Post-tetanic potentiation involves the presynaptic binding of calcium to calmodulin

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A subset of synaptic vesicles undergoes exocytosis upon Ca^{2+} influx into the presynaptic terminal. This group of vesicles constitutes a readily releasable pool (RRP) of vesicles, which is replenished from a larger reserve pool of vesicles. The RRP is thought to represent the vesicles docked to the nerve terminal membrane at active zones (Fig. 1). During a prolonged depolarizing pulse–mediated stimulation, the RRP can be divided into two separate pools: a fast-releasing pool that recovers slowly, and a slowly releasing pool that recovers quickly (Neher and Sakaba, 2008). The size of an RRP is a key determinant of a synapse's efficacy, or synaptic strength, and fluctuations in pool fullness determine in part the synaptic strength during ongoing stimulation. However, the previous history of synaptic use can alter the size of the RRP. For example, the number of vesicles released by an action potential can increase after repetitive stimulation, via an increase in release probability and/or RRP size (Zucker and Regehr, 2002). Such short-term facilitation and post-tetanic potentiation (PTP) were first recognized in the frog neuromuscular junction and were thought to be due to Ca^{2+} accumulation in the presynaptic nerve terminal (Feng, 1941; Rosenthal, 1969). Recently, an increasing number of studies indicate that diverse signaling mechanisms are involved in the trafficking, docking, and fusion of vesicles (Atwood and Karunanithi, 2002). These mechanisms thus have the potential to regulate short-term plasticity.

The calyx of Held, a giant glutamatergic synapse in the auditory brainstem, is an accessible central nervous system model synapse for studying short-term plasticity because direct patch clamp recordings can be made from the presynaptic terminal and its postsynaptic neuron, the principal cell of the medial nucleus of the trapezoid body. During high-frequency repetitive activity, excitatory postsynaptic currents at the calyx of Held undergo severe frequency-dependent depression (Borst et al., 1995). This short-term depression has a presynaptic origin because it is due to synaptic vesicle pool depletion (von Gersdorff et al., 1997). Interestingly, after a prolonged 5-second train of action potentials at 100 Hz, this synapse also exhibits PTP (Habets and Borst, 2005; Korogod et al., 2005). What is the mechanism underlying PTP? An increase in release probability (Pr), or an increase in RRP, or an increase in both? These two parameters can be modulated independently during PTP as indicated by the finding that a small depolarization of the calyceal terminal (resulting in a submicromolar elevation of basal calcium) can cause a significant increase in Pr with no effect on the RRP size (Awatramani et al., 2005). Likewise, Lee et al. (2008) assessed the contribution of resting Ca^{2+} to the post-tetanic increase in Pr and in the RRP size and found that these two parameters are regulated by two independent mechanisms during PTP in the calyx of Held. Resting calcium and activation of myosin light chain kinase (MLCK; a regulator of myosin activity through phosphorylation) independently mediate the post-tetanic increases in Pr and RRP size, respectively. Collectively, the level of Ca^{2+} increase in the presynaptic terminal can manifest itself via different roles, such as direct action on the presynaptic release machinery or alteration in the trafficking of synaptic vesicles. Several Ca^{2+}-activated proteins serve as regulating elements in the cascade that links electrical activity, elevation of the free intracellular Ca^{2+} concentration, and short-term synaptic enhancements such as facilitation and PTP. Strong evidence has also been presented that PTP is mediated in part by activation of PKC and an increase in the Ca^{2+} sensitivity of exocytosis at the calyx of Held (Korogod et al., 2007), although some PKC inhibitors have no effect on PTP (Lee et al., 2008). In addition, an increase in calcium influx also contributes to PTP at the calyx of Held synapse (Habets and Borst, 2006). The long-lasting increase in presynaptic Ca^{2+} originates from mitochondria, where it builds up during the tetanus (Lee et al., 2008). However, PTP has not been observed with paired pre- and postsynaptic whole cell recordings (Forsythe et al., 1998; Korogod et al., 2005). This has limited further mechanistic studies of PTP. Moreover, the reason for the absence of PTP during presynaptic whole cell recordings has remained a mystery. Remarkably, in this issue, Lee et al. report that by including calmodulin (CaM) in their presynaptic

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recording pipettes, they are able to rescue PTP and thus further study its underlying mechanism.

CaM is a Ca^{2+}-binding protein that can bind to and regulate several different protein targets (most notably ion channels and kinases), thereby affecting many different cellular functions. In the calyx, CaM mediates the rapid recruitment of fast-releasing synaptic vesicles (Sakaba and Neher, 2001). Many of the proteins to which CaM binds are unable to bind Ca^{2+} directly, and as such use CaM as a calcium sensor and signal transducer. First, Ca^{2+} must bind to CaM and then CaM can bind to its multiple target proteins. This binding will activate, for example, MLCK, which may go on to phosphorylate the myosin light chain. This will enable the myosin cross-bridge to bind to the actin filament and allow vesicle mobility (Fig. 1). Thus, CaM may control or modulate the traffic of synaptic vesicles at the calyx of Held.

Surprisingly, MLCK inhibition by ML-9, ML-7, and wortmannin has been shown to augment the pool of fast-releasing vesicles under low-frequency action potential stimulation conditions, or under short trains of action potential–stimulating conditions, at the mouse calyx of Held (Srinivasan et al., 2008), a finding that is now confirmed by Lee et al. (2010) in the rat calyx of Held. But in cultured sympathetic neurons, MLCK inhibitory peptide and wortmannin inhibited synaptic transmission (Mochida et al., 1994). Lee et al. (2010) have used a deconvolution method to discriminate the role of the fast RRP and slow RRP in PTP, and they find that MLCK activation is necessary for PTP. These seemingly paradoxical findings may reveal differential roles of MLCK depending on the stimulus paradigm used to elevate presynaptic Ca^{2+} levels. Such differential effects were also observed in smooth muscles where the velocity of the movement of actin filaments decreased with increasing MLCK concentration, but when incubated with Ca^{2+} and CaM, MLCK was no longer inhibitory, but slightly stimulatory (Kohama et al., 1996). Because MLCK is composed of actin binding, kinase- and myosin-binding domains, one could speculate distinct roles assigned to different domains. So it will be important to determine with greater certainty the role of different inhibitors such as ML-7, ML-9, wortmannin, and MLCK-inhibitory peptides with respect to their site of action. In smooth muscle, the roles of independent domains of MLCK have been addressed to some extent. Such studies are needed in the presynaptic terminals to pinpoint the

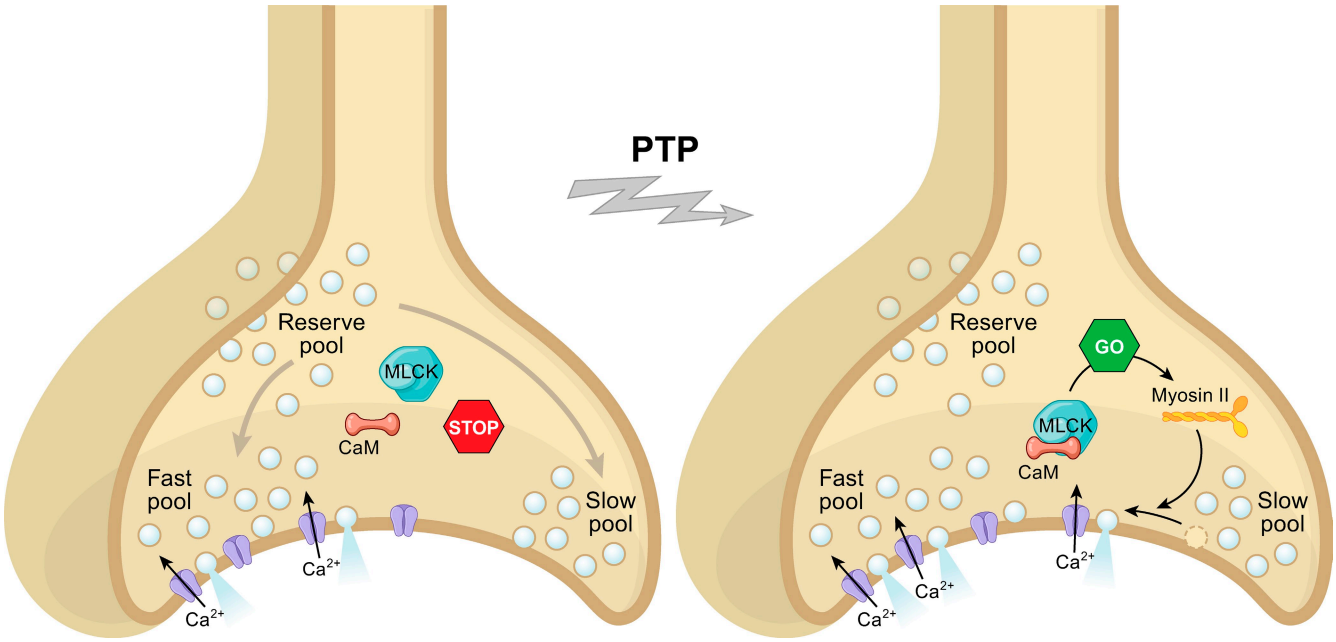

Figure 1. PTP and the RRP of synaptic vesicles at the calyx of Held nerve terminal. The calyx of Held presynaptic nerve terminal has two types of RRPs of vesicles: a fast-releasing pool that recovers slowly from pool depletion and a more slowly releasing pool of vesicles that recovers quickly from vesicle pool depletion. The fast-releasing pool of vesicles probably constitutes those vesicles docked at the plasma membrane that are primed for exocytosis and also tightly coupled to Ca^{2+} channels, whereas the slowly releasing pool of vesicles may constitute docked vesicles that are located at larger distances from Ca^{2+} channels (loosely coupled vesicles). Under low-frequency stimulation conditions, the free Ca^{2+} concentration level in the nerve terminal may not reach sufficiently high levels for the activation of significant amounts of CaM and MLCK. However, after a prolonged (4–5-s) and high-frequency (100 Hz) action potential tetanic stimulation of the calyx nerve terminal, high free Ca^{2+} concentration levels apparently activate significant amounts of CaM, which then activates MLCK. Now MLCK can activate myosin II, a molecular motor that may drive the translocation of vesicles in the slowly releasing pool toward locations where they are more tightly coupled to Ca^{2+} channels. The size of the fast-releasing pool of vesicles is thus increased at the expense of the slowly releasing pool size. The amplitude of the excitatory postsynaptic current is thereby potentiated.

role of MLCK under various conditions. In addition, studying the role of other CaM-dependent proteins in the presence of active CaM will shed light onto its regulation of synaptic transmission. The study of Lee et al. (2010) thus implies that the function of MLCK is differentially modulated under normal stimulating conditions and during PTP in the calyx of Held. Finally, Lee et al. (2010) suggest that prolonged presynaptic Ca^{2+} elevation in the presence of CaM during tetanic stimulation activates MLCK and then myosin II, which in turn may facilitate the translocation of slowly releasing synaptic vesicles toward the fast RRP of docked synaptic vesicles (Fig. 1).

Given the perennial uncertainties of specificity for certain pharmacological drugs (like ML-9 or ML-7) and for small peptides (like the MLCK-inhibitory peptide), a future conditional knockout of neuronal MLCK at the mouse calyx of Held would be very useful in certifying that MLCK is indeed involved in PTP and vesicle trafficking. In conclusion, the new studies of Lee et al. (2010) clearly show that CaM, as a Ca^{2+}-dependent protein activator, has multiple functions in short-term plasticity, depending on the calcium levels in the presynaptic terminal.

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