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Short reviews

## Long-term potentiation as synaptic dialogue

Patricia A. Colley and Aryeh Routtenberg

Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60208 (USA)

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We have proposed a testable model of the physiological and biochemical events underlying LTP that offers the following novel features. (1) The focus is not on a single mechanism or synaptic site, but rather on the integration and interaction of mechanisms occurring on both sides of the synapse. (2)  $\beta$  PKC plays a critical presynaptic role in LTP, while  $\gamma$  PKC functions postsynaptically. (3) These stages can be ordered in a time-delimited sequence of post- then presynaptic molecular events based on the period of effectiveness of inhibitor compounds. (4) The distinction is made between the time when kinase activation occurs and the time when the potentiated response requiring this kinase activation is observed.

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### 1. INTRODUCTION

Current hypotheses concerning the mechanisms of long-term potentiation (LTP) and related forms of synaptic enhancement have typically localized the critical cellular processes to either a presynaptic<sup>6,7,57</sup> or postsynaptic<sup>34,65</sup> locus. In the present review we propose a model of LTP that explicitly requires the participation of both presynaptic and postsynaptic mechanisms as well as ongoing communication between the

two sides<sup>85</sup>. We have focussed on the effects of kinase inhibitors to emphasize the necessity of particular molecular events and to reveal the time frame when they are essential.

A growing body of evidence suggests that protein kinases play a critical role in the regulation of LTP<sup>47,53,55,56,79,81</sup>. Since there is converging evidence from several laboratories that points to a necessary role for protein kinase C (PKC) in persistent synaptic enhancement<sup>1,2,23,36,42,59,72,86,88</sup> we have centered our

attention on this kinase. In later sections we discuss the role of other kinases, their indirect regulation by PKC as well as kinase-independent events.

In what follows, the sequence of events outlined in the model (Fig. 1) are described.

**2. PRESYNAPTIC Ca<sup>2+</sup> INFLUX (EVENT 1) LEADS TO POST-TETANIC POTENTIATION (EVENT 2)**

LTP induced by tetanic stimulation<sup>8,9</sup> overlaps in time with an increase in synaptic response due to Ca<sup>2+</sup>

influx triggered by depolarization of presynaptic terminals. This rapid enhancement termed post-tetanic potentiation (PTP) is PKC-independent, i.e., is not blocked by PKC inhibitors. PTP is thought to be mediated by an increase in neurotransmitter mobilization<sup>62</sup>.

**3. ACTIVATION OF POSTSYNAPTIC RECEPTORS (EVENT 3)**

Glutamate activation of the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and *N*-methyl-

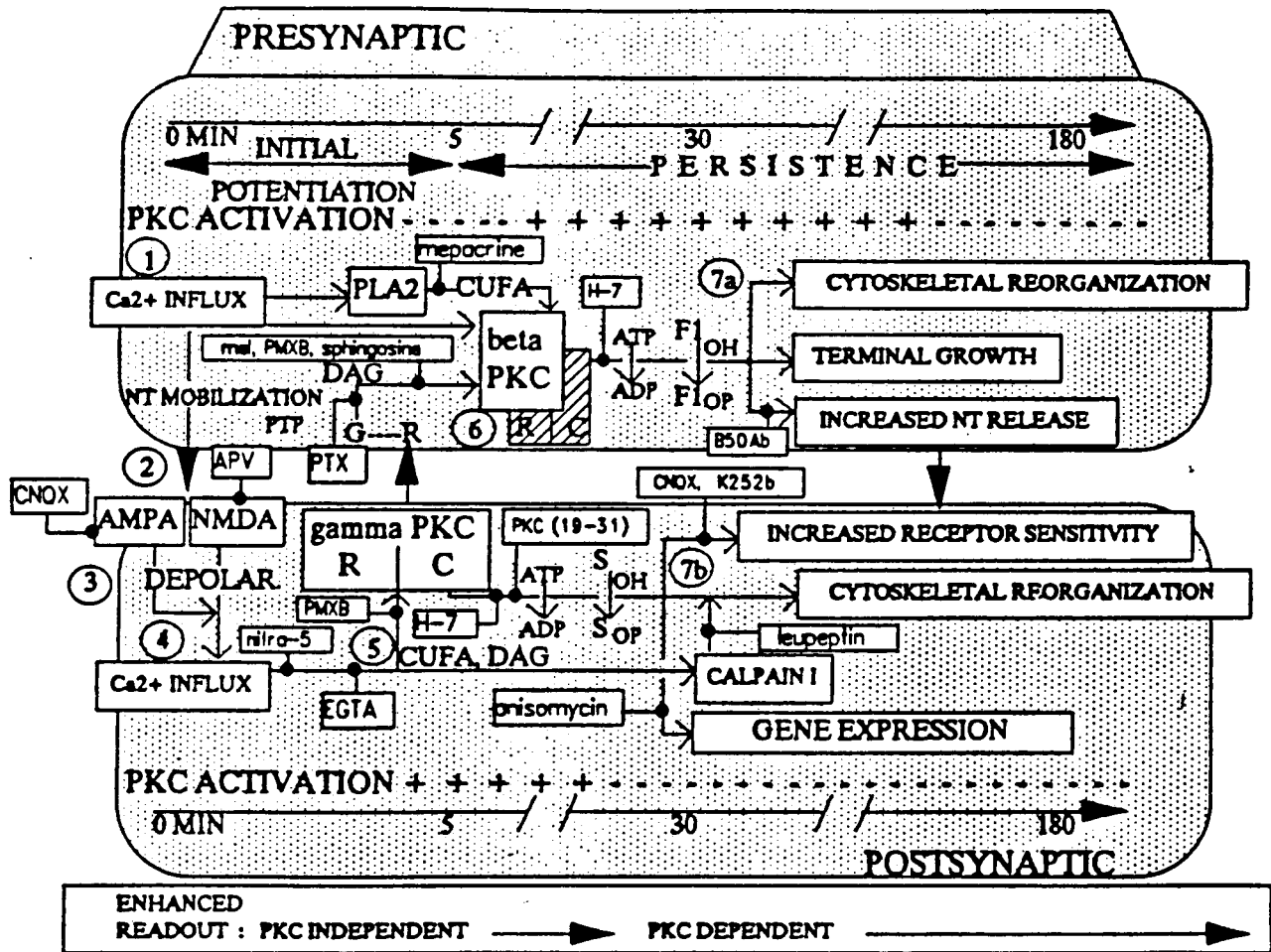


Fig. 1. Long-term potentiation (LTP) requires the sequential occurrence of events on both sides of the synapse. Enzymes regulating the events important for enhanced readout are highlighted in green. The events themselves are highlighted in purple. Inhibitors which block LTP are printed in red in a white box and the arrow leading to the mechanism inhibited is 'blocked' by a red dot. Note that the model portrays LTP mechanisms in the context of both space (synaptic location) and time (sequence) revealing that at certain time points in the sequence both presynaptic and postsynaptic events necessary for LTP are occurring simultaneously. Nonetheless, for expository purposes we have provided a sequence of numbered events. LTP is initiated by high frequency stimulation of presynaptic afferents triggering an influx of Ca<sup>2+</sup> into the presynaptic terminal (Event 1). An increase in NT mobilization, underlies the short-lasting PTP. Activation of postsynaptic glutamate receptors (Event 2; yellow) and the subsequent postsynaptic depolarization (Event 3) leads to Ca<sup>2+</sup> influx (Event 4; blue) across the postsynaptic membrane through ion channels which are gated by both the NMDA receptor and a voltage-dependent Mg<sup>2+</sup> block. This Ca<sup>2+</sup> influx, along with CUFA and DAG, triggers the activation of PKC (Event 5). Activation of presynaptic PKC (Event 6) is triggered by the presynaptic influx of Ca<sup>2+</sup> in combination with other second messengers (CUFA, DAG) released through presynaptic receptor mediated processes. Subsequent phosphorylation of the PKC substrate, protein F1/GAP43 regulates increases in neurotransmitter release and triggers cytoskeletal reorganization and axonal terminal growth (Event 7a). Postsynaptically, the activation of protein kinases and subsequent substrate phosphorylation result in increased receptor sensitivity and cytoskeletal reorganization (Event 7b). In addition, activation of PKC localized to the nuclear membrane may be responsible for the regulation of protein synthetic events. For explanation of abbreviations, see p. 120.

D-aspartate (NMDA) receptor subtypes<sup>14,17,18</sup> are pivotal events in the development of LTP. The activation of NMDA channels is required for the induction of LTP<sup>14</sup> while enhanced conductance through AMPA-associated channels, but not NMDA channels<sup>14</sup>, is important for the persistence of the LTP response<sup>34,65,66</sup>. AMPA receptors and associated channels are also primarily responsible for basal synaptic response<sup>65,66</sup>.

There is a growing awareness that the mossy fiber-CA3 synapse in the *stratum lucidum* both has a different molecular composition and shows a different form of LTP<sup>97,105</sup>. The presynaptic terminal lacks the PKC substrate protein F1/GAP43<sup>63</sup>, while the postsynaptic membrane lacks the NMDA receptor<sup>30</sup>. It may be the case, however, that, since PKC is present at these synapses, other substrates and receptors, still regulated by PKC<sup>100</sup>, provide the different form of LTP seen at the mossy fiber synapse.

A distinction is made here between the actual electrophysiological response requiring activation of postsynaptic receptors – which we call the ‘readout’ in Fig. 1 – and the mechanisms required to enhance that response. Thus, the readout after LTP may be enhanced, but the synaptic changes needed to produce enhancement may not be at the site of the postsynaptic receptor. This distinction allows for a delay in the readout of synaptic changes occurring at distant sites. Thus, the mechanisms responsible for enhanced readout may be rapid and brief, whereas a change in readout may require time to develop and be more persistent. This will be discussed with respect to the PKC enhancement mechanism in section 8.

#### 4. LTP REQUIRES POSTSYNAPTIC $Ca^{2+}$ (EVENT 4)

Activation of NMDA receptors allows for a significant influx of  $Ca^{2+}$  into the postsynaptic site<sup>32,60</sup>, an event which has been shown to be necessary for LTP, since postsynaptic injection of  $Ca^{2+}$  chelators (EGTA, nitr-5) block LTP<sup>50,54</sup>. High extracellular concentrations of  $Ca^{2+}$ , presumably elevating intracellular levels, will produce an APV-sensitive synaptic enhancement which has a similar time course to LTP<sup>81,101</sup>. An APV-insensitive LTP can also be induced via activation of postsynaptic voltage-dependent  $Ca^{2+}$  channels<sup>28,35</sup>. The postsynaptic  $Ca^{2+}$  signal, as a second messenger, is shown in Fig. 1 to activate calcium-dependent kinases and proteases.

#### 5. ACTIVATION OF $\gamma$ PROTEIN KINASE C POSTSYNAPTICALLY (EVENT 5)

Both a  $Ca^{2+}$ -stimulated and receptor-mediated rise in second messengers is proposed in the model to

activate postsynaptic PKC leading to substrate phosphorylation. When injected postsynaptically, PKC inhibitors will block the persistence of LTP, but not its induction<sup>53</sup>. A recently completed study from our laboratory<sup>31</sup>, using intracellular iontophoretic injection of inhibitors, suggests that the onset of postsynaptic PKC activation occurs immediately after LTP induction and persists for less than 5 min.

In the hippocampal synapses the  $\gamma$  PKC subtype may be restricted to the postsynaptic element<sup>73</sup> and possibly to the postsynaptic density<sup>104</sup>. Since the  $\gamma$  subtype is in highest abundance in membrane fractions<sup>73,90</sup>, it could be activated without a translocation step and would thus phosphorylate substrates rapidly.

Is the activation of NMDA receptors leading to influx of  $Ca^{2+}$  which, in turn, triggers  $Ca^{2+}$ -dependent postsynaptic events sufficient to maintain LTP? Since a depolarizing dose of NMDA alone does not produce persistent enhancement<sup>33</sup>, NMDA depolarization of postsynaptic cells is not sufficient. Perhaps NMDA needs to be applied in a fashion that more precisely mimics glutamate release after tetanic stimulation. Or, other postsynaptic mechanisms in combination with events resulting from NMDA activation are required for LTP. We favor a third view shown in Fig. 1 that after postsynaptic activation, presynaptic mechanisms are required to promote persistent LTP. In our view, activation of the NMDA receptor alone would not be sufficient to activate the presynaptic mechanism.

#### 6. ACTIVATION OF $\beta$ PROTEIN KINASE C SUBTYPE PRESYNAPTICALLY (EVENT 6)

There is converging evidence pointing to a role in LTP for the  $\beta$  PKC subtype in the presynaptic terminal. This subtype has been shown to preferentially phosphorylate protein F1/GAP43<sup>92</sup>, a presynaptic PKC substrate<sup>25</sup> that is increased in its phosphorylation after LTP<sup>45,46,87</sup>. It is reasonable to predict that this increase is mediated by  $\beta$  PKC. Furthermore, phorbol esters enhance  $K^{+}$ -stimulated release of transmitter in synaptosomes containing  $\beta$  and  $\gamma$  PKC but have no effect on synaptosomes containing only  $\gamma$  PKC<sup>73</sup>. This predicts that the  $\beta$  PKC subtype could regulate increases in presynaptic transmitter release associated with LTP<sup>6,7,10,57</sup>.

Since the increase in F1/GAP43 phosphorylation is not detected until 5 min after LTP induction<sup>46</sup>, one would expect that the activation of presynaptic  $\beta$  PKC would be delayed relative to postsynaptic  $\gamma$  PKC activation. One can also predict that the activation of presynaptic  $\beta$  PKC will be distinct from postsynaptic  $\gamma$  PKC activation not only by its delayed activation but

also by its persistence of activity<sup>13,31,55,56</sup>. These expectations are consistent with the fact that extracellular application of PKC inhibitors block LTP even when intracellular injections of inhibitor, acting postsynaptically, are no longer effective<sup>31</sup>.

As shown in Fig. 1, presynaptic  $\beta$  PKC has three effectors:  $Ca^{2+}$ , DAG and *cis*-unsaturated fatty acids (CUFAs)<sup>69</sup>, that regulate its activity. CUFAs are released from presynaptic membrane phospholipids after calcium-elevated phospholipase A2 activation<sup>68,69</sup>. The involvement of this system in LTP is suggested by the fact that mepacrine, a PLA2 inhibitor, blocked the persistence of LTP and that the phosphorylation of protein F1/GAP43 was increased after CUFA administration<sup>41,43</sup>.

Until recently, information on the interaction among these three effectors on PKC activity was not known. Chen and Murakami<sup>11</sup> and Shinomura et al.<sup>94</sup> have now demonstrated a synergistic relation among these three PKC effectors. It is attractive to think that LTP is persistent in direct relation to the extent of synergism, and hence PKC activation, among these three factors. Moreover, intermediate or abbreviated forms of LTP (see ref. 84 for review) may occur because this synergism is muted.

If PKC is responsible for LTP expression then PKC activators and inhibitors should have differential effects on potentiated and control pathways. It has been reported that prior induction of LTP did not occlude synaptic enhancement produced by phorbol esters, and H-7 inhibited control as well as potentiated responses<sup>67</sup>. It was concluded that PKC is not responsible for the expression of LTP. In this study, however, high levels of phorbol ester (15  $\mu$ M) were used and the specificity and dose of the H-7 used need to be evaluated\*. Lovinger and Routtenberg clearly showed that lower doses of phorbol ester had a synergistic potentiating effect on the pathway while leaving the control response unchanged<sup>48</sup>. Similarly, Huang et al. showed no effect on baseline responses or resting potential of a low dose of H-7 applied intracellularly<sup>31</sup>. The observation that phorbol esters enhance synaptic transmission in a manner which is not identical to that of tetanic stimulation<sup>29,67</sup> does not exclude a critical role of PKC in the expression of LTP. Indeed, we have previously

stated that PKC is necessary, but not sufficient, to produce long-lasting synaptic change<sup>83</sup>. We propose here a model of LTP in which *several* mechanisms, some of which may be dependent only in part, if at all, on PKC, are coordinated both spatially and temporally to produce a long-lasting enhancement.

## 7. RETROGRADE SIGNAL

The proposed activation sequence of postsynaptic  $\gamma$  PKC followed by presynaptic  $\beta$  PKC is consistent with previous proposals of a 'retrograde' signal from the postsynaptic element to the presynaptic terminal<sup>10,42</sup>. The existence of a retrograde factor was suggested by the observations that APV, though acting on postsynaptic receptors, will block both LTP-associated increases in neurotransmitter release<sup>22</sup> and protein F1/GAP43 phosphorylation<sup>44</sup>. However, the identification of such a factor, which is both released into the synaptic cleft from the postsynaptic cell and binds to presynaptic receptors, has not yet been made though two interesting candidates have been suggested: arachidonic acid, or one of its metabolites<sup>52,102,103</sup>, and nitric oxide<sup>74,89</sup>. It is worth noting that arachidonic acid both activates PKC<sup>69</sup> and enhances LTP persistence<sup>41</sup>.

Another mechanism in addition to a retrograde factor may be exogenous neurotransmitters acting at presynaptic autoreceptors<sup>16,61</sup>. In particular, the metabotropic glutamate receptor has been shown to be linked to PI metabolism<sup>71,99</sup> via a G-protein<sup>99</sup>. Increased glutamate release from the presynaptic terminal after LTP would feed back onto the same terminal to regulate presynaptic PKC activity and/or neurotransmitter release via the G-protein mechanism<sup>5</sup>.

Activation of a presynaptically located pertussis toxin (PTX)-sensitive G-protein has been suggested to regulate LTP<sup>27</sup>. In the present model, activation of a presynaptic receptor stimulates G-protein-mediated phospholipid turnover and diacylglycerol (DAG) production, activating PKC<sup>38</sup>. There is an interesting feedback loop that may be present given that protein F1/GAP43 has been suggested to regulate G-protein function<sup>98</sup>. It would be predicted that, since activation of presynaptic mechanisms underlying synaptic enhancement require a postsynaptic signal, application of  $Ca^{2+}$  chelators

\* As indicated in 'The Messenger' from LC Services Corp. (1992, Vol. 1, 4-5) H-7 from Sigma used by Muller et al.<sup>67</sup> (also known as iso-H-7) is "at a minimum, 4-fold weaker against PKC than the Seikagaku H-7, and, since the Sigma H-7 does not inhibit some phorbol ester effects even at a very high concentration, it might possibly be inactive against one or more particular PKC isoforms (p. 5)". The bulletin also notes that it "thus appears that many of the conclusions regarding 'PKC-independence' ... will have to be reevaluated in light of the questions surrounding the nature of the H-7 used ...". Furthermore, any effects of the Sigma H-7 may be attributed to non-specific actions of the drug.

restricted to the postsynaptic cell will inhibit both increased protein F1 phosphorylation and enhanced neurotransmitter release.

#### 8. PROTEIN KINASE C-DEPENDENT PRESYNAPTIC MECHANISMS RESPONSIBLE FOR PERSISTENT ENHANCED READOUT (EVENT 7a)

As proposed in Fig. 1, sustained presynaptic PKC activity leads to three consequences that would enhance readout. First, F1/GAP43 phosphorylation would regulate the increase in neurotransmitter release after LTP induction<sup>6,7,57</sup>. This mechanism can be blocked by an antibody to the B50 (a.k.a. F1/GAP43) substrate<sup>20</sup>. Second, protein F1/GAP43 regulates the rate of axon extension and growth<sup>63,95</sup>, a putative mechanism for synaptic enhancement<sup>83</sup>. These two consequences may be related. Enhanced exocytosis while increasing neurotransmitter release also adds vesicle membrane to the plasma membrane, thereby increasing the surface area of axon terminals<sup>78</sup>. Third, protein F1/GAP43 would also regulate the stability of membrane skeleton<sup>3,95</sup> to allow greater access of vesicles to the plasma membrane and provide linkages between the cytoskeleton and new membrane. Although not yet established in mammals<sup>75</sup>, but suggested for an invertebrate system<sup>26</sup>, the synthesis and transport of newly synthesized proteins such as F1/GAP43 could support these presynaptic mechanisms.

These three persistent presynaptic changes are then read out by the postsynaptic AMPA receptor ion channel complex. This statement re-emphasizes the distinction (see Section 3) we wish to make between enhanced readout and the mechanism underlying synaptic change. It is logically possible that the postsynaptic receptor itself would remain unaltered, yet enhanced readout, caused by presynaptic alterations, would still occur. In LTP, however, a change in the postsynaptic mechanism has been observed so that enhanced readout is here proposed to be a consequence of persistent changes in both presynaptic and postsynaptic processes.

#### 9. POSTSYNAPTIC MECHANISMS RESPONSIBLE FOR PERSISTENT, ENHANCED READOUT (EVENT 7b)

Three postsynaptic PKC-dependent mechanisms are depicted in the model: increased sensitivity to AMPA, cytoskeletal change and increased protein synthesis. The evidence that PKC can regulate these processes is briefly summarized here. First, an increased sensitivity to AMPA<sup>15,19</sup> after LTP is blocked by the PKC inhibitor K252-b<sup>80</sup>. The enzyme PLA2 has also been

shown to modulate AMPA receptors<sup>58</sup> and could do so via PKC activation. Second, cytoskeletal elements are altered through a PKC-calpain interaction<sup>37</sup>. It has been proposed that stimulation-induced increase in postsynaptic Ca<sup>2+</sup> activates the protease calpain I which by cleaving fodrin filaments alters postsynaptic structure<sup>49</sup>. One consequence of this may be enhanced sensitivity to AMPA<sup>15,19</sup>. Third, the required increase in protein synthesis lasting hours after LTP inferred from inhibitor studies<sup>24,39,75,76,96</sup> could be regulated by PKC activity via the phosphorylation of transcription factors<sup>4,40,64</sup>.

#### 10. ROLE OF CALMODULIN KINASE

Postsynaptic CaM kinase II, as a regulator of receptor sensitivity, cytoskeletal reorganization and/or protein synthetic events might be expected to play an important role in LTP. Although there is no direct evidence to our knowledge that CaM kinase II enzyme activity itself is enhanced after LTP, there is evidence from CaM kinase inhibitors that predicts such a change<sup>53,56,79</sup>. In contrast to PKC, CaM-dependent processes have been proposed to be important for the initial but not the persistent change of LTP<sup>59,77,79</sup>.

CaM kinase could be regulated by PKC in the presynaptic terminal since protein F1/GAP43 (neuro-modulin) binds CaM<sup>2,12</sup> and then releases it when phosphorylated by PKC or in the presence of elevated Ca<sup>2+</sup> levels. Ca<sup>2+</sup> influx into the presynaptic terminal could therefore trigger an elevation of intra-terminal CaM levels. The increase in phosphorylation of putative CaM kinase II presynaptic substrates, IIIa and IIIb, after LTP<sup>70</sup> is consistent with this scenario. Furthermore, combined PKC and CaM-dependent actions have been proposed to regulate the increase in neurotransmitter release after LTP<sup>51</sup>.

#### 11. CONCLUSIONS

The present model proposes that both presynaptic and postsynaptic mechanisms are critical for the persistence of LTP. It would be unusual if such a complex regulatory event as synaptic plasticity were related to a single molecule. Indeed, Fig. 1 emphasizes the point that different mechanisms would need to be recruited in sequence. Nonetheless, it appears that during this sequential activation, alternatives to the PKC pathway may not exist, since inhibitors whose common action is on PKC eliminate persistent synaptic enhancement. Yet inhibition of PKC has no influence on basal synaptic transmission<sup>12,47,53,55,56,63,79,81</sup>. Thus, the role of PKC

is to regulate synaptic change rather than basal synaptic activity<sup>85,93</sup>.

This model has focussed on the synaptic change of hippocampal LTP. There is reason to suspect that this synaptic dialogue may have application to the readjustment of synapses in other systems. Evidence for this comes from recent studies of visual cortex<sup>21,91</sup> as well as different forms of learning<sup>23</sup>.

#### ABBREVIATIONS

Ab	antibody
ADP	adenosine diphosphate
AMPA	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionate
APV	2-amino-5-phosphovalerate
ATP	adenosine triphosphate
CaM	calmodulin
CNOX	6-cyano-2,3-dihydroxy-7-nitroquinoxaline
CUFA	cis-unsaturated fatty acid
DAG	diacylglycerol
Depolar	depolarization
EGTA	ethylenedis(oxyethylenenitrilo)tetraacetic acid
H-7	1-(5-isoquinoliny)sulfonyl)-2-methylpiperazine
LTP	long-term potentiation
mel	mellitin
NMDA	<i>N</i> -methyl- <i>D</i> -aspartate
NT	neurotransmitter
PKC	protein kinase C
PLA <sub>2</sub>	phospholipase A <sub>2</sub>
PMXB	polymyxin B
PTP	post-tetanic potentiation
PTX	pertussis toxin
S	substrate

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