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COMMENTARY

NITRIC OXIDE AND HIPPOCAMPAL SYNAPTIC PLASTICITY

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Long-term potentiation and retrograde messengers

The mechanisms underlying learning and memory in the mammalian CNS are uncertain but thought to involve changes in the efficacy of synaptic transmission. A convenient model for studying synaptic changes that may be important in memory is long-term potentiation (LTP†). LTP refers to a persisting use-dependent synaptic enhancement that typically follows either brief high frequency stimulation of certain synapses or the pairing of postsynaptic depolarization with lower frequency synaptic activation [1, 2]. In these stimulation paradigms, the induction of LTP depends on coincidental presynaptic and postsynaptic activity. In many regions that are presumed to use glutamate as an excitatory neurotransmitter, particularly the Schaffer collateral-commissural pathway synapses on CA1 hippocampal pyramidal neurons and the perforant path synapses on granule cells in the dentate gyrus, LTP depends on activation of the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors. In other regions, most notably the mossy fiber-CA3 hippocampal pyramidal neuron synapses, NMDA receptors are not required for LTP [2]. In this commentary, we will focus primarily on NMDA receptor-dependent LTP.

At synapses where NMDA receptors are involved in LTP, several events occur in postsynaptic neurons that are necessary for inducing a lasting synaptic enhancement. These include glutamate binding, membrane depolarization and increases in intracellular calcium. If depolarization of the postsynaptic neuron is prevented during presynaptic activation [3] or if postsynaptic calcium levels are heavily buffered by calcium chelators [4, 5], LTP is prevented. The postsynaptic depolarization is required to relieve the voltage-dependent block of NMDA ion channels by magnesium ions [6] and allows glutamate to gate current flow through these channels. NMDA channels are highly permeable to calcium [7] and are thought to serve as a major source of the postsynaptic calcium increases that are necessary for LTP [1, 2]. Calcium, in turn, is believed to activate a variety of biochemical processes that ultimately produce the changes in synaptic efficacy. These biochemical changes include the activation of serine-threonine kinases [calmodulin-dependent protein kinases and protein kinase C (PKC)], tyrosine kinases, phospholipase A_2 and possibly nitric oxide synthase [8, 10].

Recent studies using quantal analysis techniques indicate that the enhanced synaptic responses result from changes in both the release of glutamate from presynaptic terminals [11, 12] and the postsynaptic response to glutamate [13–17]. The critical dependence of LTP on increases in postsynaptic calcium and the observed changes in presynaptic transmitter release have prompted the hypothesis that there is generated in postsynaptic neurons a calcium-dependent signal that, in some fashion, alters presynaptic function [18]. For a messenger to accomplish this task it must be permeable to (or released from) postsynaptic neurons, diffuse across synapses, and interact with receptors on presynaptic membranes (or permeate presynaptic membranes) to activate biochemical changes that alter the release characteristics of the presynaptic terminal. O'Dell and colleagues [19] specified nine criteria that should be met in order for an agent to be considered a retrograde messenger in pathways exhibiting NMDA receptor-dependent LTP:

1. Messenger is synthesized in postsynaptic neurons.
2. Messenger is released in response to NMDA receptor activation.
3. Inhibition of messenger synthesis blocks LTP.
4. A removal or degradation pathway for the messenger exists.
5. Exogenous application of the messenger mimics LTP.
6. Effects of exogenous application do not depend on NMDA receptor activation.
7. Actions of the messenger are rapid.
8. Messenger does not occlude other forms of presynaptic facilitation.
9. Actions are synapse specific.

Most importantly, the retrograde messenger should be synthesized in postsynaptic neurons, released extracellularly in response to NMDA receptor

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† Abbreviations: AA, arachidonic acid; APV, 2-amino-5-phosphonovalerate; CO, carbon monoxide; EPSPs, excitatory postsynaptic potentials; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; L-NMMA, L-N^G-monomethylarginine; L-NOArg, L-N^G-nitroarginine; LTD, long-term depression; LTP, long-term potentiation; mEPSCs, miniature excitatory postsynaptic currents; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PKC, protein kinase C; PAF, platelet-activating factor; PI, phosphoinositide; and SNP, sodium nitroprusside.

activation, and augment synaptic responses presynaptically by a mechanism that is insensitive to NMDA receptor antagonists. Currently, there are two leading candidates for such a messenger, arachidonic acid (AA) and nitric oxide (NO).

Arachidonic acid as a retrograde messenger

AA has several characteristics that are consistent with a role as an intercellular messenger in LTP. It is produced in neurons by activation of NMDA receptors through the actions of calcium-dependent phospholipase A_2 [20]. AA readily diffuses across cell membranes and has been shown to be released extracellularly in the dentate gyrus during stimulations that induce LTP [21]. In both CA1 and the dentate gyrus, several studies have documented that phospholipase inhibitors block LTP [2, 19, 22]. Additionally, Williams *et al.* [23] demonstrated that AA fails to produce a lasting potentiation of responses in the dentate gyrus when administered in conjunction with low frequency synaptic stimulation. However, when coupled with weak tetanic stimulation, a slowly developing long-term enhancement of synaptic responses occurs. Williams *et al.* [23] interpreted the slow development of LTP in AA-treated slices as indicating that this agent could serve as a messenger for the late phases of LTP but that another messenger, possibly NO, was likely to contribute to the early presynaptic changes. In the CA1 hippocampal region, O'Dell *et al.* [19] reported that AA produces LTP when administered in conjunction with a weak tetanus but not when administered alone. The weak tetanus by itself was insufficient to induce LTP. However, the enhancement produced by the combination of AA and a weak tetanus was inhibited by 2-amino-5-phosphonovalerate (APV), a competitive NMDA receptor antagonist, indicating that activation of NMDA receptors was necessary for the LTP. Similar APV-sensitive LTP produced by AA in the presence of low frequency synaptic stimulation and low extracellular concentrations of magnesium has been described in the CA1 region by Kato *et al.* [24]. The dependence of AA-mediated LTP on NMDA receptors diminishes the likelihood that AA serves as a primary retrograde messenger in the CA1 region. Furthermore, Miller *et al.* [25] found that AA augments responses mediated by NMDA receptors in cultured cerebellar granule cells, providing a possible explanation for the APV-sensitive LTP produced by AA. It should be noted, however, that the LTP seen in the presence of AA in the dentate gyrus was APV-insensitive [23], making it possible that AA has different actions in different regions.

Another problem with the studies demonstrating a role for AA in LTP is that the effects have been produced by concentrations of AA (50–200 μM) [19, 23, 24] that may exceed those achieved extracellularly in the nervous system during synaptic activation. However, a recent finding of possible importance in interpreting the role of AA in LTP is that low concentrations of AA augment the function of a presynaptic metabotropic glutamate receptor that promotes glutamate release from synaptosomes [26]. Metabotropic glutamate receptors are coupled by a guanine nucleotide binding protein (G-protein) to specific second messenger systems, including the phosphoinositide-protein kinase C (PI-PKC) system [27]. PKC activators are known to enhance synaptic transmission by a presynaptic mechanism [28, 29] and there is evidence supporting a role for PKC in LTP [2, 30, 31]. Taken together, the available data suggest that AA could play a retrograde messenger role in dentate gyrus LTP, but the story is far from complete.

Nitric oxide as a retrograde messenger

The second candidate for a retrograde messenger in NMDA receptor-dependent LTP is NO. This agent has been shown to serve as an intercellular messenger mediating a variety of physiological effects including smooth muscle relaxation, vasodilation and neurotransmission [32, 33]. Furthermore, NO appears to participate in cytotoxic events including the calcium-dependent delayed excitotoxic neuronal damage produced by NMDA receptor activation in the CNS [34, 35]. NO is produced from either of the two terminal guanidinium nitrogens of L-arginine by a calcium and calmodulin-dependent enzyme, NO synthase (NOS) [36, 37]. This 150 kDa enzyme also requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) and catalyzes the formation of L-citrulline and a group of NO-related molecules [38]. Interest in NO as a CNS and LTP messenger was fueled by the observation that NMDA promotes the calcium-dependent release of NO from cultured cerebellar neurons [39] and cerebellar and hippocampal slices [40].

Consistent with its proposed role as an intercellular messenger, NO is freely diffusible, membrane permeable and highly reactive with a half-life of several seconds [41]. NO synthesis depends on increases in intracellular calcium, and thus NO production could be interpreted as reflecting cellular activation. Based on the chemical properties of NO and the events required for LTP, Gally and colleagues [42] hypothesized that NO could serve as a principal determinant of synaptic strength based on rules that require a correlation between postsynaptic depolarization and NO levels. Furthermore, local diffusion of NO could help to coordinate the activity of neurons in a volume of tissue and to alter the efficacy of some synapses that are not directly activated during LTP induction. While such local diffusion seems to contradict the synapse specificity of LTP [1, 2], it has the ability to explain the observation by Bonhoeffer and colleagues [43] that synapses within about 150 μm of a directly activated connection show potentiation following LTP induction in hippocampal slice cultures. If a freely permeable retrograde messenger is involved in LTP, it might be expected that some non-activated synapses would exhibit potentiation depending on the distance over which the messenger diffuses.

In late 1991 and early 1992, four groups presented evidence consistent with a role for NO in CA1 LTP [19, 44–46]. This evidence is based on several observations. First, competitive NOS inhibitors, including L-N^G-monomethylarginine (L-NMMA) and L-N^G-nitroarginine (L-NOArg), inhibit the induction of LTP but do not reverse established LTP. The

inhibition produced by these agents is reversed by high concentrations of L-arginine, the natural substrate for NOS, but not by D-arginine. D-Isomers of the NOS inhibitors, which are ineffective enzyme antagonists, do not block LTP. Second, hemoglobin, an agent that binds NO, blocks LTP when administered extracellularly prior to tetanic stimulation. This action is not mimicked by methemoglobin, an agent that is much less effective than hemoglobin in binding NO. Third, application of NO produces an APV-insensitive increase in the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSCs) in cultured hippocampal neurons [19]. Such an effect is consistent with a presynaptic enhancement of transmitter release. More recently, preliminary evidence has been presented that NO produces LTP in hippocampal slices in an APV-insensitive fashion when administered in conjunction with a weak presynaptic stimulus [47]. Similarly, Bohme and colleagues [44, 48] found that 1 mM sodium nitroprusside (SNP) and 1 mM hydroxylamine, agents that spontaneously release NO, produce LTP in the CA1 region. The LTP produced by these agents occludes tetanus-induced LTP, suggesting that common mechanisms are involved in the enhancement.

The possible retrograde messenger role of NO in CA1 LTP is supported by three observations in these studies. First, the block of LTP by bath-applied hemoglobin suggests that NO must be released extracellularly to exert its actions since hemoglobin is likely restricted to the extracellular space when bath applied [19, 45, 46]. Second, competitive NOS inhibitors block LTP in individual neurons when injected into the postsynaptic cell [19, 45]. This suggests that the NO needed for LTP is formed in postsynaptic neurons. However, these studies must be interpreted cautiously since the NOS inhibitors are membrane permeable and could have exerted effects on cells in the local environment. Third, the ability of NO to increase the frequency of mEPSCs is consistent with a presynaptic site of action [19].

Nitric oxide and long-lasting synaptic change

Taken together, the data from these four groups strongly suggest that NO is involved in CA1 LTP and are consistent with NO acting as a retrograde messenger. However, many questions about NO remain. Because of its short half-life and highly reactive chemical nature, it is likely that NO activates longer-lived cellular processes that produce the long-term changes in transmitter release. NO exerts several biochemical effects that could have long-lived effects on synaptic transmission. These include the formation of peroxynitrite anions, activation of the soluble guanylate cyclase-cyclic GMP second messenger system, and ADP ribosylation of certain proteins [49]. Presently there is little evidence to support the role of peroxynitrite anions or other free radicals formed from NO in the generation of LTP, although hydrogen peroxide-derived radicals may hasten the decline of potentiated responses in CA1 [50]. In contrast, cyclic GMP has multiple effects on cellular biochemistry that could contribute to long-term synaptic changes. These include the activation of protein kinases that phosphorylate specific cellular proteins. In the cerebellum, NO, or a related messenger, is thought to be a prime mediator of the effect of excitatory amino acids on cyclic GMP production [51]. However, the situation in hippocampal neurons is less certain. Haley *et al.* [46] found that dibutyryl cyclic GMP, a membrane permeable cyclic GMP analogue, does not alter baseline synaptic responses in CA1 but partially overcomes the effect of a competitive NOS inhibitor on LTP. In contrast, Schuman and Madison [45] found that 8-bromo-cyclic GMP, another membrane permeable analogue, has no effect on CA1 synaptic responses and that dibutyryl cyclic GMP fails to augment synaptic responses when administered in conjunction with strong presynaptic activity in the presence of APV [52]. Furthermore, an inhibitor of cyclic GMP-dependent protein kinase fails to block LTP [52]. Although the latter studies do not exclude a role for cyclic GMP, it presently seems unlikely that cyclic GMP mediates the LTP effects attributed to NO.

An alternative possibility is that NO promotes the covalent modification of proteins involved in excitatory synaptic transmission via ADP ribosylation. Schuman and colleagues [52] recently presented preliminary evidence that nicotinamide, luminol and vitamin K_1, inhibitors of mono-ADP ribosyltransferase (ADPRT), block CA1 LTP. However, these agents could have other effects on cellular functions and the nature of the proteins that might be ADP ribosylated is uncertain. One possibility is the 37 kDa protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which Zhang and Snyder [53] have shown is ADP ribosylated following NO release. GAPDH plays an important role in glycolysis but has an uncertain function in the release of neurotransmitters. ADP ribosylation of GAPDH inhibits the enzyme [54], and iodoacetate, an inhibitor of GAPDH, irreversibly diminishes CA1 hippocampal population spikes [55] and excitatory postsynaptic potentials (EPSPs) (Izumi Y and Zorumski CF, unpublished observations). Other possible substrates for ADP ribosylation include G-proteins, which are known to undergo long-term changes in function following pertussis toxin- and cholera toxin-induced ADP ribosylation [56]. Previous studies have shown that G-proteins are involved in synaptic plasticity but that their presumed ADP ribosylation by pertussis toxin actually inhibits CA1 LTP [57]. Recently, Coggins *et al.* [58] demonstrated that protein B50/GAP-43, a membrane phosphoprotein that is believed to be important in neuronal development and possibly synaptic transmission [59], is also modified by ADP ribosylation. Again, it is presently uncertain whether this action influences LTP generation, but this protein (also known as protein F1) is present in presynaptic terminals in the hippocampus [60] and has been shown to be phosphorylated by PKC during LTP in the dentate gyrus [61, 62].

Problems with the nitric oxide hypothesis

One problem in assigning a retrograde messenger role to NO in CA1 LTP concerns the localization of NOS. For NO to be a prime messenger in LTP, it should be synthesized in postsynaptic CA1 pyramidal

neurons. Indeed, the studies demonstrating a block of LTP by postsynaptic injections of NOS inhibitors are consistent with this. However, attempts to localize NOS by immunocytochemical or NADPH-diaphorase staining [63] have found that the enzyme is primarily localized to interneurons in the hippocampus [64–66]. These studies have either failed to demonstrate significant amounts of the enzyme in CA1 pyramidal neurons [64, 65] or have found that only some pyramidal neurons contain NOS [66]. It is possible that a novel form of the enzyme exists in CA1 that is not detected by current methods, but this is speculative at present. There may also be species differences in whether the currently identified forms of NOS are present in the hippocampus as mice, but not rats, appear to have the enzyme in CA1 pyramidal neurons [67]. Three of the four groups demonstrating a role for NO in CA1 LTP used hippocampal slices prepared from rats (Table 1).

A second problem with the NO story has been the failure of some groups to replicate the earlier reports [1]. Recent studies suggest that NO may contribute to LTP only under certain circumstances. Gribkoff and Lum-Ragan [68] found that a single 100 Hz × 1 sec tetanus at a stimulus intensity that evoked a 50% maximal synaptic response produced robust LTP in the CA1 region but that the enhancement was insensitive to either a NOS inhibitor or APV. In contrast, delivery of two 100 Hz × 1 sec stimulus trains at maximal stimulus intensity induced APV- and L-NOArg-sensitive LTP. This suggests that NO contributes to LTP only during intense stimulation paradigms. The unusual finding in this study was the failure of APV to block LTP produced by the lower intensity stimulus. The earlier studies demonstrating a role for NO in LTP used repeated 1-sec tetanic stimulations or repeated brief bursts of synaptic activation to induce LTP (Table 1). The temperature at which experiments are conducted may also be a contributing variable. In a preliminary study, Li *et al.* [69] recently observed that LTP in the dentate gyrus is blocked by NO inhibitors at room temperature but not at higher temperatures. Two groups demonstrating a role for NO in CA1 LTP worked at room temperature (Table 1).

Another complicating variable concerns the possible role of NO release in NMDA-mediated LTP inhibition. Previously, several groups have found that untimely activation of NMDA receptors either by synaptic stimulation in low magnesium solutions [70] or weak tetani [71] blocks the subsequent induction of LTP by a strong tetanus. In hippocampal slices prepared from 30-day-old rats, Izumi *et al.* [72] demonstrated that low concentrations of NMDA (1 μM) block CA1 LTP when administered either immediately before or following delivery of a single 100 Hz × 1 sec tetanus to the Schaffer collateral pathway. This NMDA-mediated LTP inhibition is reversed by NOS inhibitors and hemoglobin and is mimicked by 10 μM SNP in a hemoglobin-sensitive fashion [73]. One possibility that could explain this effect is that the released NO blocks tetanus-induced activation of NMDA responses. Previously, NO and agents that release NO have been shown to inhibit NMDA responses in several preparations [74–76]. However, two observations argue against this explanation. First, neither 1 μM NMDA nor 10 μM SNP decreased the NMDA component of CA1 synaptic responses recorded extracellularly, although both blocked LTP. Second, NMDA, unlike NMDA receptor antagonists, blocks LTP when administered immediately following the tetanic stimulation [72, 73].

These observations suggest that untimely NO release interferes with LTP generation downstream of NMDA receptor activation and can be interpreted as either supporting or contradicting a role for NO in CA1 LTP. It is possible that NMDA receptor activation down-regulates biochemical processes necessary for LTP by inappropriately releasing NO. However, a recent observation suggests that NO may actually be a negative modulator of synaptic transmission. This is based on the finding that NO mediates a form of homosynaptic long-term depression (LTD) in CA1. Previously, Dudek and Bear [77] reported that 1 Hz stimulation of the Schaffer collateral pathway for 15 min produces APV-sensitive LTD. Mulkey and Malenka [78] showed that this LTD is dependent on calcium entry into postsynaptic neurons and is not due to excitotoxic synaptic fatigue. In preliminary experiments, Izumi and Zorumski observed that the LTD following 1 Hz stimulation is reversed by NOS inhibitors and hemoglobin (Fig. 1) and can be mimicked by 15-min applications of 100 μM SNP or 100 μM *S*-nitrosocysteine, agents that release NO. Furthermore, Fujii *et al.* [79] found that persistent 1 Hz stimulation blocks CA1 LTP in an APV-sensitive fashion when administered either before or following tetanic stimulation. This block of LTP was also reversed by NO inhibitors (Izumi Y and Zorumski CF, unpublished observations).

Taken together, these recent observations suggest that NO produces long-lasting increases or decreases in synaptic transmission depending on the timing and perhaps degree of release. Interestingly, two other factors—NMDA receptor activation and calcium influx into postsynaptic neurons—are also involved in both LTP and LTD in the CA1 region. This situation is, in some ways, analogous to the visual cortex where Artola and colleagues [80] have shown that a tetanus can induce either LTP or LTD depending on the degree of postsynaptic depolarization. Greater degrees of depolarization and presumably greater calcium influx are needed to induce LTP. Similarly, Xie *et al.* [81] found that a given synaptic stimulus elicited either LTP or LTD of the NMDA component of synaptic responses in the rabbit dentate gyrus depending on the post-synaptic membrane potential. A notable difference is that LTD in the visual cortex is insensitive to APV and thus not mediated by NMDA receptors [80]. NMDA receptor-independent forms of homo-synaptic LTD have also been described in the CA1 region [82]. Whether NO is involved in the various forms of LTP and LTD in hippocampus and cortex is unclear at present. In the cerebellum, LTD of the parallel fiber-Purkinje cell synapse that occurs when climbing fibers and parallel fibers are stimulated conjunctively is reversed by NO inhibitors and

Table 1. Nitric oxide inhibitors and LTP

Reference	Species	Age/ weight	LTP stimulus	Temperature	NO inhibitors	Drug exposure	Effect
O'Dell *et al.* [19]	Guinea pig		(a) 100 Hz × 1 sec × 2, 20 sec apart at sub PS intensity (b) Pairing: 1 Hz × 15–20 sec with depol. to 0 mV	30°	50 μM L-NoArg 20 μM Hb	30 min before and 10 min after LTP stimulus	LTP blocked
Schumann and Madison [45]	Rat	300 g (adult)	(a) 100 Hz × 1 sec × 4–5, 15–30 sec apart at sub PS intensity (b) Pairing: sustained depol. + 1 Hz × 30–45 sec	22°	10 μM L-MeArg 10 μM L-NoArg 100 μM Hb	1.5–2 hr before LTP stimulus	LTP blocked
Bohme *et al.* [44]	Rat	140–200 g (~60 days)	100 Hz × 1 sec × 2 at 2 × baseline intensity (baseline = sub PS intensity)	32°	0.1 μM L-NoArg	15 min before and 5 min after LTP stimulus	LTP blocked
Bon *et al.* [48]	Rat	140–200 g	Same as Bohme *et al.*	32°	L-NoArg L-NMMA L-NAME Hb	15 min before and 5 min after LTP stimulus	LTP blocked
Haley *et al.* [46]	Rat		40 msec bursts at 100 Hz × 10,200 msec apart at sub PS intensity	22°	100 μM L-NAME 100 μM L-Hb	15 min before LTP stimulus	LTP blocked
Gribkoff and Lum-Ragan [68]	Rat	30–90 days	(a) 100 Hz × 1 sec at 50% max EPSP intensity (b) 100 Hz × 1 sec at max intensity × 2, 60 sec apart	32°	50–100 μM L-NoArg 100 μM L-NMMA	1 hr before LTP stimulus	LTP blocked only with intense stimulus

Abbreviations: L-NoArg, L-N^G-nitroarginine; L-MeArg, L-N^G-methylarginine; Hb, hemoglobin; L-NAME, L-N^G-nitroarginine methylester; L-NMMA, L-N^G-monoethylarginine; and PS, population spike.

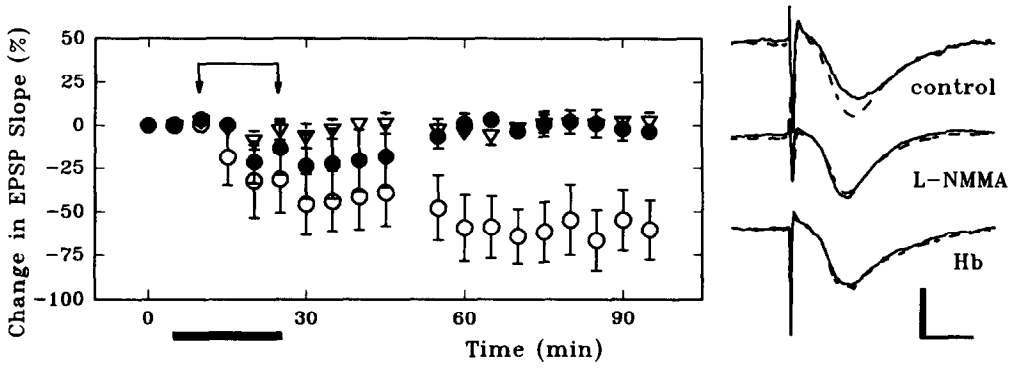

Fig. 1. Mediation of hippocampal LTD by NO. The graph shows the time course of change (± SEM) in EPSP slope for slices stimulated at 1 Hz for 15 min without [(○), N = 5] and with 30 μM L-NMMA [(●), N = 5] or 0.1 μM hemoglobin [Hb, (△), N = 6]. L-NMMA and Hb were administered for 5 min before and during the 1 Hz stimulation (solid bar). The connected arrows denote the period of 1 Hz stimulation. The traces depict representative EPSPs taken prior to (dashed traces) and 1 hr following (solid traces) the 1 Hz stimulation. Calibration bar: 1 mV, 5 msec for control and Hb and 2 mV, 5 msec for L-NMMA.

mimicked by NO [83], providing a parallel line of evidence that NO plays a role in synaptic depression.

The possible involvement of similar factors in both synaptic enhancement and depression is consistent with previous theoretical models in which it has been proposed that the degree of correlation between presynaptic and postsynaptic neuronal activation, and presumably the degree of postsynaptic calcium rise, determines whether synaptic weights will increase or decrease [84, 85]. According to the model proposed by Lisman [85], low to moderate calcium rises are associated with synaptic depression (anti-Hebb processes) while large rises in calcium promote potentiation (Hebb processes). If NO is a mediator of the calcium-dependent changes, release of this substance would potentiate responses when presynaptic activation and postsynaptic depolarization are closely correlated. Such a situation would be expected during a high frequency tetanus and during the pairing of postsynaptic depolarization with presynaptic stimulation. When presynaptic activation and postsynaptic depolarization are less closely coupled, as might occur during 1 Hz stimulation or during tetanic stimulation of neurons voltage clamped near their resting membrane potential, synaptic depression or inhibition of LTP would result. Thus, as proposed by Gally *et al.* [42], the degree of NO release may be a biochemical index of the correlation between presynaptic and postsynaptic activity.

Other retrograde messenger candidates

Although there are questions concerning both AA and NO as retrograde messengers, it is reasonable to pursue their roles in synaptic plasticity. It is also reasonable to look for other retrograde messenger candidates. Lowenstein and Snyder [49] recently postulated that other volatile molecules could serve roles attributed to NO. In particular, carbon monoxide (CO), which is produced by heme oxygenase in the breakdown of heme to biliverdin, may serve as a mediator of cyclic GMP production in the CNS. This hypothesis is based on the observation that heme oxygenase is more closely linked to the neuronal populations that contain guanylate cyclase than is NOS [86]. Furthermore, Lowenstein and Snyder [49] suggest that a principal CNS function of NO may be the ADP ribosylation of specific proteins. Interestingly, the NOS molecule contains a CO-binding heme moiety, raising the possibility that CO may alter the production of NO [87].

Another candidate that merits study as part of a putative retrograde signalling cascade is platelet-activating factor (PAF), a lipid mediator released from glycero-3-phosphocholine by the action of calcium-dependent phospholipase A_2 [88]. This enzyme also catalyzes the release of AA, and phospholipase inhibitors have been shown to block LTP [2, 19, 22]. In peripheral systems, PAF serves as an intercellular messenger [89], and there is evidence that PAF can be released extracellularly from neurons [90]. Three groups have presented evidence consistent with a role for PAF in CA1 LTP [91–94]. This evidence is based largely on the ability of PAF receptor antagonists to block LTP. Recently, Clark *et al.* [95] demonstrated that a PAF analogue augments glutamate-mediated excitatory synaptic transmission in cultured hippocampal neurons by a presynaptic mechanism. Furthermore, although PAF alone does not produce LTP [92], a persisting enhancement of CA1 responses occurs when PAF is administered in conjunction with moderate presynaptic activation [93] in the presence of APV. Whether these effects are mediated by PAF or a PAF-derived messenger is uncertain, but the effects are inhibited by an antagonist of PAF receptors localized to synaptic regions of the CNS [96]. It is notable that PAF promotes PI turnover and increases in intracellular calcium [89], effects that could lead to increased neurotransmitter release. Additionally, PAF promotes release of AA and, in smooth muscle,

certain PAF effects may be mediated by NO [97]. Thus PAF could serve as a link in a retrograde messenger biochemical cascade involving AA and NO.

Summary

The dependence of NMDA receptor-dependent LTP on postsynaptic depolarization and increases in postsynaptic calcium, coupled with evidence supporting presynaptically mediated increases in transmitter release accompanying LTP, suggest that a retrograde transsynaptic messenger participates in the synaptic enhancement. Although many questions remain unanswered, the available evidence suggests a role for NO as such a messenger in certain LTP paradigms. It is unclear, however, whether NO contributes to LTP under differing experimental conditions and whether other messengers, acting in concert with or independent of NO, contribute to a retrograde signalling system. Furthermore, the conditions under which NMDA receptor activation, postsynaptic calcium increases and NO contribute to synaptic enhancement, synaptic depression and excitotoxic neuronal injury need to be clarified. Furthermore, efforts aimed at clarifying the molecular targets of NO must remain a priority of this line of research.

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