

CALCIUM AND SHORT-TERM SYNAPTIC PLASTICITY

ROBERT S. ZUCKER

Molecular and Cell Biology Department, University of California, Berkeley, CA 94720, U.S.A.

ABSTRACT

The roles of Ca in phasic transmitter release and short-term synaptic plasticity are reviewed. An action potential evokes phasic release solely by elevating presynaptic Ca concentration locally and briefly to high levels (about 100 μ M) at release sites. Multiple Ca ions bind rapidly to a low affinity site near Ca channels to trigger exocytosis of neurotransmitter-containing vesicles. The localized action of Ca near channel mouths accounts for the lower apparent stoichiometry of Ca action when spikes are broadened or depolarizations increased than when external Ca concentration is increased. At crayfish neuromuscular junctions, several forms of short-term plasticity depend on the action of residual Ca in nerve terminals following conditioning stimulation. Augmentation and potentiation are due to residual Ca acting with slow kinetics (300 ms) at a high affinity site to enhance release to action potentials, while facilitation is due to residual Ca acting at a different site with much faster kinetics. The durations of these processes reflect the time that residual Ca persists at these sites of action after conditioning activity. At mammalian cortical synapses, postsynaptic Ca activates long-term potentiation within a time period of about 2 s.

Experiments on mechanisms underlying synaptic plasticity must be interpreted in terms of a model for the mechanism of transmitter release. Therefore, this paper begins by an outline of our understanding of the role of calcium in synaptic transmission.

A Rise in Internal Calcium is Needed to Evoke Transmitter Release

In the nineteenth century, Sydney Ringer showed that the vagus ceased its inhibition of the frog heart when calcium was omitted from the bathing medium, thus establishing that synaptic transmission requires external calcium. Katz and Miledi (15) showed that, to be effective, calcium must be present during presynaptic depolarization of frog neuromuscular junctions, suggesting that calcium enters terminals during action potentials to act internally. This idea was strengthened by the observation (16) that strong depolarization delayed transmitter release until the end of the pulse, as if calcium influx could be retarded by

potentials more positive than the equilibrium potential for this ion. This indicated that release was tied to calcium influx rather than depolarization *per se*. Llinás and coworkers (20) showed a close association between presynaptic calcium influx at the squid giant synapse, measured by voltage clamping the presynaptic terminal, and transmitter release measured as the excitatory postsynaptic potential (EPSP), even during the 'off-EPSP' at the end of a large presynaptic depolarization.

Calcium may not Act Alone

However, certain problems surrounded the hypothesis that calcium acts alone to cause secretion of neurotransmitter. Our measurements of presynaptic calcium concentration ($[Ca^{2+}]_i$) at the squid giant synapse using the colorimetric dye arsenazo III (6) revealed that the rise in average presynaptic $[Ca^{2+}]_i$ lasts for seconds following presynaptic action potentials, while the release of transmitter lasts only a couple milliseconds.

Similar measurements were obtained with the fluorescent dye fura-2 at crayfish neuromuscular junctions (10). When the external calcium concentration ($[Ca^{2+}]_e$) was reduced sufficiently to block transmitter release at neuromuscular junctions, transmission to spikes was restored when $[Ca^{2+}]_i$ was elevated by photolysis of the caged calcium chelator nitr-5, which had been injected into motor nerve terminals (12). This suggested that action potentials could evoke phasic transmitter release in the presence of a slightly elevated $[Ca^{2+}]_i$ that by itself was insufficient to do more than raise the frequency of miniature EPSPs. It was as if both calcium and depolarization were needed for phasic transmission.

Calcium could Act Alone

Meanwhile, a number of recent observations indicated that local high $[Ca^{2+}]_i$ transients at sites of transmitter release could cause phasic release: 1) Robitaille *et al.* (28) labelled presynaptic calcium channels at frog neuromuscular junctions and showed that they were clustered exclusively at transmitter release sites. 2) The brief 200 μ s delay between the opening of presynaptic calcium channels and the beginning of transmitter release (20) showed that calcium must act very near channel mouths if it alone evokes release. 3) Simulations of presynaptic calcium diffusion (33) predicted that $[Ca^{2+}]_i$ reaches very high levels (about 100 μ M) for only a very brief time (a few milliseconds) after an action potential. 4) Presynaptic injection of the slow calcium buffer EGTA had little effect on synaptic transmission at squid giant synapses (30) and crayfish neuromuscular junctions (8), indicating that calcium must bind quickly to its receptor and reach equilibrium during an action potential. 5) Because transmitter release increases on increasing $[Ca^{2+}]_e$ (11), the calcium binding site is not saturated by the high local level of $[Ca^{2+}]_i$ and so must have a low affinity for calcium. 6) Only high levels of fast buffers injected presynaptically block transmitter release (1), indicating that the effective $[Ca^{2+}]_i$ transient must reach quite high levels (100 μ M or more). 7) Release depends quite cooperatively on $[Ca^{2+}]_i$ when this is altered by changing $[Ca^{2+}]_e$ (36). 8) Simulations of a model for calcium ions diffusing from clusters of calcium channels to vesicles docked at release sites and acting cooperatively to trigger secretion

(33) replicate the observed time course of transmitter release at fast synapses. These observations showed that local intense changes of $[Ca^{2+}]_i$ near calcium channel mouths, called 'Ca domains', could account for phasic transmitter release without the need to invoke another factor activated by membrane potential, and would still permit measured average presynaptic $[Ca^{2+}]_i$ transients to greatly outlast transmission.

Calcium does Act Alone

That presynaptic calcium alone triggers transmitter release was shown in a number of preparations: 1) At inhibitory cholinergic synapses between cultured neurons of the fresh water snail *Helisoma*, presynaptic membrane potential and $[Ca^{2+}]_i$ were controlled independently by voltage clamp and use of 'caged calcium' chelators respectively (37). A rise in presynaptic $[Ca^{2+}]_i$ was needed to release transmitter, while varying presynaptic potential was without effect when calcium influx was blocked in a calcium-free medium. 2) Similar techniques and procedures were used at the squid giant synapse (9) to show that action potentials were without effect on calcium-evoked release. 3) Injecting the calcium indicator fura-2 presynaptically, Mulkey and Zucker (24) found that the low- $[Ca^{2+}]_e$ solutions used by Hochner *et al.* (12) did not fully block calcium influx or transmitter release during action potentials. When a truly zero- $[Ca^{2+}]_e$ medium was employed, influx was fully blocked, and then action potentials had no effect on transmitter release evoked by elevating $[Ca^{2+}]_i$ by photolysis of the presynaptically injected 'caged calcium' chelator DM-nitrophen. 4) Partial flash photolysis of this substance evokes a $[Ca^{2+}]_i$ 'spike' when the chelator is partially loaded with calcium, due to the rapid photolysis kinetics but slow rebinding of released calcium to unphotolyzed DM-nitrophen (34). Such a 'calcium spike' evokes phasic transmitter release without changes in presynaptic potential at squid giant synapses (9) and crayfish neuromuscular junctions (35).

Other Consequences of Transmitter Release by Local 'Calcium Domains'

This view of how synaptic transmission works helps explain a number of otherwise curious observations: 1) When calcium influx during

action potentials is increased by raising $[Ca^{2+}]_e$, transmitter release increases very nonlinearly with calcium influx, due to the cooperativity of calcium action at release sites. 2) Similar results are obtained when the 'calcium spike' evoked by flash photolysis of DM-nitrophen is increased (18). 3) Broadening presynaptic action potentials with potassium channel blockers leads to a much shallower relationship between transmitter release and calcium influx (3, 36). This is probably because prolonged action potentials open additional calcium channels after the first ones to open begin to close. This has the effect of recruiting additional 'calcium domains', with less of an increase in the $[Ca^{2+}]_i$ at individual release sites. 4) For the same reason, increasing calcium influx by increased depolarization also generates a shallower relation between transmitter release and total calcium influx than when $[Ca^{2+}]_e$ is changed (4). 5) Only the 'calcium spike' obtained on photolyzing DM-nitrophen evokes phasic transmitter release (36); the more moderate step in $[Ca^{2+}]_i$ on photolyzing nitr-5 only causes a modest increase in MEPP frequency (12).

Augmentation and Potentiation Correlate with Residual $[Ca^{2+}]_i$

If this view of synaptic transmission is accepted, it may be used as a foundation on which to build an understanding of short-term synaptic plasticity. During a train of action potentials, the amount of transmitter released by each spike increases with several time constants. The fastest occur within tens and hundreds of milliseconds to a second, and are called fast and slow components of facilitation. Next comes augmentation, which takes many seconds. Finally, if the tetanus lasts long enough and is of sufficiently high frequency, there is potentiation, which grows over a period of a few minutes. Test pulses after the train show an enhanced release that decays with the same combination of rates.

Some time ago, we showed (6) that injecting calcium presynaptically at the squid giant synapse increases spike-evoked release (as well as MEPP frequency), but it is unclear which of the above processes are being activated. Measurements of the decay of residual $[Ca^{2+}]_i$ after a tetanus show a close linear correlation to the decay of potentiation (or post-tetanic potentiation: PTP) (10). When shorter trains are used, a

similar correlation holds for augmentation (7), even when calcium removal by transport pumps is showed by lowering temperature or injecting calcium buffers presynaptically. A simple model of synaptic enhancement holds that it is a consequence of calcium cooperativity at the site triggering secretion and the summation of calcium influx with residual calcium (26, 38). However, in conflict with our understanding of transmitter release outlined above, this model of synaptic enhancement predicts that the peak $[Ca^{2+}]_i$ at a release site reaches less than a micromolar in an action potential (10). The linear relationship between PTP or augmentation and residual $[Ca^{2+}]_i$ also contradicts the model of simple summation of entering and residual calcium acting at one site. If $[Ca^{2+}]_i$ reaches peaks of 100 μM , this model predicts from the measured level of residual $[Ca^{2+}]_i$ far less augmentation or PTP than are actually observed. This suggests that residual calcium acts at a site separate from that causing exocytosis in generating these processes.

Sodium Ions and Potentiation

Sodium ions also accumulate presynaptically in a tetanus (K. Delaney, unpublished), and are pumped out slowly afterwards. Sodium ions also affect PTP, which is enhanced and prolonged when Na^+/K^+ exchange is blocked (2, 27). Mulkey and Zucker (25) showed that $[Na^+]_i$, elevated by tetanic stimulation does not release calcium from internal stores nor reverse the Na^+/Ca^{2+} exchange, but rather slows the extrusion of accumulated intracellular calcium to enhance and prolong potentiation.

Calcium is Also Involved in Facilitation

Working on frog neuromuscular junctions, Katz and Miledi (17) showed that external calcium must be present not only for an action potential to evoke transmitter release, but also for a spike to facilitate subsequent release. At the squid giant synapse, we demonstrated that facilitation occurs without an increase in presynaptic calcium influx or enhancement of the $[Ca^{2+}]_i$ transient in a spike (6). Simulations of calcium diffusion and transmitter release (33) showed that the high levels of facilitation observed could not be due entirely to residual calcium summing with entering calcium at the site of exocytosis, and

proposed that calcium acts locally at a separate site to facilitate release. This might be the same site involved in augmentation and potentiation, or a different one.

Calcium Binding Sites in Short-term Synaptic Plasticity

EGTA injection prevents augmentation at the squid giant synapse (30; but see ref. 31, for conflicting results using BAPTA-AM at frog neuromuscular junction). EGTA and BAPTA injection reduce facilitation at frog and crayfish neuromuscular junctions (8, 13, 31). These results are consistent with a presynaptic calcium requirement for these processes, although the slowing of facilitation by diffusible buffer (29) is not expected if residual free $[Ca^{2+}]_i$ sums with entering calcium at the exocytosis trigger to facilitate release.

Recent experiments by Dr. Haruyuki Kamiya in my laboratory (14) have addressed the question of the kinetics of calcium binding to sites generating augmentation and facilitation. Using the photolysable calcium chelator diazo-2, we found that augmentation was eliminated by sudden chelation of residual calcium after a tetanus. This effect takes about 500 ms, indicating either slow equilibration of calcium with the site controlling augmentation, or the involvement of additional second messenger or enzymatic processes, perhaps calmodulin, Ca/CaM kinase II, and synapsin I (19). Facilitation, on the other hand, was eliminated rapidly (within 10 ms) by sudden chelation of residual calcium, which had little effect on baseline levels of spike-evoked release. We also looked at the effects of reversibly elevating $[Ca^{2+}]_i$ by steady photolysis of the caged calcium compound DM-nitrophen (34). Spike-evoked release was enhanced during the light pulse, probably reflecting a mixture of the processes underlying facilitation and augmentation. Following reversal of the elevation of $[Ca^{2+}]_i$, spike-evoked release returned to control levels only after about 500 ms, apparently reflecting decay of the augmentation process.

Since both facilitation and augmentation are dependent on micromolar levels of residual $[Ca^{2+}]_i$, they are likely to be activated by high affinity calcium binding sites different from the low affinity site triggering exocytosis. Equilibration with the facilitation site occurs in milliseconds, but activation of the augmentation site

takes almost 1 second, so these are apparently two distinct sites. The normally longer durations of augmentation and potentiation (tens of seconds to minutes) are due to the persistence of posttetanic $[Ca^{2+}]_i$, and are determined by the processes regulating calcium removal.

Long-term Potentiation, Another Calcium-Activated Synaptic Enhancement

At many mammalian brain synapses, a brief high frequency tetanus evokes a very long lasting (hours to days) enhancement of synaptic transmission that requires postsynaptic depolarization and calcium entry (5). This process is best studied at synapses onto hippocampal CA1 pyramidal cells, where calcium influx through NMDA receptors is involved, and it appears to be mechanistically unrelated to so-called long-term potentiation or long-lasting facilitation in sympathetic ganglia and neuromuscular junctions (10, 23, 32). Using nitr-5, we showed (21) that LTP is blocked by postsynaptic calcium chelation, and can be induced by elevating postsynaptic $[Ca^{2+}]_i$. Recently, we used diazo-4 to determine that the minimum duration of a postsynaptic $[Ca^{2+}]_i$ rise needed to establish LTP is about 2-3 seconds (22), presumably long enough for a calcium-dependent kinase to phosphorylate a protein in the cascade of events initiating LTP.

Calcium is a major player in many forms of short-term and long-term synaptic plasticity. The next problem to be tackled is the identification of the molecular targets of calcium action. Hopefully these kinetic characterizations of these sites will help narrow down the search.

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