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Short reviews

## Long-term potentiation as synaptic dialogue

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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-

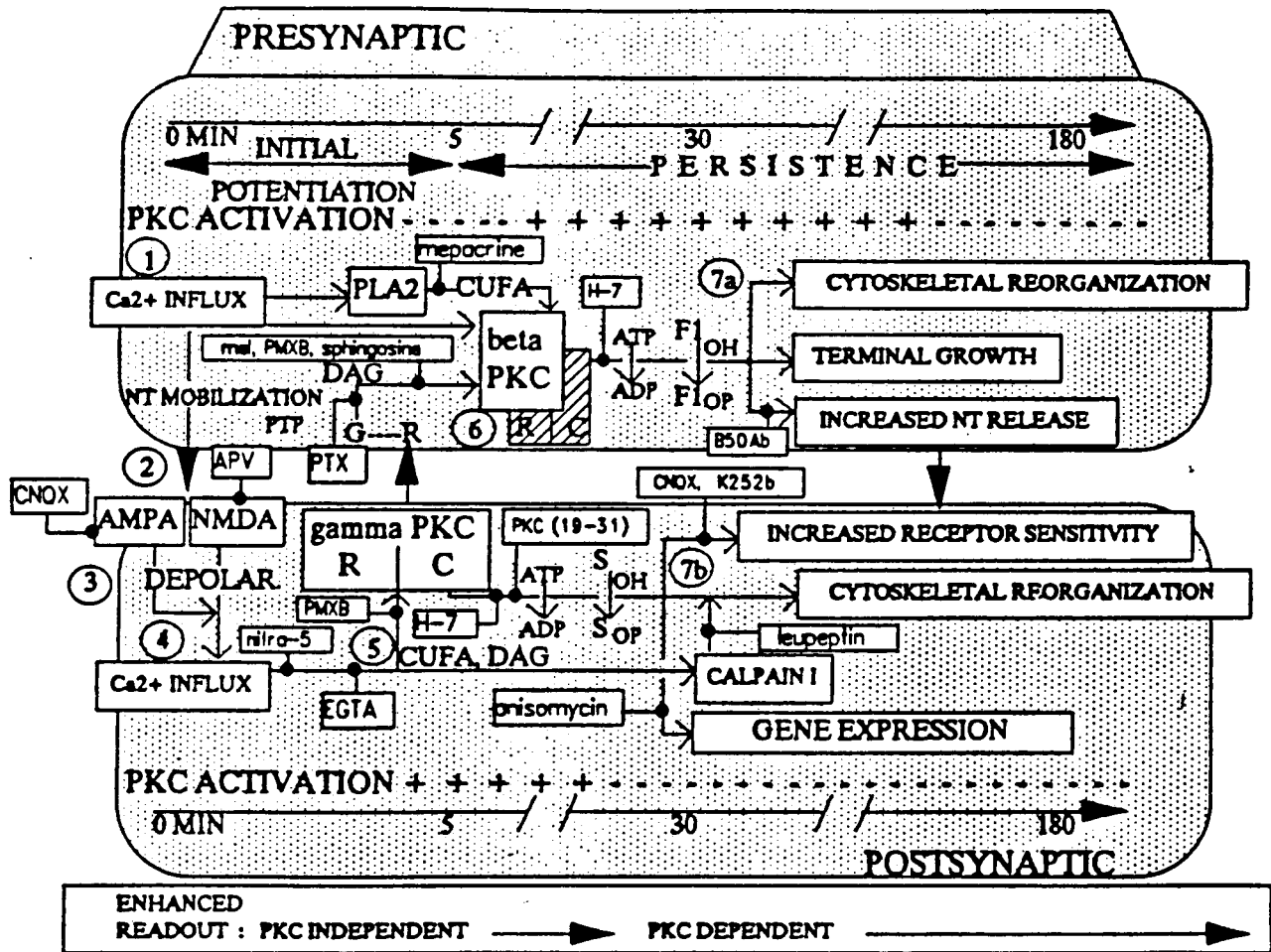


Fig. 1. Long-term potentiation (LTP) requires the sequential occurrence of events on both sides of the synapse. Enzymes regulating the events important for enhanced readout are highlighted in green. The events themselves are highlighted in purple. Inhibitors which block LTP are printed in red in a white 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

