MECHANISMS UNDERLYING LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION

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Introduction

A curious property of excitatory synapses in the hippocampus and some other neural tissues is that when they are heavily used, they undergo a long-lasting increase in their efficacy. Brief repetitive activation of hippocampal excitatory synapses results in a substantial increase in synaptic strength that can last for several hours and has been detected even weeks after induction. This use-dependent strengthening of a synapse is known as long-term potentiation, or more commonly, LTP (Bliss & Lomo 1973, Lomo 1966, Bliss & Lynch 1988). LTP occurs most prominently in the hippocampus, where consolidation of experience into long-term memory is thought to occur. LTP is the most compelling and widely studied model

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for a cellular mechanism related to learning and memory in the mammalian brain.

Long-term potentiation is produced in hippocampal slices by stimulating a population of presynaptic fibers with a short-duration train of high-frequency stimulation (Bliss & Lomo 1973). Typically these trains consist of electrical shocks delivered at 100 Hz for 1 sec. Immediately following this stimulus, the strength of the tetanized synaptic connections (as tested with single shock stimuli) increases up to about fivefold. Most of this increase decays to a level about 150–200% of baseline within a few minutes after the tetanus. The early, short-lasting phase of potentiation of synaptic transmission is called post-tetanic potentiation, or PTP. By analogy to a similar phenomenon seen at the neuromuscular junction and in α-afferents to motoneuron synapses, PTP is believed to be caused by a short-lasting accumulation of free calcium ions in the presynaptic terminal (Delaney et al 1989).

Methods of Studying Long-Term Potentiation

The strength of synaptic transmission is typically assayed in the hippocampus by recording the size of the excitatory postsynaptic potential (epsp) that results from stimulation of presynaptic fibers with electrical shocks. The hippocampus is a highly laminated structure, and it is therefore a simple matter to place stimulating electrodes on presynaptic fiber tracts, and recording electrodes near the sites of synaptic termination (Andersen et al 1971, e.g. Malinow et al 1988). Synaptic strength has typically been measured in one of three ways: the extracellular epsp field potential, the extracellular population spike, and the intracellularly recorded epsp (Andersen 1975). Unlike the two epsp measures, however, the population spike is an indirect measure of synaptic transmission that can be influenced by several other factors, including the magnitude of inhibitory transmission and general changes in postsynaptic neuronal excitability.

Although LTP was first described in anesthetized animals, the fact that it can reliably be induced in thin slices of hippocampus, maintained in vitro, has been a major technological advance. When slices are placed in a recording chamber having stable perfusion, and with mechanically stable tissue and electrodes, an extremely reproducible synaptic response is obtained. With single stimuli delivered at a frequency below 0.1 Hz, the synaptic strength varies only a few percent over many hours of testing (e.g. Malinow et al 1988). Thus, changes in synaptic strength caused by experimental manipulation are relatively easy to detect. Three major excitatory hippocampal synaptic connections have generally been used to study LTP: the perforant path (origin in entorhinal cortex) to dentate granule cell synapse, the mossy fiber (dentate granule cell axon) to CA3

pyramidal cell synapse, and the Schaffer collateral/commissural (CA3 pyramidal cell axon) to CA1 pyramidal cell synapse (Andersen 1975, Bliss & Lomo 1973). For the most part, perforant path and Schaffer collateral LTP appear to be quite similar, whereas mossy fiber to CA3 synapses exhibit a substantially different form of LTP (Harris & Cotman 1986, Zalutsky & Nicoll 1990, Staubli et al 1990). This review focuses primarily on LTP in the Schaffer collateral/commissural synapse.

Induction of Long-Term Potentiation

Two aspects of the tetanic stimulation are important in the induction of LTP. First the stimulus must be of sufficient strength to reach a threshold for eliciting LTP. This threshold requirement, known as cooperativity, reflects the need for a certain number of presynaptic fibers to be activated simultaneously for LTP induction to occur (McNaughton et al 1978). Second, the LTP-inducing stimulus must be delivered at high frequency (Dunwiddie & Lynch 1978). Frequency and stimulus strength interact such that increasing one decreases the requirement for the other. For example, a tetanic stimulation delivered at low frequency will not produce LTP, but increasing the frequency can render the weak stimulus effective (Bliss & Lomo 1973). Conversely, increasing the stimulus strength decreases the frequency requirement for producing LTP.

Another striking feature of LTP is that it occurs only in the synapses that were tetanized (Barrionuevo & Brown 1983, Gustafsson & Wigstrom 1986, Kelso et al 1986, Larson & Lynch 1986, Sastry et al 1986). A given postsynaptic cell receives a great many afferent synapses. Only a fraction of these afferents will receive tetanic stimulation during a typical experiment. Although LTP is produced in the tetanized synapses, a test of other, non-tetanized afferents to the same postsynaptic cell reveals that these synapses have not undergone any potentiation. This "synapse specificity" of LTP places important constraints on possible mechanisms for the induction of LTP.

Synapse specificity notwithstanding, different afferents to the same post-synaptic cell can interact in ways very significant for understanding the mechanism for LTP induction. Experimentally, it is possible to place two stimulating electrodes in the hippocampal slice that activate two entirely separate subpopulations of Schaffer collateral/commissural afferents terminating on the same postsynaptic cell (Barrionuevo & Brown 1983, Gustafsson & Wigstrom 1986, Kelso et al 1986, Larson & Lynch 1986, Sastry et al 1986). Delivering a weak tetanus to one of these afferent populations, through one stimulating electrode, fails to produce LTP. Strong tetanic stimulation delivered through only one of the stimulating electrodes fails to produce LTP in afferents subsequently stimulated by

the other electrode (synapse specificity). If, however, one electrode delivers a weak tetanus at the same time that a strong tetanus is given by the other, both sets of afferents will potentiate. Thus, some form of communication occurs between the two afferent populations. The most likely path for this communication is through the postsynaptic cell, since this is the anatomical structure that connects the two sets of afferents. This so-called associative LTP can occur even when the two groups of afferents are spatially separated by several hundred microns (Larson & Lynch 1986). Indeed, associative LTP can be produced by the pairing of afferents terminating on dendritic trees originating from opposite sides of the cell body (Gustafsson & Wigstrom 1986, Larson & Lynch 1986). For successful pairing of two inputs to occur, their activation must be temporally correlated. If the weak and strong inputs are separated in time by more than about 100 ms, no associative LTP will result (Gustafsson & Wigstrom 1986, Levy & Steward 1983). This places severe constraints on the nature of the associative messenger and makes the polarization state of the postsynaptic membrane the most likely messenger.

Depolarization of the postsynaptic membrane has indeed been found to be critical in the induction of LTP. If the postsynaptic cell is held at a hyperpolarized level by the injection of current through an intracellular recording electrode, LTP can be prevented in that cell (Malinow & Miller 1986). Hyperpolarization of the membrane also prevents the association of weak and strong inputs (Kelso et al 1986). Perhaps more significantly, depolarized postsynaptic cells lack a frequency requirement for LTP induction. In depolarized postsynaptic cells, single-shock presynaptic activation produces small amounts of LTP (Gustafsson et al 1987, Wigstrom et al 1986). Continued delivery of single shocks to the presynaptic fibers, paired with depolarization of the postsynaptic cell, eventually results in a level of LTP equal to that evoked by tetanic stimulation. Taken together, these experiments indicate that the postsynaptic cell must be depolarized during the tetanic stimulation to induce LTP. Based on the results of the experiments that paired single shocks with depolarization, it has been deduced that associative LTP is produced in weak inputs because the strong input depolarizes the entire postsynaptic cell. Weak tetani cannot themselves depolarize the cell enough to support the induction of LTP.

Frequency Requirement of Long-Term Potentiation

A tetanic train of stimulation obviously consists of a rapid series of single shocks, each of which presumably releases transmitter. If the frequency of tetanic stimulation is high enough, sequential epsps produced by the train will overlap and summate (Gamble & Koch 1987). The amount of epsp summation, and hence ultimately the level of depolarization reached, will

vary directly with the frequency of the tetanus and also with the membrane resistance (Gamble & Koch 1987). The probability of reaching sufficient depolarization to produce LTP depends primarily on the size of individual epsps (determined by stimulus strength) and the amount of overlap and summation of epsps (determined by stimulus frequency). This, then, is a plausible explanation for the dependence on these two factors, stimulus strength and frequency, in LTP induction.

Though depolarization of the postsynaptic cell is necessary for the induction of LTP, depolarization alone is not sufficient. This can be shown quite simply by experiments in which postsynaptic cells are strongly de-polarized, either by injection of current, or by causing them to fire long-duration calcium action potentials during blockade of potassium currents. Such postsynaptic depolarization alone does not produce LTP. Only at synapses in which depolarization is paired with activation of the synapse does LTP occur (Gustafsson et al 1987, Wigstrom et al 1986). Thus, synaptic activation must also contribute at least one necessary factor besides depolarization to the induction of LTP. In particular, binding of the excitatory amino acid transmitter to a postsynaptic receptor is required.

The NMDA Receptor and Long-Term Potentiation

The neurotransmitter at most, if not all, excitatory synapses in the hippo-campus is an excitatory amino acid, probably glutamate (Cotman & Nadler 1981). Glutamate can bind to at least four subtypes of receptor protein, three of which are coupled to transmembrane ion channels. The ionophore-associated receptors are defined by the selective agonists active at each type, N-methyl-D-aspartate (NMDA), quisqualate, and kainate. Since it has been difficult to distinguish between the quisqualate and kainate receptors, these two types are referred to collectively as the non-NMDA receptors. Following synaptic release, glutamate binds to both postsynaptic NMDA and non-NMDA receptors (Collingridge et al 1983), but the epsp during low-frequency stimulation is primarily supported by current flow through the non-NMDA channel (Collingridge 1985, Herron et al 1985). Though the NMDA receptor binds glutamate, the associated channel is largely prevented from passing current into the postsynaptic cell because the channel pore is immediately blocked by magnesium ions from the extracellular fluid (Ascher & Nowak 1988, Mayer et al 1984, Mayer & Westbrook 1987, Nowak et al 1984).

Though postsynaptic current flows primarily through the non-NMDA receptor channel at normal resting potentials, opening of the NMDA ionophore is required for the induction of LTP (Collingridge & Bliss 1987). Antagonists of the NMDA receptor, such as 4-amino-5-phosphonovaler-ate (APV), prevent the induction of LTP when present at the time of tetanic

stimulation (Collingridge et al 1983). This and other specific NMDA antagonists do not interact with the non-NMDA receptor and have little effect on the size of the normal epsp (Collingridge et al 1983). Blockers of the NMDA-associated ionophore itself, which do not interact with the glutamate binding site, agents such as MK-801 (Huettner & Bean 1988), also prevent the induction of LTP (Coan et al 1987).

The NMDA receptor channel exhibits a strong voltage-dependence that allows current to pass only when the postsynaptic cells are depolarized (Mayer et al 1984, Mayer & Westbrook 1987, Nowak et al 1984). When the postsynaptic membrane is hyperpolarized, electrical forces are favorable for the divalent $(2+)$ magnesium ion to bind in the channel. When the cell is depolarized, these forces decrease and magnesium is less likely to occupy its binding site in the channel. Thus, when the postsynaptic cell is depolarized, magnesium is less likely to occupy the channel, and other ions can flow into the cell. The apparent voltage-sensitivity of the NMDA channel is secondary to the voltage-dependence of the magnesium block.

The experiments discussed above have demonstrated that two factors are necessary for the induction of LTP: (a) The NMDA ionophore must open (in response to glutamate binding); (b) the postsynaptic cell must be depolarized (to allow current flow through the NMDA ionophore). Both of these requirements can be boiled down to a single condition: *LTP induction will only occur when a critical level of current flows through the NMDA receptor channel in the postsynaptic cell* (Collingridge & Bliss 1987, Gustafsson & Wigstrom 1988). This hypothesis is probably the single most influential idea in understanding LTP induction. Examination of its correlates help to explain most of the induction properties of LTP. Stimulus threshold, frequency requirements, and associative LTP are explained by the need to provide sufficient depolarization to remove magnesium blockade from NMDA channels. Synapse specificity results because synapses not active during tetanic stimulation have no glutamate bound to their NMDA receptors. In addition to these conclusions, these experiments make it very certain that the induction mechanisms for LTP reside, at least in part, in the postsynaptic cell. This is apparent because manipulations that affect only the postsynaptic cell are able to prevent LTP induction.

Properties of the NMDA Receptor Ionophore

The above stated hypothesis does not provide an explanation of what critical factor is provided by NMDA channel current that can trigger LTP. Comparisons of whole cell and single channel currents generated by quisqualate and by NMDA application to cultured hippocampal neurons showed that both receptor channels are fairly nonselective monovalent cation-carrying pores (Jahr & Stevens 1987, Mayer et al 1987). The non-

NMDA receptor channels can be permeated by sodium and potassium ions, but calcium and other divalent ions cannot pass through this channel. The NMDA channel also carries sodium and potassium, and in addition passes calcium ions into the cell (Ascher & Nowak 1988, Jahr & Stevens 1987, Mayer et al 1987). Work in isolated CNS neurons has shown that calcium flux through the NMDA channel can cause significant elevations of intracellular calcium (MacDermott et al 1986, Mayer et al 1987). This also has been demonstrated in the dendrites of CA1 pyramidal cells in brain slices (Regehr & Tank 1990).

Calcium and Long-Term Potentiation

Direct evidence of the importance of postsynaptic calcium entry in the induction of LTP was provided by two experiments. First it was shown that injection of calcium chelators such as EGTA into postsynaptic neurons prevented the induction of LTP in those neurons (Lynch et al 1983). Second, release of calcium into postsynaptic neurons by the caged calcium compound Nitr-5 produced a potentiation of synaptic transmission (Malenka et al 1988). These two experiments, taken together, strongly support the idea that an elevation of postsynaptic calcium is the critical triggering factor in LTP.

The Importance of Dendritic Spines in the Induction of Long-Term Potentiation

If a general postsynaptic calcium influx were the critical trigger for LTP, the synapse specificity of LTP would be difficult to understand, since tetanic stimulation produces widespread increases in dendritic calcium supported in part by voltage-dependent calcium channels (Regehr et al 1989). Yet inactive synapses have been shown to be immune to the influence of such calcium elevations generated in the dendrites (Gustafsson et al 1987, Kelso et al 1986, Malenka et al 1989b). Thus, during a tetanus, there must be some way a particular synapse is able to provide its own private source of calcium distinct from that provided by depolarization alone.

    The NMDA-ionophore could provide an ideal private source of calcium related to synapse activity, especially tetanically induced activity. This idea is made particularly attractive because excitatory synapses on cortical pyramidal cells are formed on dendritic spines. Dendritic spines are small protuberances that are connected to the main dendritic shaft through a thin neck (Harris & Landis 1986, Landis & Reese 1983, Landis 1988, Harris & Stevens 1989). This neck may serve as a barrier to prevent calcium from the main dendritic shaft from reaching the subsynaptic region (cf. Landis 1988). Active synapses can provide their own calcium if NMDA receptors are located on these spines. Furthermore, the small size of these

spines could serve to concentrate (Gamble & Koch 1987) the calcium entering though NMDA receptor channels because of their large surface-to-volume ratios.

Are Additional Factors Required for the Induction of Long-Term Potentiation?

The idea that a privileged rise in intraspine calcium triggers LTP allows for the critical role of NMDA receptors while also accounting for synapse specificity. General depolarization of the postsynaptic dendrite does not trigger LTP in non-tetanized synapses, because each spine must generate its own calcium load, a function that requires local activation of NMDA receptors. This hypothesis is extremely powerful in explaining LTP induction, but one particular experimental result raises the possibility that it is an incomplete solution for the initiation of LTP. Application of NMDA or glutamate directly onto postsynaptic cells, through an iontophoresis micropipette, provides the agonist to bind to postsynaptic receptors and also strongly depolarizes the cell. Thus, application of exogenous NMDA or glutamate would appear to fulfill both the depolarization and glutamate-binding requirements for LTP induction. Applying NMDA or glutamate in this manner, however, produces a short-lived potentiation (Collingridge et al 1983, Kauer et al 1988b). Thus, although calcium flux through the NMDA receptor is certainly necessary to induce LTP, it may not be sufficient. One interpretation of this result is that, in addition to releasing glutamate and depolarizing the postsynaptic cell, presynaptic fiber activity provides some additional, as yet unidentified, component that is critical for eliciting nondecremental LTP (Kauer et al 1988b; but see Thibault et al 1989). Alternatively the postsynaptic calcium increase elicited by agonist application might be different from that elicited by synaptically released glutamate. For example, NMDA application could result in a smaller or slower rise in calcium than that elicited by a tetanus, and perhaps some larger, critical level of postsynaptic calcium increase is required to convert a decremental form of potentiation into a longer-lasting, nondecremental form. Another possibility is that NMDA application may cause long-lasting calcium increases in postsynaptic cells (see Connor et al 1988) that are qualitatively different from those caused by more physiological stimuli. Nevertheless, the inability of exogenous NMDA or glutamate application to mimic LTP precisely is perhaps the most glaring example of data that appear to be inconsistent with the presented model of LTP induction.

Biochemical Mechanisms Underlying Long-Term Potentiation

Given that a rise in calcium within the dendritic spine is an essential step in the induction of LTP, a question of great interest is: What are the

biochemical processes activated by calcium that are responsible for LTP? One of the first reasonably comprehensive hypotheses concerning the biochemical mechanisms underlying LTP proposed that the transient elevation of calcium in the spine activated a membrane-associated calcium-activated neutral protease, i.e. calpain, that in turn degraded the cytoskeletal protein fodrin (Lynch & Baudry 1984). It was proposed that this may result in the unmasking of a covert population of glutamate receptors or produce alterations in dendritic spine ultrastructure. Although an intriguing hypothesis, the only direct experimental evidence supporting a role for calpain in LTP is the finding from in vivo experiments that chronic administration of the protease inhibitor, leupeptin, blocks LTP (Staubli et al 1988). A further difficulty with this theory is that calpain may not be present in the region of axo-spinous synapses in the hippocampus (Hamakubo et al 1986).

Stronger experimental evidence exists of a critical role for protein kinases in LTP. A battery of nonselective kinase blockers including H-7 (Lovinger et al 1987, Malinow et al 1988), sphingosine (Malinow et al 1988), polymyxin B (Lovinger et al 1987, Reymann et al 1988a,b), and K-252b (Reymann et al 1990) have been shown to suppress LTP. Furthermore, intracellular injection of H-7 into the postsynaptic cell suppressed LTP (Malenka et al 1989a, Malinow et al 1989), thus suggesting that postsynaptic kinase activity, presumably activated by the rise in calcium in the dendritic spine, is required for the induction of LTP.

Most of the initial experimental work on kinase activity and LTP focused on the calcium- and phospholipid-dependent protein kinase C (PKC). In dentate gyrus, LTP has been shown to be associated with a translocation of PKC from cytosol to the membrane (Akers et al 1986) and increased phosphorylation of a substrate protein of PKC (B-50, F-1, GAP-43, or P-57) (Nelson & Routtenberg 1985, Lovinger et al 1986). Furthermore, activation of PKC by phorbol esters enhances synaptic transmission (Malenka et al 1986, Gustafsson et al 1988), as does injection of PKC directly into postsynaptic cells (Hu et al 1987). There is evidence that differences exist between LTP and phorbol ester-induced potentiation (Muller et al 1988, Gustafsson et al 1988, Malinow et al 1988), but this does not rule out a role for PKC in LTP. On the contrary, direct evidence supporting a critical role for PKC is found in the finding that injection into postsynaptic cells of a specific pseudosubstrate peptide inhibitor of PKC blocks LTP (Malinow et al 1989).

An increase in phosphoinositide turnover is one mechanism whereby PKC may be activated during LTP, since an increase in PI turnover, perhaps involving activation of a G protein, is associated with LTP (Lynch et al 1988) (although it is important to note that this work was done

primarily in the CA3 region). Whether a pertussis toxin-sensitive G protein is required for LTP generation in the CA1 region is controversial (Ito et al 1988, Goh & Pennefather 1989). If ligand-activated PI turnover is required for LTP, one receptor that may mediate this turnover is the so-called metabotropic quisqualate receptor (cf. Miller & Murphy 1988). An alternative biochemical pathway that may activate PKC and play a role in LTP involves the generation of *cis*-fatty acids from membrane phospholipids by phospholipase A2 (Linden & Routtenberg 1989).

Another kinase that has been proposed as an important element in the mechanism producing LTP is the calcium-calmodulin-dependent kinase type II (CAMKII). This kinase is found in great abundance in the hippocampus, particularly in the postsynaptic dendritic spine (Kennedy et al 1983, Kelly et al 1984), and therefore should be exposed to the rise in calcium within the dendritic spine caused by tetanic stimulation. Bath application of calmodulin antagonists has been reported to block LTP (Dunwiddie et al 1982, Finn et al 1980, Turner et al 1982, Reymann et al 1988a), but many of these compounds lack specificity. More convincing and direct evidence for a role for postsynaptic CaMKII in LTP comes from experiments in which LTP was blocked by injection into postsynaptic cells of peptides that bind to and inhibit calmodulin (Malenka et al 1989a) or a peptide that directly blocks CAMKII activity (Malinow et al 1989).

The proposal was made several years ago that a kinase capable of autophosphorylation could serve as a "molecular switch" that could store information for long periods of time (Lisman 1985, Miller & Kennedy 1986, Saitoh & Schwartz 1985). Evidence that such a constitutively active kinase may participate in maintaining LTP was obtained by taking advantage of the distinct mechanisms of actions of the kinase blockers sphingosine and H-7 (Malinow et al 1988). Sphingosine inhibits the activation of both PKC and CaMKII by competing with diacylglycerol and calmodulin, respectively. It blocked LTP when present at the time of the tetanus but not if applied subsequently (Malinow et al 1988). In contrast, H-7, which inhibits the catalytic activity of activated protein kinases by competing with ATP, not only blocked LTP when present at the time of the tetanus but also reversibly diminished previously established LTP while having minimal effects on basal synaptic transmission (Malinow et al 1988, but see Muller et al 1988, 1990). Taken together, these results have been used to suggest that a constitutively active kinase, not dependent on the continued presence of activators, is required for the persistence of LTP.

Both CAMKII and PKC have properties that may allow them to be converted into constitutively active forms. CAMKII exhibits the biochemical property of no longer requiring calcium or calmodulin to maintain its kinase activity following calmodulin-dependent auto-

phosphorylation (Saitoh & Schwartz 1985, Miller & Kennedy 1986). A constitutively active form of PKC, known as PKM, can be generated by proteolytic cleavage in other cell types. Interestingly, PKM can be produced in neutrophils by calpain (Pontremoli et al 1986), the protease that has been suggested to play an important part in LTP induction (Staubli et al 1988). Whether PKM can be produced in the hippocampus is unknown.

Although there is evidence that activation of both postsynaptic CaMKII and PKC are required for the generation of LTP, the exact mechanisms of their actions remain to be determined. For example, it is still not known whether they are activated sequentially or in parallel and whether important interactions occur between them. The identity of their substrates also remains to be determined.

An intriguing finding is that in the presence of any of the kinase inhibitors discussed to this point, LTP-inducing stimuli still cause a decremental enhancement of synaptic transmission that is remarkably similar to the potentiation caused by NMDA application. The resilience of this decremental form of potentiation raises several important questions. Is it completely independent of kinase activity (even though it is dependent on NMDA receptor activation)? Does its presence indicate that LTP was still "induced" but the processes responsible for the "maintenance" of LTP were inhibited? Alternatively, was the induction of LTP completely blocked, thus suggesting that this form of potentiation represents a parallel event? An intriguing notion is that the decremental potentiation represents a process during which a decision can be made whether or not to more permanently alter synaptic strength (cf. Stevens 1989).

Protein Synthesis in Long-Term Potentiation

The long duration of LTP along with the finding that protein synthesis inhibitors disrupt the consolidation of memory has prompted several investigators to study the effects of protein synthesis inhibitors on LTP. Unfortunately, the results of these experiments are somewhat inconsistent (cf. Deadwyler et al 1987, Dragunow et al 1989, Frey et al 1988, Krug et al 1984, Stanton & Sarvey 1984). Anisomycin, applied in vivo to the dentate gyrus (Krug et al 1984) or in vitro while recording from the CA1 region (Frey et al 1988), was reported to block a "late phase" of LTP beginning 4–8 hours after the LTP-inducing tetanus. In contrast, Stanton & Sarvey (1984) reported that emetine, cycloheximide, and puromycin blocked LTP at 30 minutes but that anisomycin was unable to block LTP even though it was as effective in preventing the incorporation of [3H]valine into protein. The most reasonable interpretation of these later results is that these compounds blocked LTP by some mechanism independent of protein synthesis inhibition. Based on experiments dealing with the role of protein

synthesis on memory consolidation, and in simple forms of learning in *Aplysia* (cf. Goelet et al 1986), it would seem most reasonable that if protein synthesis is necessary for LTP, then it would be the later phases of LTP that would most likely require this synthesis.

Gene Expression in Long-Term Potentiation

Neuronal activity can induce expression of a number of genes, most notably the immediate early genes *c-fos* and *c-jun* (Morgan & Curran 1989), thus raising the question of whether NMDA-receptor activation during LTP results in transcriptional activation. Recently mRNA encoding the transcription factors *zif/268*, *jun-B*, and possibly *c-fos* have been found to rapidly increase in the dentate gyrus following high frequency stimulation of the perforant path and these increases require NMDA receptor activation (Cole et al 1989). The role of these factors in LTP remains uncertain, as several immediate-early genes (including *zif/268*, *jun-B*, and *c-fos*) can be activated in the absence of LTP (Wisden et al 1990). Nonetheless, these findings raise the possibility that changes in gene expression may be required for maintaining LTP, as has been reported for synaptic plasticity in invertebrates (Goelet et al 1986).

Pre- Versus Postsynaptic Mechanisms in Long-Term Potentiation

At least some of the critical events responsible for triggering LTP occur in the postsynaptic cell. Much confusion, however, has been generated by the experiments attempting to determine the sites and mechanisms that are ultimately responsible for the continued enhancement of synaptic strength. Initially the experimental evidence was consistent with the hypothesis that LTP induction resulted in a long-lasting enhancement of transmitter release. Samples of extracellular fluid collected from the dentate gyrus, in vivo, via a push-pull cannula, were found to contain more glutamate following LTP induction (Bliss et al 1986, Dolphin et al 1982, but see Aniksztejn et al 1989) and this increase was blocked by APV (Errington et al 1987). In addition, release of glutamate induced by high $[K^+]_o$-induced depolarization, measured in vitro from tissue prepared from the dentate gyrus or hippocampus of rats in which LTP had been induced, was greater than that in tissue taken from control rats (Feasey et al 1986, Lynch & Bliss 1986).

The mechanisms for LTP induction are now known to reside, at least in part, within the postsynaptic cells. If an increase in transmitter release accounts for the expression of LTP, then the postsynaptic cell must communicate with the presynaptic terminal, perhaps by releasing some factor that acts on recently activated presynaptic terminals to affect subsequent

transmitter release (Williams et al 1989). A prominent candidate for such a messenger is arachidonic acid or one of its metabolites (cf. Piomelli et al 1987), because these compounds can readily cross cell membranes. In support of this idea, NDGA, an inhibitor of lipoxygenase and phospholipase A2, have been found to block LTP in vivo in the dentate gyrus (Lynch et al 1989) or in vitro in the CA1 region (Okada et al 1989, Williams et al 1989). This agent also blocks the associated increase in extracellularly detected glutamate (Lynch et al 1989). Mepacrine, a phospholipase A2 inhibitor, has also been reported to inhibit the persistence of LTP in vivo in the dentate gyrus (Linden et al 1987). Additional direct evidence for the potential role of arachidonic acid in LTP comes from the findings that an increase in extracellular arachidonic acid can be measured in vivo from the dentate gyrus during LTP (Lynch et al 1989) and application of arachidonic acid can enhance synaptic transmission both in vivo and in vitro (Williams et al 1989). Interestingly, application of arachidonic acid does not enhance low-frequency synaptic transmission by itself, but requires concomitant activation of the presynaptic fibers by a (weak) tetanus. Even then, synaptic transmission did not immediately increase but, instead, slowly increased over the course of 1–3 hours, at which point tetanus-induced LTP was occluded. The requirement for the increased rates of stimulation in the presence of arachidonic acid to produce LTP is in contrast to LTP produced in depolarized cells in which very low rates of stimulation are sufficient. Previously either arachidonic acid or oleic acid were reported to prolong the time course of LTP in vivo in the dentate gyrus (Linden et al 1987). The slow time course of the arachidonic acid effect was taken to suggest that arachidonic acid could act as a retrograde messenger for later phases of LTP but that an earlier phase must be mediated by a distinct retrograde messenger (Williams et al 1989). One possible site at which arachidonic acid could act is on a glial glutamate uptake mechanism (Barbour et al 1989). Reduced uptake could act to increase the amount of glutamate in the extracellular fluid and perhaps also in the synaptic cleft. This would not be a strictly presynaptic mechanism but would result in an increase in glutamate measured in the extracellular fluid.

Other evidence is also consistent with a role for presynaptic mechanisms in LTP. As stated above, injection of H-7 into postsynaptic cells before LTP-inducing stimuli blocks LTP (Malenka et al 1989a, Malinow et al 1989). In contrast, if cells are impaled with H-7 filled electrodes after LTP induction, no effect on established LTP is observed, even though in the same cells LTP can still be prevented in an untetanized pathway (Malinow et al 1989). Furthermore, bath application of H-7 to the hippocampal slice containing the H-7-filled cell does depress previously established LTP.

This evidence suggests that persistent kinase activity, not localized to the postsynaptic cell, is required for the persistence of LTP (Malinow et al 1989). Similarly, injection of GTP-γ-S into postsynaptic cells failed to reduce LTP, even though LTP was reported to be sensitive to pertussis toxin. This led to the suggestion that a G protein not localized to the postsynaptic cell was required for LTP (Goh & Pennefather, 1989).

Recently, a more direct electrophysiological approach has been used to address the issue of possible presynaptic mechanism in LTP. Taking advantage of the superior signal-to-noise conditions offered by whole-cell recording, two groups have performed an analysis of LTP (Bekkers & Stevens 1990, Malinow & Tsien 1990). These experiments are based on the quantal nature of transmitter release and involve a statistical analysis of the trial-to-trial variability in the size of small excitatory postsynaptic currents (epscs) recorded under voltage-clamp conditions. The results of this analysis were consistent with a presynaptic mechanism being primarily responsible for the expression of LTP.

Although there is strong evidence for a role for presynaptic mechanisms in LTP, other experimental findings are more easily explained by a post-synaptic modification. During LTP, the epsp component mediated by CNQX-sensitive non-NMDA receptors is increased, whereas the NMDA-receptor-mediated component of the epsp is little affected (Kauer et al 1988a, Muller & Lynch 1988, Muller et al 1989). Furthermore manipulations known to enhance transmitter release result in an increase in both components, thereby demonstrating that the NMDA component of the epsp is sensitive to increases in transmitter release. This differential effect on the two components of the epsp during LTP is difficult to reconcile with a persistent increase in transmitter release and is more compatible with some postsynaptic modification.

Additional evidence for a postsynaptic change underlying LTP is that the postsynaptic response to exogenously applied AMPA, a quisqualate receptor agonist, has been reported to increase during LTP (Davies et al 1989). The increase does not appear immediately following LTP induction however, but instead gradually appears over the course of 0.5 to 2 hours. This delay may explain why other investigators have not previously seen this increase (Lynch et al 1976, Taube & Schwartzkroin 1988). The slow growth of the response to AMPA led to the conclusion that immediately following LTP induction, presynaptic mechanisms account for the increase in synaptic strength, but that gradually postsynaptic mechanisms take over (Davies et al 1989). This process would require precise timing between the decrement of the presynaptically mediated potentiation and the enhancement of the postsynaptic mechanism, as LTP normally does not demon-

strate any abrupt discontinuities. Taken together, the data concerning the pre- or postsynaptic locus of LTP shows that significant strides have been made toward settling this issue. It is also clear however, that data supporting either a pre- or postsynaptic mechanism will need to be reconciled before a complete resolution of the question of the sub-synaptic localization of LTP is at hand.

Conclusion

The last several years have seen remarkable progress in understanding LTP. During the course of research on synaptic potentiation it has become increasingly clear that the nervous system has a battery of mechanisms available for modulating synaptic efficacy. Understanding this, perhaps unanticipated, complexity remains one of the premier challenges facing neuroscientists.

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