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REVIEW ARTICLES

# Calmodulin modulation of ion channels and receptors*

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**Abstract** Ion channels and receptors are the structural basis for neural signaling and transmission. Recently, the function of ion channels and receptors has been demonstrated to be modulated by many intracellular and extracellular chemicals and signaling molecules. Increasing evidence indicates that the complexity and plasticity of the function of central nervous system is determined by the modulation of ion channels and receptors. Among various mechanisms, $Ca^{2+}$ signaling pathways play important roles in neuronal activity and some pathological changes. $Ca^{2+}$ influx through ion channels and receptors can modulate its further influx in a feedback way or modulate other ion channels and receptors. The common feature of the modulation is that $Ca^{2+}$/calmodulin (CaM) is the universal mediator. CaM maintains the coordination among ion channels/receptors and intracellular $Ca^{2+}$ homeostasis by feedback modulation of ion channels/receptors activity. This review focuses on the modulating processes of ion channels and receptors mediated by CaM, and further elucidates the mechanisms of $Ca^{2+}$ signaling.

**Keywords: calmodulin, receptor, ion channel, inactivation, facilitation.**

$Ca^{2+}$ is an important signaling molecule in cells and is a ubiquitous regulator of many cellular processes. Eukaryotic cells have evolved a complex system of transmembrane molecules, including ionic channels, pumps and exchangers, which maintain intracellular $Ca^{2+}$ homeostasis[1]. Many intracellular signaling events are triggered by transient changes in the intracellular free $Ca^{2+}$ concentration ($[Ca^{2+}]_i$). $Ca^{2+}$-triggered events are critical for both normal cellular activity and pathophysiological changes in cell functions. $Ca^{2+}$ binding protein is now believed to mediate most $Ca^{2+}$-dependent cellular processes. Among various $Ca^{2+}$-binding proteins, calmodulin (CaM) is the most important one. CaM plays a role in the modulation of many cellular functions in eukaryotic cells, such as muscle contraction, synaptic transmission, synthesis and release of neurotransmitter, secretion of hormone and gene expression[2~6]. A lot of studies indicate that CaM also plays a pivotal role in the functional modulation of ion channels and receptors.

## 1 CaM mediation of the $Ca^{2+}$-dependent inactivation and facilitation of voltage-dependent $Ca^{2+}$ channels (VDCCs)

$Ca^{2+}$ influx through VDCCs acts as a messenger for most intracellular signaling events, including feedback processes that regulate activity of the channel itself. Such feedback includes inactivation which closes the channels, and facilitation which enhances channels opening[7]. Both forms of auto-regulation have a significant impact on the amount of $Ca^{2+}$ entering the cell during repetitive activity, and on the consequences downstream. A number of studies have reached the surprising conclusion that CaM mediates both inactivation and facilitation of $Ca^{2+}$ channels[8~16].

### 1.1 Modulation of L-type $Ca^{2+}$ channels by CaM

Over 20 years ago, Brehm et al.[8] reported a curious finding: VDCCs are not only opened by membrane depolarization, but also inactivated during a sustained depolarization. This inactivation is $Ca^{2+}$-dependent and caused by the very $Ca^{2+}$ that enters through the opening $Ca^{2+}$ channels (Fig. 1). The fa-

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cilitation of VDCCs mediated by $Ca^{2+}$ is also found recently.

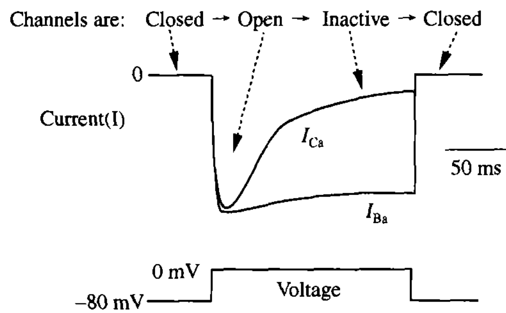


Fig. 1. $Ca^{2+}$-dependent inactivation of VDCCs. Upon membrane depolarization from − 80 mV to 0 mV, VDCCs open and $Ca^{2+}$ flows into the cell. When $Ca^{2+}$ is used as the charge carrier, the $Ca^{2+}$ current ($I_{Ca}$) decreases with time during the depolarization, as a result of $Ca^{2+}$-dependent inactivation of the VDCCs. $Ba^{2+}$ permeates VDCCs even better than $Ca^{2+}$ does but does not cause inactivation. Hence, when $Ba^{2+}$ is used as the charge carrier, the $Ba^{2+}$ current ($I_{Ba}$) is sustained throughout the membrane depolarization. These experiments indicate that the inactivation is indeed $Ca^{2+}$-dependent. (Modified from Levitan[13]).

Among the many types of VDCCs, L-type $Ca^{2+}$ channels particularly display inactivation and facilitation, both of which are closely linked to the earlier entry of $Ca^{2+}$. This channel is critical for diverse phenomena such as contraction of cardiac muscle, hormone secretion, regulation of gene expression in neurons and initiation of transcriptional events associated with learning and memory, and hence its autoregulation by $Ca^{2+}$-dependent inactivation and facilitation is of fundamental biological significance. Several recent studies about the molecular mechanism of $Ca^{2+}$-dependent inactivation and facilitation of L-type $Ca^{2+}$ channels have suggested that the ubiquitous $Ca^{2+}$ sensor CaM plays a central role in the $Ca^{2+}$-dependent inactivation and facilitation of L-type $Ca^{2+}$ channels[12].

Both Reuter's group[9,10,15] and Yue's group[12] have found that there is a putative CaM-binding isoleucine-glutamine (IQ) motif in the carboxy terminus of the $\alpha_{1C}$ subunit (Fig. 2). The work of the two groups indicates that the nature of the modulatory effect of CaM on L-type $Ca^{2+}$ channel inactivation and facilitation depends on residues within the IQ motif which are important for CaM binding. Mutation of the isoleucine residue Ile1624 to alanine will inhibit $Ca^{2+}$-dependent inactivation and unmask overt $Ca^{2+}$-dependent facilitation during trains of pulses, whereas its conversion to glutamate will eliminate both effects (Fig. 2). They also found that $Ca^{2+}$-dependent inac-

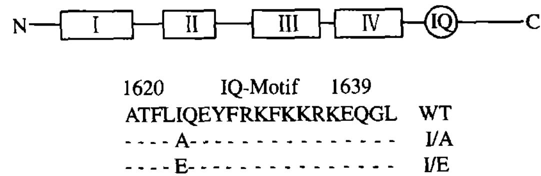


Fig. 2. Putative model for the $\alpha_{1C}$ subunit of the L-type $Ca^{2+}$ channels. The $\alpha_{1C}$ subunit includes four transmembrane domains (I ~ IV) and a consensus CaM-binding IQ motif (Leu1624-1635Leu) in cytoplasmic C-terminus. Binding of CaM to IQ motif is involved in $Ca^{2+}$-dependent inactivation and facilitation of L-type $Ca^{2+}$ channels. (Modified from Zühlke et al.[10]).

tivation and facilitation of L-type $Ca^{2+}$ channels depend on the binding of wild-type CaM to IQ motif. CaM has been identified to contain four EF hands from N-terminus. $Ca^{2+}$-dependent inactivation and facilitation are essentially abolished upon co-expression of L-type channels with mutant CaM in which aspartate has been mutated to alanine in any three EF hands. Thus, CaM-dependent inactivation and facilitation can be eliminated either by mutation of 1624Ile in the $\alpha_{1C}$ IQ motif that interferes with its binding to CaM[17] or by mutation in CaM that diminishes its affinity for $Ca^{2+}$[12]. It is therefore the interaction of $\alpha_{1C}$ motif and CaM that leads to the L-type $Ca^{2+}$

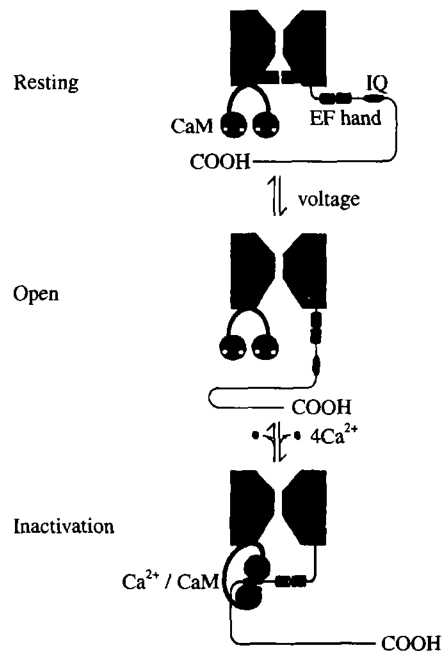


Fig. 3. $Ca^{2+}$-dependent inactivation of L-type $Ca^{2+}$ channels. In the resting state, the IQ-like domains are not bound by CaM. Upon membrane depolarization, the channels transit to the open state and $Ca^{2+}$ influxes. Accumulation of intracellular $Ca^{2+}$ at the inner mouth of the channels leads to the activation of CaM. Interaction of $Ca^{2+}$/CaM with the IQ-like region induces channel inactivation. The open circle within CaM indicates the $Ca^{2+}$ binding site. (Modified from Peterson et al.[12]).

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channel inactivation and facilitation. Any changes in structure and function of either IQ motif or CaM will alter the properties of the channels (Fig. 3). Taken together, these results indicate that CaM can either positively or negatively modulate ion channels[7,10]. However, it is not very clear to date the exact site that CaM acts. CaM could bind directly to the pore-forming $\alpha_{1C}$ subunit of the channel (Figs. 2 and 3), or it may have other targets, such as protein kinases or phosphatases, that regulate $Ca^{2+}$ channels[7]. Peterson et al.[12] have found that CaM could also bind to the second binding site, a putative EF hand, and lead to a weak $Ca^{2+}$-dependent inactivation. This binding is also essential for long-term $Ca^{2+}$-dependent facilitation. More recently, they demonstrate that while CaM serves as $Ca^{2+}$ sensor for inactivation, the EF-hand motif of $\alpha_{1C}$ may support the transduction of $Ca^{2+}$-CaM binding into channel inactivation[16].

### 1.2 Modulation of N-, P/Q- and R-type $Ca^{2+}$ channels by CaM

Neurotransmitter release at many central synapses is initiated by an influx of $Ca^{2+}$ ions through N-, P/Q-, and R-type $Ca^{2+}$ channels[11]. Because neurotransmitter release is proportional to the fourth power of $Ca^{2+}$ concentration[18], regulation of its entry can profoundly influence neurotransmission. Apart from the L-type $Ca^{2+}$ channels, N-, P/Q-, and R-type $Ca^{2+}$ channels are also modulated by CaM[7,11], suggesting that CaM-mediated effects may be a general regulator in the VDCCs family.

It has been reported by Lee[11] and Peterson[12] that there are pore-forming subunits corresponding to $\alpha_{1C}$ in R-type ($\alpha_{1E}$) and P/Q-type ($\alpha_{1A}$) $Ca^{2+}$ channels. C-terminus of both R-type and P/Q-type channels contain IQ-like motifs. Although IQ-like motifs from these channels bind CaM in a $Ca^{2+}$-dependent manner, both R-type and P/Q-type channels are absent of $Ca^{2+}$-dependent inactivation. This suggests that the absence of $Ca^{2+}$-dependent inactivation in N-, P/Q-, and R-type channels does not result from a failure to bind CaM but rather a failure to transduce CaM binding into inactivation. It seems that CaM binding to N-, P/Q-, and R-type channels is transduced into other, as yet unknown functional sequelae. A 32 amino acids CaM-binding domain (CBD) whose amino acid sequences are very similar to adenylyl cyclase type 8 (AC8) was found in the downstream of IQ motif in the C-terminus of the $\alpha_{1A}$ subunit of P/Q-type channels[11]. The function of CBD of P/Q-type channels is similar to the second CaM-binding site of L-type channels. $Ca^{2+}$/CaM binding to the CBD leads to a weaker $Ca^{2+}$-dependent inactivation and unmasks long-term facilitation. These results further suggest that there are more than one CaM-binding site in VDCCs. CaM binding to different sites produces different physiological effects. The results of Lee et al.[11] support a model in which $Ca^{2+}$ entering through P/Q-type channels promotes $Ca^{2+}$/CaM binding to the second binding site of $\alpha_{1A}$ subunit. The association of $Ca^{2+}$/CaM with the channel would accelerate inactivation, enhance recovery from inactivation and augment further $Ca^{2+}$ influx by facilitating the $Ca^{2+}$ current so that it is greater after full recovery from inactivation. These relatively slow modulatory effects may interact to sharpen the response of P/Q-type channels to repeated trains of stimuli. Accumulation of intracellular $Ca^{2+}$ during a train of impulses would increase inactivation, shorten $Ca^{2+}$ transients and contribute to terminating the $Ca^{2+}$ signal following the train. The overall effect would be briefer, more intense $Ca^{2+}$ signals in dendrites and nerve terminals with P/Q-type channels.

In conclusion, recent works indicate that $Ca^{2+}$ entry through L-type $Ca^{2+}$ channels binds to the IQ motif of the pore-forming $\alpha_{1C}$ subunit leading to $Ca^{2+}$-dependent inactivation and facilitation in a feedback manner. As the actual $Ca^{2+}$ sensor, CaM plays a central role. The possible mechanism is that $Ca^{2+}$ binds to the CaM tethered in the pore-forming subunits, and then $Ca^{2+}$/CaM interacts with the IQ motif in the pore-forming $\alpha_{1C}$ subunits leading to $Ca^{2+}$-dependent inactivation and facilitation[12](Fig. 3). Alternatively, it is difficult to block $Ca^{2+}$-dependent inactivation with $Ca^{2+}$ chelators, suggesting that the site of $Ca^{2+}$ binding for inactivation must be very close to the channel pore through which the $Ca^{2+}$ enters[13]. However, the role of IQ-like motif in N-, P/Q- and R-type $Ca^{2+}$ channels is not clear to date. The $Ca^{2+}$-dependent inactivation and facilitation depends on the second CaM binding site in these $Ca^{2+}$ channels.

## 2 CaM mediation of the $Ca^{2+}$-dependent inactivation of *N*-methyl-D-aspartate receptors (NMDARs)

NMDARs are heteromeric complexes composed of both NR1 and NR2 subunits. NMDARs play critical roles in such fundamental phenomena as synaptic transmission, synaptic plasticity, synapse formation,

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and excitotoxic cell death in mammalian central nervous system (CNS). A key property of the NMDARs is that they exhibit a high permeability to $Ca^{2+}$. Works by Huganir et al.[19,20] and Westbrook et al.[21] have demonstrated that receptor-mediated $Ca^{2+}$ influx leads to rapid $Ca^{2+}$-dependent inactivation of NMDARs. This $Ca^{2+}$-dependent inactivation serves as a negative feedback control system to regulate $Ca^{2+}$ influx[22](Fig.4).

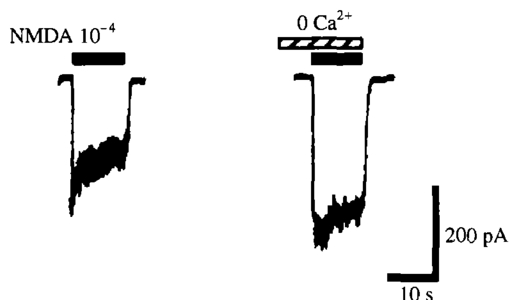


Fig.4. $Ca^{2+}$-dependent auto-regulation of NMDA currents. Enhancement of NMDA-induced currents in $Ca^{2+}$-free external solution suggests the $Ca^{2+}$-dependent inactivation of NMDARs (Modified from Xu et al.[22]).

Recently, several molecular biology strategies have shown that the long intracellular C-terminus on the NR1 subunit can be divided into three domains: C2, C1, C0 (numbered from C-terminus). The NR1 subunit contains two CaM binding sites in the C-terminus, designated CBS (CaM binding site) 1 and CBS2. CBS1 (Lys839~Gln863) and CBS2 (Lys875~Lys898) are located in C0 and C1 region, respectively[20](Fig.5). Two groups led by Huganir[19,20] and Westbrook[21] found respectively that mutation of amino acids 847~850 (QMQL) in the CBS1 of NR1 to alanines reduced the affinity for CaM only slightly,

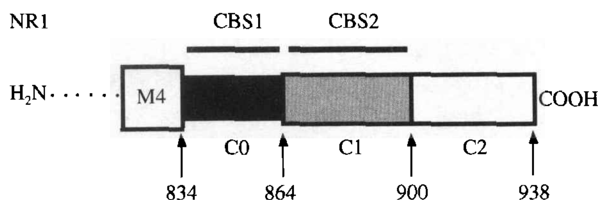


Fig.5. Schematic diagram of the C-terminus of the NR1 subunit. The NR1 subunit contains three domains in the C-terminus. Two CaM binding sites (CBS1 and CBS2) are located in C0 region and C1 region, respectively. (Modified from Zhang et al.[20]).

and did not affect $Ca^{2+}$-dependent inactivation, whereas mutation of the same residues to glutamates completely abolished CaM binding and thus markedly reduced $Ca^{2+}$-dependent inactivation. Furthermore, intracellular infusion of a CaM inhibitory peptide could inhibit the $Ca^{2+}$-dependent inactivation, suggesting that the $Ca^{2+}$-dependent inactivation of NMDARs is modulated by the binding of CaM to C0 domain of NR1 subunit. Subsequent studies further suggest that the inactivation of NMDARs is also involved in another binding partner of NR1 subunit: the actin-associated protein (for example, α-actinin2). In addition to binding CaM, the C0 region of NR1 also interacts with α-actinin2, and the binding of these two molecules to the C0 region of NR1 is competitive. The C-terminus of the NR1 subunit is normally anchored to the actin cytoskeleton by its direct interaction with α-actinin2. Upon activation of NMDARs, $Ca^{2+}$ influx activates CaM, which in turn competes with α-actinin2 binding to the C0 region of the NR1 subunit. CaM binding to NR1 will then displace α-actinin2, resulting in the dissociation of the NR1 subunit from the cytoskeleton (Fig.6). The free C-terminus of the NR1 subunit may then cause a

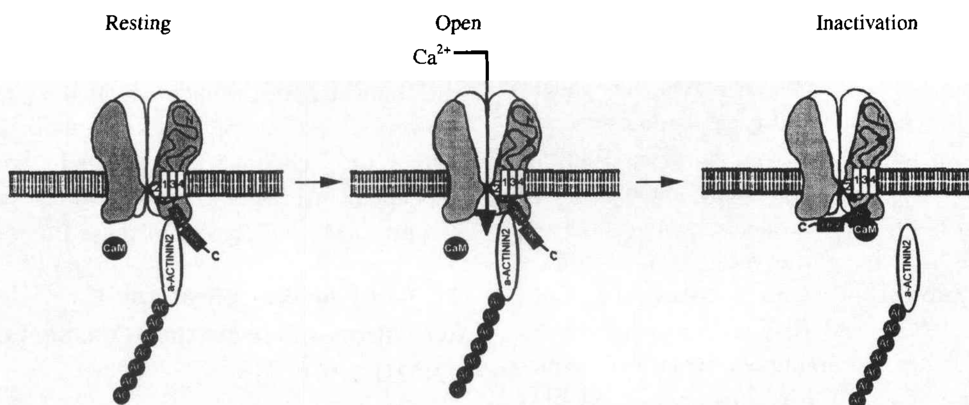


Fig.6. $Ca^{2+}$-dependent inactivation of NMDARs. The CBS1 region is normally anchored to the actin cytoskeleton (AC) by its direct interaction with α-actinin 2. Activation of the NMDARs increases intracellular $Ca^{2+}$ level, which then activates CaM. CaM, in turn, competes with α-actinin 2 binding to the CBS1 region, resulting in dissociation of the C-terminus from the actin cytoskeleton, and then leads to the NMDARs inactivation (Modified from Zhang et al.[20]).

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conformational change in the receptor, or possibly directly interact with other regions of the receptor, thereby decreasing the open probability of NMDARs, which leads to $Ca^{2+}$-dependent inactivation[20,23]. The role of CBS2 in NMDARs function is not clear to date. It may determine the location of NR1[23].

Recent work has suggested that in addition to the NR1 subunits, NR2 subunits also play a role in $Ca^{2+}$-dependent inactivation. CaM binding to the C0 region of NR1 subunits might be regulated by the specific NR2 subunits present in the receptor complex, or perhaps that the C-terminus of NR1 specifically interacts with certain NR2 subunits in a $Ca^{2+}$-dependent manner[24].

## 3 CaM mediation of the activation of $Ca^{2+}$-activated $K^+$ channels ($K_{Ca}$)

$K_{Ca}$ is a kind of $Ca^{2+}$-dependent $K^+$ channel that is voltage-dependent and intracellular $Ca^{2+}$-dependent. $K_{Ca}$ can be activated by depolarization and increase of $[Ca^{2+}]_i$. $K_{Ca}$ is subdivided into three classes: SK (small conductance), IK (intermediate conductance) and BK (big or large conductance).

The slow afterhyperpolarization that follows an action potential is generated by the activation of SK channels. The slow afterhyperpolarization limits the firing frequency of repetitive action potentials and is essential for normal neurotransmission. SK channels play a fundamental role in regulating neuronal excitability[25,26]. The SK-channel family contains SK1, SK2 and SK3. The amino acid sequences of the three known members of the SK-channel family show that the channels share high overall structural homology, with little similarity to members of other $K^+$-channel subfamilies. SK channels are voltage-independent and high-affinity $Ca^{2+}$ sensors. They are activated by submicromolar concentrations of intracellular $Ca^{2+}$ and transduce fluctuations in $[Ca^{2+}]_i$ into changes in membrane potential[26]. The activation of SK channels is not caused by the binding of $Ca^{2+}$ to the pore forming α-subunit of the channels, but is involved in CaM. Cytoplasmic C-terminus of α-subunit contains four repetitive sequences ($S_7$, $S_8$, $S_9$, $S_{10}$) that provide a shell to be bound by CaM. The binding of CaM to the shell is $Ca^{2+}$-independent. CaM is an integral component of SK channels, even can be taken as β-subunit. The functional SK channels are heteromeric complexes with CaM[27,28]. Using yeast two-hybrid assay and biochemical techniques, Xia et al.[27] showed that mutation of aspartate to alanine in the third and fourth EF hands ($D_{EF3,4}A$), or in the second, third and fourth EF hands ($D_{EF2,3,4}A$), or in all four EF hands ($D_{EF1,2,3,4}A$) all reduced the affinity of CaM for $Ca^{2+}$. Co-expression of these mutant CaM and SK channels downregulated the $Ca^{2+}$ sensitivity of SK channels, suggesting that CaM is involved in the modulation of SK channels. Xia et al.[27] demonstrated that the SK channels could be activated by the binding of $Ca^{2+}$ to constitutively SK-associated CaM, and the conformational changes in CaM initiated by $Ca^{2+}$-binding were translated to the channel α-subunits, resulting in $Ca^{2+}$-dependent gating.

A similar result has been reported recently for IK and BK in many peripheral cell types including hematopoietic cells. CaM regulates IK channel function by binding directly to the channels. A mutant CaM, defective in $Ca^{2+}$ sensing but retaining binding to the channel, dramatically reduces current amplitudes[29,30]. A domain in the extended C-terminal region of the α-subunit in BK channels, termed as "$Ca^{2+}$ bowl", is a novel $Ca^{2+}$-binding motif that contains a string of conserved aspartate residues. The "$Ca^{2+}$ bowl" is highly selective to $Ca^{2+}$ and is involved in the $Ca^{2+}$ sensitivity of BK channels, which is mediated by CaM[29,31]. However, there is no direct evidence yet that CaM plays a role in the activation of BK channels.

## 4 CaM mediation of the $Ca^{2+}$-dependent inhibition of cyclic nucleotide-gated (CNG) ion channels

Phototransduction in retinal rod photoreceptors and olfactory transduction in olfactory sensory neurons are mediated by members of the family of CNG ion channels, whose opening requires the binding of cAMP or cGMP to the channel. CNG channels are permeable to both $Na^+$ and $Ca^{2+}$ and regulate the entry of these ions into the sensory cells, as a result of signal-induced changes in the intracellular concentrations of cAMP (olfactory neurons) or cGMP (rod photoreceptors). $Ca^{2+}$ has long been known to play a key role in both visual and olfactory adaptation. But the mechanism is not clear. Recent work from several laboratories suggests the involvement of CaM in the regulation of CNG channel activity[32~36].

CaM affects the rod and olfactory CNG channels in a similar way: it decreases the channel activity by

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decreasing the sensitivity of channel gating to cyclic nucleotides. However, there are some interesting differences in the details. For example, CaM binds to the α-subunit of the olfactory channels, whereas it is the β-subunit of the rod channel that interacts with CaM[35]. Although the α-subunit of rod CNG channels has a CaM binding site, the homomeric channel that it forms is not functionally modulated by CaM, whereas the homomeric olfactory CNG channels composed of α-subunit is functionally modulated by CaM. Varnum et al.[33] found that CaM binds to the N-terminus of the α-subunit and decreases the open probability of the channels by interfering the interaction between the N-terminus and the cyclic nucleotide binding domain in the C-terminus (Fig. 7). Scott et al.[34] showed that in *Drosophila* photoreceptor cell, $Ca^{2+}$ influx through CNG channels bound to CaM and activated the CaM in response to the light. Activated CaM in turn contributed to the negative feedback regulation by initiating inactivation of the CNG channels.

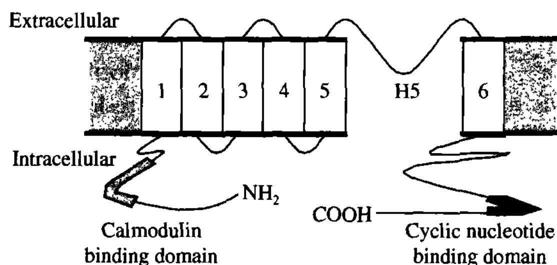


Fig. 7. Predicted topology of the α-subunit of CNG channel. The α-subunit has six putative transmembrane domains (1~6) and a hydrophobic loop (H5). Both N and C termini are located in the cytoplasm. There is a CaM-binding site in the N-terminus and a CNG binding domain in the C-terminus.

The recently identified CNG ion channels from some plants have the ability to bind CaM, and the CaM binding site is located in the C-terminus. This is in contrast to CNG channels from animals, implying that different mechanisms for CNG channel's modulation have evolved in animals and plants[36]. In conclusion, the modulation of CNG channels by CaM provides a negative feedback through CNG channel inactivation, which is very important for physiological function.

## 5 Universality of the modulation of ion channels and receptors by CaM

Many ion channels and receptors other than the mentioned ones are also regulated by CaM, such as nicotinic acetylcholine receptor (nAChR)[37], α-amino-3-hydroxy- 5-methyl-4-isoxazole-propionic acid receptor (AMPAR)[38,39], kainate receptor (KAR)[40], γ-aminobutyric acid receptor ($GABA_AR$)[41], glycine receptor (GlyR)[42,43] and voltage-dependent $Na^+$ channel (VDSC)[44,45].

nAChRs containing α7 subunits have a high relative permeability to $Ca^{2+}$ and influence numerous $Ca^{2+}$-dependent cellular events. Liu et al.[37] have shown that the responses from α7-containing receptors undergo substantial rundown when the receptors are repeatedly challenged with nicotine, which is termed "activity-dependent rundown". The rundown depends on the activation of CaM-dependent kinase II (CaM KII) by $Ca^{2+}$ influx through the receptors or $Ca^{2+}$ release from the intracellular $Ca^{2+}$ pools. Calcineurin (CaN) prevents the rundown and CaM facilitates the rundown. Ghetti et al.[40] suggested that synaptic activity mediated by NMDARs or other routes of $Ca^{2+}$ influx might modulate the function of KARs in neonatal hippocampal neurons. We recently found that $Ca^{2+}$ influx through NMDARs inhibits $GABA_AR$, which is CaM-dependent, in rat sacral dorsal commissural neurons[41]. We also demonstrated that $Ca^{2+}$ influx through NMDAR and AMPAR modulates GlyR by co-activation of CaMKII and CaN, in which CaM plays an important role[42,43] (Fig. 8).

Saimi et al.[44] reported that CaM is a physiological factor critically required for $Na^+$ channel activation, and is the $Ca^{2+}$ sensor of the $Na^+$ channel gating machinery. Mori et al.[45] found a novel interaction between the C-terminus domain and CaM by applying yeast two-hybrid screening to the cytoplasmic C-terminus domain of the main pore-forming α-subunit in VDSC. The interaction site of VDSC in a C-terminus region is composed of 38 amino acid residues and contains both IQ-like and Baa motifs. Baa motif binds only to $Ca^{2+}$-bound CaM, whereas IQ motif binds to both $Ca^{2+}$-bound CaM and $Ca^{2+}$-free CaM. These observations suggest the possibility that VDSC is functionally modulated through the direct interaction between CaM or $Ca^{2+}$/CaM and IQ-like motif or Baa motif in the C-terminal domain, and then leads to the $Ca^{2+}$-dependent conformational transition of the channel complex.

In conclusion, ion channels and receptors are widely modulated by CaM. In most voltage-dependent channels, CaM acts by binding to the IQ-like motif in the cytoplasmic C-terminus of the channels.

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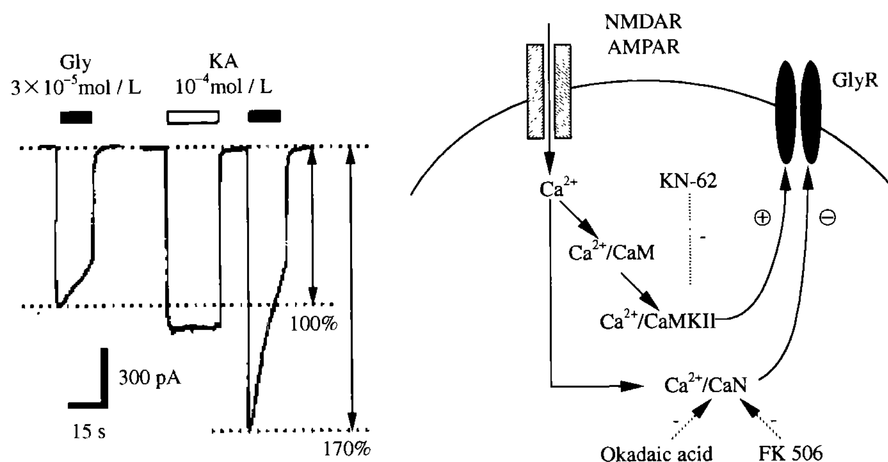


Fig. 8. CaM-mediated modulation of GlyR. $Ca^{2+}$ influx through NMDAR/AMPAR activates CaM and CaN. Activated CaM upregulates GlyR through CaMKII, which is inhibited by KN-62; activated CaN downregulates GlyR, which is inhibited by okadaic acid and FK506. Thus, GlyR is co-modulated by CaM and CaN. Gly, glycine; KA, kainic acid.

## 6 Concluding remarks

$Ca^{2+}$ influx feeds back to limit its own entry into the cell or modulates other ion channels and receptors. An important feature common to all of these examples is that CaM plays a key role in the processes. In the case of the L-type $Ca^{2+}$ channels, the CNG channels and the NMDAR, this negative feedback is an obvious consequence of channel inactivation (Fig. 9). It is also widely believed that at least some $K_{Ca}$ are located in close proximity to VDCCs, and so can act directly as sensors of the $Ca^{2+}$ that flows in through the VDCCs[27,29]. And then, the fluctuation of $[Ca^{2+}]_i$ transits to the change of membrane voltage, by which $K_{Ca}$ acts as feedback regulators of $Ca^{2+}$ entry (Fig. 9). In addition, $Ca^{2+}$/CaM also modulates many other ion channels and receptors. $Ca^{2+}$/CaM keeps the coordination among ion channels/receptors and intracellular $Ca^{2+}$ homeostasis by modulating ion channels/receptors activity.

Future studies must determine whether the presence of IQ motif is universal in the ion channels/receptors which are modulated by CaM; whether the action of IQ motif is similar in different ion channels/receptors; whether the IQ motif alone acts as both a tethering and an effector site? The future work must also focus on the modulation of ion channels/receptors in pathological conditions, such as ischemia, and the mechanism of disease occurrence. Furthermore, the acting sites of CaM on ion channels/receptors will be hopeful targets for developing new drugs.

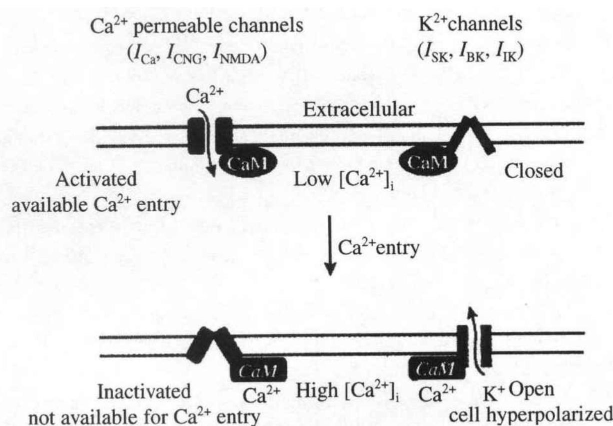


Fig. 9. CaM as a negative feedback regulator of $Ca^{2+}$ entry into cells. At resting state (top), when $[Ca^{2+}]_i$ is low and CaM is not activated, $Ca^{2+}$-permeable channels are available for $Ca^{2+}$ entry and $K_{Ca}$ is closed. Hence, $Ca^{2+}$ entry is enabled. After $Ca^{2+}$ entry, CaM binds $Ca^{2+}$ and is activated (bottom). As a result, the $Ca^{2+}$-permeable channels are inactivated, and $K_{Ca}$ are open and hyperpolarize the cell and thereby oppose voltage-dependent $Ca^{2+}$ entry. A common feature of these actions of CaM on different ion channels is that it acts as a $Ca^{2+}$ sensor and limits the amount of $Ca^{2+}$ that is allowed to enter the cell. $I_{Ca}$, $I_{CNG}$, and $I_{NMDA}$ refer to $Ca^{2+}$ currents carried through VDCCs, CNG channels, and NMDAR, respectively. $I_{SK}$, $I_{IK}$ and $I_{BK}$ refer to $K^+$ currents carried through the small, intermediate and big conductance $K_{Ca}$, respectively (Modified from Levitan[13]).

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