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Review Article

Ca^{2+}/Calmodulin and Presynaptic Short-Term Plasticity

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Synaptic efficacy is remodeled by neuronal firing activity at the presynaptic terminal. Presynaptic activity-dependent changes in transmitter release induce postsynaptic plasticity, including morphological change in spine, gene transcription, and protein synthesis and trafficking. The presynaptic transmitter release is triggered and regulated by Ca^{2+}, which enters through voltage-gated Ca^{2+} (Ca_V) channels and diffuses into the presynaptic terminal accompanying action potential firings. Residual Ca^{2+} is sensed by Ca^{2+}-binding proteins, among other potential actions, it mediates time- and space-dependent synaptic facilitation and depression *via* effects on Ca_V2 channel gating and vesicle replenishment in the readily releasable pool (RRP). Calmodulin, a Ca^{2+}-sensor protein with an EF-hand motif that binds Ca^{2+}, interacts with Ca_V2 channels and autoreceptors in modulation of SNARE-mediated exocytosis.

1. Introduction

For memory formation in a neuronal circuit, the primary function of presynaptic terminals is the firing activity-dependent release of neurotransmitters and subsequent recycling of their carrier synaptic vesicles, processes which critically depend on ATP and Ca^{2+}. Presynaptic firing of action potentials activates voltage-gated Ca^{2+} (Ca_V) channels, and Ca^{2+} entry initiates release of neurotransmitters. Ca^{2+} dependence on fast neurotransmitter release is thought to be conferred by the synaptotagmin, a family of Ca^{2+} sensors that interact with SNAREs [1]. Synaptotagmin 1 and 2 are synaptic vesicle proteins with tandem C2 domains that bind Ca^{2+} and ensure the synchronization of Ca^{2+}-dependent exocytosis with the presynaptic action potential [2–5]. Neuronal firing activity also controls other protein functions and dynamically remodels synaptic efficacy. Ca^{2+}-binding proteins sensing residual Ca^{2+}, which accumulates locally in the presynaptic terminal during trains of action potentials, may act as potential effectors for these reactions. Considerable evidence supports a role for calmodulin (CaM), another family of Ca^{2+} sensors with an EF hand motif that binds Ca^{2+}, in modulation of SNARE-mediated exocytosis [6, 7] and endocytosis [8, 9]. Targets of CaM include multiple proteins implicated in exocytosis (e.g., Ca^{2+} channels [10], Ca^{2+}/ CaM kinase II [11], rab3 [12], and Munc13 [13]), and endocytosis (e.g., calcinulin [14]). Another Ca^{2+}-binding protein with an EF hand motif, parvalbumin, acts as a mobile presynaptic Ca^{2+} buffer that accelerates withdrawal of residual Ca^{2+} and decay of short-term facilitation in the calyx of held [15] and GABAergic synapses between interneurons and Purkinje cells [16]. Calretinin is upregulated in the calyx of held during postnatal development and may act as a Ca^{2+} effector or Ca^{2+} buffer to regulate transmitter release probability [17].

A large number of proteins are involved in presynaptic function; their synthesis, transport, and function appear to be regulated by presynaptic firing activity [18–20], Sympathetic superior cervical ganglion (SCG) neurons, which form a well-characterized cholinergic synapse in long-term culture [21, 22], are an ideal cell model [21, 23] with which to investigate these processes. The SCG neuron has a large cell body and nucleus that allows for the manipulation of gene expression and function in mature neurons *via* acute microinjection of cDNA, small interfering RNA (siRNA), dominant-negative transgenes, peptides, antibodies, and metabolites [21, 24–27], an approach not technically feasible for cultured neurons from the central nervous system. In addition, synaptic activity and short-term plasticity, as it

relates to the size and replenishment of functional synaptic vesicle pools, can be accurately monitored by recording excitatory postsynaptic potentials (EPSPs) evoked by paired or repetitive action potentials in presynaptic neurons. Using this approach, we recently uncovered a critical role for CaM in presynaptic short-term plasticity [26, 28]. Our new findings concerning activity-dependent regulation of synaptic efficacy by modulating synaptic vesicle exocytosis which underlies memory formation in the brain are summarized in this paper. CaM is a Ca^{2+} effector sensing residual Ca^{2+} that mediates time- and space-dependent synaptic depression and facilitation *via* effects on Ca_V2 channel gating [26] and vesicle replenishment in the readily releasable pool (RRP) [28]. To maintain synaptic remodeling, mitochondrial ATP biogenesis supports synaptic transmission, including efficient mobilization of synaptic vesicles into the RRP for the generation of short-term plasticity [29].

2. $Ca_V2.1$ Channel Regulation and Short-Term Plasticity

2.1. Ca^{2+}/CaM Modulates $Ca_V2.1$ Channel Activity. At most fast synapses in the central nervous system, $Ca_V2.1$ channels are densely clustered [30], and Ca^{2+} entered through $Ca_V2.1$ channels initiates synaptic transmission [31, 32]. $Ca_V2.1$ channels are Ca^{2+}-dependently regulated by CaM [10, 33–36] and related to neuron-specific Ca^{2+}-binding proteins, calcium-binding protein 1 and visinin-like protein-2 [37–39]. CaM and the Ca^{2+}-binding proteins interact with a bipartite regulatory site in the intracellular C terminus of the $\alpha_1 2.1$ subunit [36] called the IQ-like motif, which begins with the sequence isoleucine-methionine (IM) rather than isoleucine-glutamine (IQ); they also interact with the nearby downstream CaM-binding site. Alanine substitutions in the IQ-like domain ($\alpha_1 2.1_{IM-AA}$) blocked Ca^{2+}-dependent facilitation of $Ca_V2.1$ channels [34, 36], whereas Ca^{2+}-dependent inactivation was blocked in channels lacking the adjacent CaM-binding domain (CBD; $\alpha_1 2.1_{\Delta CBD}$) [10, 35, 36, 38, 39].

Ca^{2+}/CaM-dependent inactivation of $Ca_V2.1$ channels is dependent on global elevations of Ca^{2+} [35], which in turn are dependent on the density of Ca^{2+} channels, local Ca^{2+} buffers, the volume of the intracellular compartment, and other differences in the cellular context in which the channels are located. Ca^{2+}-dependent inactivation of $Ca_V2.1$ channels is observed in transfected cells overexpressing $Ca_V2.1$ channels [10, 34, 35] and in the nerve terminals of the calyx of Held [40, 41], where $Ca_V2.1$ channels are densely localized and large Ca^{2+} transients are generated. In contrast, Ca^{2+}-dependent inactivation is not reliably observed in the large neuronal cell bodies of Purkinje neurons [42] or SCG neurons [26].

2.2. Ca^{2+}/CaM-Mediated $Ca_V2.1$ Channel Modulation, Synaptic Facilitation and Depression. Paired-pulse facilitation (PPF) and paired-pulse depression (PPD) are both mediated by Ca^{2+}/CaM-dependent regulation of $Ca_V2.1$ channels [26]. EPSPs recorded by pairs of action potentials with varied stimulation intervals show PPD and PPF in synaptically connected SCG neurons in which the presynaptic neuron was transfected with the $\alpha_1 2.1_{WT}$. PPD with short interstimulus interval (<40 ms) was blocked by the $\alpha_1 2.1_{\Delta CBD}$ transfection, while PPF with intermediate interstimulus interval (50–100 ms) was blocked by the $\alpha_1 2.1_{IM-AA}$transfection. Thus, temporal regulation of $Ca_V2.1$ channels contributes to PPD and PPF by changing the release probability in an activity-dependent manner in response to Ca^{2+} entry during the first action potential of the pair, suggesting a spatial change in Ca^{2+} concentration at the active zone and also regulates $Ca_V2.1$ channel activity.

Neural information is encoded in bursts of synaptic activity *in vivo*. During bursts of action potentials, the CaM-dependent regulation of $Ca_V2.1$ is involved in the short-term synaptic plasticity (Figure 1). Repetitive stimulation produces frequency-dependent regulation of EPSPs in SCG neurons. As the frequency of stimulation was increased, synaptic transmission mediated by the $\alpha_1 2.1_{WT}$ showed increased facilitation followed by depression during the trains of 1 s (Figure 1(a), WT). This form of short-term synaptic plasticity resembles that recorded at the calyx of Held in response to repetitive stimuli [40, 41, 43]. The facilitation is reduced by the IM-AA mutation (Figure 1(a), IM-AA) but not by the △CBD mutation (Figure 1(a), △CBD), suggesting that enhancement of neurotransmission following 1 s trains requires CaM binding to the IQ-like domain. In contrast to the facilitation followed by depression of synaptic transmission observed during trains for WT, the EPSP size increased steadily during trains (<30 Hz) for △CBD (Figure 1(a), ΔCBD). Facilitation of the ΔCBD mutant was greater than the WT and the IM-AA mutant throughout the train, and depression was much slower, suggesting that, similar to paired-pulse experiments, CaM-dependent inactivation of $Ca_V2.1$ channels shapes the time course of short-term synaptic plasticity by determining the timing of the peak of synaptic facilitation during the train as well as the steady-state level of synaptic depression at the end of the train.

Two forms of presynaptic short-term enhancement of synaptic transmission, termed augmentation and posttetanic potentiation (PTP), last for seconds to minutes [44]. In SCG neurons, augmentation is elicited with 10 s trains [45], and PTP is elicited with 60 s trains [46, 47]. Augmentation observed with 10 Hz, 20 Hz, and 40 Hz trains is significantly reduced by the IM-AA mutation, whereas the ΔCBD mutation has little effect on the magnitude of the augmentation [26]. Thus, Ca^{2+}/CaM-dependent facilitation of Ca^{2+} entry significantly contributes to an intermediate-length form of synaptic enhancement and augmentation, which has a time course of tens to hundreds of milliseconds [26]. In contrast to augmentation, the Ca^{2+} signal that induces PTP does not require Ca^{2+}/CaM-dependent facilitation. Consistent with this idea, previous reports suggested that PTP, but not augmentation, resulted from the slow efflux of mitochondrial Ca^{2+} accumulated during titanic stimulation [48] and also involved activation of protein kinase C [49, 50].

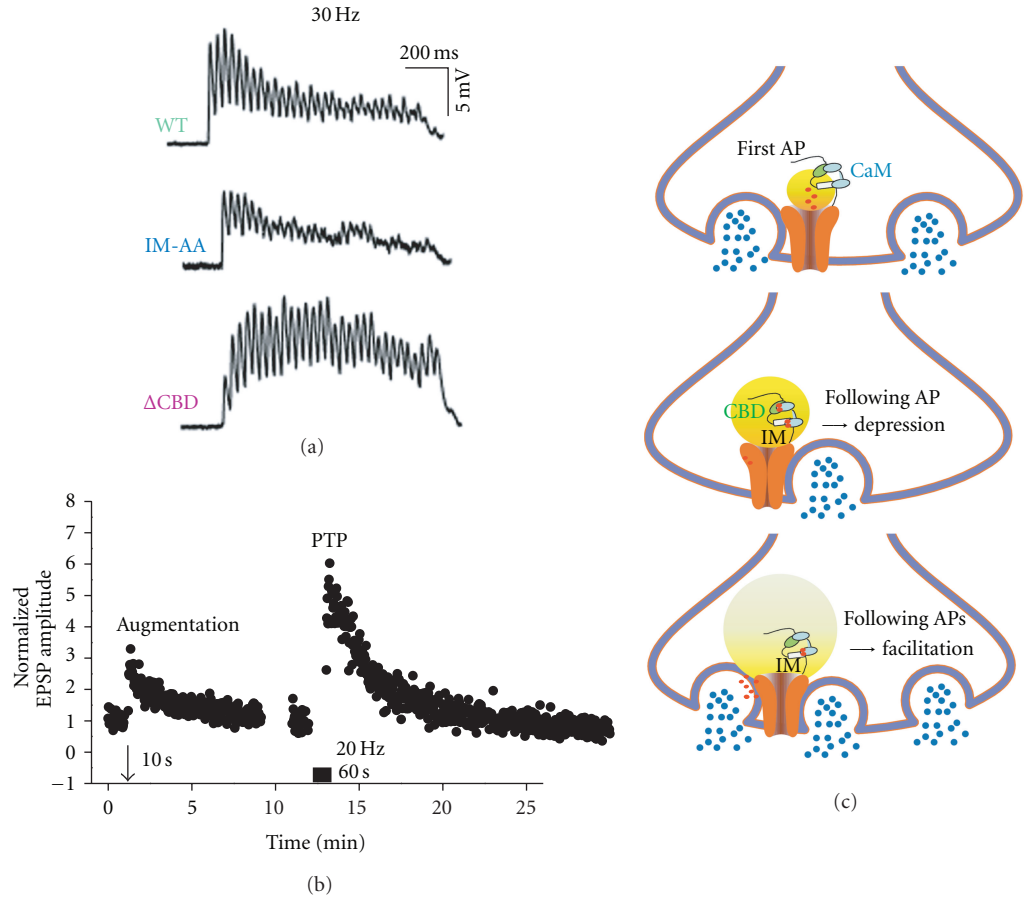

Figure 1: Presynaptic facilitation and depression mediated by $Ca_V2.1$ channel facilitation and inactivation. (a) Averaged trace of EPSPs ($n = 5 - 12$), in which $Ca_V2.1$ channels were the only active channels in the presence of ω-conotoxin GVIA, evoked by action potentials with 1 s train at 30 Hz. (b) Normalized amplitudes of EPSPs recorded every 2 s in the presence of ω-Conotoxin GVIA. Conditioning stimuli were applied at the indicated times at 20 Hz for 10 s to evoke augmentation and at 20 Hz for 60 s to induce PTP. Adapted from Mochida et al., 2008 [26]. (c) Model illustrating $Ca_V2.1$-mediated mechanisms of synaptic depression, facilitation, and augmentation. In synaptic depression, CaM sensing local Ca^{2+} interacts with the CaM-binding domain (CBD) to cause channel inactivation and reduce Ca^{2+} entry, thus, reducing neurotransmitter release. In synaptic facilitation and augmentation, CaM sensing global Ca^{2+} interacts with the IQ-like motif to cause channel facilitation and increase in Ca^{2+} entry, and subsequently neurotransmitter release increases.

2.3. Ca^{2+}/CaM-Mediated $Ca_V2.1$ Channel Modulation and Presynaptic Short-Term Plasticity. Short-term synaptic plasticity is crucial for encoding information in the nervous system [51]. Changes in the concentration of residual Ca^{2+} are temporally sensed by a Ca^{2+} sensor CaM, resulting in Ca^{2+}-dependent facilitation and inactivation of presynaptic $Ca_V2.1$ currents. These currents can mediate short-term synaptic facilitation and depression, which are conserved forms of plasticity at many different types of synapses [44]. Evidently, by causing facilitation and inactivation of $Ca_V2.1$ channels, residual Ca^{2+} can actually control "instantaneous" Ca^{2+} during an action potential, shape the local Ca^{2+} transient at the active zone, and cause facilitation, augmentation, and depression of synaptic transmission. The postsynaptic response is, therefore, controlled by Ca^{2+}/CaM-dependent modulation of presynaptic Ca^{2+} entry, which acts to encode the information contained in the frequency of presynaptic firing of action potentials for transmission to the postsynaptic cell. Thus, presynaptic Ca^{2+} channel regulation by CaM is a major molecular mechanism underlying information processing in the nervous system.

3. CaM, mGluR and Munc 18-1 Interaction and Synaptic Facilitation

3.1. Presynaptic mGluRs. Presynaptic group III metabotropic glutamate receptors (mGluR4, mGluR7, and mGluR8) are expressed in the presynaptic active zone and reduce synaptic vesicle release upon stimulation by agonists [52–55]. A prominent enigma concerning the function of group III mGluRs is depicted in the case of mGluR4: short-term synaptic facilitation is impaired in mGluR4-KO mice although the expression of the plasticity is not reduced by pharmacological blockade of mGluR4 [52, 56]. These findings suggest the existence of some ligand-independent functions of the receptor linked to synaptic vesicle release [52]. It has been shown that Ca^{2+}-dependent CaM binds to the intracellular C-terminal tail

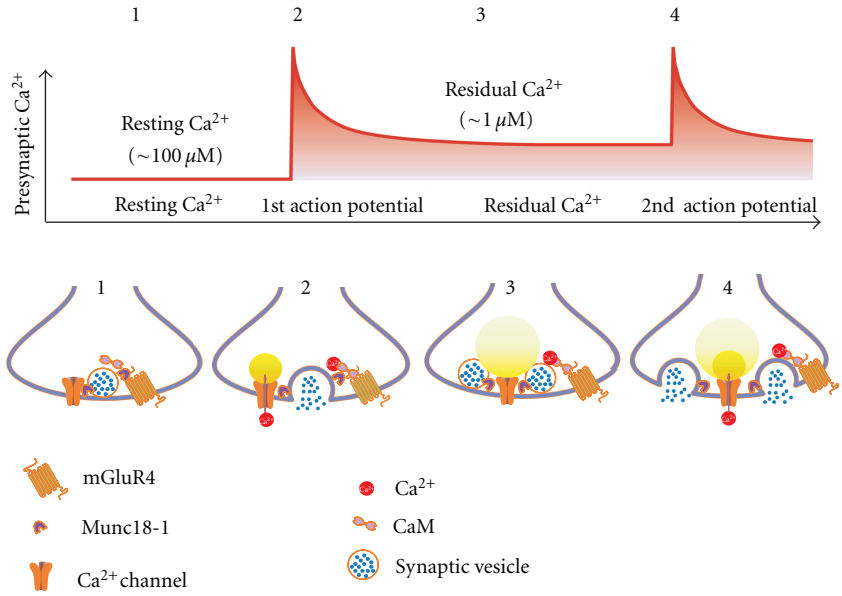

Figure 2: Model illustrating presynaptic short-term facilitation by Ca^{2+}/CaM-mediated Munc18-1 release from mGluR4. Step 1: At the resting Ca^{2+} level, Munc18-1 is sequestered by mGluR4. Step 2: Owing to the low availability of Munc18-1 for SNARE, the initial synaptic vesicle release is small. Steps 2-3: Following the action potential, CaM sensing residual Ca^{2+} liberates Munc18-1 from mGkuR4. Step 4: The higher availability of Munc18-1 for SNARE enables a greater release of synaptic vesicles in response to the second action potential, which is derived in rapid succession. Adapted from Nakajima et al., 2009 [28].

(ct) of group III mGluRs [28, 57]. Because the affinity of CaM for Ca^{2+} [58] corresponds to the required residual Ca^{2+} level for the expression of short-term facilitation, a molecule that can bind to the ct region of group III mGluRs and have an interaction with the receptor that is regulated by Ca^{2+}-activated CaM is a good candidate for mGluR-mediated expression of short-term synaptic facilitation.

3.2. CaM and Munc18-1 Interactions with Presynaptic mGluRs. Munc18-1 is a protein essential for neurotransmission [59] and promotes SNAREs-mediated vesicle fusion [60]. Coimmunoprecipitation and *in vitro* binding experiments indicated that either mGluR4 or mGluR7 directly interacted with Munc18-1 in the brain. Group II mGluRs are also expressed presynaptically and modulate synaptic transmission [53–55]. However, group II mGluRs are localized in the preterminal portions of axons but not in the active zone [55] and do not interact with Munc18-1. Munc18-1 can be divided into three domains [61]; it binds to ct-mGluR4 through domain 1 [28].

CaM binds to the membrane-proximal region of group III ct-mGluRs [57, 62]. Munc18-1 binds to this region of ct-mGluR4; the interaction is disrupted by Ca^{2+}-activated CaM at a concentration of residual Ca^{2+} [44]. Although Munc18-1 does not have an apparent Ca^{2+}-binding motif, the interaction between ct-mGluR4 and Munc18-1 is enhanced with increasing concentrations of Ca^{2+}, with saturation at 3 μM. In the presence of CaM, mGluR4 binds Munc18-1 at the resting Ca^{2+} level of 0.1 μM and releases it above the Ca^{2+} level of 1 μM, corresponding to the residual Ca^{2+} level after an action potential.

3.3. mGluR4-Mediated Regulation of Basal Transmitter Release and Synaptic Facilitation. Function of the mGluR4/Munc18-1 interaction in synaptic transmission is the reduction of basal transmitter release [28]. At synapses of cultured SCG neurons, where mGluR4 is not expressed [63], the injection of mGluR4 peptide, residues 849–889, reduced gradually the amplitude of EPSPs. The time course and extent of inhibition were similar to that caused by the injection of the domain 1 of Munc18-1, suggesting that the mGluR4/Munc18-1 interaction is inhibitory to synaptic transmission.

Generally, a decrease in the initial probability of transmitter release leads to a larger synaptic enhancement or a reduction of synaptic depression [44, 64]. From the above inhibitory role of mGluR4 peptide in synaptic transmission and the operating range of the Ca^{2+}-sensing mechanism, mGluR4C appears to be responsible for the expression of short-term synaptic facilitation; at the very least it reduces short-term synaptic depression. Acutely, PPD, but not PPF, was suppressed in the presence of mGluR4C peptide, suggesting that mGluR4 peptide introduces a facilitating property to these synapses which is apparent when these synapses exhibit PPD. This evidence is consistent with the idea that the absence in the ct region of mGluR4 accounts for the impaired expression of PPF in mGluR4-KO mice [56].

3.4. The Role of Ca^{2+}/CaM, Munc18, and mGluR Interactions in Activity-Dependent Synaptic Transmission. An accumulation of evidence suggests a model for the mGluR4/Munc18-1 interaction in the regulation of synaptic transmission in the brain. When neurons are inactive, Munc18-1 is sequestered by mGluR4; therefore, basal synaptic transmission is kept

low. After the action potential, residual Ca^{2+} activates CaM, which in turn liberates Munc18-1 from mGluR4, causing short-term synaptic facilitation (Figure 2). This Ca^{2+}-sensing mechanism demonstrates a function for mGluR4 in the expression of short-term facilitation and explains the discrepancy between pharmacological blockade and gene targeting of mGluR4, revealed clearly at the parallel fiber synapses onto cerebellar Purkinje cells [52, 56]. Another interesting implication of our findings is that the presence of mGluR4 is responsible for the expression of certain synaptic properties (e.g., mGluR4-positive facilitating parallel fiber synapses and mGluR4-negative depressing climbing fiber synapses onto the same cerebellar Purkinje cells [44, 51, 65]). Although Munc18-1 is known to be an essential protein for synaptic transmission, evidence for both positive and negative roles of Munc18-1 in synaptic transmission has been reported [59]. If Munc18-1 has a negative role in synaptic transmission, then our data suggests that the ct-mGluR4/Munc18-1 interaction augments the inhibitory function of Munc18-1. In this case, disruption of this negative complex by Ca^{2+}/CaM would relieve Munc18-1-mediated inhibition and consequently lead to synaptic facilitation. Thus, no matter whether Munc18-1 had a positive or negative role in synaptic vesicle release, the Ca^{2+}-sensing system would operate in the expression of short-term synaptic facilitation.

4. Conclusion

Neuronal firing activity controls the functions of synaptic proteins and dynamically remodels synaptic efficacy. Ca^{2+}-binding proteins that sense residual Ca^{2+}, which temporally and spatially accumulates in the active zone during trains of action potentials, act as potential effectors for these reactions at the presynaptic terminal. Two pieces of evidence are shown in this article (Figures 1 and 2). First, CaM interacts with a bipartite regulatory site of the $\alpha_1 2.1$ subunit at the mouth of Ca^{2+} channels, senses firing-dependent temporal changes in local and global elevations of Ca^{2+}, and regulates activity of Ca^{2+} channels for SNAREs-mediated synaptic vesicle exocytosis [33, 66]. The CaM regulation of Ca^{2+} channels results in presynaptic depression and facilitation (Figure 1). Secondly, CaM regulates synaptic efficacy by controlling the supply of Munc 18-1 at the active zone (Figure 2). Munc18-1, an essential protein for SNAREs-mediated synaptic vesicle membrane fusion [59], binds to mGluR4 in the active zone [53–55] at the resting state. With elevation of Ca^{2+}, Ca^{2+}/CaM binds to mGluR4 in place of Munc18-1 [57, 62]. The Ca^{2+}/CaM regulation of the mGluR4/Munc18-1 interaction results in presynaptic facilitation. The presynaptic events shown here control synaptic efficacy and encode postsynaptic activity that underlie generation of the synaptic plasticity resulting in regulation of neural circuits.

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