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Retrograde Signaling in the Development and Modification of Synapses

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Fitzsimonds, Reiko Maki, and Mu-ming Poo. Retrograde Signaling in the Development and Modification of Synapses. *Physiol. Rev.* 78: 143–170, 1998.—Retrograde signaling from the postsynaptic cell to the presynaptic neuron is essential for the development, maintenance, and activity-dependent modification of synaptic connections. This review covers various forms of retrograde interactions at developing and mature synapses. First, we discuss evidence for early retrograde inductive events during synaptogenesis and how maturation of presynaptic structure and function is affected by signals from the postsynaptic cell. Second, we review the evidence that retrograde interactions are involved in activity-dependent synapse competition and elimination in developing nervous systems and in long-term potentiation and depression at mature synapses. Third, we review evidence for various forms of retrograde signaling via membrane-permeant factors, secreted factors, and membrane-bound factors. Finally, we discuss the evidence and physiological implications of the long-range propagation of retrograde signals to the cell body and other parts of the presynaptic neuron.

I. INTRODUCTION

Bidirectional communication between a neuron and its postsynaptic cell is essential for the development, maintenance, and activity-dependent modulation of synaptic connections. Neurotransmitters, neuropeptides, and other protein factors released from the presynaptic nerve terminal are known to produce immediate electrical actions as well as long-term structural and metabolic changes in the postsynaptic cell. Reciprocal retrograde influences of the postsynaptic cell on the survival, differentiation, and functioning of the presynaptic neuron are also present in many parts of the nervous system. This review summarizes the evidence for the existence of retrograde interactions and the identity, production, as well as the mechanism of action of retrograde factors involved in these interactions. Various aspects of this topic have been covered by a number of recent reviews (56, 69, 90, 125, 149, 154, 319).

A synaptic retrograde factor may be defined by several criteria analogous to those used for the identification of neurotransmitters. The factor must be synthesized in the postsynaptic cell. It must be presented to the presynaptic neuron at the appropriate time for retrograde interaction. In addition, if the factor is a diffusible or secreted substance, exogenous application of the factor should mimic the retrograde action produced by the postsynaptic cell. In contrast to neurotransmitter signal-

ing, however, retrograde effects may be mediated by direct physical interaction between membrane-bound factors across the synapse. Moreover, retrograde factors may be present constitutively over a relatively long duration, although the production, secretion, or action of the retrograde factor could be regulated by neuronal activity.

The molecular understanding of target-derived retrograde factors began with the discovery of nerve growth factor (NGF) and its potent effects on the survival and growth of sympathetic and sensory neurons in culture (66, 212). That NGF is required for the survival of sympathetic and sensory ganglion neurons was demonstrated by the dramatic atrophy of these ganglia in newborn mice and rats injected with antiserum against NGF (213). Immunoassays showed that NGF is indeed produced by the targets of sympathetic neurons in amounts proportional to the density of innervation (199). Moreover, targets innervated by sympathetic neurons contain mRNA for NGF, whereas many other tissues do not (324). Thus NGF has largely satisfied the criteria for a retrograde factor. The search for other protein factors with similar actions on central neurons has led to the discovery of a family of NGF-like molecules, each specific for different but overlapping populations of neurons. Other members of this NGF family of factors, or neurotrophins, now include brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3, NT-4/5, and NT-6 (213, 215). In addition to their traditional role as survival and differentiation factors, recent studies have shown acute modulatory effects of neurotrophins on axonal branching and arborization, ion channel function, and synaptic efficacy (24, 223). Furthermore, the expression and secretion of neurotrophins are regulated by electrical activity (341), suggesting that many activity-dependent retrograde interactions at the synapse may be mediated by neurotrophins. Neurotrophins have thus become prototypic retrograde factors for both long-term and acute actions on presynaptic neurons. In addition to neurotrophins, other families of growth factors and cytokines can also regulate the survival and growth of presynaptic neurons as well as modulate neuronal and synaptic functions (24, 280). However, it remains largely unknown whether these factors act as circulating hormones, with wide spread influences on many neuronal populations, or serve as localized synaptic retrograde factors for selective presynaptic neurons.

In addition to the effect on the growth and survival of presynaptic neurons, target-derived factors are also responsible for inducing presynaptic development. Contact with a specific target cell triggers a transformation of the presynaptic nerve ending from a motile growth cone to a stable nerve terminal, which is capable of efficient transmitter secretion in response to action potentials. In some systems, the characteristics of the transmitter secretion machinery at different nerve terminals of the same presynaptic neuron match the type of target cell they innervate (30, 89, 90, 122), suggesting retrograde influences of the postsynaptic cell on secretory function. The transmitter phenotype used by the presynaptic neuron can be regulated by target-derived factors, as shown by the switch from noradrenergic to cholinergic phenotype in sympathetic neurons after innervation of the sweat gland (206, 207, 319). Target contact made by one axonal process can regulate the growth and projection pattern of other processes of the same neuron (124, 129) and change the global membrane excitability or transmitter sensitivity of the neuron (202). The target cell innervated by the axonal terminal (226, 297) regulates even synaptic connections formed on the presynaptic neuron. Finally, studies of activity-dependent plasticity of developing and mature synapses have shown that presynaptic secretion properties are susceptible to rapid modification by retrograde factors originating from the postsynaptic cell, and such modulation can lead to long-term changes in synaptic efficacy (32, 85, 205, 269).

The findings on synapse development and plasticity underscore several important features of retrograde signaling. Retrograde signals consist of not only trophic factors that maintain the health and survival of the presynaptic neuron, but also serve as instructive signals that trigger selective gene expression. Moreover, presynaptic functions are under the regulation of retrograde signals in an activity-dependent manner with a rapid time course. Finally, although retrograde signals can exert localized presynaptic regulation, long-range presynaptic propagation of the retrograde signals must occur in some cases, both in conveying regulatory signals to the nucleus and in affecting functions of distant parts of the neuron. The present review discusses these features of retrograde signaling, with special attention on the early inductive actions of the target cell on presynaptic differentiation, the role of retrograde signaling in activity-dependent modulation at developing synapses, the cellular mechanisms involved in retrograde signaling, and the nature of presynaptic spread of retrograde signals.

II. RETROGRADE SIGNALING AT DEVELOPING SYNAPSES

Retrograde interactions at the synapse begin with the contact of the growth cone with the surface of the target cell. These interactions are likely to be responsible for the modulation of growth cone motility, the induction and maturation of transmitter secretion machinery, and activity-dependent selective stabilization and elimination of nerve connections. Most of the information concerning retrograde signaling at developing synapses comes from studies of peripheral synapses, neuromuscular synapses in particular (17, 86, 145, 149).

At electrical synapses, intercellular communication via gap junctions provides an obvious avenue for retrograde signaling. Gap junctions have been observed during the early phase of nerve-muscle synapses in culture (5, 118) and could mediate cell-cell signaling at early contacts. In developing cortex, injections of the intercellular tracer neurobiotin reveal extensive coupling via gap junctions between groups of cortical neurons. These groups of neurons exhibit synchronous bursts of intracellular Ca^{2+} changes (366, 367). Elevation of Ca^{2+}, inositol 1,4,5-trisphosphate ($InsP_3$), adenosine 3′,5′-cyclic monophosphate (cAMP), or other small molecules in a postsynaptic neuron can result in a spread of these messengers to the presynaptic neuron via the gap junctions (183, 310). Evidence for cell-specific gap junction coupling in the cortex, retina, and adult hippocampus points to the possibility that bidirectional intercellular communication via gap junctions may play a role in development and maintenance of synapses. A variety of cell adhesion molecules, including E-cadherin, neural cell adhesion molecule (NCAM), and N-cadherin appear to be necessary for the formation of functional gap junctions (182, 191, 254). During the early phase of synaptogenesis, selective cell-cell adhesion may lead to gap junction-mediated communication and exchange of specific intracellular messengers, which in turn serve to functionally group specific cells and to direct the formation of stable chemical synapses between appropriate partners.

A. Early Inductive Events in Synaptogenesis

Synaptogenesis involves the transformation of a highly motile growth cone into a stable nerve ending, equipped with the capacity for efficient transmitter secretion. The first step in synaptogenesis is the recognition of target cells by the nerve growth cone. Target cell surface molecules responsible for cell-cell recognition may also trigger the transformation of the nerve growth cone. Growing nerve processes are guided by pathway cues in the developing tissue to reach their targets (143, 152, 193). Upon arrival of these processes at the target zone, selective interaction between the growth cone and specific target cells may take place. In developing chick embryos, there is strong evidence for specific recognition between subsets of afferent sensory axons and their target motoneurons in the ventral spinal cord (123). In *Drosophila* larva, different motoneurons make highly specific innervation of different subsets of body wall muscles. Studies by genetic manipulation have shown the involvement of a number of subset-specific muscle surface proteins in nerve-muscle recognition. For example, fasciclin III contributes to positive interaction between motoneuron RP3 and its target muscle, since ectopic expression of fasciclin III on all muscles caused RP3 to recognize other nontarget muscles (62). Two other surface proteins, semaphorin II and connectin, were found to prevent formation of selective innervation by the proper motoneuron when they were expressed at high levels on the target muscle fibers (244, 272), suggesting a repulsive action of these molecules on the growth cone of specific motoneurons. These results from the *Drosophila* neuromuscular system have shown that the growth cone from the contacted cells may receive both positive and negative retrograde signals before the decision on synapse formation is made. The presynaptic receptors for these muscle surface proteins and the downstream transduction mechanism involved in growth cone responses are unknown. Because elevation of Ca^{2+} usually results in an inhibition of growth cone motility (188), which is a required step for the transition into a stable nerve terminal, Ca^{2+} may serve as a second messenger for an early retrograde signal. In *Xenopus* and *Helisoma* cell cultures, fluorescence Ca^{2+} imaging has shown that contact by the target muscle indeed induces Ca^{2+} elevation in the presynaptic neuron (81, 374).

The use of cell cultures has greatly facilitated the study of early physiological events during synaptogenesis, since the timing of nerve-muscle contacts can be more precisely determined. In *Xenopus* nerve-muscle cultures, both spontaneous (miniature) and nerve-evoked synaptic potentials are observed within seconds after nerve-muscle contact (63, 114, 194, 362). The rapid onset of synaptic transmission is possible in this system, because the growth cone is capable of efficient evoked transmitter release before contact with the muscle cell (338). Muscle contact, nevertheless, exerts a marked retrograde effect on the properties of transmitter secretion. This was shown by the immediate induction of elevated spontaneous (action potential-independent) acetylcholine (ACh) secretion from the growth cone by the muscle contact. As shown by an excised patch of muscle membrane placed near the growth cone as a detector for ACh, the growth cone exhibits very little spontaneous secretion before the muscle contact, and the induction of spontaneous release is specific to the muscle cell. The induction is not due to secreted factors from the muscle, since the excised patch of muscle membrane by itself is capable of inducing spontaneous ACh secretion from the growth cone (362). These studies support the notion that interaction between membrane-bound surface components is capable of providing a retrograde signal during the early phase of synaptogenesis. The precise nature of the surface component responsible for the induction of spontaneous ACh secretion is unknown, but elevation of spontaneous secretion could result from a contact-induced increase of cytosolic Ca^{2+} at the growth cone (81). Consistent with the specific nature of the nerve-muscle surface interaction, a subset of spinal neurons showed neither an increase in transmitter release nor an elevation of Ca^{2+} after muscle cell contact. Similar induction of elevation of intracellular Ca^{2+} levels

is observed after contact of presynaptic neurites of *Helisoma* B19 motoneurons with their target supralateral radular tensor (SLT) muscles (374). *Helisoma* motoneuron B19 will form functional chemical connections only with its normal synaptic partner SLT muscle, and not with novel targets (155, 373). This contact-mediated Ca^{2+} accumulation in the B19 nerve terminals appears to result from a SLT muscle-derived retrograde messenger, which acts via a presynaptic cAMP-dependent protein kinase to enhance action potential-evoked Ca^{2+} influx (128).

Ultrastructural studies of early neuromuscular contacts revealed close membrane appositions between the nerve and muscle membranes, with an extracellular gap of $<$10 nm (48, 204). Thus direct interactions between plasma membrane components could be responsible for the initial nerve-muscle recognition and serve as the trigger for the induction of spontaneous transmitter release. Application of suramin, a small sulfonated polyanion that interferes with cell surface receptors, suppresses contact-mediated ACh release as well as muscle contact-induced elevation of intracellular Ca^{2+} (81). Muscle membrane extracts also elicited changes in intracellular Ca^{2+} in *Helisoma* B19 motoneurons, and the effect was abolished by enzymatic treatment of the membrane extracts (374). Cell adhesion molecules or extracellular matrix (ECM) proteins (31, 77) are likely candidates for mediating contact-dependent presynaptic changes during the early phase of synaptogenesis. Contact with latex beads coated with basic fibroblast growth factor (bFGF), a heparin-binding growth factor associated with the ECM of skeletal muscle (98, 142), mimics the muscle target in inducing elevation of presynaptic Ca^{2+} and clustering of synaptic vesicles. The effects can be blocked by Ca^{2+}-free medium or suramin (82). Taken together, these studies indicate those direct interactions of surface-bound factors, rather than target-derived diffusible factors, are likely to be responsible for the early retrograde inductive events during synaptogenesis. As discussed in section IV*C*, retrograde interaction through direct physical contact may be a general mechanism for *trans*-synaptic signaling.

B. Maturation of Presynaptic Structure and Function

After the initial establishment of synaptic contacts, the nerve terminal undergoes structural and functional maturation over a protracted period (192, 198, 204). At early nerve-muscle contacts in *Xenopus* cultures, functional synaptic transmission occurs without apparent presynaptic differentiation, but the efficacy of synaptic transmission shows improvement with time, in parallel to the development of presynaptic structures (48). Ultrastructural studies during the first few days of synaptogenesis revealed a gradual formation of the active zone, with clustering of synaptic vesicles around a region of the presynaptic plasmalemma characterized by membrane thickening (48, 262). The formation of the active zone involves the localization and assembly of components of transmitter secretion machinery, including voltage-dependent Ca^{2+} channels and other membrane proteins involved in vesicle docking, as well as cytoplasmic synaptic vesicles and associated cytoskeletal elements. Because the site of postsynaptic contact dictates the site of active zone formation, it is reasonable to expect that signals from the postsynaptic cell provide the trigger for initiating the localization of presynaptic components. Using specific antibodies, Lupa and Hall (229) observed a redistribution of proteins that form neurofilaments and synaptic vesicles during the formation of mouse neuromuscular junctions. The appearance of synaptic vesicle-containing neurites on the surface of the early myotubes correlates temporally with the formation of ACh receptor clusters and the first synaptic potentials. Further studies (228) showed that the preferential association of synaptic vesicle containing neurites with myotubes is specific for muscle cells and was still observed when the myotubes were previously fixed with paraformaldehyde. Moreover, the preferential association disappeared in cocultures of neurons with variant strains of the myotube that are deficient in proteoglycans, suggesting that proteoglycans on the surface of the myotube may play an inductive role in the differentiation of presynaptic neurons.

The role of basal lamina in synaptic development was demonstrated by an elegant series of studies on synapse regeneration in the absence of muscle cells (243, 315). Although isolation of the muscle segments by transection causes the fibers to degenerate, the basal lamina remains intact, allowing identification of the original end-plate site. These basal lamina "ghosts" were shown to contain signals not only for postsynaptic clustering of ACh receptors (49), but also for induction of presynaptic differentiation (138). Surprisingly, motor nerve terminal maintenance continues for many months in the absence of muscle fibers (107), suggesting that presynaptic function may be maintained by long-lasting target-derived signals persisting either in the nerve terminal or in the remaining basal lamina. Alternatively, target-derived factors may not be needed for the long-term maintenance of presynaptic function after selective damage to the muscle at mature neuromuscular junctions, but rather that retrograde signaling occurs only during the early phase of synapse formation or reinnervation of the target. Agrin, a component in the basal lamina responsible for ACh receptor clustering (42, 250), may also be involved in presynaptic differentiation (52). In knock-out mice lacking agrin, there is profuse outgrowth of presynaptic nerve terminal arbors. This effect on presynaptic nerve terminals may be indirect, resulting from impaired retrograde signaling associated with postsynaptic differentiation normally induced by

agrin. Extensive axonal arborization is also observed when the ACh receptor-associated cytoskeletal protein rapsyn is deleted by homologous recombination (132) and in mice with homozygous gene deletions of the agrin receptor MuSK (93). Inadequate presynaptic differentiation in these mutants is therefore likely to be due to the failure of the muscle to provide appropriate retrograde signals resulting from the lack of specialized postsynaptic signaling machinery (313).

In analogy to the role of agrin on postsynaptic ACh receptors, a retrograde factor provided by the postsynaptic cell may trigger the presynaptic clustering of Ca^{2+} channels and other membrane components associated with the active zone. Like agrin, the retrograde factor may be bound to the ECM, which may account for the requirement for muscle surface proteoglycan described above (228). Basic fibroblast growth factor, which associates with ECM, was capable of inducing active zonelike structures in cultured spinal neurons (82). Thus it is a good candidate for a retrograde clustering factor. In addition to secreted factors, integral membrane proteins are also potential candidates for triggering plasma membrane localization. The close apposition of plasma membranes during the early phase of synaptogenesis allows intercellular bonding and coclustering of pre- and postsynaptic membrane components. In such a coclustering mechanism, anterograde and retrograde signaling are two sides of the same process. At mature neuromuscular junctions, clusters of Ca^{2+} channels in the presynaptic nerve terminals are precisely aligned with postsynaptic ACh receptor clusters (307). This precise colocalization must result from either a coclustering process or be induced by a common clustering factor during development. A number of synapse-specific ECM molecules have been identified in the synaptic cleft, including S-laminin, agrin, collagens, nexins, acetylcholinesterase, heparan sulfate proteoglycan, and synapse-specific carbohydrates (149). These ECM molecules may play an important function in the clustering and alignment of pre- and postsynaptic channels and receptors at neuromuscular junctions. In addition, they may serve as a physical link for transmitting retrograde signals from post- to presynaptic cells, resulting in changes in structural and functional properties of the nerve terminal during development as well as in the mature state (see sect. IV*C*). Among various synapse-specific ECM molecules, S-laminin is of particular interest. Fragments of this synapse-specific form of laminin promote binding of ciliary motoneurons to culture substrate, and the binding is due to the presence of a specific tripeptide leucine-arginine-glutamate (LRE; Refs. 171, 172). Interestingly, some other synaptic molecules (agrin and acetylcholinesterase) also contain LRE sequence, but the extrasynaptic form of laminin B1 does not (170). The selective binding with the nerve terminal could account for the trapping of these ECM molecules at the synaptic. Moreover, the adhesion of S-laminin to motoneurons may serve to inhibit neurite outgrowth (292) and act as a retrograde signal to initiate nerve terminal differentiation. Indeed, in mice with targeted mutations of the S-laminin gene, nerve terminals were relatively unbranched, with no active zones and abnormal distribution of synaptic vesicles, and most strikingly, a significant reduction in frequency of spontaneous transmitter release (271). Many aspects of postsynaptic development were normal in the mutant neuromuscular junction. However, whether the synapse-specific ECM molecules appear at the muscle surface at an appropriate time to serve as retrograde signals for nerve terminal differentiation remains to be determined.

In contrast to the intrinsic propensity for evoked ACh secretion from *Xenopus* spinal neurons (338), the appearance of evoked transmitter release from motoneuron B19 requires several hours of SLT muscle contact in *Helisoma* cultures (373). The contact-dependent induction apparently involves a change in the responsiveness of the presynaptic secretory machinery to Ca^{2+}, as well as in the amount of depolarization-evoked Ca^{2+} influx. The retrograde signal from the target SLT muscle apparently activates cAMP-dependent protein kinase in the presynaptic B19 neuron to produce enhanced evoked Ca^{2+} influx (128). Whether the activated protein kinase modulates the number, distribution, or functional properties of plasma membrane Ca^{2+} channels is unknown. The dependence of presynaptic differentiation on the postsynaptic target cell is even more pronounced at hippocampal synapses. Presynaptic specializations and functional synaptic transmission between hippocampal neurons do not appear during the first 4 days in culture, although numerous neuritic contacts are made within the first day after cell plating (120). The appearance of functional synaptic transmission is correlated with the expression of ω-conotoxin GVIA-sensitive Ca^{2+} channels and the appearance of the punctate immunoreactivity to synaptic proteins rab3a, synapsin I, and synaptotagmin, which is indicative of presynaptic vesicle clusters at active zones (16). Using "heterochronic" cultures made by plating newly dissociated "young" hippocampal neurons on more mature hippocampal neurons in 4-day-old cultures, Fletcher et al. (121) found a rapid appearance of synaptic vesicle clusters in young neurons. This suggests that the propensity for presynaptic differentiation exists in young neurons, and the presence of a retrograde signal from the postsynaptic neurons appears to be the determining step in the timing of presynaptic differentiation. It appears that postsynaptic retrograde signals for induction of presynaptic differentiation become available in hippocampal neurons only after 4 days in culture.

Calcium is not only essential for regulated transmitter secretion from presynaptic nerve terminals but is likely to be an essential intracellular messenger for triggering

presynaptic differentiation, which depends on selective gene regulation (135, 327, 328). As discussed above, elevation of presynaptic Ca^{2+} level triggered by target contact may initiate a cascade of events leading to maturation of transmitter secretion machinery. The frequency of nerve-muscle contacts and the number of neuritic terminals on the myocyte are greatly reduced when Ca^{2+} is removed from the extracellular milieu, suggesting involvement of Ca^{2+} in neuritic arborization and maturation (158). Voltage-dependent Ca^{2+} influx is required to trigger Ca^{2+}-induced release of Ca^{2+} from intracellular stores, which is necessary for normal differentiation of ion channels in *Xenopus* spinal neurons in culture (96, 165, 166).

Target-derived factors have long been postulated to be responsible for the regulation of the types of transmitter and neuropeptide used by the neuron and the synapses the neuron receives on its dendrites (54, 125, 280, 319), but the molecular identification of the factors has been difficult to achieve. The neurotrophin BDNF was shown to specifically enhance the expression of neuropeptides both in vivo (265) and in vitro (263). Treatment of neuronal cultures with neurotrophins also enhances the expression of glutamate receptors and the synaptic vesicle protein synaptophysin (264). Although many neurotrophins are likely to originate from the target tissue, whether they are presented to the responsive neurons as retrograde factors is yet to be determined. In addition to neurotrophins, neuropoietic factors or cytokines are known to affect neuronal differentiation. A notable example is cholinergic differentiation factor (CDF)/leukemia inhibitory factor (LIF), which regulates gene expression in postmitotic sympathetic and sensory neurons without affecting their growth (266). The CDF/LIF mRNA is selectively enhanced in sweat glands of rat footpads, which are normally innervated by sympathetic neurons. It appears, however, that the noradrenergic to cholinergic switch of transmitter phenotype involves factors other than CDF/LIF, since innervation of sweat glands is phenotypically normal in CDF/LIF-deficient mice (301). A different sweat gland factor, which is related to ciliary neurotrophic factor (CNTF), has also been identified (300). It is likely that a number of factors with overlapping but nonidentical presynaptic actions are produced by the target cell, and a combination of them is required for the complete spectrum of presynaptic modulation. In addition to CDF/LIF and CNTF-related factors, other previously known cytokines and growth factors are known to regulate neuronal gene expression. For example, activin A induces somatostatin gene expression in cultured ciliary ganglion neurons (76), similar to the somatostatin-inducing activity originally identified in the conditioned medium of the target cell (the choroid layer of the chick eye) of these neurons (75), and activin A mRNA was indeed found in cultured choroid cells (76). Thus many factors have been identified as potential retrograde factors for regulating presynaptic phenotype of transmitter and neuropeptide. However, most of them have yet to fully satisfy the criteria of an established synaptic retrograde factor.

III. ACTIVITY-DEPENDENT RETROGRADE SYNAPTIC MODIFICATION

A. Synapse Competition and Elimination During Development

Although activity-independent events may underlie target recognition and synapse formation, stabilization or elimination of the early connections in the developing nervous systems is known to depend on electrical activity (143). The pioneering work of Hubel, Weisel, and colleagues (168, 169, 210, 211) has shown that visual inputs during a critical period in development were crucial for the formation of ocular dominance columns in the primary visual cortex. Early in development, the initial projections from the eye-specific layers of the lateral geniculate nucleus (LGN) to the visual cortex completely overlap. The segregation of LGN projections into eye-specific columns depends on the activity in the two eyes. The projections remain intermixed if the activity is blocked. When the activity in the two eyes becomes unequal, the LGN projections from the eye with higher activity occupy a larger share of the territory in the visual cortex. Thus axonal terminals of LGN neurons apparently compete for their postsynaptic target by an activity-dependent mechanism. An attractive cellular mechanism for activity-dependent synapse rearrangement is based on Hebb's postulate (156), which states that correlated pre- and postsynaptic activity leads to strengthening of the synapse, whereas uncorrelated activity leads to synapse weakening (73, 143, 332). The LGN projections carrying activity of adjacent retinal neurons of the same eye are likely to be active synchronously and drive postsynaptic cells in a correlated fashion, and their synapses onto the common group of postsynaptic cells are strengthened and stabilized. In contrast, projections carrying inputs from distant retinal cells or from a different eye are likely to be active asynchronously, and their connections will be weakened and eliminated. Cellular mechanisms underlying this Hebbian modulation of synaptic strength have been suggested by the findings of activity-dependent long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus and cortex (see sect. III*B*). There is strong evidence that these forms of long-term synaptic modulation may involve retrograde signaling from the activated postsynaptic cell to the presynaptic nerve terminal, resulting in persistent changes in the efficacy of evoked transmitter secretion. It is possible that such retrograde modulation may eventually lead to stabilization or elimination of the pre-

synaptic nerve terminal. Although LTP and LTD have been found in the visual cortex (45, 195–197, 318, 351, 364; for review, see Ref. 18), a clear link between functional modulation and structural rearrangement of synaptic connections remains to be established.

Activity-dependent modulation of synaptic connectivity has been extensively studied at developing neuromuscular junctions (for reviews, see Refs. 69, 349). Neonatal skeletal muscles in mammals are innervated by axons from several motoneurons, and the number of motor inputs to each muscle fiber is gradually reduced to one within the first few postnatal weeks (20, 47, 303). The level of electrical activity (21, 273, 330, 342, 343) can influence the extent and rate of synapse elimination at the neuromuscular junction. The majority of evidence indicates that activity strengthens the stability of the active nerve terminal at the expense of neighboring less active ones (see Refs. 28, 349). In neonatal sternomastoid muscle of mice, there is a progressive loss of ACh receptors at synaptic sites preceding the retraction of the presynaptic nerve terminals at multiply innervated neuromuscular junctions (9). This process was also found to be activity dependent in a competitive manner. Local application of the irreversible ACh receptor antagonist ω-bungarotoxin to a portion of the neuromuscular junction led to selective loss of the ACh receptors and withdrawal of the overlying nerve branches in that area, whereas blockade of the whole junction had no effect on selective elimination (10). Recent work has demonstrated that in newborn and adult mouse muscles, synapse elimination is associated with both a decrease in quantal content and a reduction in mini amplitude, which appears to be due to a reduction in postsynaptic ACh receptor density (70). It remains unclear, however, whether local changes in postsynaptic receptor density or changes in presynaptic release are the initial trigger for the cascade of events leading to synaptic elimination. How could postsynaptic receptor loss lead to the eventual withdrawal of nerve terminals? One possibility is that the loss of receptors results in a reduction of nerve-muscle adhesion. The ACh receptors could be linked through ECM molecules to presynaptic membrane components, thus serving for the adhesive interaction themselves. Alternatively, nerve withdrawal could be induced by retrograde actions of diffusible factors. The production or release of these factors may depend on the electrical activity mediated by ACh receptors, and their range of action may be spatially restricted. In either case, retrograde signaling is clearly involved. Based on the finding that perimembrane electric fields may move and induce aggregation of ACh receptors (278, 288), electrokinetic redistribution of postsynaptic receptors in the plane of plasma membrane by synaptic currents has been proposed as a potential mechanism for strengthening active synapses and weakening inactive ones (290). This appears to be an attractive mechanism to account for the depletion of ACh receptors at inactive nerve terminals described above.

Betz and colleagues (27, 305) monitored activity-dependent changes in synaptic efficacy at dually innervated rat lumbricle muscles. They found that a single conditioning stimulation applied to one nerve reduces the synaptic response to test stimulation of the other nerve. The observed inhibition was maximal when the interval between the conditioning stimulus and test stimulus was short ($<$50 ms). Such heterosynaptic inhibitory interactions are a potential mechanism for activity-dependent competition between coinnervating inputs and may initiate the process of synapse elimination. In *Xenopus* nerve-muscle cultures, Lo and Poo (224) observed that a more long-lasting heterosynaptic depression ($>$1 h) can be induced by a train of action potentials in one of two inputs to an innervated myocyte. This heterosynaptic depression was due to a reduced evoked transmitter release and could be prevented if both inputs coinnervating the myocyte were synchronously stimulated with the same train of stimuli. Further studies indicated that repetitive elevation of Ca^{2+} in the postsynaptic myocyte is both necessary and sufficient for the induction of such synaptic depression (55, 85, 225). Moreover, the induction of this heterosynaptic depression satisfies the Hebbian rule: synaptic efficacy was stable or strengthened if pre- and postsynaptic activities are coincident and weakened if they are noncoincident. Postsynaptic activity induced by either heterosynaptic input or direct activation of postsynaptic ACh receptors (by ACh iontophoresis) resulted in synaptic depression if the presynaptic neuron was inactive or active in a nonsynchronous manner. These studies have delineated the following sequence of events: activation of postsynaptic ACh receptors leads to Ca^{2+} influx into the myocyte, which triggers a cascade of events in the postsynaptic cytoplasm, eventually resulting in a retrograde signal to suppress the presynaptic release mechanism. The nature of the retrograde signal and the mechanism by which coincident presynaptic activity protects the nerve terminal from the retrograde effect remain unknown. There is evidence that nitric oxide (NO) may mediate the synaptic depression induced by prolonged postsynaptic depolarizations of the muscle cell in *Xenopus* nerve-muscle cultures (355). However, NO does not seem to be responsible for the heterosynaptic depression in this system (Y. Lo and M-m. Poo, unpublished observation; see also Ref. 55). An important unresolved question is whether activity-induced persistent synaptic depression eventually contributes to the physical withdrawal of the inactive nerve terminal.

A trophic hypothesis has been proposed to account for activity-dependent synapse rearrangement in developing nervous systems (296). Survival of a nerve terminal may depend on the reception of a certain amount of "trophic factor" produced by the target cell. The release of the factor depends on postsynaptic depolarization, whereas the uptake of the factor depends on the presynaptic activity. Thus stronger or more active inputs may pos-

sess an advantageous condition for capturing the necessary factor, whereas the inactive or weaker inputs fail to acquire the factor. To account for temporal specificity in synaptic competition, the presence or effectiveness of the trophic factor must be temporally restricted to a duration defined by that of postsynaptic excitation. For example, postsynaptic activity could trigger the secretion of the trophic factor locally at the synapse, and the effectiveness of the secreted factor could be temporally limited by a steep concentration dependence in its action. To account for the competitive nature of synapse elimination, additional mechanisms need to be postulated by which active terminals destabilize the less active ones, either by direct interaction or via other "negative" retrograde signals from the postsynaptic cell to the adjacent nerve terminals. The results on heterosynaptic depression described above are consistent with this negative influence on the functional integrity of the inactive synapses. Depletion of ACh receptors (9, 10), local secretion of protease (116), or remodeling of basal lamina at the synapse (6) may serve to trigger the withdrawal of the inactive nerve terminal, whereas the active nerve terminals are somehow protected from this negative effect. Protease inhibitors can indeed reduce activity-induced loss of nerve terminals at multineuronally innervated developing rat soleus muscle fibers (71, 274) and cultured myotubes (222).

An attractive candidate for the hypothesized trophic factor involved in synapse rearrangement is neurotrophin. There are two forms of potential actions of neurotrophins on synaptic connections. In a long-term "global" action, the neurotrophin can promote the health and survival of the pre- or postsynaptic neuron, thus indirectly affecting the stabilization and maintenance of selective connections. In a local acute action, neurotrophins could modulate the functional efficacy, terminal arborization, and structural integrity of synaptic connections, without affecting neuronal survival. The two types of actions are not necessarily linked. Growth factors are known to regulate various neuronal functions of some cell types without affecting their survival (280). Cabelli et al. (50) have found that infusion of NT-4/5 or BDNF, but not NGF or NT-3, into cat primary visual cortex locally inhibited formation of ocular dominance columns. Excessive amounts of the trophic factors at the synapse could have prevented the normal rearrangement process by substituting for endogenous retrograde factors. Local modulatory effects of neurotrophins on neuronal morphology have been directly observed. Application of NT-4/5 and BDNF to the organotypic slice cultures of developing ferret visual cortex increases dendritic length, branching pattern, and spine outgrowth in a laminar-specific manner (248). Local injection of BDNF into optic tectum of *Xenopus* tadpole resulted in increased axonal branching, whereas injection of neutralizing antibodies to BDNF produced the opposite effect, suggesting that endogenous BDNF may act to modulate axonal patterning (67). In the latter study, the effect on axonal branching was observed within 2 h after injection of BDNF; thus this is likely to be a local modulatory action of the neurotrophin. There is also evidence for a more global action of neurotrophins on the visual system. In monocularly deprived ferrets, Riddle et al. (304) found that neurotrophin could prevent the atrophy of the cell bodies of the lateral geniculate neurons that receive inputs from the deprived eye, suggesting a more global action of neurotrophins on neuronal survival. In the latter study, neurotrophins were applied at the nerve terminals of LGN cells in the cortex; thus long-range retrograde transport of the neurotrophin may be required for the effect on the cell body.

The action of neurotrophins can be linked to electrical activity in a number of ways. The expression and secretion of neurotrophins from the source neuron, and the binding, signal transduction, or uptake of neurotrophin in the responsive neuron could all depend on activity. There is ample evidence for activity-dependent regulation of neurotrophin expression in various parts of the nervous system (reviewed in Ref. 218). For example, depolarization of cultured hippocampal neurons by high potassium, by activation of non-*N*-methyl-D-aspartate (NMDA) glutamate receptors, or activation of muscarinic ACh receptors resulted in upregulation of BDNF and NGF mRNA; downregulation is induced by γ-aminobutyric acid $(GABA)_A$ receptor activation (23, 218). Epileptiform activity markedly increased NGF and BDNF mRNA levels in the hippocampus (113, 177). Induction of LTP in the hippocampus selectively increased the mRNA levels of BDNF and NT-3, both in vitro (281) and in vivo (58). Neuromuscular activity also modulates muscle-derived NT-4 mRNA level and immunoreactivity (127). Activity-induced Ca^{2+} influx appears to play a key role in regulating gene expression. In cell cultures and in vivo, induction of increased BDNF mRNA expression in hippocampal and cortical neurons required Ca^{2+} influx through voltage-dependent L-type Ca^{2+} channels (23, 218, 356). Activation of these Ca^{2+} channels resulted in an increase in BDNF expression and survival of cultured cortical neurons, and the survival effect was prevented by neutralizing BDNF antibodies (134). In contrast to the expression of neurotrophins, the evidence for activity-dependent secretion of neurotrophins is rather slim. In cell cultures, Blochl and Thoenen (35) have shown that NGF secretion from rat hippocampal neurons was triggered by high K^+, carbachol, glutamate, or veratridin, suggesting activity-dependent regulation. Extracellular Ca^{2+} was not required, but Ca^{2+} elevation due to release from internal stores was necessary. Under physiological conditions, electrical activity may induce Ca^{2+} influx as well as Ca^{2+} release from internal stores, leading to regulated secretion of neurotrophins. At present, we are unaware of any evidence that suggests that the binding of neurotrophin to their receptors can be in-

fluenced by electrical activity. However, signal transduction cascades triggered by neurotrophin binding are likely to be affected by electrical activity. Membrane depolarization and Ca^{2+} influx have been shown to stimulate MEK and mitogen-activated protein (MAP) kinases (309), major effectors of neurotrophin signaling. As discussed above, for the trophic theory to account for Hebbian modulation of synaptic connections, spatial and temporal specificity of the neurotrophin secretion or action must be satisfied. It is thus important to know more precisely the site and kinetics of activity-dependent release of neurotrophins. If activity indeed modulates neurotrophin signal transduction, we need to determine the precise kinetics of such modulation. Activity-dependent neurotrophin release from postsynaptic cells (35), followed by the acute action of neurotrophins on the efficacy of presynaptic transmitter release (184, 227), could account for some form of activity-dependent synaptic strengthening. Such modulation of synaptic efficacy may represent a prelude to more long-term structural stabilization of synaptic connections.

B. Synaptic Modification at Mature Synapses

In many parts of the nervous system, repetitive synaptic activity induces a persistent increase or decrease of synaptic efficacy, known as LTP or LTD, respectively. Although these long-term changes have been described for glutamatergic, GABAergic, and cholinergic synaptic transmission in a variety of central and peripheral synapses (203, 217), the hippocampus has been the most popular model system for elucidating the underlying mechanisms. Hippocampal LTP and LTD at glutamatergic synapses are of particular interest, since their induction by activity exhibited temporal specificity analogous to that of associative learning, and clinical evidence indicates a critical role of the hippocampus in the formation of long-term memory (329). Various aspects of hippocampal LTP and LTD have been extensively reviewed (14, 32, 68, 109, 205, 217, 269). We focus here on the involvement of retrograde signaling, as well as the production and mechanism of action of various retrograde signals.

The phenomenon of LTP was first fully characterized by Bliss and Lomo (34). High-frequency trains of activation of perforant path fibers to the dentate area of the hippocampus in rabbits produced a selective increase of synaptic efficacy that could last for up to 10 h. Long-term potentiation was later described at synapses in CA3 and CA1 areas of the hippocampus (3, 323). At Schaffer collateral-commissural pathway synapses on CA1 pyramidal neurons, brief tetanic bursts of stimulation or pairing of postsynaptic depolarization with low-frequency presynaptic activation result in robust expression of LTP (see Ref. 32). The NMDA receptor is of critical importance for the induction of LTP at these CA1 synapses, since it allows influx of Ca^{2+} under conditions of sufficient postsynaptic depolarization, which removes Mg^{2+} block of the NMDA channel. Postsynaptic elevation of Ca^{2+} is necessary to trigger the induction of LTP, a process that involves a variety of kinases, including Ca^{2+}/calmodulin-dependent kinase II (CaM kinase II), protein kinase C, and one or more tyrosine kinases (for review, see Refs. 150, 220, 339). Evidence for the involvement of retrograde signaling was obtained mostly for synaptic modulation observed in the CA1 area of the hippocampus, which is the main focus of this section.

Activity-induced LTD has been described throughout the central and peripheral nervous systems (for review, see Ref. 217). Homosynaptic LTD in area CA1 of the hippocampus, a form of LTD that is closely related to LTP in the CA1 area described above, has been recently examined in detail (38, 104, 260, 334, 335). This form of LTD is induced by prolonged low-frequency afferent stimulation and appears to represent a reversal of the processes involved in LTP (see Refs. 237, 334). In the CA1 area of the hippocampus, induction of LTD, like LTP, depends on activation of NMDA receptors and subsequent increases in intracellular Ca^{2+}. How does NMDA receptor-mediated Ca^{2+} influx account for the bidirectional control of synaptic efficacy resulting in LTP or LTD? It has been proposed that different properties of the Ca^{2+} signal (e.g., magnitude, temporal pattern) can lead to differential activation of phosphatases and kinases, thereby controlling the phosphorylation state of target proteins involved in LTP or LTD (219, 220, 237, 302). Although enhanced CaM kinase II activity is required for LTP induction (240, 247, 326), a reduction of CaM kinase II activity by dephosphorylation may underlie LTD (220). Experimental evidence indeed has demonstrated that induction of LTD involves a protein phosphatase cascade, including calcineurin and protein phosphatase I, which eventually leads to dephosphorylation of CaM kinase II (258, 259). The balance of kinase and phosphatase activity in the postsynaptic cell thus appears to be critical in the induction of synaptic modulation.

Although there is general agreement for a postsynaptic locus of induction of LTP and LTD in the CA1 area of the hippocampus, whether the long-term cellular changes underlying synaptic modification reside in the pre- or postsynaptic cell has been a controversial issue (32). Substantial evidence has been accumulated for an increased postsynaptic transmitter sensitivity after LTP induction (205, 269; see also Ref. 276). On the other hand, there is also convincing evidence for presynaptic changes in the efficacy of transmitter release (32). Measurements of glutamate release in vivo (33, 102) and in vitro (115, 232, 234) have shown an increase in evoked transmitter secretion after LTP induction. Quantal analysis of the fluctuation of synaptic currents and the failure rate before and after induction of LTP in the CA1 region of hippocampal slice preparations as well as in hippocampal culture indicate

that the potentiation involves at least in part a presynaptic increase in the probability of transmitter release (19, 39, 241, 334). Persistent increases in the frequency of miniature excitatory postsynaptic currents can be induced by local glutamate application in cultured hippocampal neurons (239), and this LTP-like presynaptic modulation is accompanied by an increase in synaptic vesicle recycling, as measured by the rate of labeling of the presynaptic terminal with an antibody to the intraluminal domain of a synaptic vesicle protein synaptotagmin I (238). Recent studies have demonstrated that LTD in neonatal hippocampal CA1 neurons is also maintained via long-lasting decreases in the probability of presynaptic transmitter release (38, 140, 334, 361).

The above findings led to the conclusion that induction of LTP and LTD at CA1 hippocampal neurons is triggered by processes in the postsynaptic cell, whereas the expression involves persistent changes in the efficacy of presynaptic transmitter release. This requires a retrograde signal from the post- to presynaptic cell. The retrograde factor could be a membrane-permeant diffusible messenger that is released by the postsynaptic cell, diffuses across the synaptic cleft, and acts on the presynaptic terminal. As discussed in section IV, evidence has been obtained in support of a number of membrane-permeable molecules and gases as potential retrograde factors in LTP and LTD. These include arachidonic acid, platelet-activating factor, NO, and carbon monoxide (CO) (for reviews, see Refs. 139, 153, 375). In analogy to anterograde signaling, activity-dependent exocytic secretion of soluble factors from postsynaptic cells could in principle provide retrograde signals to the nerve terminal. In addition, through direct physical linkage between pre- and postsynaptic membranes, molecular activities initiated in the postsynaptic cytoplasm could be conveyed through the postsynaptic density and synaptic cleft to the presynaptic secretion machinery. However, at present, there is very little evidence that the latter two mechanisms of retrograde signaling are involved in long-term synaptic modulation.

The transduction cascades induced by retrograde signals in the presynaptic cell and the identity of the molecular targets of retrograde modulation in LTP and LTD remain largely unknown. Diffusible factors NO, CO, and arachidonic acid (AA) have all been shown to activate soluble guanylyl cyclase and increase guanosine 3′,5′-cyclic monophosphate (cGMP) levels in neural tissues (for review, see Ref. 153). Inhibitors of guanylyl cyclase or cGMP-dependent kinase blocked LTP, whereas bath application of 8-bromo-cGMP or injection of cGMP into the presynaptic neuron produced activity-dependent long-lasting potentiation at hippocampal CA1 synapses (371) and in hippocampal cultures (7). Recently, it has been proposed that ADP-ribosyltransferase is another potential presynaptic target for the retrograde modulation in LTP (105, 322). The retrograde signal itself or effector molecules in the second messenger pathway activated by the retrograde factor can affect transmitter release properties by their action on a large number of potential targets. These include presynaptic voltage-dependent ion channels, synaptic vesicle proteins, plasma membrane vesicle docking proteins, as well as cytosolic factors involved in regulating fusion of synaptic vesicles (51).

IV. MECHANISMS OF RETROGRADE SIGNALING

There are three forms of retrograde signaling at the synapse: *1*) signaling by membrane permeant molecules, *2*) signaling by secreted factors, and *3*) signaling by membrane-bound factors (see Fig. 1 and sect. IV, *A*–*C*).

A. Signaling by Membrane-Permeant Factors

Among various membrane-permeable factors, AA was an early candidate for the retrograde signal in hippo-

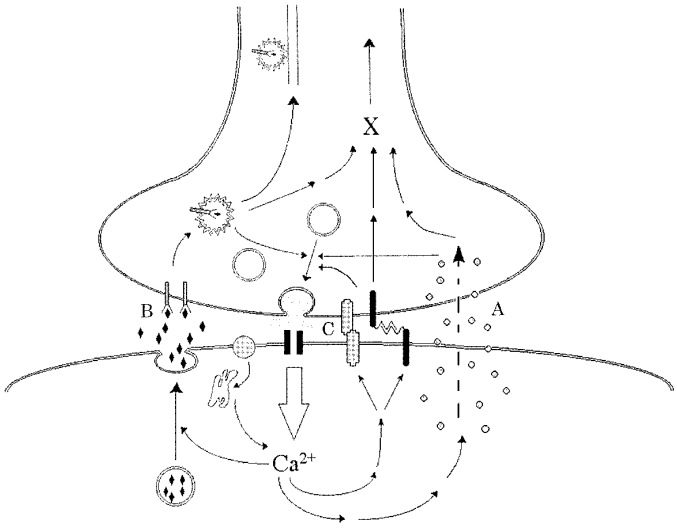

FIG. 1. Three forms of retrograde signaling at synapse. Activation of postsynaptic transmitter receptors results in an influx of Ca^{2+} (through transmitter receptor channels or voltage-dependent Ca^{2+} channels) or a release of Ca^{2+} from internal stores. Elevation of Ca^{2+} triggers a cascade of events (broken arrowed lines) that eventually leads to 3 forms of retrograde signaling to presynaptic neuron: production of membrane-permeant diffusible factors (e.g., nitric oxide and arachidonic acid) that diffuse from post- to presynaptic neuron (*A*), exocytic secretion of soluble factors (e.g., neurotrophins) that diffuse across synaptic cleft to activate presynaptic membrane receptors, which may in turn be internalized and transported to nucleus and other parts of neuron (*B*), and modulation of postsynaptic membrane proteins, which are physically linked to presynaptic membrane receptors either directly or indirectly (via extracellular matrix molecules), resulting in activation of presynaptic receptors (*C*). All 3 forms of presynaptic actions may lead to modulation of transmitter secretion machinery or production of downstream cytosolic factors (X) for long-range retrograde propagation to nucleus and other parts of neuron.

campal LTP. Postsynaptic Ca^{2+} entry through the NMDA channel liberates AA from membrane phospholipids via activation of phospholipase A_2 (PLA_2) and phospholipase C (106). Prevention of AA synthesis by inhibitors of PLA_2 blocked LTP in the dentate gyrus and CA1 region of the hippocampus (275, 358). Induction of LTP by tetanic stimulation or activation of NMDA receptors stimulated release of AA in the dentate gyrus (235). Exogenous application of AA in the dentate gyrus (359) and AA application coupled with a weak tetanic stimulus in the CA1 region of the hippocampus (275) produced a slow-developing long-lasting potentiation. Expression of LTD at CA1 synapses of neonatal rat hippocampal slices appears to involve AA as a retrograde signal (38), since application of AA either alone at high concentrations or at lower concentrations with coapplication of the metabotropic glutamate receptor agonist ACPD induced LTD, whereas a PLA_2 inhibitor blocked induction of LTD. The role of AA as a retrograde signal has been questioned because of the high concentrations (50–200 μM) of exogenous AA required to produce synaptic modulation and the slow time course of its effect (190, 275, 359; for review, see Ref. 153). For LTP in the CA1 region, AA is unlikely to be the retrograde factor involved, since the synaptic enhancement by AA could be blocked by 2-amino-5-phosphonovalerate (APV), a competitive NMDA antagonist, indicating that AA acts upstream of NMDA receptor activation (190).

Activation of PLA_2 also produces other products that are membrane-permeant molecules. Platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; PAF) is one of them. Activation of PLA_2 by Ca^{2+} releases AA from the precursor alkyl choline phosphoglyceride, creating the intermediate lyso-PAF, which is acetylated to produce PAF. A number of studies have shown that application of PAF enhanced excitatory synaptic transmission in hippocampal slices (65, 189, 357) and that PAF antagonists blocked LTP induction (95). The lipoxygenase metabolites of AA cascades, 12-hydroperoxyeicosatetraenoic (12-HPETE), 12- and 5-hydroxyeicosatetraenoic acids (12-HETE and 5-HETE) were reported to play a role in presynaptic modulation of sensory neurons in *Aplysia* (286), and are potential candidates for retrograde signaling in LTP or LTD. The inhibitor nordihydroguaiaretic acid (NDGA) used to inhibit PLA_2 activity also inhibits the activity of lipoxygenase, the enzyme that converts AA to 12- and 5-HETE and 12-HPETE. Induction of LTP in the dentate gyrus of the hippocampus resulted in an increase of AA in the perfusate (235), as well as a similar persistent increase in the perfusate concentration of 12-HPETE and 12-HETE (233). Arachidonic acid, 12-HETE, and 12-HPETE significantly increased K^+-stimulated release of ^3H in synaptosomes prepared from hippocampal tissue (236). In nerve-muscle cocultures of developing *Xenopus* spinal cord, postsynaptic loading of nonhydrolyzable GTP analogs resulted in increased spontaneous transmitter release, and this effect was mimicked by postsynaptic loading of AA and blocked by an inhibitor of the lipoxygenase pathway (151). Indeed, although exogenous application of AA had little effect on synaptic activity, exogenous application of the lipoxygenase metabolite 5-HPETE caused a similar potentiating effect on presynaptic release as postsynaptic loading of GTP analogs, indicating that 5-HPETE may serve as the retrograde signal at these synapses.

Two other candidates for membrane-permeant diffusible retrograde messengers are the gases NO and CO (for review, see Refs. 92, 130, 153, 375). Nitric oxide is synthesized from the amino acid L-arginine by the enzyme NO synthase and released in a Ca^{2+}-dependent manner upon activation of NMDA receptors (108, 131). Carbon monoxide is produced during the conversion of heme to biliverdin by the enzyme heme oxygenase. Carbon monoxide has been recently studied as a retrograde signal in LTP because it has cellular effects similar to NO in other systems (see Ref. 92), and the enzyme heme oxygenase is localized in hippocampal pyramidal cells. Several characteristics of NO and CO make them attractive candidates as intercellular messengers. They are extremely diffusible in both aqueous and lipid environments, allowing rapid *trans*-synaptic spread of the signal. The range of NO and CO action is limited by their concentration and by their short lifetime, with a half-life of only a few seconds (253).

Application of competitive NO synthase inhibitors or extracellular application of hemoglobin, an agent which binds NO and does not penetrate cells, inhibits the induction of LTP but does not reverse established LTP (37, 148, 275, 320). Nitric oxide is synthesized in the postsynaptic cell, as shown by the blockade of LTD after postsynaptic injection of competitive NO synthase inhibitors (37, 275). Application of sodium nitroprusside and hydroxylamine, agents that spontaneously release NO, produce LTP in the hippocampal CA1 region (37, 40). Exogenously applied NO enhances transmitter release in an activity-dependent, NMDA receptor-independent fashion (372) and increases the frequency of spontaneous miniature excitatory postsynaptic currents in cultured hippocampal neurons (275). There is also evidence that NO is necessary for LTD induction in the cerebellum (79, 325) and hippocampus (178, 371), where NO-releasing compounds induce LTD and hemoglobin or NO synthase inhibitors attenuate LTD. At developing neuromuscular synapses in culture, depression of presynaptic ACh secretion induced by prolonged repetitive postsynaptic depolarizations also appears to be mediated by NO (355).

Long-lasting increases in the amplitude of evoked synaptic potentials are observed when CO is paired with weak tetanic stimulation, even in the presence of the NMDA receptor antagonist APV (372). The heme oxygenase inhibitor zinc protoporphyrin IX (ZnPP-9) blocks

the induction of LTP in a dose-dependent manner (333, 372). In addition, unlike NO, CO paired with low-frequency stimulation (0.25 Hz) produced long-lasting potentiation of the excitatory postsynapitc potential (372). However, the heme oxygenase inhibitor ZnPP-9 is found to be a nonselective compound that can inhibit NO synthase (252), and mutant mice expressing deficiency in heme oxygenase show no difference from wild-type mice in basal synaptic transmission or in the amount of synaptic potentiation produced by a variety of LTP induction protocols (293).

It is unclear how a single retrograde messenger, such as AA or NO, can produce both LTP and LTD. It is likely that other pre- and postsynaptic factors (e.g., different intracellular Ca^{2+} levels or activities of different subsets of second messenger cascades) play an essential role in integrating information leading to synaptic enhancement and depression (220). The findings that exogenously applied NO and CO require the presence of presynaptic activity for their long-lasting effects on synaptic transmission (153, 214) suggest that some aspect of presynaptic activity may prime the presynaptic terminal for modulation by these gases. When the very short half-life of the membrane-permeant factors is considered, local and efficient transduction processes must be activated by these retrograde signals in the presynaptic cell, which in turn produces long-term changes in transmitter release.

B. Signaling by Secreted Factors

Vesicular exocytosis is a common form of anterograde signaling at the synapse. Small classical transmitters and neuropeptides are packaged into secretory vesicles and exocytosed upon Ca^{2+} entry in response to membrane depolarization. Can postsynaptic cells use a similar mechanism for secreting retrograde factors? The existence of dendrodendritic synapses (283) and the detection of dendritic release of dopamine (133) clearly indicate the capability of postsynaptic site for regulated exocytosis. Recent evidence indicates that Ca^{2+}-regulated exocytosis is present ubiquitously in eukaryotic cells. When exogenous ACh was loaded into the cytoplasm of a myocyte, ACh became trapped into sealed membrane compartments, and spontaneous release of packets of ACh was subsequently observed (84). This quantal release was elevated in response to membrane depolarization or other means of elevating cytosolic Ca^{2+}. Similar Ca^{2+}-regulated exocytosis was also observed in fibroblasts (137, 257) and sea urchin eggs (29, 331). At excitatory synapses, synaptic activity is associated with a substantial elevation of postsynaptic Ca^{2+}, as a result of Ca^{2+} influx through transmitter-activated channels (94) and voltage-dependent Ca^{2+} channels, or due to Ca^{2+} release from internal stores (180, 186, 285). It is thus reasonable to expect that synaptic activity would trigger the exocytic secretion of retrograde factors packaged in secretory vesicles.

Among various secreted factors, neurotrophic factors are known to be involved in maintaining neuronal survival and in regulating presynaptic neuronal phenotype. Recent findings of the acute effects of these factors on synaptic efficacy have raised the intriguing possibility that activity-dependent secretion of these factors may play a part in modulating transmitter secretion (see reviews, see Refs. 24, 223, 341). Application of neurotrophins BDNF or NT-3 to *Xenopus* nerve-muscle cultures induced a potentiation of both spontaneous and nerve-evoked transmitter releases at developing neuromuscular synapses (227). These two factors also induce a long-lasting potentiation of Schaffer collateral-CA1 synapses when applied to the hippocampal slices (184). In the latter study, it was found that neurotrophin-induced potentiation did not occlude further induction of LTP by tetanic stimulation, whereas previous tetanus-induced LTP only slightly occluded potentiation by neurotrophins. Thus neurotrophin does not participate directly in the induction of conventional LTP. However, in BDNF knock-out mice, both heterozygous and homozygous animals showed marked impairment of tetanus-induced LTP, although the basal synaptic transmission was not different from the wild-type mouse (200). Moreover, replacement of the BDNF gene via adenovirus-mediated gene transfer into CA1 neurons of hippocampal slices of the BDNF knock-out mice restores LTP (see Ref. 341). A useful clue to the possible role of BDNF in long-term synaptic plasticity is provided by the recent work of Figurov et al. (117). These authors found that application of exogenous BDNF to neonatal hippocampal slices had no effect on basal synaptic transmission in Schaffer collateral-CA1 synapses at low-frequency stimulation. However, it potentiates the synaptic response to tetanic stimulation and allows induction of LTP by tetanus at a developmental stage when tetanus normally fails to induce LTP. Interestingly, at adult hippocampal synapses, reduction of endogenous BDNF with specific scavenger TrkB-immunoglobulin G fusion protein significantly inhibited tetanus-induced LTP, suggesting the presence of secreted BDNF is required for the induction or maintenance of LTP even after the synapse matures. In the latter study, prolonged (2.5 h) incubation of neonatal hippocampal slices with exogenous BDNF is required to observe the effect on the induction of LTP. Thus, although BDNF is unlikely to be involved in the mechanism of LTP induction, its constitutive presence may be required to endow the secretory machinery with sustainable efficacy, which is critical for the induction of LTP by tetanic stimuli.

How is the secretion of neurotrophic factors regulated? Blochl and Thoenen (35, 36) have found both a constitutive and a regulated pathway of NGF secretion from rat hippocampal neurons. The release of NGF was triggered by high K^+, carbachol, glutamate, or veratridin,

suggesting activity-dependent regulation. Surprisingly, regulated NGF release depended on extracellular Na^+ but was independent of extracellular Ca^{2+}. Nevertheless, elevation of intracellular Ca^{2+} appears to be the trigger for secretion, since buffering cytosolic Ca^{2+} at a low level with Ca^{2+} chelator or depleting Ca^{2+} stores with thapsigargin or dantrolene inhibited the release. Moreover, tetanus toxin, a neurotoxin that specifically cleaves the vesicular protein synaptobrevin, blocks activity-dependent secretion of NGF (see Ref. 35), indicating that release of NGF involves mechanisms similar to that of regulated vesicular exocytosis. Thus, under physiological conditions, depolarization-triggered Ca^{2+} influx and Ca^{2+} release from internal stores are both likely to contribute to trigger the exocytosis of NGF-containing vesicles. Interestingly, secreted NGF from these cultured neurons was detected on the external somatodendritic surface by immunostaining, suggesting that neurotrophins may bind to ECM and act as a retrograde factor in the surface-bound form. Indeed, trapping of secreted factors by ECM at the synapse may provide a mechanism for concentrating the factor and for delivering the factor more persistently to the presynaptic neuron.

Activity-dependent exocytosis of post-Golgi vesicles at the postsynaptic site may offer an opportunity for incorporating new receptors and channels into the postsynaptic membrane (87). The expression of LTP in CA1 regions of the hippocampus may involve the conversion of a silent synapse into an active one by insertion of DL-α-amino-3-hydroxy-5-methylisoxazole-propionic acid (AMPA) glutamate receptors (176, 216), which could result from a Ca^{2+}-dependent exocytosis of post-Golgi vesicles containing AMPA receptors. In addition, incorporation of other membrane proteins or ECM components from the postsynaptic cell could serve for retrograde interactions with the presynaptic nerve terminal as a bound factor at the synaptic cleft (see sect. III*C*).

In addition to protein factors, there is the possibility that postsynaptic secretion of conventional transmitters may also serve as retrograde signals. The variety of presynaptic transmitter receptors is suited for reception of transmitters released from both the presynaptic nerve terminal and the postsynaptic cell. There is evidence for the existence of secretory vesicles and transmitters in dendrites (270), at regions that show no presynaptic differentiation (e.g., active zone and vesicle clustering). We now know that depolarization-evoked transmitter secretion can occur in undifferentiated growing neurites (48, 338), cell bodies of acutely dissociated adult hippocampal neurons (83), muscle cells (84), and fibroblasts (257) as long as sufficient concentrations of the transmitter are present in the cytoplasm. Whether and how cytosolic transmitters are packaged into secretory vesicles at these nonconventional sites is yet to be fully resolved (see Ref. 289), but the existence of depolarization-evoked transmitter release from nonconventional sites is firmly established.

C. Signaling by Membrane-Bound Factors

A structural characteristic of central synapses is the tight association between pre- and postsynaptic membranes. In electron micrographs, the synaptic cleft of central synapses is 10–20 nm wide and is filled with electron-dense filamentous material (173, 283). Strong molecular bonding between the two membranes prevents their separation during tissue homogenization and preparation of synaptosomes. Isolated synaptosomes are frequently found to be associated with a piece of postsynaptic density (PSD). This tight physical association prompted the suggestion that such a physical linkage between pre- and postsynaptic elements form a "structural unit," allowing coordinated pre- and postsynaptic modifications (221). Chemical modifications of proteins in the PSD, such as those resulting from elevated kinase and phosphatase activities associated with the induction of LTP or LTD, may trigger conformation changes of the linkage molecules, which in turn convey the retrograde signal to the presynaptic nerve terminal. This mechanism offers higher spatial and temporal specificity than that associated with signaling by diffusible factors. During excitation-contraction in skeletal muscle, depolarization of the t-tubule membrane may trigger a conformational change in the "foot" structure linking dihydropyridine receptors in the t-tubule membrane with ryanodine receptors in the sarcoplasmic reticulum (SR) membranes, leading to the opening of Ca^{2+} release channels in the SR membrane. In an analogous fashion, *trans*-synaptic membrane linkage may exert rapid modification of presynaptic Ca^{2+} channel activity, leading to changes in the efficacy of transmitter release mechanism.

Induction of LTP/LTD may involve modification of the physical linkage at the synapse, which initiates signaling cascades in the presynaptic nerve terminal, leading to sustained changes in transmitter release. Modification of the linkage can be achieved by introduction of new synaptic components (e.g., postsynaptic receptors or ECM molecules) or conformational changes of existing ones. There is evidence that surface adhesion molecules at the synapse can affect synaptic efficacy and plasticity. In *Aplysia*, cell- and site-specific changes in the levels of an NCAM-like cell surface adhesion molecule (apCAM) at synapses of sensory neurons influence the formation and elimination of presynaptic connections with the motor target, which may be relevant to long-term synaptic modulation (282, 369, 370). In CA1 neurons of hippocampal slices, LTP was reduced by blocking neural cell adhesion molecules L1 and NCAM with specific antibodies, recombinant

L1 fragments, or oligomannosidic carbohydrates and NCAM peptides that caused dissociation of the L1/NCAM complexes (230). Mice deficient in neural adhesion molecules (NCAM) show deficits in spatial learning when tested in the Morris water maze (78). Experimental perturbations of cell surface adhesion molecules could interfere with the structural integrity of the synapse, leading to deleterious effects on the induction or maintenance of synaptic modification. At this time, there is no definitive evidence that *trans*-synaptic physical linkage plays an active role in mediating activity-dependent long-term synaptic modulation.

Close apposition of pre- and postsynaptic membranes is also observed during the early phase of formation of neuromuscular junctions, before the deposition of basal lamina material (48). The synaptic cleft is widened at the mature neuromuscular synapse. Filamentous attachments of the pre- and postsynaptic membranes to the intervening basal lamina were observed (164). A number of synapse-specific basal lamina proteins have been identified (312). Of particular interest is S-laminin (314), which is known to bind to nerve terminals and could serve for retrograde signaling (171). As discussed in section III*A*, activity-dependent synapse elimination at neonatal skeletal muscle fibers may involve a *trans*-synaptic adhesive interaction between the presynaptic nerve terminal and postsynaptic muscle membrane, an interaction that depends on the presence of postsynaptic ACh receptors (9). In *Xenopus* nerve-muscle cultures, persistent presynaptic depression of evoked ACh secretion was induced by elevation of Ca^{2+} in the postsynaptic muscle cell (55), and rapid local perfusion of the synapse with fresh culture medium had no effect on the induction of presynaptic depression (57). This is consistent with a direct physical interaction rather than diffusible factors in mediating the retrograde signaling, although it is possible that extremely localized transfer of diffusible factors across the synaptic cleft is unaffected by extracellular clearance.

V. PRESYNAPTIC PROPAGATION OF RETROGRADE SIGNALS

The actions of retrograde factors vary widely in their spatial and temporal characteristics. The factor may act locally at the nerve terminal and modify presynaptic functions in a transient or sustained manner. Cellular activities at the cell body or other parts of the neuron may also be affected after a spread of the signal in the presynaptic neuron. The signal could be the retrograde factor itself or in the form of internalized factor-receptor complexes. Alternatively, downstream effector molecules activated by localized retrograde factor may further spread in the presynaptic neuron to propagate the signal. Long-range effects induced by retrograde factors include regulation of gene expression and protein synthesis at the cell body and changes in the global neuronal properties, in the efficacy and morphology of synaptic inputs received by the neuron, as well as of synaptic outputs by other axonal terminals.

At least three types of potential mechanisms can be utilized for long-range presynaptic spread of retrograde signals. The ATP-driven motor proteins associated with microtubule (347) or actin (208) could transport the signals. The signal may be carried by regenerative waves of cytosolic second messengers, e.g., Ca^{2+}, $InsP_3$, and cAMP (25, 26, 157, 187, 256). Finally, the signal could spread by passive diffusion of second messengers and their downstream effector enzymes in the cytoplasm. The rate of signal propagation varies widely among various forms of molecular transport. Consideration of the characteristic time of the spread of retrograde signaling will help to determine potential cellular mechanisms involved. For example, unlike that of active transport, the characteristic time for diffusion increases with the square of distance. Over longer distances, the diffusional transport becomes progressively more inefficient. For soluble components, e.g., small second messengers and globular proteins, diffusion is as effective as active transport for distances shorter than 100 μm. However, diffusion of molecules or organelles with sizes larger than 10 nm is highly restricted in the neuronal cytoplasm (see sect. IV*C*), because of the presence of cytoskeletal meshwork. Active propagation of the signal in the form of cytoskeleton-based transport or second messenger waves is required for cytoplasmic spread of the signal.

A. Cytoskeleton-Based Axonal Transport

Signal transduction cascades are rapidly activated after binding of neurotrophins and cytokines to their cell surface receptors (144, 174, 175). Acute actions of these factors on the morphology and physiological functions of the presynaptic neuron (53, 72, 147, 184, 227; for review, see Ref. 24) are likely to result from the localized cellular activity triggered by receptor activation. However, long-term effects of neurotrophins on cell survival and differentiation must involve retrograde signaling to the cell body and regulation of gene expression and protein synthesis (for reviews, see Refs. 13, 159, 277). The presence of this long-range signaling is also vividly shown by the reduction in the efficacy of synaptic transmission onto dendrites of the axotomized neuron. In sympathetic ganglia, axotomy of postganglionic fibers results in the withdrawal of synaptic contacts on ganglionic cells, and the contacts are restored when postganglionic axons regenerate in the periphery (245, 294, 297). Because similar synaptic loss is found after colchicine treatment, which interrupts axonal transport but leaves axons intact, the synaptic loss after

axotomy appears to result from a lack of retrograde signals transported from the axon, rather than the axotomy-induced injury (284, 295). The retrograde factor received by the ganglionic neurons at the periphery appears to be NGF, since exogenous application of NGF to sympathetic ganglion cells after axotomy prevented the synaptic loss induced by axotomy, whereas treatment with antiserum to NGF induced synaptic loss in the absence of axotomy (297).

Using ^{125}I-labeled NGF, Hendry et al. (160) first showed that binding and uptake of NGF at the presynaptic nerve terminal of sensory neurons are followed by retrograde axonal transport of NGF. More recently, receptor-mediated retrograde axonal transport has been demonstrated for a variety of neurotrophic factors, including NGF, BDNF, NT-3 (100, 353), NT-4 (80), and LIF (346), in both the peripheral and central nervous systems. Antibodies to the Trk family of neurotrophin receptors have revealed that the receptors, in addition to the neurotrophins, are retrogradely transported by neurons (100, 181; for review, see Ref. 161). Because these neurotrophic factors are internalized together with their receptors through receptor-mediated endocytosis and transported as receptor-ligand complexes in endocytic vesicles, it would be of interest to know whether the factor or the receptor carries the propagated signal, or both. Heumann et al. (163) demonstrated that NGF alone is not the intracellular retrograde signal, since intracellular injection of NGF did not mimic the NGF receptor-mediated responses. Furthermore, injection of antibodies to NGF into the cytoplasm of PC12 cells had no effect on the cellular effects of NGF (162). These findings indicate that the active retrograde signal is not the neurotrophin released to the cytoplasm, but likely is the neurotrophin-receptor complexes contained within vesicles.

In addition to Trk receptors, neurotrophins bind with low affinity to a transmembrane protein called p75NTR (60, 299). The p75NTR protein may exert its cellular actions independently of the Trk receptors (101, 298). It may also interact with the Trk receptor to affect the binding affinity and specificity of neurotrophins, efficiency of neurotrophin signal transduction, or internalization and transport of neurotrophins (59, 61). It has been shown recently that in the isthmo-optic nucleus (ION) of chick embryos, transport of BDNF alone does not promote the survival of ION neurons when axonal TrkB is inactivated by a Trk inhibitor K252a (353). It is possible that all cellular effects, including long-term survival and differentiation, require only Trk receptor activation at the axonal surface and retrograde axonal transport of neurotrophin, and its receptors bear no functional significance. Alternatively, neurotrophin-receptor complexes in the transported vesicle need to remain active during and after its transport to the cell body. In this manner, endocytic vesicles can be viewed as parcels of activated plasmalemma that spread cytosolic transducing activity of the neurotrophin-receptor complexes to distant parts of the neuron, with the help of active transport machinery. The advantage of this mechanism of propagating retrograde signals is obvious, in view of the slow rate of diffusional spread of cytosolic downstream effector molecules over long distances (see sect. IV*C*). It is interesting to note that the transport mechanism for shipping neurotrophin-containing endocytic vesicles is not polarized only in the retrograde direction; anterograde microtubule-based transport of BDNF from retinal ganglion cells to the optic tectum has recently been reported (352). Upon arrival at axonal terminals, BDNF is apparently further released and internalized by tectal neurons in a functional form. Long-range retrograde transport of neurotrophins and other ligand-receptor complexes may thus also be viewed as merely an intermediate step in an extensive intercellular exchange and propagation of protein signals within the neuronal network.

B. Second Messenger Waves

Another potential mechanism for propagating retrograde signals over long distances is the use of regenerative waves of second messengers in the cytoplasm. These waves can be generated at the local sites of reception of retrograde factors and propagate over long distances across the entire cell, with a speed in the range of 8–100 μm/s (256). The most dramatic example is the Ca^{2+} wave during fertilization, which originates from the site of sperm entry and sweeps across the fertilized egg, resulting in a wave of cortical reaction (136). Calcium waves can be generated in oocytes by internal release of Ca^{2+} by activation of muscarinic receptors or by injection of $InsP_3$ (46, 209). Calcium waves were also observed in neurons (146, 180) and in large networks of cells coupled by gap junctions, e.g., developing cortex (367), vascular endothelial cells (179, 311), myocytes (340), hepatocytes (308), and astrocytes (74, 88).

A messenger wave can be generated if mechanisms for a positive-feedback amplification of the messenger exist in the cell. Diffusional spread of the elevated messenger and subsequent messenger-triggered production of itself in the adjacent region results in a propagated wave. For example, Ca^{2+} waves can be generated by Ca^{2+}-induced Ca^{2+} release from adjacent Ca^{2+} stores. Alternatively, generation of a Ca^{2+} wave may be coupled to the action of $InsP_3$; $InsP_3$-induced Ca^{2+} release from internal stores leads to cytosolic Ca^{2+} elevation, and elevated Ca^{2+} activates phospholipase C and production of $InsP_3$, which then diffuses to adjacent regions to activate further release of Ca^{2+} (see Ref. 25). In the latter mechanism, an $InsP_3$ wave is also generated together with the Ca^{2+} wave. Repetitive concentric cAMP waves are generated in the extracellular space by a population of *Dictyostelium dis-*

coideum cells, serving as the chemotactic signal for cell migration and formation of the cell aggregate (97). There is a Ca^{2+}-sensitive form of adenylate cyclase in the nervous system (360), and Ca^{2+} release or influx could be regulated by protein phosphorylation via cAMP-dependent protein kinase. Whether appropriate rates of production and diffusion of cAMP and Ca^{2+} exist in the nervous system to generate cAMP-Ca^{2+}-coupled waves remains to be determined.

C. Diffusional Spread of Cytosolic Transduction Cascades

All retrograde signals presented by the postsynaptic cell, either diffusible or membrane bound, are likely to trigger a cascade of biochemical events in the presynaptic neuron. Spread of second messengers or their downstream effector molecules within the presynaptic cytoplasm will result in a spread of the retrograde effect. In the case of membrane-permeant diffusible retrograde factors, additional effects of extracellular diffusion of the retrograde factor itself will further extend the range of potential spread to nearby neurons unrelated to the active synapse. Given the rapid reduction in the concentration by volume dilution and the limited lifetime of some of the known diffusible factors (see sect. IV*A*), the spread due to extracellular diffusion of retrograde factors themselves may be less significant than that resulting from the cytosolic diffusion of its downstream effector molecules. Not only the retrograde signal is amplified by the transduction cascade; the effective concentration of signaling molecules will also persist over longer distances due to more restricted dilution within the cytoplasm.

The spread by a cytosolic signal is limited by the rate of transport and the lifetime of the signal. The diffusion rate of Ca^{2+} is greatly slowed by the presence of high concentrations of relatively immobile buffering proteins, and its lifetime as a signal is greatly reduced by effective Ca^{2+} sequestration (see Ref. 344). Direct measurements of diffusion coefficient (D) of Ca^{2+} in the cytosolic extract from *Xenopus* oocytes have yielded a D value from 13 to 65 $\mu m^2/s$ when the free Ca^{2+} concentration was raised from 90 nM to 1 μM, which corresponds roughly to fully buffered and free Ca^{2+}, respectively (4). These D values are close to that for protein diffusion in neuronal cytoplasm (291), consistent with diffusion limited by protein buffers. Assuming a typical on-rate of Ca^{2+} binding to its buffer, 108 $M^{-1} \cdot s^{-1}$ (306), and a concentration of Ca^{2+} buffers of 300 μM, Allbriton et al. (4) estimated the lifetime of free Ca^{2+} to be only 30 μs. For buffered Ca^{2+}, the lifetime is limited by the rate of sequestration into internal stores, which is ~1 s (344). When the measured D values for free and buffered Ca^{2+} are considered, the range of action of Ca^{2+} signal is limited to 0.1 and 5 μm. Thus Ca^{2+} is ideally suited for a localized signal. Fluorescence imaging of Ca^{2+} elevation in postsynaptic dendritic spines (110, 242, 365) indeed showed limited Ca^{2+} diffusion in the cytoplasm after localized Ca^{2+} influx. In contrast to Ca^{2+}, the diffusion coefficients of other small cytosolic messengers, e.g., $InsP_3$ and cAMP, and cGMP are much closer to that found for free diffusion of small molecules in aqueous solution, and their diffusional spread becomes substantial within their lifetimes. For cAMP diffusing in *Aplysia* axons, the measured D is 780 $\mu m^2/s$ (8). With the assumption of a lifetime of ~1 min, cAMP signal can spread over a distance of 300 μm. The typical lifetime for $InsP_3$ in cells has been estimated to be ~1 s (255, 354), and the diffusion coefficient measured in oocyte extract was 280 $\mu m^2/s$. This would yield a spread of 24 μm within its lifetime. In addition to cytoplasmic signals, activated receptor proteins or bioactive lipids residing in the plasma membrane could also spread via lateral diffusion in the plane of the membrane. However, the diffusion of membrane components is at least two to three orders of magnitude slower than that of soluble molecules in the cytoplasm, in the range of 0.1–1 $\mu m^2/s$ for membrane lipids and 0.01–0.1 $\mu m^2/s$ for membrane proteins, respectively (249). Their contribution to long-range signaling in neurons is likely to be insignificant.

The diffusion rates of soluble macromolecules of various sizes in the neuronal cytoplasm have been measured quantitatively by Popov and Poo (291). For molecules with hydrodynamic radius <10 nm, which encompass most effector molecules in signal transduction cascades, the diffusion coefficient in neuronal cytoplasm is reduced by three- to fivefold, as compared with that in aqueous solution. In contrast, larger molecules experience increasingly disproportional higher viscous resistance. The diffusion rate of larger molecules (but not of smaller molecules) can be reduced significantly by treatments that disrupt microfilaments, suggesting that the cytoplasmic meshwork (43, 44) imposes a clear size limit for unrestricted diffusion of large particles within the cytoplasm. When the diffusion coefficient of a typical globular protein is considered (e.g., serum albumin, D = 70 $\mu m^2/s$, see Ref. 291), the average time for diffusional transport over a distance of 100 μm is ~70 s, comparable to that achieved by fast axonal transport (0.5–3 μm/s; Refs. 347, 348). However, for a signal received at the nerve terminal to be relayed to the cell body located at distances a few millimeters away, the time required is likely to become longer than the lifetime of the activated molecule. For long-range signaling, the effector molecules must be carried by some active transport mechanism, or they must be generated at distant sites after active transport of retrograde factor-receptor complexes (see sect. V*A*).

Among various second messengers, Ca^{2+} appears to play a central role in mediating the presynaptic actions of a number of retrograde factors. As described in section

IIA, Ca^{2+} elevation in the presynaptic nerve terminal appears to be a signal after nerve-muscle contact in *Xenopus* and *Helisoma* cell cultures. Exogenous application of BDNF and NT-3 induces a transient elevation of cytosolic Ca^{2+} in cultured hippocampal neurons (22). Potentiation of Schaffer collateral-CA1 synapses in hippocampal slices by these neurotrophins was antagonized by nifedipine, a blocker of L-type voltage-dependent Ca^{2+} channels, and by thapsigargin, an inhibitor of Ca^{2+} release from internal stores (185). At *Xenopus* neuromuscular synapses in culture, BDNF also induced a rise in cytosolic Ca^{2+} in parallel with synaptic potentiation (336). These studies are all carried out using uniform exogenous application of neurotrophins over the entire neuron. It would be of interest to determine the spatial as well as temporal characteristics of Ca^{2+} elevation upon localized and physiological reception of neurotrophins at the nerve terminal, and to determine whether Ca^{2+} waves can be generated by retrograde factors.

The existence of long-range intracellular signaling after local elevation of cAMP dependent activity was vividly demonstrated in a simple system of developing spinal neurons in culture. Zheng et al. (368) observed that local application of a membrane-permeable cAMP analog or forskolin at the growth cone of one of the neurites of a multipolar neuron resulted in growth inhibition at the growth cone of other neurites located 150–200 μm away. In contrast, cAMP itself stimulates growth at the site of local perfusion or when applied uniformly in the bath. It appears that the retrograde cAMP signal arriving to the cell body had instructed a redistribution of cellular activity that led to the inhibition of the untreated neurite. That the spreading retrograde signal from one nerve process induces different and "compensatory" activity in other processes of the same neuron is a recurring phenomenon in many systems. In cultured hippocampal neurons, transection of a differentiated axon leads to reinitiation of rapid growth of dendritic processes, which in some cases leads to respecification of the axon (103). In bifurcating axonal branches of identified *Aplysia* neurons in culture, the growth of one branch becomes inhibited after the sibling branch contacts a target cell (317). Two branches show opposite changes in axonal transport of organelles, with increased transport activity in the target-contacted branch and reduced activity in the other (141). In the developing nervous system of the leech, interruption of retrograde signals received by one neurite from the target cell (by severing the neurite or removing the target) leads to changes in growth and projection pattern of other neurites (11, 129).

The cAMP-dependent signal transduction pathway is now recognized to be of crucial importance to activity-dependent synaptic modulation and to memory formation in the nervous system. Formation of long-term memory requires gene activation and protein synthesis, since transient application of inhibitors of mRNA and protein synthesis selectively blocks induction of long-term memory without affecting short-term memory (see Ref. 91). Studies of *Aplysia* memory in gill- and siphon-withdrawal reflexes have shown that long-term facilitation of sensory-motor synapses requires new protein synthesis and cAMP-dependent gene expression through the activation of cAMP-responsive element-binding protein (CREB)-1 and the relief from repression of CREB-2 (2, 15). Localized application of serotonin, a neurotransmitter known to mediate synaptic facilitation, to the sensory neuron cell body and proximal synapses induced long-term facilitation in distal synapses that were not exposed to serotonin, again suggesting the role of nuclear action in the induction of long-term facilitation (111). In rat hippocampus, the persistence of a late phase (>3 h) of LTP requires RNA and new protein synthesis and is also mediated by cAMP (126, 167, 246, 267, 268). Behavioral studies of olfactory learning in *Drosophila* also showed that long-term memory requires gene expression initiated by CREB (345, 363). With the assumption that induction of long-term memory begins with activity-dependent synaptic modification, a retrograde signal in the cAMP signal transduction cascade must be transported from the nerve terminal to the nucleus at the cell body. Bacskai et al. (8) have measured directly the dynamics of cAMP diffusion in *Aplysia* sensory neurons in culture or in intact cell clusters, using cAMP activated dissociation and loss of fluorescence energy transfer between fluorescently conjugated protein kinase A subunits. Application of serotonin produced a much higher elevation of cAMP in the nerve processes than in the cell body. Such a gradient could drive a diffusional flux of cAMP to the cell body, since injected cAMP showed high diffusibility. Furthermore, physiological applications of serotonin also induced an increase in perinuclear cAMP concentration, which was followed in some cells by a slow translocation of the catalytic subunit of cAMP-dependent kinase into the nucleus, where it presumably acts to phosphorylate transcription factors, e.g., CREB. Diffusional transport of cAMP could be an efficient way of retrograde signaling over long distances, provided that phosphodiesterase activity in the cytoplasm does not severely reduce its concentration.

In hippocampal neurons, depolarization-induced presynaptic depression of inhibitory transmitter release was blocked by pretreatment with pertussis toxin (287). Because loading postsynaptic cell with nonhydrolyzable GTP analogs had no effect on presynaptic depression, pertussis toxin probably works by blocking a presynaptic G protein, which mediates the downstream action of a retrograde signal. Diffusible membrane-permeant retrograde factors NO, CO, and AA have all been shown to activate soluble guanylyl cyclase and increase cGMP levels in neuronal tissues (for review, see Ref. 153). Inhibitors of guanylyl cyclase or of cGMP-dependent kinase blocked LTP at hip-

pocampal synapses in slice preparations (371) as well as in culture (7). In addition, application of cGMP analogs results in long-lasting enhancement of synaptic transmission. To distinguish a pre- versus postsynaptic site of action, Arancio et al. (7) demonstrated that presynaptic injection of cGMP paired with tetanic stimulation resulted in immediate and long-lasting enhancement of the postsynaptic current. In analogy to cAMP, retrograde diffusional transport of cGMP could further induce regulatory action on gene expression or posttranslational modification through cGMP-dependent kinase at the cell body and other parts of the neuron.

Neurotrophins bind with high affinity to the Trk family of receptor tyrosine kinases (for reviews, see Refs. 12, 144, 161). Activation of Trk receptors results in ligand-mediated oligomerization of receptors at the cell surface and autophosphoryation of tyrosine residues. These phosphotyrosine residues serve as binding sites for activating src homology 2 (SH2) domain-containing signaling proteins, which in turn stimulate other effector molecules including phospholipase C, GTP binding protein Ras, GTPase-activating protein, and multiple serine/threonine kinases of the Ras/Raf/MAP kinase pathway (99). Although the immediate actions of these effector enzymes on cytosolic and plasma membrane components could account for acute effects of neurotrophins at the local site of receptor activation, the eventual consequence is a change in the activity of transcription factors in the nucleus. Diffusional spread or active translocation of second messengers and effector proteins in the neuronal cytoplasm could account for efficient retrograde signaling over distances less than a few millimeters. Retrograde active transport of endocytic vesicles containing internalized neurotrophin-receptor complexes is required for signaling over longer distances (see sect. v*A*). Finally, we note that long-range signaling is important not just for gene regulation in the nucleus. Protein phosphorylation of ion channels and receptors by activated kinases throughout the presynaptic neuron could induce global changes in the membrane excitability and in the efficacy of synaptic connections. For example, potentiation of transmitter release at developing neuromuscular synapses by local exposure of the synapse to CNTF appears to require signaling with the neuronal cell body that does not involve mRNA or new protein synthesis (337), perhaps for redirecting the supply of synaptic material from the cell body. Synaptic potentiation by BDNF, in contrast, is a local presynaptic modulation that does not involve long-range signaling (336, 337).

D. Intra- and Intercellular Spread of Synaptic Modulation

Activity-dependent synaptic modification occurs not only at the active synapse but also affects other nearby synapses. If the spread of synaptic modulation is due to intracellular signals during the induction or expression of the synaptic changes, only synapses associated with the pre- or postsynaptic cells will be affected. However, secreted or membrane-permeant diffusible factors produced by the active synapse may spread through the extracellular space to affect nearby synapses on different populations of cells. Indeed, both intra- and intercellular spread of synaptic modulation has been reported. Such spread directly affects the specificity of activity-induced synaptic changes and are likely to be important for developmental and adult synaptic plasticity.

In cerebellar Purkinje cells and hippocampal pyramidal neurons, depolarization-induced increases in Ca^{2+} concentration led to a transient presynaptic reduction of evoked GABA release from inhibitory presynaptic nerve terminals, presumably by the action of a retrograde signal (287, 350). Simultaneous recordings from two neighboring Purkinje cells showed that repetitive depolarization of one cell reduces not only the inhibitory postsynaptic currents recorded in that cell, but also those recorded in the neighboring cell (350). In *Xenopus* nerve-muscle cultures, when one input on a doubly innervated myocyte is tetanized, the other synapse on the same postsynaptic myocyte was affected only if it is within a limited distance from the active synapse (224). This spatial restriction is apparently related to the spread of postsynaptic Ca^{2+} elevation from the active synapse within the myocyte (55). Furthermore, when persistent presynaptic depression of evoked ACh secretion was induced by elevation of Ca^{2+} in the postsynaptic muscle cell, distant synapses made by the same presynaptic neuron on other muscle cells were also depressed (57). The time of onset of depression showed a distance dependence, with a 10- to 15-min delay of onset for synapses located at 250–400 μm away. The spread of synaptic depression at these neuromuscular synapses appears to be mediated by a cytoplasmic signal, since clearance of extracellular medium by fast perfusion of fresh medium across the presynaptic site had no effect on the spread of depression to distant synapse. The nature of the retrograde interaction that induces the presynaptic depression at the active synapse and the signal responsible for the presynaptic spread remain unknown. Over the limited range of distances tested in this culture system, the time course of presynaptic spread is consistent with either passive diffusion (of a cytosolic second messenger or protein) or a cytoskeletal-based axonal transport. It is not clear whether the spread eventually dissipates at longer distances and whether such spread is a general property of all forms of retrograde synaptic modulation, including potentiation. The presynaptic spread of retrograde modulation has direct consequence on the spatial and temporal specificity in activity-dependent modulation. It affects the effectiveness of specific synapse stabilization/elimination of developing connections and the synap-

tic plasticity of mature neural circuits. For example, at polyneuronally innervated neonatal muscles, presynaptic spread of retrograde signals associated with synaptic stabilization/elimination will lead to more global influence on the presynaptic neuron, affecting more distant axonal terminals of the same motor unit.

In the hippocampus, LTP induced in one synaptic pathway will lead to potentiation of synapses on the same or different postsynaptic neuron (41, 112, 201, 321). In the study by Schuman and Madison (321), simultaneous intracellular recordings were made from two adjacent CA1 pyramidal neurons, and excitatory postsynaptic potentials resulting from stimulation of Schaffer collaterals were made. When LTP was induced in one cell ("paired" cell) by pairing low-frequency stimulation with postsynaptic depolarization, smaller but significant LTP was also detected in adjacent neurons whose cell body is within ~150 μm from that of the paired cell. No LTP was detected when the distance between the two cells was beyond 500 μm. Inhibition of NO synthase in the paired cell prevented the LTP in the paired cell as well as that of the adjacent cell. However, it appears that the spread of LTP is not due simply to the action of NO on the presynaptic nerve terminals of the adjacent neuron, since postsynaptic dialysis, Ca^{2+} chelation, and hyperpolarization of the adjacent neuron prevented LTP at that cell. This suggests that the postsynaptic cell plays an active role in the enhancement of the adjacent synapses, perhaps by releasing another retrograde signal that corroborates with NO released by the paired neuron. However, using a local superfusion technique in hippocampal slice cultures, Engert and Bonhoeffer (112) recently demonstrated that LTP spreads to other synapses within a distance of 70–100 μm which have not experienced presynaptic activity, whereas synapses far away show no potentiation. Thus susceptibility to the actions of a diffusible retrograde signal due to repetitive activation of a presynaptic terminal does not explain the loss of input specificity at neighboring synapses.

Long-term potentiation generated at one synaptic input to a CA1 hippocampal neuron was found to result in LTD in more distant neurons (251, 316). The spread of synaptic modification can be mediated by signaling within the neuron. Induction of LTP in hippocampal CA1 neurons is known to produce heterosynaptic depression of neighboring inactive synapses on the same postsynaptic cell (1, 64, 231, 261, 279, 316). A signaling process within the postsynaptic cells whereby an active potentiated synapse sends a "depressive" signal to adjacent inactive postsynaptic sites may account for this depression. Scanziani et al. (316) recently observed heterosynaptic LTD in different populations of postsynaptic cells after induction of LTP in CA1 neurons by tetanic burst stimulation of the Schaffer afferent. An intercellular diffusible signal must be released by the potentiated neuron and spread throughout the extracellular space to induce LTD in the neighboring cells. Interestingly, the appearance of this heterosynaptic LTD requires postsynaptic activation of protein phosphatase 1 in the neighboring cells, similar to homosynaptic LTD, but does not require increases in postsynaptic Ca^{2+}. Compared with the spread of LTP described above, heterosynaptic LTD appeared to cover more extensive population of cells. These two opposing influences on adjacent synapses are clearly mediated by different diffusible factors. On the basis of the effect of NO synthase inhibitor, the spread of LTP appears to be mediated by NO, whereas heterosynaptic LTD is not. The identity of intercellular message for inducing heterosynaptic LTD is unknown. It could be either a secreted or membrane-permeant factor, but different from that proposed for the retrograde signal involved in LTP. Scanziani et al. (316) proposed that the extensive nonspecific spread of heterosynaptic LTD helps to sharpen the LTP and enhance the information stored in the activated pathway. In a similar sense, a more restricted spread of LTP may further help to amplify the localized activation.

Using triple whole cell recording from small networks of cultured hippocampal neurons, Fitzsimonds et al. (119) recently found that induction of LTD at glutamatergic synapses is accompanied by a retrograde spread of depression to synapses on the dendrites of the presynaptic neuron (back-propagation). The depression was also found to spread laterally to synapses made by divergent outputs of the presynaptic neuron (presynaptic lateral propagation) or to convergent inputs on the postsynaptic neurons (postsynaptic lateral propagation). In contrast, there was no evidence for forward-propagation of depression to output synapses of the postsynaptic neuron. Moreover, there is no evidence for back-propagation or presynaptic lateral propagation of depression resulting from LTD induced at GABAergic synapses. The contrasting results of the lack of spread of depression in GABAergic neurons point to the possibility that retrograde signaling is not involved in GABAergic plasticity. However, it appears that a retrograde signal is generated by the induction of glutamatergic LTD, which is capable of spreading or inducing a signal, which then propagates to other axonal outputs and the dendritic inputs of the presynaptic neuron. Taken together, these results demonstrated an extensive yet selective distribution of activity-induced synaptic changes involving intra- and intercellular retrograde signaling within the neural network. These findings on the intra- and intercellular spread of synaptic modulation have further revealed the intricacy of synaptic plasticity within the neuronal network, and their full implications are yet to be fully understood.

VI. CONCLUDING REMARKS

Since the discovery of chemical synaptic transmission, our perception of what is being transmitted at the

synapse has been continuously evolving. For anterograde transmission, we have now recognized that, in addition to fast-acting classical neurotransmitters, there are a wide range of coreleased proteins and peptides, which serve for structural and functional regulation of the postsynaptic cell with a slower time course. The list of proteins released by the presynaptic nerve terminal is growing with time, and a most recent addition is BDNF (352). It is interesting to note that neurotrophin has long been considered to be a prototypic retrograde signal released by the postsynaptic cell, a view that now appears to be biased. As reviewed here, a large number of diffusible and bound factors are shown to be likely candidates for retrograde signals, with presynaptic actions including long-term trophic influences as well as more rapid physiological effects. In view of the increasingly apparent symmetry of bidirectional communication at the chemical synapse, it would not be surprising if small classical neurotransmitters and neuropeptides are found to be released by the postsynaptic cell and serve as retrograde signals for presynaptic regulation.

Most previous attention on retrograde signaling has focused on diffusible factors. It appears now that direct contact-mediated retrograde interactions are likely to serve significant roles, not only during the early phase of synaptogenesis, but also in activity-dependent synaptic modification. Further progress in this area will require molecular dissection of the cell surface components involved in specific cell-cell recognition, the composition of synaptic cleft material, and their linkage and interactions with pre- and postsynaptic membrane components. Physiological and biophysical techniques need to be devised to probe the dynamics of the physical linkage at the synapse with a time resolution on the order of seconds or shorter.

Because retrograde inputs not only act locally but also affect other parts of the presynaptic neuron, the mechanisms by which signals are propagated in the neuronal cytoplasm are of great interest. At present, we know very little about how the movement and localization of cellular components are regulated. It is within the general context of the cell biology of molecular transport and localization that the problem of long-range retrograde signaling will eventually be solved. On the other hand, given the acute requirement for organized molecular transport and long-range signaling in neurons, the study of neuronal retrograde signaling may prove to be advantageous in revealing cell biological mechanisms for molecular transport, signaling, and localization in the cytoplasm.

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