normal human heart. *Circ Res*. 1993;72:1337–1344.
- De Jongh KS, Warner C, Catterall WA. Subunits of purified calcium channels:  $\alpha_2$  and  $\delta$  are encoded by the same gene. *J Biol Chem*. 1990;265:14738–14741.
- Jay SD, Sharp AH, Kahl SD, Vedvick TS, Harpold MM, Campbell KP. Structural characterization of the dihydropyridine-sensitive calcium channel  $\alpha_2$ -subunit and the associated  $\delta$  peptides. *J Biol Chem*. 1991;266:3287–3293.
- Ellis SB, Williams ME, Ways NR, Brenner R, Sharp AH, Leung AT, Campbell KP, McKenna E, Koch WJ, Hui A, Schwartz A, Harpold MD. Sequence and expression of mRNAs encoding the  $\alpha_1$  and  $\alpha_2$  subunits of a DHP-sensitive calcium channel. *Science*. 1988;241:1661–1664.
- Klugbauer N, Lacinova L, Hübner M, Hofmann F. Molecular diversity of the calcium channel  $\alpha_2\delta$  subunit. *J Neurosci*. 1999;19:684–691.
- Bangalore R, Mehrke G, Gingrich K, Hofmann F, Kass RS. Influence of L-type Ca channel  $\alpha_2\delta$  subunit on ionic and gating current in transiently transfected HEK293 cells. *Am J Physiol*. 1996;39:H1521–H1528.
- Campbell DL, Strauss HC. Regulation of calcium channels in the heart. *Adv Second Messenger Phosphoprotein Res*. 1995;30:25–88.

40. Yatani A, Brown AM. Rapid  $\beta$ -adrenergic modulation of cardiac calcium channel currents by a fast G protein pathway. *Science*. 1989; 245:71–74.
41. Hartzell HC, Mery PF, Fischmeister R, Szabo G. Sympathetic regulation of cardiac calcium current is due exclusively to cAMP-dependent phosphorylation. *Nature*. 1991;351:573–576.
42. Xiao R-P, Cheng H, Zhou Y-Y, Kuschel M, Lakatta EG. Recent advances in cardiac  $\beta_2$ -adrenergic signal transduction. *Circ Res*. 1999; 85:1092–1100.
43. Steinberg SF. The molecular basis for distinct  $\beta$ -adrenergic receptor subtype actions in cardiomyocytes. *Circ Res*. 1999;85:1101–1111.
44. Altschuld RA, Starling RC, Hamlin RL, Billman GE, Hensley J, Castillo L, Fertel RH, Hohl CM, Robitaille P-ML, Jones LR, Xiao R-P, Lakatta EG. Response of failing canine and human heart cells to  $\beta_2$ -adrenergic stimulation. *Circ Res*. 1995;92:1612–1618.
45. Xiao R-P, Lakatta EG.  $\beta_1$ - and  $\beta_2$ -adrenoceptor stimulation and  $\beta_2$ -adrenoceptor stimulation differ in their effects on contraction, cytosolic  $\text{Ca}^{2+}$ , and  $\text{Ca}^{2+}$  current in single rat ventricular cells. *Circ Res*. 1993;73:286–300.
46. Jurevicius J, Fischmeister R. cAMP compartmentation is responsible for a local activation of cardiac  $\text{Ca}^{2+}$  channels by  $\beta$ -adrenergic agonists. *Proc Natl Acad Sci U S A*. 1996;93:295–299.
47. Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac  $\beta_2$ -adrenergic signal transduction. *Circ Res*. 1999;85: 1092–1100.
48. Hool LC, Harvey RD. Role of  $\beta_1$ - and  $\beta_2$ -adrenergic receptors in regulation of  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  channels in guinea pig ventricular myocytes. *Am J Physiol*. 1997;273:H1669–H1676.
49. Laflamme MA, Becker PL. Do  $\beta_2$ -adrenergic receptors modulate  $\text{Ca}^{2+}$  in adult rat ventricular myocytes? *Am J Physiol*. 1998;274: H1308–H1314.
50. Mery PF, Abi-Gerges N, Vandecasteele G, Jurevicius J, Eschenhagen T, Fischmeister R. Muscarinic regulation of the L-type calcium current in isolated cardiac myocytes. *Life Sci*. 1997;60:1113–1120.
51. Herzig S, Meier A, Pfeiffer M, Neumann J. Stimulation of protein phosphatases as a mechanism of the muscarinic-receptor-mediated inhibition of cardiac L-type  $\text{Ca}^{2+}$  channels. *Pflugers Arch*. 1995;429: 531–538.
52. Han X, Kuboto I, Feron O, Opel DJ, Arstall MA, Zhao Y-Y, Huang P, Fishman MC, Michel T, Kelly RA. Muscarinic cholinergic regulation of cardiac myocyte  $I_{\text{Ca-L}}$  is absent in mice with targeted disruption of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A*. 1998;95: 6510–6515.
53. Vandecasteele G, Eschenhagen T, Scholz H, Stein B, Verde I, Fischmeister R. Muscarinic and  $\beta$ -adrenergic regulation of heart rate, force of contraction and calcium current is preserved in mice lacking endothelial nitric oxide synthase. *Nat Med*. 1999;5:331–334.
54. Aprigliano O, Rybin VO, Pak E, Robinson RB, Steinberg SF.  $\beta_1$ - and  $\beta_2$ -adrenergic receptors exhibit differing susceptibility to muscarinic accentuated antagonism. *Am J Physiol*. 1997;272:H2726–H2735.
55. Sculptoreanu A, Rotman E, Takahashi M, Scheuer T, Catterall WA. Voltage-dependent potentiation of the activity of cardiac L-type calcium channel  $\alpha_1$  subunits due to phosphorylation by cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A*. 1993;90:10135–10139.
56. Johnson BD, Scheuer T, Catterall WA. Voltage-dependent potentiation of L-type  $\text{Ca}^{2+}$  channels in skeletal muscle cells requires anchored cAMP-dependent protein kinase. *Proc Natl Acad Sci U S A*. 1994;91: 11492–11496.
57. Pietrobon D, Hess P. Novel mechanism of voltage-dependent gating in L-type calcium channels. *Nature*. 1990;346:651–655.
58. Wiechen K, Yue DT, Herzig S. Two distinct functional effects of protein phosphatase inhibitors on guinea-pig cardiac L-type  $\text{Ca}^{2+}$  channels. *J Physiol*. 1995;484:583–592.
59. Bourinet E, Charnet P, Tomlinson WJ, Stea A, Snutch TP, Nargeot J. Voltage-dependent facilitation of a neuronal  $\alpha_{1C}$  L-type calcium channel. *EMBO J*. 1994;13:5032–5039.
60. Dai S, Klugbauer N, Zong X, Seisenberger C, Hofmann F. The role of subunit composition on prepulse facilitation of the cardiac L-type calcium channel. *FEBS Lett*. 1999;442:70–74.
61. Kamp TJ, Hu H, Marbán E. Voltage-dependent facilitation of cardiac L-type Ca channels expressed in HEK-293 cells requires  $\beta$ -subunit. *Am J Physiol (Heart Circ Physiol)*. 2000;278:H126–H136.
62. Hell JW, Yokoyama CT, Breeze LJ, Chavkin C, Catterall WA. Phosphorylation of presynaptic and postsynaptic calcium channels by cAMP-dependent protein kinase in hippocampal neurons. *EMBO J*. 1995;14: 3036–3044.
63. Davare MA, Dong F, Rubin CS, Hell JW. The A-kinase anchor protein MAP2B and cAMP-dependent protein kinase are associated with class C L-type calcium channels in neurons. *J Biol Chem*. 1999;274: 30280–30287.
64. Gao T, Yatani A, Dell'Acqua ML, Sako H, Green SA, Drascal A, Scott SD, Hosey MM. cAMP-dependent regulation of cardiac L-type  $\text{Ca}^{2+}$  channels requires membrane targeting of PKA and phosphorylation of channel subunits. *Neuron*. 1997;19:185–196.
65. Yoshida A, Takahashi M, Nishimura S, Takeshima H, Kokubun S. Cyclic AMP-dependent phosphorylation and regulation of the cardiac dihydropyridine-sensitive Ca channel. *FEBS Lett*. 1992;309:343–349.
66. Haase H, Karczewski P, Beckert R, Krause EG. Phosphorylation of the L-type calcium channel  $\beta$  subunit is involved in  $\beta$ -adrenergic signal transduction in canine myocardium. *FEBS Lett*. 1993;335:217–222.
67. Haase H, Bartel S, Karczewski P, Morano I, Krause EG. In-vivo phosphorylation of the cardiac L-type calcium channel  $\beta$ -subunit in response to catecholamines. *Mol Cell Biochem*. 1996;163–164:99–106.
68. Gerhardtstein BL, Puri TS, Chien AJ, Hosey MM. Identification of the sites phosphorylated by cyclic AMP-dependent protein kinase on the  $\beta_2$  subunit of L-type voltage-dependent calcium channels. *Biochemistry*. 1999;38:10361–10370.
69. Bunemann M, Gerhardtstein BL, Gao T, Hosey MM. Functional regulation of L-type calcium channels via protein kinase A-mediated phosphorylation of the  $\beta_2$  subunit. *J Biol Chem*. 1999;274:33851–33854.
70. Rubin CS. A kinase anchor protein and the intracellular targeting of signals carried by cyclic AMP. *Biochim Biophys Acta*. 1994;1224: 467–479.
71. Gray PC, Scott JD, Catterall WA. Regulation of ion channels by cAMP-dependent protein kinase and A-kinase anchoring proteins. *Curr Opin Neurobiol*. 1998;8:330–334.
72. Edwards AS, Scott JD. A-kinase anchoring proteins: protein kinase A and beyond. *Curr Opin Cell Biol*. 2000;12:217–221.
73. Vallee RB, DiBartilomeis J, Theurkauf WE. A protein kinase bound to the projection portion of MAP 2 (microtubule-associated protein 2). *J Cell Biol*. 1981;90:568–576.
74. Yang J, Drazba JA, Ferguson DG, Bond M. A-kinase anchoring protein 100 (AKAP100) is localized in multiple subcellular compartments in the adult rat heart. *J Cell Biol*. 1998;142:511–522.
75. Carl SL, Felix K, Caswell AH, Brandt NR, Ball WJ Jr, Vaghy PL, Meissner G, Ferguson DG. Immunolocalization of sarcolemmal dihydropyridine receptor and sarcoplasmic reticular triadin and ryanodine receptor in rabbit ventricle and atrium. *J Cell Biol*. 1995;129:673–682.
76. Gray PC, Tibbs VC, Catterall WA, Murphy BJ. Identification of a 15-kDa cAMP-dependent protein kinase-anchoring protein associated with skeletal muscle L-type calcium channels. *J Biol Chem*. 1997;272: 6297–6302.
77. Gray PC, Johnson BD, Westenbroek RE, Hays G, Yates JR III, Scheuer T, Catterall WA, Murphy BJ. Primary structure and function of an A kinase anchoring protein associated with calcium channels. *Neuron*. 1998;20:1017–1026.
78. Bean BP, Nowycky MC, Tsien RW.  $\beta$ -Adrenergic modulation of calcium channels in frog ventricular heart cells. *Nature*. 1984;307: 371–375.
79. Tsien RW, Bean BP, Hess P, Lansman JB, Nilius B, Nowycky MC. Mechanisms of calcium channel modulation by  $\beta$ -adrenergic agents and dihydropyridine calcium agonists. *J Mol Cell Cardiol*. 1986;18: 691–710.
80. Herzig S, Patil P, Neumann J, Staschen C-M, Yue DT. Mechanisms of  $\beta$ -adrenergic stimulation of cardiac  $\text{Ca}^{2+}$  channels revealed by discrete-time Markov analysis of slow gating. *Biophys J*. 1993;65: 1599–1612.
81. Yue DT, Herzig S, Marbán E.  $\beta$ -Adrenergic stimulation of calcium channels occurs by potentiation of high activity gating modes. *Proc Natl Acad Sci U S A*. 1990;87:753–757.
82. Hartzell HC, Hirayama Y, Petit-Jacques J. Effects of protein phosphatase and kinase inhibitors on the cardiac L-type Ca current suggest two sites are phosphorylated by protein kinase A and another protein kinase. *J Gen Physiol*. 1995;106:393–414.
83. Ono K, Fozzard HA. Phosphorylation restores activity of L-type calcium channels after rundown in inside-out patches from rabbit cardiac cells. *J Physiol*. 1992;454:673–688.
84. Ono K, Fozzard HA. Two phosphatase sites on the  $\text{Ca}^{2+}$  channel affecting different kinetic functions. *J Physiol*. 1993;470:73–84.

85. Frace AM, Hartzell HC. Opposite effects of phosphatase inhibitors on L-type calcium and delayed rectifier currents in frog cardiac myocytes. *J Physiol*. 1993;472:305–326.
86. Davare MA, Horne MC, Hell JW. Protein phosphatase 2A is associated with class C L-type calcium channels and antagonizes channel phosphorylation by cAMP-dependent protein kinase. *J Biol Chem*. 2000. Available at: <http://www.jbc.org>. Accessed October 30, 2000.
87. Dorn GW, Brown JH. Gq signaling in cardiac adaptation and maladaptation. *Trends Cardiovasc Med*. 1999;9:26–34.
88. Berridge MJ. Elementary and global aspects of calcium signaling. *J Physiol*. 1997;499:291–306.
89. Bkaily G, Wang S, Bui M, Menard D. ET-1 stimulates Ca<sup>2+</sup> currents in cardiac cells. *J Cardiovasc Pharmacol*. 1995;26:S293–S296.
90. He J-Q, Pi Y-Q, Walker JW, Kamp TJ. Endothelin-1 and photoreleased diacylglycerol increase L-type Ca<sup>2+</sup> current by activation of protein kinase C in rat ventricular myocytes. *J Physiol*. 2000;524:807–820.
91. Cheng TH, Chang CY, Wei J, Lin CI. Effects of endothelin 1 on calcium and sodium currents in isolated human cardiac myocytes. *Can J Physiol Pharmacol*. 1995;73:1774–1783.
92. Thomas GP, Sims SM, Karmazyn M. Differential effects of endothelin-1 on basal and isoprenaline-enhanced Ca<sup>2+</sup> current in guinea-pig ventricular myocytes. *J Physiol*. 1997;503:55–65.
93. Delpech N, Soustre H, Potreau D. Endothelin-1 inhibits L-type Ca<sup>2+</sup> current enhanced by isoprenaline in rat atrial myocytes. *J Cardiovasc Pharmacol*. 1997;29:136–143.
94. Woo SH, Lee CO. Effects of endothelin-1 on Ca<sup>2+</sup> signaling in guinea-pig ventricular myocytes: role of protein kinase C. *J Mol Cell Cardiol*. 1999;31:631–643.
95. Liu SJ, Kennedy RH.  $\alpha_1$ -Adrenergic activation of L-type Ca current in rat ventricular myocytes: perforated patch-clamp recordings. *Am J Physiol*. 1998;274:H2203–H2207.
96. Watanabe T, Endoh M. Characterization of the endothelin-1-induced regulation of L-type Ca<sup>2+</sup> current in rabbit ventricular myocytes. *Arch Pharmacol*. 1999;360:664.
97. Kurata S, Ishikawa K, Iijima T. Enhancement by arginine vasopressin of the L-type Ca<sup>2+</sup> current in guinea pig ventricular myocytes. *Pharmacology*. 1999;59:21–33.
98. Kelso E, Spiers P, McDermott B, Silke B. Dual effects of endothelin-1 on the L-type Ca<sup>2+</sup> current in ventricular cardiomyocytes. *Eur J Pharmacol*. 1996;308:351–355.
99. Pi Y, Walker JW. Role of intracellular Ca<sup>2+</sup> and pH in positive inotropic response of cardiomyocytes to diacylglycerol. *Am J Physiol*. 1998;275:H1473–H1481.
100. Dosemeci A, Dhallan RS, Cohen NM, Lederer WJ, Rogers TB. Phorbol ester increases calcium current and simulates the effects of angiotensin II on cultured neonatal rat heart myocytes. *Circ Res*. 1988;62:347–357.
101. Lacerda AE, Rampe D, Brown AM. Effects of protein kinase C activators on cardiac Ca<sup>2+</sup> channels. *Nature*. 1988;335:249–251.
102. Schreur KD, Liu S. 1,2-Dioctanoyl-*sn*-glycerol depresses cardiac L-type Ca<sup>2+</sup> current: independent of protein kinase C activation. *Am J Physiol*. 1996;270:C655–C662.
103. Tseng GN, Boyden PA. Different effects of intracellular Ca and protein kinase C on cardiac T and L Ca currents. *Am J Physiol*. 1991;261:H364–H379.
104. Walsh KB, Kass RS. Regulation of a heart potassium channel by protein kinase A and C. *Science*. 1988;242:67–69.
105. Zhang X, Anderson JW, Fedida D. Characterization of nifedipine block of the human heart delayed rectifier, hKv1.5. *J Pharm Exp Ther*. 1997;281:1256.
106. Asai T, Shuba LM, Pelzer DJ, McDonald TF. PKC-independent inhibition of cardiac L-type Ca<sup>2+</sup> channel current by phorbol esters. *Am J Physiol*. 1996;270:H620–H627.
107. Hartzell HC, Rinderknecht A. Calphostin C, a widely used protein kinase C inhibitor, directly and potently blocks L-type Ca channels. *Am J Physiol*. 1996;270:C1293–C1299.
108. Puceat M, Brown JH. Protein kinase C in the heart. In: Kuo JF, ed. *Protein Kinase C*. Oxford, UK: Oxford University Press; 1994: 249–268.
109. Rybin VO, Buttrick PM, Steinberg SF. PKC- $\lambda$  is the atypical protein kinase C isoform expressed by immature ventricle. *Am J Physiol*. 1997;272:H1636–H1642.
110. Cohen MV, Downey JM. Myocardial preconditioning promises to be a novel approach to the treatment of ischemic heart disease. *Annu Rev Med*. 1996;47:21–29.
111. Disatnik M-H, Buraggi G, Mochly-Rosen D. Localization of protein kinase C isozymes in cardiac myocytes. *Exp Cell Res*. 1994;210:287–297.
112. Huang XP, Pi Y, Lokuta AJ, Greaser ML, Walker JW. Arachidonic acid stimulates protein kinase C- $\epsilon$  redistribution in heart cells. *J Cell Sci*. 1997;110:1625–1634.
113. Mochly-Rosen D, Gordon AS. Anchoring proteins for protein kinase C: a means for isozyme selectivity. *FASEB J*. 1998;12:35–42.
114. Zhang Z-H, Johnson JA, Chen L, El-Sherif N, Mochly-Rosen D, Boutjdir M. C2 region-derived peptides of  $\beta$ -protein kinase C regulate cardiac Ca<sup>2+</sup> channels. *Circ Res*. 1997;720–729.
115. Johnson JA, Mochly-Rosen D. Inhibition of the spontaneous rate of contraction of neonatal cardiac myocytes by protein kinase C isozymes: a putative role for the  $\epsilon$  isozyme. *Circ Res*. 1995;76:654–663.
116. Puri TS, Gerhardstein BL, Zhao X-L, Ladner MB, Hosey MM. Differential effects of subunit interactions on protein kinase A- and C-mediated phosphorylation of L-type calcium channels. *Biochemistry*. 1997;36:9605–9615.
117. Bourinet E, Fournier F, Lory P, Charmet P, Nargeot J. Protein kinase C regulation of cardiac calcium channels expressed in *Xenopus* oocytes. *Pflugers Arch*. 1992;421:247–255.
118. Singer-Lahat D, Gershon E, Lotan I, Hullin R, Biel M, Flockerzi V, Hofmann F, Dascal N. Modulation of cardiac Ca<sup>2+</sup> channels in *Xenopus* oocytes by protein kinase C. *FEBS Lett*. 1992;306:113–118.
119. Bouron A, Soldatov NM, Reuter H. The  $\beta_1$ -subunit is essential for modulation by protein kinase C of a human and a non-human L-type Ca<sup>2+</sup> channel. *FEBS Lett*. 1995;377:159–162.
120. Shistik E, Ivanina T, Blumenstein Y, Dascal N. Crucial role on N terminus in function of cardiac L-type Ca<sup>2+</sup> channel and its modulation by protein kinase C. *J Biol Chem*. 1998;273:17901–17909.
121. McHugh D, Sharp EM, Scheuer T, Catterall WA. Inhibition of cardiac L-type calcium channels by protein kinase C phosphorylation of two sites in the N-terminal domain. *Proc Natl Acad Sci USA*. 2000;97:12334–12338.
122. Boutjdir M, Restivo M, Wei Y, El-Sherif N.  $\alpha_1$ - and  $\beta$ -adrenergic interactions on L-type calcium current in cardiac myocytes. *Pflugers Arch*. 1992;421:397–399.
123. Ono K, Tsujimoto G, Sakamoto A, Eto K, Masaki T, Ozaki Y, Satake M. Endothelin-A receptor mediates cardiac inhibition by regulating calcium and potassium currents. *Nature*. 1994;370:301–304.
124. Ai T, Horie M, Obayashi K, Sasayama S. Accentuated antagonism by angiotensin II on guinea-pig cardiac L-type Ca-currents enhanced by  $\beta$ -adrenergic stimulation. *Pflugers Arch*. 1998;436:168–174.
125. Mitarai S, Reed TD, Yatani A. Changes in ionic currents and  $\beta$ -adrenergic receptor signaling in hypertrophied myocytes overexpressing G $\alpha_q$ . *Am J Physiol*. 2000;279:H139–H148.
126. Boixel C, Tessier S, Pansard Y, Lang-Lazdunski L, Mercadier JJ, Hatem SN. Tyrosine kinase and protein kinase C regulate L-type Ca<sup>2+</sup> current cooperatively in human atrial myocytes. *Am J Physiol*. 2000;278:H670–H676.
127. Hool LC, Middleton LM, Harvey RD. Genistein increases the sensitivity of cardiac ion channels to  $\beta$ -adrenergic receptor stimulation. *Circ Res*. 1998;83:33–42.
128. Gomez AM, Kerfant BG, Vassort G. Microtubule disruption modulates Ca<sup>2+</sup> signaling in rat cardiac myocytes. *Circ Res*. 2000;86:30–36.