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# The Role of Postsynaptic Calcium in the Induction of Long-Term Potentiation

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## Abstract

Long-term potentiation (LTP), a long-lasting, activity-dependent increase in the strength of synaptic transmission, is one of the most intensively studied forms of synaptic plasticity in the mammalian brain. In the CA1 region of the hippocampus, the induction of LTP is likely to require a rise in postsynaptic calcium levels. The main source for this calcium is influx through the NMDA receptor ionophore, although other potential sources include voltage-dependent calcium channels and release from intracellular stores. Dendritic spines, the sites of synaptic contact, may function to isolate and amplify synaptically mediated increases in postsynaptic calcium. Recent evidence indicates that the magnitude of postsynaptic calcium increase is a critical variable controlling the duration of synaptic enhancement. Although a number of calcium-dependent biochemical processes have been implicated in LTP, determining their exact role remains a challenging experimental problem.

**Index Entries:** Long-term potentiation (LTP); short-term potentiation (STP); *N*-methyl-D-aspartate (NMDA); calcium; CA1; hippocampus; voltage-dependent calcium channel; intracellular store.

## Introduction

Activity-dependent changes in the strength of synaptic transmission are likely to play important roles as cellular substrates for learning and memory. Long-term potentiation (LTP), originally described in the dentate gyrus in vivo (Bliss and Lømo, 1973; Lømo, 1966), has become the most intensively studied and well understood model for such activity-dependent synaptic enhancements. This brief article will focus on the experimental evidence supporting a role for postsynaptic calcium in the induction of LTP at the synapse between Schaffer collateral/commissural fibers and CA1 pyramidal cells.

## Postsynaptic Calcium Is Required for LTP Induction

It is well accepted that the induction of LTP in the CA1 region of hippocampal slices requires activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors by synaptically released glutamate during concomitant postsynaptic depolarization. The evidence in support of this hypothesis has been the subject of numerous reviews (Gustafsson and Wigström, 1988,1990; Madison et al., 1991; Malenka et al., 1989; Nicoll et al., 1988) and will not be discussed here. The first direct evidence supporting a role for postsynaptic calcium in the induction of LTP came from an experiment in which it was demonstrated that injection of the calcium chelator, EGTA, into postsynaptic cells prevented the generation of LTP (Lynch et al., 1983). Later work replicated this finding using the more rapid and potent calcium chelator, Nitr-5 (Malenka et al., 1988). If a rise in postsynaptic calicum is required for the generation of LTP, then it should be possible to enhance synaptic transmission by directly increasing postsynaptic calcium. It is technically quite difficult to inject calcium from an intracellular recording electrode and have it spread throughout the cell. However, an alternative way of increasing calcium was accomplished taking advantage of the special properties of Nitr-5. Nitr-5 is a photolabile, "caged" calcium compound that, when exposed to UV light, undergoes photolysis, resulting in a 40-fold decrease in its affinity for calcium. Thus calcium-loaded Nitr-5 can serve as a source for calcium. Injection of calcium-loaded Nitr-5 into CA1 pyramidal cells and subsequent photolysis did in fact produce a potentiation of synaptic transmission (Malenka et al., 1988).

The aforementioned results are consistent with the hypothesis that an increase in postsynaptic calcium level is required for LTP induction. However, it is conceivable that the calcium chelating agents lowered ambient calcium levels and that this affected biochemical processes critical for the generation of LTP. In addition, the potentiation of synaptic transmission produced by photolysis of calcium-loaded Nitr-5 was not demonstrated to be the same as physiologically induced LTP. Nevertheless, these experiments constitute reasonably strong evidence for a role of postsynaptic calcium in LTP.

## Sources for Postsynaptic Calcium Increases

### *NMDA Receptors*

Assuming that a rise in postsynaptic calcium is necessary for LTP generation, an obvious and important question is: What structures provide the requisite increase in postsynaptic calcium? Much experimental work suggests that the most likely source is influx of calcium through the NMDA receptor ionophore. It is clear that activation of NMDA receptors is required for LTP induction (Collingridge et al., 1983) and that the NMDA receptor ionophore is permeable to calcium (Ascher and Nowak, 1988; Jahr and Stevens, 1987; Mayer et al., 1987). Calcium imaging studies in isolated CNS neurons (MacDermott et al., 1986; Mayer et al., 1987) and more recently in the

CA1 region of hippocampal slices (Regehr and Tank, 1990) have also shown that calcium flux through the NMDA channel can cause significant elevations of intracellular calcium. More direct evidence demonstrating that calcium influx is required for LTP comes from experiments examining the effects on LTP of manipulating membrane potential during the induction protocol. Since the calcium influx through the NMDA channel can be decreased at positive voltages that reduce the electrochemical gradient for calcium entry (Mayer et al., 1987), strongly depolarizing the cell during LTP induction should prevent or reduce the magnitude of calcium influx. Such manipulations were found to inhibit the generation of LTP (Malenka et al., 1988; Perkel et al., 1991).

## Voltage-Dependent Calcium Channels

Although the NMDA receptor ionophore may provide all the calcium required for LTP induction, another source for increasing intracellular calcium levels is voltage-dependent calcium channels (VDCCs). It is clear that hippocampal pyramidal cell dendrites contain VDCCs (Jones et al., 1989; Westenbroek et al., 1990) and that like NMDA receptors, their activation can result in significant increases in intradendritic calcium level (Regehr et al., 1989). Recently it has been reported that, in the presence of the NMDA receptor antagonist, APV, strong tetanic stimulation (Grover and Teyler, 1990) or application of potassium channel blockers (Aniksztejn and Ben-Ari, 1991) can produce long-lasting enhancements of synaptic transmission. Both these forms of synaptic enhancement could be reduced or blocked by pharmacological agents that block VDCCs. In one study (Grover and Teyler, 1990), it was also reported that injection of the calcium chelator, BAPTA, into postsynaptic cells prevented the tetanus-induced, APV-insensitive synaptic potentiation. Both studies appropriately conclude that calcium influx through VDCCs may be important for LTP induction.

If VDCCs provide calcium, which can activate processes leading to synaptic enhancement, it should be possible to activate these channels directly and observe an increase in the recorded EPSPs. The first attempt at performing this experiment resulted in no discernable change in synaptic transmission (Malenka et al., 1989). However, using a different stimulation protocol, it has recently been found that repetitive depolarization of CA1 cells in the absence of synaptic stimulation can produce a transient potentiation of synaptic transmission (Perkel et al., 1991).

It therefore appears likely that calcium influx through VDCCs can, under some circumstances, result in an increase in synaptic efficacy. A critical question remains, however: Is this source of calcium required for the generation of "normal" physiologically induced LTP? One possibility is that during normal LTP induction, influx of calcium through VDCCs is not necessary, but under unusual experimental circumstances, this calcium can overcome endogenous buffering mechanisms and activate biochemical processes normally activated by calcium entering through NMDA channels. Alternatively, calcium entry via VDCCs could activate distinct processes, resulting in a form of LTP mechanistically different from NMDA receptor-dependent LTP. More experimental work should be able to determine which alternative is correct.

VDCCs in CA1 pyramidal cells have also been suggested to play an important role in a completely different form of synaptic plasticity, known as heterosynaptic long-term depression (Wickens and Abraham, 1991; Lisman, 1989). This suggestion is difficult to reconcile with the previous results indicating a role for VDCCs in causing synaptic potentiation, although it is conceivable that different populations of VDCCs are involved or that there are different calcium thresholds for producing synaptic depression vs synaptic potentiation (Lisman, 1989).

### Intracellular Stores

Calcium can also be increased in neurons by release from at least two intracellular stores: one activated by inositol 1,4,5-trisphosphate ($IP_3$) (Mayer and Miller, 1990) and the other a caffeine-sensitive store that may correspond to a brain ryanodine receptor (McPherson et al., 1991). The role that these intracellular sources of calcium play in LTP is unclear, although it has been reported that dantrolene-Na, a compound that in other preparations can inhibit calcium release from sarcoplasmic reticulum, prevents LTP induction (Obenaus et al., 1989).

## Role of Dendritic Spines

Excitatory synapses on hippocampal pyramidal cells are formed on dendritic spines which are small protuberances that are connected to the main dendritic shaft through a thin neck (Harris and Stevens, 1989). Because of the experimental inaccessibility of dendritic spines and their likely importance for synaptic plasticity, several groups have developed biophysical models to predict the consequences of synaptic activation on intraspinous calcium levels (Gamble and Koch, 1987; Holmes, 1990; Holmes and Levy, 1990; Wickens, 1988; Zador et al., 1990). The essential conclusions from these models are that spines may serve both to isolate and amplify synaptically mediated calcium increases. Preventing a calcium increase in one spine from significantly influencing adjacent synapses is important in accounting for the synapse specificity of LTP. The amplification of the modest calcium influx is owing to the small volume of spines and may be important for activating calcium-dependent biochemical processes. All these models demonstrate that the final concentration of calcium achieved during synaptic activation and the duration of the elevation depend on many factors, including the concentration and localization of endogenous buffers, the rate of sequestration into intracellular pools, the rate of extrusion of calcium by membrane pumps, and the rate of passive diffusion into the main dendritic shaft, which may be impeded by the narrow spine neck.

A critically important feature of dendritic spines for controlling calcium levels is the exact localization of ion channels. Several years ago, it was proposed that NMDA and non-NMDA receptors may be colocalized on spines (Wigström and Gustafsson, 1985), a suggestion supported by recent evidence from cultured cells (Bekkers and Stevens, 1989). Having NMDA receptors localized to spines is an attractive hypothesis, because it would provide each spine with a private source of calcium that could only be activated by synaptically released glutamate and not by nonspecific dendritic depolarization. However, it is still unknown whether VDCCs are found on or adjacent to spines, and whether calcium through these channels can access intraspinous biochemical processes.

## Calcium Levels May Control the Duration of LTP

A number of physiological stimuli can result in a transient, decremental synaptic enhancement sometimes known as short-term potentiation (Anwyl et al., 1989; Larson et al., 1986; Malenka, 1991; Racine and Milgram, 1983). A similar decremental synaptic potentiation is elicited by iontophoretic application of NMDA (Collingridge et al., 1983; Kauer et al., 1988) or by applying LTP-inducing stimuli in the presence of kinase inhibitors (*see* Malenka and Nicoll, 1990 for review of these studies). Recent work has demonstrated that the magnitude of the calcium influx through the NMDA receptor ionophore may be a critical variable controlling whether STP or LTP is elicited (Malenka, 1991). Specifically, it was proposed that stable LTP requires a critical "threshold" level of postsynaptic calcium and that, if smaller increases in calcium are generated, STP (but not LTP) results. The higher threshold level of calcium may activate processes not activated by lower calcium concentrations. Alternatively, since changes in calcium level can be greatly amplified

by calcium-dependent proteins (e.g., calmodulin; *see* Gamble and Koch, 1987), the stabilization of LTP may require activation of a threshold concentration of some calcium-dependent process.

## Calcium-Dependent Biochemical Processes

If a rise in postsynaptic calcium is the critical trigger for generating LTP, what are the specific biochemical processes activated by this calcium? This topic has also been the subject of numerous recent reviews (Lynch et al., 1990; Madison et al., 1991; Malenka and Nicoll, 1990) and will not be disussed in detail here. Suffice it to say that the standard approach has been to apply, either extracellularly or intracellularly via an intracellular recording electrode, agents that specifically inhibit the activity of one or more enzymes and determine whether LTP can be generated. Experiments taking this approach have implicated a number of calcium-dependent proteins, including (but not limited to) calmodulin, calcium/calmodulin-dependent protein kinase II, calpain, and protein kinase C.

Although the results of such studies are consistent with the hypothesis that the inhibited protein is critically important for LTP induction, these studies all have significant limitations in terms of proving that the rise in calcium activates a specific biochemical process and that this activation is necessary for LTP. One limitation is the lack of specificity of the applied inhibitory agent at the concentrations required to block LTP in a slice preparation. Perhaps a more important limitation is that all of the proteins studied thus far likely play important roles in a variety of normal cell functions. Thus, inhibiting their activity could affect other "downstream" processes, any number of which may be important for the generation of LTP. Thus at this point, there are many promising candidates for calcium-dependent proteins that play roles in LTP, but ferreting out the ones activated by the rise in calcium remains a challenging experimental problem.

## Summary

In this article, I have attempted briefly to summarize the current state of knowledge of the role that postsynaptic calcium plays in the induction of LTP. It seems likely that a rise in calcium is a necessary, and perhaps sufficient, trigger for LTP and that at least one important source for this calcium is the NMDA receptor localized to dendritic spines. The importance for LTP of calcium entry via VDCCs or release of calcium from intracellular stores will require more definitive experimental work. The most challenging problem remains the elucidation of the biochemical processes activated by calcium and required for LTP.

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