Determinants for Calmodulin Binding on Voltage-dependent Ca\textsuperscript{2+} Channels*  

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Calmodulin, bound to the α\textsubscript{1} subunit of the cardiac L-type calcium channel, is required for calcium-dependent inactivation of this channel. Several laboratories have suggested that the site of interaction of calmodulin with the channel is an IQ-like motif in the carboxyl-terminal region of the α\textsubscript{1} subunit. Mutations in this IQ motif are linked to L-type Ca\textsuperscript{2+} current (I\textsubscript{L}) facilitation and inactivation. IQ peptides from L, P/Q, N, and R channels all bind Ca\textsuperscript{2+}-calmodulin but not Ca\textsuperscript{2+}-free calmodulin. Another peptide representing a carboxyl-terminal sequence found only in L-type channels (designated the CB domain) binds Ca\textsuperscript{2+}-calmodulin and enhances Ca\textsuperscript{2+}-dependent I\textsubscript{L} facilitation in cardiac myocytes, suggesting the CB domain is functionally important. Calmodulin blocks the binding of an antibody specific for the CB sequence to the skeletal muscle L-type Ca\textsuperscript{2+} channel, suggesting that this is a calmodulin binding site on the intact protein. The binding of the IQ and CB peptides to calmodulin appears to be competitive, signifying that the two sequences represent either independent or alternative binding sites for calmodulin rather than both sequences contributing to a single binding site.

Calcium dynamically regulates L-type Ca\textsuperscript{2+} current (I\textsubscript{L}) through opposing processes of facilitation (1) and inactivation (2). Although the mechanisms for these processes remain incompletely understood, critical molecular determinants for Ca\textsuperscript{2+}-dependent facilitation and inactivation of the L-type calcium channel have been attributed to the cytoplasmic carboxyl-terminal tail of the α\textsubscript{1} subunit, which contains a putative Ca\textsuperscript{2+} binding EF hand motif (3) and an “IQ-like” motif (4–7). The latter resembles the IQ domains that bind Ca\textsuperscript{2+}-calmodulin and enhances Ca\textsuperscript{2+}-dependent facilitation in cardiac myocytes, suggesting the CB domain is functionally important. Calmodulin blocks the binding of an antibody specific for the CB sequence to the skeletal muscle L-type Ca\textsuperscript{2+} channel, suggesting that this is a calmodulin binding site on the intact protein. The binding of the IQ and CB peptides to calmodulin appears to be competitive, signifying that the two sequences represent either independent or alternative binding sites for calmodulin rather than both sequences contributing to a single binding site.

Ca\textsuperscript{2+} channels

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The abbreviations used are: apoCaM, apocalmodulin; Ca\textsuperscript{2+}-free calmodulin; CaM, calmodulin; Ca\textsuperscript{2+}-CaM, Ca\textsuperscript{2+}-bound calmodulin; CB peptide, CaM-binding peptide representing amino acids 1484–1509 of the skeletal muscle α\textsubscript{1} subunit L-type channel and 1627–1652 of the cardiac L-type channel; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonate; DHPR, dihydropyridine receptor; EF hand, Ca\textsuperscript{2+} binding motif; IQ, motif for binding CaM; MOPS, 4-morpholinepropanesulfonic acid.

necessary for calcium-dependent inactivation of the cardiac channel. P/Q channels have also been shown to be modulated by calmodulin (10).

The IQ-like domain in the L-type channels appears to bind primarily Ca\textsuperscript{2+}-CaM rather than apoCaM (5). An isoleucine to alanine mutation in this motif results in loss of Ca\textsuperscript{2+}-dependent inactivation and unmask a strong facilitation by CaM (11, 12). If the isoleucine is changed to a glutamate, the effects of CaM (inactivation and facilitation) are lost (11). A double mutation of I1624A/Q1625A (Swiss Prot Q0815) produced an even more pronounced facilitation (12). These investigators concluded that Ca\textsuperscript{2+}-dependent inactivation requires strong binding of CaM to the IQ motif. Peterson et al. (5) find that a mutant CaM that cannot bind Ca\textsuperscript{2+} at any of the four Ca\textsuperscript{2+} binding sites blocks the effects of Ca\textsuperscript{2+}-CaM on the L-type Ca\textsuperscript{2+} channel, suggesting that both the Ca\textsuperscript{2+}-free and Ca\textsuperscript{2+}-bound forms of CaM bind to this channel. However, only the Ca\textsuperscript{2+}-bound form can induce inactivation (5). In particular, it appears that Ca\textsuperscript{2+}-binding sites 3 and 4 of CaM are required for Ca\textsuperscript{2+}-dependent inactivation (5).

Other studies suggest that the IQ motif may not be the only determinant necessary for Ca\textsuperscript{2+}-dependent inactivation of the channel (7, 13, 14). Adams and Tanabe (15) replaced the I-II loop of the cardiac channel with the corresponding loop of the skeletal channel, which slowed Ca\textsuperscript{2+}-dependent inactivation. An effect of replacing the II-III loop was also observed. The role of the EF hands of the carboxyl-terminal region of the α\textsubscript{1} subunit of the L-type channel in Ca\textsuperscript{2+}-dependent inactivation has been controversial (6, 7, 14, 16). Bernatchez et al. (16) found that mutations in the EF hand altered Ca\textsuperscript{2+}-dependent inactivation, but Zhou et al. (6) found that mutant channels containing a triple mutation that disrupted Ca\textsuperscript{2+} coordinating activity was still inhibited by Ca\textsuperscript{2+}. Recently, Peterson et al. (14) demonstrated that replacing four amino acids (VVTI) in the F helix of the putative EF hand of α\textsubscript{1C} with those of the α\textsubscript{1E} (MYEM) channel completely abolished Ca\textsuperscript{2+}-dependent inactivation. In contrast, mutating the residues presumably involved in coordinating Ca\textsuperscript{2+} reduced the inactivation only about 2-fold. These authors suggested that the EF hand plays a role in transducing the signal generated by Ca\textsuperscript{2+} binding to CaM into channel inactivation. Other regions of the carboxyl tail are also likely to be involved in Ca\textsuperscript{2+}-dependent inactivation. Soldatov et al. (13) found that mutating regions between the EF hand and the IQ motif (IKTEG and LLDQV) eliminated Ca\textsuperscript{2+}-dependent inactivation. They suggested that a cooperative interaction between these two regions contributed to Ca\textsuperscript{2+}-dependent inactivation. Zulke and Reuter (7) used deletions to show that three different domains (the putative EF hand, the IQ domain, and the two-amino acid motif, NE, at amino acids 1630 and 1631 of the cardiac L-type channel) are important for
the inactivation process. Sequences within the region between amino acids 1572 and 1651 have also been suggested to be important for regulating targeting, conductance, and open probability of the channel (17).

Peterson et al. (5) compared the binding of Ca\(^{2+}\)-CaM to the IQ-like motifs from N-, P/Q-, and R-type calcium channel \(\alpha\) subunits. In their study, the P/Q-type (\(\alpha_{1\lambda}\)), R-type (\(\alpha_{1\gamma}\)), and N-type (\(\alpha_{1\beta}\)) calcium channels bound Ca\(^{2+}\)-CaM, but \(\alpha_{1\beta}\) had a much lower affinity. P/Q-type calcium channels display a small amount of calcium-dependent inactivation, whereas R and N do not. If Ca\(^{2+}\)-CaM binding to the \(\alpha_{1\gamma}\) is responsible for Ca\(^{2+}\)-dependent inactivation, this result raises the question of the functional role of CaM when bound to R- and N-type calcium channels, since they do not undergo Ca\(^{2+}\)-dependent inactivation.

In this study we show that the IQ domain of the L-type binds partially Ca\(^{2+}\)-saturated calmodulin, whereas the IQ domains of P/Q, R, and N have a much lower apparent affinity for the partially Ca\(^{2+}\)-saturated calmodulin. We also show that another sequence (CB) found in the carboxyl-terminal region of L-type channels binds partially and fully Ca\(^{2+}\)-saturated CaM and enhances \(I_{Ca}\) facilitation in cardiac myocytes, suggesting that CB can participate in Ca\(^{2+}\)-dependent modulation of \(I_{Ca}\).

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MATERIALS AND METHODS

**Reagents**—Bovine brain calmodulin (95% pure) was purchased from Sigma, solubilized in 10 mM MOPS, 1 mM EGTA, 0.02% NaN\(_3\), pH 7.4, and quantified by absorption from 320 to 277 nm to obtain stock solutions of about 300 \(\mu\)M. Peptides were synthesized at the protein lab facility at Baylor College of Medicine and diluted into 200 mM MOPS, pH 7.4, for assays. The antipeptide antibody was prepared by immunization of rabbits with a peptide representing amino acids 1484–1509 of skeletal L-type channel, and the second antibody was goat anti-rabbit antibody coupled to hors eradish peroxidase. The blots were developed using the SuperSignal chemiluminescence substrate (Pierce). For competition experiments, CaM (2 \(\mu\)g) was incubated with the blot for 6–8 h at 4 °C before the addition of the first antibody.

**Calcium Currents in Cardiac Myocytes**—Voltage clamp experiments were performed in whole cell mode (22) with freshly isolated rabbit ventricular myocytes, (23). L-type Ca\(^{2+}\) current (\(I_{Ca}\)) was isolated by adding Ca\(^{2+}\) and tetraethylammonium chloride and reducing Na\(^+\) and K\(^+\) in the pipette and bath solutions. Elimination of the residual current by nifedipine (10 \(\mu\)M) confirmed that the identity of active current was \(I_{Ca}\) (not shown). \(I_{Ca}\) was activated (0.5 Hz) by voltage command pulses from −30 mV to +10 mV at 24 °C. Total charge movement was determined by integrating inward \(I_{Ca}\) during the command step using pClamp 6.2 (Axon Instruments) and expressed as a ratio of the nth to the 1st stimulated “beat.” The pipette (intracellular) solution was 120.0 mM CsCl, 10.0 mM EGTA, 10.0 mM HEPES, 10.0 mM tetraethylammonium chloride, 5.0 mM phosphocreatine, 3.0 mM CaCl\(_2\), 1.0 mM MgATP, 1.0 mM NaGTP, and pH was adjusted to 7.2 with 1.0 N CsOH. The calculated resting free [Ca\(^{2+}\)] was about 250 nM in the pipette solution. In some experiments, the CaM-binding peptide CB-L-B (100 \(\mu\)M) was included in the pipette solution, and all cells were dialyzed for at least 5 min before initiating experiments. The bath (extracellular) solution was N-methyl-D-glucamine 137.0 mM, 25.0 mM CsCl, 10.0 mM HEPES, 10.0 mM glucose, 1.8 mM CaCl\(_2\), 0.5 mM MgCl\(_2\), and the pH was adjusted to 7.4 with 12 N HCl. For experiments designed to eliminate CaM binding, Ca\(^{2+}\) was omitted, EGTA was substituted with BAPTA in the pipette solution, and Ba\(^{2+}\) was substituted for Ca\(^{2+}\) in the bath solution. The null hypothesis was rejected for \(p < 0.05\) using the unpaired Student’s \(t\) test or analysis of variance as appropriate, and data were expressed as means ± S.E.

**RESULTS**

**Binding of CaM to Peptides from the Different Voltage-dependent Ca\(^{2+}\) Channels**—Partial sequences for the carboxyl-terminal tails of the different voltage-dependent Ca\(^{2+}\) channels are shown in Fig. 1. IQ peptides corresponding to the IQ motif of the different voltage-dependent channels are underlined and italicized in this figure. The cardiac IQ motif has been shown to...
CaM band at increasing peptide to CaM ratios in the presence of CaM and increasing molar ratios of CB-L-A, CB-L-B, CB-L-C, and CB-L-D. The data represent three independent determinations. 

The sequences of these peptides are shown in Table I. All of the peptides were able to interact with CaM (Fig. 2), suggesting that only the sequence LRAIKKIKWRTSMKL was required for CaM binding. The two shorter peptides (CB-L-C and CB-L-D) both produced multiple bands in the presence of CaM. The reason for this is not clear. It may be that, under these conditions, more than one peptide can bind to the CaM molecule, possibly by binding at each of the two CaM lobes. We chose CB-L-B, whose sequence is the same in the cardiac and skeletal muscle L-type channels and has similar affinity for CaM as CB-L-A, for subsequent studies because of its simpler gel pattern and because it was shorter and hence less expensive to prepare than CB-L-A.

A Comparison of the IQ and CB Peptides for Binding CaM—We next synthesized two sets of peptides: 1) peptides matching the IQ motifs from cardiac L-, skeletal L- and the P/Q-, N-, and R-type voltage-dependent calcium channels and 2) peptides matching the regions that most closely align with the CB region of these same channels. The sequences of these peptides are shown in Table I. We assessed and compared the interaction of these peptides (both IQ and CB) with CaM on nondenaturing gels. These data for the IQ and CB peptides are summarized in Fig. 3, A and B, respectively. We found, in agreement with Peterson et al. (5), that the IQ domains from all of the different channels bound CaM in the presence of CaM.
Calcium Channels and Calmodulin Sites

Dependence of the Interaction of the Peptides with CaM—Previous studies suggest that the L-type channel (cardiac) can bind CaM at low Ca$^{2+}$ (5). None of the peptides used in our study were able to bind to CaM if the gels were electrophoresed in the presence of 1 mM EGTA with no added Ca$^{2+}$ (data not shown). This binding site, therefore, does not represent a binding site for apoCaM. We next examined the ability of the various peptides to bind to CaMs that are mutated in the first two (B12), the second two (B34), or all four Ca$^{2+}$ binding sites (B1234) on CaM. All mutations in CaM involve Glu to Gln substitutions at the $z$ positions for coordinating Ca$^{2+}$, resulting in a greatly decreased affinity of the EF hand for Ca$^{2+}$ (25). All gels were electrophoresed in the presence of 200 $\mu$M Ca$^{2+}$. Consistent with the findings with apoCaM, none of the peptides could bind to the B1234 mutant (data not shown). However, as can be seen in Fig. 4, both the IQ peptide from the cardiac L-type channel and CB-L-B peptide were able to bind B12 with an affinity similar to that seen with the wild type CaM. The IQ-P/Q and IQ-R peptides could also bind B12, but to a lesser extent. The cardiac IQ peptide was also able to bind B34. None of the other peptides bound B34 as well as IQ-Lc. The mutation of either the amino- or carboxyl-terminal Ca$^{2+}$ binding sites in CaM greatly reduced the affinity of the IQ domains of the skeletal L-type, R-type, and N-type to bind CaM, suggesting that these sites prefer fully Ca$^{2+}$ saturated CaM. The other CB-L peptides (A, C, and D) bound the mutant CaMs similar to CB-L-B (data not shown).

To quantify the Ca$^{2+}$ dependence of the cardiac L-type CB peptide-CaM interaction we examined the change in tryptophan fluorescence of the CB peptide upon binding CaM. The emission spectra for the peptide and CaM, alone and in combination, are shown in Fig. 5A. The binding of CaM to the peptide increases the emission and results in a shift of the peak to shorter wavelengths. The Ca$^{2+}$ dependence of this interaction is shown in Fig. 5B. The $EC_{50}$ for the Ca$^{2+}$-dependent enhancement of the CB-L-B peptide-CaM interaction is $105 \pm 5$ nM ($n = 3$). The absence of a tryptophan in the IQ peptides prevented this type of analysis of their Ca$^{2+}$ dependence.

Nondenaturing gels in which the CB-L-B peptide was added at increasing concentrations to a 2:1 mixture of IQ-Lsk (Fig. 6A) and CaM, respectively, showed that the CB-L-B peptide could apparently displace the Lsk-IQ peptide from its complex with CaM, indicating a competitive interaction of these two peptides. In contrast, a 1:1 mixture of IQ-Lc (Fig. 6B) with CaM showed very little displacement by CB-L-B. This is likely due to the higher affinity of IQ-Lc compared with IQ-Lsk for CaM. There is no band corresponding to CaM complexed simultaneously to both peptides. These data clearly show that L-IQ and CB-L-B cannot bind simultaneously to CaM. Our data support a model in which the CB and IQ regions represent either distinct or alternative binding sites rather than both sequences contributing to a single binding site.

Demonstration of the Ability of the L-type Ca$^{2+}$ Channel to Bind Calmodulin Close to the CB Site—To demonstrate that the full-length $alpha_{1C}$ subunit can bind calmodulin at a site close to the CB sequence, we prepared an anti-peptide antibody to peptide CB-L-B and examined the ability of calmodulin to block
the binding of the antibody to the α subunit of the DHPR on Western blots (Fig. 7A). The skeletal protein was used for these studies because it is more abundant than the cardiac channel, and its sequence in the CB region is identical to that of the cardiac channel. Pre-incubation of the blots with calmodulin before the addition of the antibody blocked the labeling of this subunit by antibody, suggesting that the denatured α1S protein bound calmodulin at a site close to the antibody binding site. To assess the effects of calmodulin on the binding of the antibody to a channel in the presence of a non-denaturing detergent, we partially solubilized membranes in CHAPS and examined the ability of calmodulin to block the interaction with the antibody in an enzyme-linked immunoabsorbance assay (Fig. 7B). Again, calmodulin blocked the interaction of the antibody with the DHPR.

**Calcium Channels and Calmodulin Sites**

**Fig. 5.** Effect of Ca\(^{2+}\) on the interaction of CB-L-B with CaM. CB-L-B has a tryptophan at amino acid 15 (corresponding to amino acid 1498 in the intact skeletal L-type α1 subunit), allowing its interaction with CaM (2.9 μM) to be assessed by changes in fluorescence. Excitation was at 280 nm, and emission in relative fluorescence units (RFU) was detected between 300–410 nm. Panel A, the emission spectrum of CB-L-B at high Ca\(^{2+}\) in the presence and absence of CaM. Solid triangles, buffer alone; open circles, CaM alone; closed circles, CB-L-B alone; solid squares, CaM + CB-L-B. Panel B, changes in the fluorescence of a 1:1 mixture of CaM (2.9 μM) to peptide at increasing Ca\(^{2+}\) concentrations. Triangles, CaM + CB-L-B; solid circles, peptide alone; solid squares, CaM alone.

**Fig. 6.** Competition between IQ and CB peptides for binding to CaM. CaM was incubated with IQ-L in the presence of increasing molar ratios of CB-L-B. The samples were then electrophoresed on 20% non-denaturing gels and stained with Coomassie Blue. Panel A is with IQ-L; molar ratios of IQ:CaM:CB-L-B are 0:1:20 (lane 1), 0:1:0 (lane 2), 2:1:0 (lane 3), 2:1:0.5 (lane 4), 2:1:1 (lane 5), 2:1:3 (lane 6), 2:1:5 (lane 7), 2:1:10 (lane 8), and 2:1:20 (lane 9). Panel B is with IQ-Le; molar ratios of IQ:CaM:CB-L-B are 0:1:20 (lane 1), 0:1:0 (lane 2), 1:1:0 (lane 3), 1:1:0.5 (lane 4), 1:1:1 (lane 5), 1:1:3 (lane 6), 1:1:5 (lane 7), 1:1:10 (lane 8), and 1:1:20 (lane 9). Left dashed arrow, CaM-CB-L-B; right small solid arrow, CaM alone; right large solid arrow, CaM-IQ-L complex.

**DISCUSSION**

L-type Ca\(^{2+}\) channels show Ca\(^{2+}\)-dependent inactivation that requires CaM. Recent work has centered on the IQ-like domain in the carboxyl terminus of the α subunit in Ca\(^{2+}\) channels as representing the CaM binding site. However, this is an incomplete picture since voltage-dependent Ca\(^{2+}\) channels that do not show Ca\(^{2+}\)-dependent inactivation bind CaM at their corresponding IQ-like sequences in the carboxyl terminus. This raises the question of why the binding of Ca\(^{2+}\)-CaM to the IQ domain on the L-type channel leads to Ca\(^{2+}\)-dependent inactivation, whereas the binding to IQ domains of the other channels does not. If the IQ domain is the primary binding site for CaM on these channels, the binding to the IQ domain of the L-type channel must lead to a secondary change that does not occur in the other channels. Recently Soldatov et al. (13), Zuhlke and Reuter (7), and Peterson et al. (14) found that determinants outside of the IQ motif are necessary for Ca\(^{2+}\)-dependent inactivation. Soldatov et al. (13) showed that the sequences IKTGE and LLDVQ are required, and Zuhlke and Reuter (7) demonstrated the requirement for the IQ domain and residues 1630 and 1631 for Ca\(^{2+}\)-dependent inactivation. These sequences immediately bracket the CB sequence in L-type channels that we have shown binds CaM. Petersen et al. (14) demonstrated that the exchange of the sequence VVTI in the P helix of the putative EF hand with the corresponding sequence of the R channel abolished Ca\(^{2+}\)-dependent inactivation. The widely spaced regions of the carboxyl tail involved in Ca\(^{2+}\)-dependent inactivation suggest that some of these regions are involved in the CaM binding, whereas others may contribute to the regulation of the channel that occurs after CaM binds. Other portions might be crucial for the communication between these two domains.

Our data with the different IQ and CB peptides are summarized in Table I. Consistent with the results of Peterson et al. (5), we found that the IQ peptides matching the sequence from the L, P, Q, N, and R channels all bound Ca\(^{2+}\)-CaM. However, only cardiac IQ-L was able to bind (in the presence of Ca\(^{2+}\))
CaMs mutated in the Ca$^{2+}$ binding sites (B12 and B34) with an affinity comparable with that of wild type CaM. The P/Q and R channel IQ peptides bound B12 CaM and B34 CaM with reduced affinity compared with wild type CaM, and the N and L-ek IQ peptide showed no ability to bind these mutant CaMs. None of the peptides were able to bind to CaM in less than 10 nanomolar Ca$^{2+}$ concentrations or to a CaM mutated at all four Ca$^{2+}$ binding sites. These peptides do not, therefore, represent binding sites for apoCaM. The cardiac L-type channel and, to a lesser extent, the P/Q channels show Ca$^{2+}$-dependent modulation. The other channels do not. The unique ability of the cardiac L-type IQ sequence to bind CaM that is not fully Ca$^{2+}$ saturated may allow CaM to bind to this channel under resting Ca$^{2+}$ conditions. Higher Ca$^{2+}$ concentrations could then produce Ca$^{2+}$-dependent inactivation. This is the first demonstration of a property of the IQ site on the L-type channels that sets it apart from the IQ sites on the other channels.

We also found that a domain (CB) between the EF hand and the IQ domain of L-type channels was able to bind B12 CaM and wild type CaM in the presence of Ca$^{2+}$. Mutations in Ca$^{2+}$ binding sites 3 and 4 on CaM greatly reduced the affinity of CaM for this sequence. The Ca$^{2+}$ dependence of the interaction of CB-L-B with CaM (EC$_{50}$ = 100 nM) suggests that this interaction could also take place at resting Ca$^{2+}$ levels in the cell, preparing the channel for inactivation when the Ca$^{2+}$ reaches the appropriate levels. Half-maximal inhibition of Ca$^{2+}$ channel activity by CaM occurs at about 4 μM Ca$^{2+}$ (26). The CB-L-B peptide also enhanced $I_{Ca}$ facilitation in cardiac myocytes in a Ca$^{2+}$-dependent manner, suggesting that this sequence may have functional significance for regulating L-type calcium channels. The P/Q, R, and N channels did not have comparable sequences that bound CaM. This is the second feature that distinguishes the cardiac L-type channels from the other voltage-dependent channels. The identified CaM binding sequence is within a domain previously suggested to play important roles in regulating Ca$^{2+}$-dependent inactivation (7, 13), targeting (17, 18, 27), conductance (17), and open probability (17) of the channel. These findings suggest a role for calmodulin in regulating other aspects of L-type calcium channel function.

In addition to the peptide data, other workers have found that amino acids within and bracketing this sequence are necessary for Ca$^{2+}$-dependent inactivation of the channel. Our data support these findings. We have shown that calmodulin can block the interaction of an antibody to the CB-L-B sequence with both the SDS-denatured and the CHAPS-solubilized skeletal muscle L-type channel. Although these findings support a model in which the CB sequence is a CaM binding site, we
cannot rule out the possibility that CaM binding to the nearby IQ sequence sterically hinders antibody binding to the CB sequence. Mutations of this region coupled to analysis of CaM binding will be necessary to resolve this issue.

The carboxyl-terminal tail of the L-type channels has also been suggested to play a role in membrane targeting of this protein (18). The CB sequence that we demonstrate to bind CaM is within the region that has recently been suggested to play a role in membrane targeting of the L-type channels (18). CaM binding may also contribute to this process.

The IQ and CB regions of the cardiac L-type channel could either each bind a molecule of CaM or they could both contribute to the same CaM binding site. We have demonstrated that the interactions of the L-type IQ and CB peptides with CaM are competitive. Based on our findings, we propose a model in which the IQ and CB domains in the carboxyl-terminal tails of the L-type channels represent either two distinct binding sites for CaM or alternative sites for the interaction with partially and fully saturated CaM. One possibility is that only one CaM can bind to the carboxyl tail, and which site (IQ versus CB) is occupied is controlled by factors regulating the conformation of this region (for example, Ca$^{2+}$ binding to the EF hand).

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