Chapter 15
Transsynaptic Regulation of Presynaptic Release Machinery in Central Synapses by Cell Adhesion Molecules

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Contents

Abstract Neuronal activity and resultant synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), are accompanied by a dynamic regulation of the synaptic structure. At the same time, pre- and postsynaptic structures and functions are well coordinated at the individual synapse level. For example, large postsynaptic dendritic spines have a larger postsynaptic density with higher AMPA receptor number on their surface, while juxtaposing presynaptic terminals have a larger active zone and more docked vesicles. This indicates that structural modification seen in LTP and LTD must be coordinated at both pre- and postsynaptic structure, likely as a result of coordinated assembly of specific molecules on both sides of the synaptic cleft. Interestingly, there is evidence that the postsynaptic cell may be instructive to presynaptic functions. This review focuses on the postsynaptic mechanisms that retrogradely regulate presynaptic functionality and structure, emphasizing the role of neuronal adhesion molecules.

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The synapse is a highly specialized asymmetric structure that transmits information and stores it in the brain. The majority of synapses in the central nervous system are chemical synapses, which are physically separated into pre- and postsynaptic structures at the synaptic cleft. Although the two structures are physically separated, pre- and postsynaptic structures and functions are well coordinated at the individual synapse level. For example, in excitatory synapses on hippocampal pyramidal cells, large postsynaptic dendritic spines have a larger postsynaptic density with a greater number of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors on the surface. At the same time, juxtaposing presynaptic terminals have a larger active zone and more docked vesicles (1–4). Such coordination of postsynaptic and presynaptic structure and function ensures more efficient transmission. It has been reported that neuronal activity and resultant synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), alter the efficiency of synaptic transmission in conjunction with postsynaptic structural changes (5), suggesting that synaptic plasticity can influence presynaptic structure and function. Therefore, it is extremely important to understand the mechanism of how presynaptic-postsynaptic coordination takes place in mature synapses. Most likely it is a result of the coordinated assembly of synaptic adhesion molecules on both sides of the synaptic cleft. Synaptically localized adhesion molecules have been reported as important mediators for organizing synaptic structure (6), and recent studies indicated that synaptic adhesion molecules modulate basal synaptic transmission and plasticity in matured synapses.

This chapter describes the history and possibilities of transsynaptic, especially retrograde, signaling on the expression mechanism of LTP, and discusses the molecules that support transsynaptic signaling, with an emphasis on cell adhesion molecules.

Historical Perspective on the Retrograde Regulation of Transmitter Release: Long-Term Potentiation Studies

Long-term potentiation (LTP) is a phenomenon in which a transient burst of synaptic input causes a long-lasting increase in subsequent synaptic transmission (7). It has been well established that LTP induction requires postsynaptic depolarization combined with the activation of *N*-methyl-D-aspartate (NMDA) receptors, and resultant influx of Ca^{2+}. This triggers a series of biochemical processes including the activation of calcium/calmodulin-dependent protein kinase II (CaMKII). Expression of LTP is achieved by increasing the number of AMPA receptors (AMPARs) at the synapse through the activity-dependent change of AMPAR trafficking pathway or by changing AMPAR channel properties via direct phosphorylation (8–10). Although this postsynaptic view is nowadays widely accepted, the presynaptic view was ini-

tially suggested by the observations of increased transmitter release and reduced failure rate after LTP induction, which are generally considered to reflect changes in release probability based on studies at the neuromuscular junction. Several diffusible molecules including nitric oxide (NO), arachidonic acids, carbon monoxide (CO), platelet-activating factor (PAF), and brain-derived neurotrophic factor (BDNF) have been suggested as possible retrograde messengers (11). However, these suggestions have often been questioned because either the reported results cannot be reproduced or the candidate molecules appear to lack sufficient specificities (12,13). Moreover, the studies from various knockout animals renounced the retrograde function of these molecules, at least in the context of LTP (14–16).

Although a decade of debate made the postsynaptic view of LTP current prevailing, it does not positively rule out the presynaptic view, and further new evidence is accumulating (16–19). In view of structural changes occurring at the synapse, as early as 1977, only four years after LTP was reported (7), a pioneering electron microscopic study by Fifková's group (20) reported that the induction of LTP in the dentate gyrus increases the size of dendritic spines, which is followed by an increase in the dimension of the presynaptic terminus after a transient disparity. In fact, the presynaptic structures (length of active zone and number of synaptic vesicles) and postsynaptic structures (length of postsynaptic density and the dimension of postsynaptic density) in naive tissue appear well coordinated (1–4). At some point after LTP induction, pre- and postsynaptic structures must necessarily be coordinated to maintain the proportion observed in vivo.

The presence of retrograde modulation of presynaptic function and structure is also suggested by observations outside of LTP studies. For example, analyses of connections made between a Schaffer collateral axon from a CA3 pyramidal neuron targeting CA1 pyramidal neurons or inhibitory interneurons indicate that the type of the postsynaptic target neuron can dictate the presynaptic properties (21). A similar observation was made between synapses formed between a single presynaptic pyramidal cell and two different types of postsynaptic cells (22). The presynaptic termini in a single cell can have different protein components depending on postsynaptic cell type, and this ability appears to be involved in target cell-specific presynaptic function (23). Furthermore, the alteration in the activity level of a specific postsynaptic neuron can change presynaptic properties. For example, in hippocampal dissociated culture, increasing postsynaptic CaMKII activity by transfecting its active forms results in the remodeling of presynaptic input by increasing the number of synaptic contacts between pairs of neuron, while decreasing the total number of connected cells (24). Similar retrograde action of CaMKII activity has also been reported in *Drosophila* (25,26). Postsynaptically localized synaptotagmin 4 (Syt 4), a calcium sensor for membrane fusion, is a candidate molecule for the release of retrograde messengers in *Drosophila* (27). Yoshiwara et al reported that Syt 4 triggered the release of retrograde messengers that enhanced presynaptic function through the activation of the presynaptic cyclic adenosine monophosphate (cAMP)-dependent kinase pathway. These observations exemplify the ability of a postsynaptic neuron to retrogradely influence functional properties of the presynaptic terminal. Thus, postsynaptic neurons must be equipped with mechanisms to retrogradely regulate the presynaptic

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release probability, though these mechanisms may not take place in relatively early phase of LTP (<30 min after induction).

Transsynaptic Adhesion Molecules

Cadherin-Catenin–Mediated Transsynaptic Signaling

The cadherin superfamily consists of more than 100 members in vertebrates. They are classified into subfamilies that are called classical cadherins, desmosomal cadherins, protocadherins, Flamingo/CELSRs (*c*adherin, *e*pidermal growth factor [EGF]-like, *l*aminin A globular-like [LAG], and *s*even-pass *r*eceptors), and Fat cadherin (28). Cadherins make homophilic adhesion between cells expressing the same class of cadherin through their extracellular domain containing the repetitive cadherin repeats, including the calcium-binding domain. Classical cadherins have been most extensively studied, and their cytoplasmic domain binds to β-catenin and p120 catenins (29,30). β-catenin associates with α-catenin, which is known as an actin-binding protein. These protein–protein interactions likely underlie the mechanism of cadherin-mediated synapse formation and spine stability.

Postsynaptic overexpression of the dominant-negative form of N-cadherin, which has a deletion in the extracellular domain, reduced the number of presynaptic puncta and changed spine morphology concomitant with the reduction of frequency of miniature excitatory postsynaptic currents (mEPSCs) (31). A neuronal culture differentiated from mouse embryonic stem (ES) cells lacking N-cadherin showed that the absence of N-cadherin enhanced synaptic depression in response to paired-pulse or high-frequency stimulation, although evoked postsynaptic currents (EPSCs) in response to a single stimulus and the mean amplitude of mEPSCs were indistinguishable between neurons with and those without N-cadherin (32). Synaptic structures were also not altered in neurons lacking N-cadherin, consistent with the analysis of a conditional knockout of N-cadherin in hippocampal neurons (33). These observations suggest that N-cadherin controls short-term synaptic plasticity transsynaptically. Interestingly, the same synaptic phenotypes were observed when the deficiency of N-cadherin was restricted to postsynaptic neurons in experiments of coculturing wild-type neurons and the ES cell-derived neurons, suggesting that postsynaptic N-cadherin retrogradely controls presynaptic release (32). These studies suggested that N-cadherin is involved in vesicle recruitment from the readily releasable pool to the active zone and in vesicle recycling pathways (31,32). The entire process likely involves N-cadherin binding proteins. A conditional knockout of β-catenin reduced the number of releasable vesicles and exacerbated synaptic depression during high-frequency stimulation (34). Conversely, postsynaptic overexpression of β-catenin resulted in an increase in mEPSC frequency, suggesting a retrograde regulation by postsynaptic β-catenin/cadherin interaction, although there is the alternative possibility that β-catenin overexpression increased the number of functional synapses in this case (35).

It has also been reported that spine stability and spine density are altered in knockout mice of α-N-cadherin and p120 catenin, respectively, but precise electrophysiologic analyses of these animals have not been performed (36–38). Interestingly, δ-catenin knockout mice showed reduced paired-pulse facilitation (PPF) in hippocampal neurons consistent with the ES cell study for N-cadherin (39). Like N-cadherin, other classical cadherins are also important for the formation of synapses and synaptic function. Knockout of cadherin 11 enhanced LTP in the hippocampal CA1 region without changes in paired-pulse facilitation (PPF), indicating that the absence of cadherin 11 may increase the flexibility of the synaptic structure, allowing it to receive more AMPA receptors (40). Knockout of cadherin 8, which is specifically expressed in the spinal cord, led to loss of men-
[Au2] thol-induced enhancement of AMPAR-mediated mEPSC frequency (41). RNAi-based knockdown of cadherin 11 and 13 indicates the importance of these molecules on the formation and function of the glutamatergic synapse (42).

Recently two studies showed that N-cadherin formed a protein complex with AMPA receptors in vivo (43), and the extracellular N-terminal domain of GluR2, a key subunit of several AMPA receptors, can interact directly with N-cadherin (44). It is unclear whether this interaction is mediated by *cis*- or *trans*-synaptic manner. Nevertheless, this heterophilic interaction could be an important mechanism for AMPA receptor trafficking, retrograde regulation of synaptic transmission, and coordination between pre- and postsynaptic function.

Neuroligin-Neurexin-Mediated Transsynaptic Signaling

Neurexins (Nrxns) were isolated as a family of brain membrane surface proteins that bind α-latrotoxin, which is a neurotoxin from black widow spiders and functions as a potent trigger of neurotransmitter release (45,46). Nrxns are encoded by three genes *(Nrxn1–3),* each consisting of two isoforms (α- and β-) with different product lengths. Both α- and β-Nrxns have a single transmembrane domain and bind to CASK (mLin-
[Au3] 2) intracellularly through a PDZ domain binding consensus sequence (47). CASK further interacts with Mint, syntenin, and synaptotagmin. Through these three interacting proteins, CASK is eventually linked to other proteins of the presynaptic vesicle release machinery. Extracellularly, both α- and β-Nrxns bind to neuroligin (NL) through their LNS (laminin, nectin, sex-hormone binding globulin) domains (48,49). The NLs are encoded by five different genes in humans, and they have in common one transmembrane region and an extracellular domain that is homologous to acetylcholinesterase but is catalytically inactive (46,50). Intracellularly, NLs have PDZ domain binding consensus sequences that bind to PSD-95, SAP102, Shank, S-SCAM, PICK1, SPAR, and GOPC, which are major components of the postsynaptic structure (51–54). Through these interactions, Nrxns and NLs bridge the presynaptic release machinery and the postsynaptic receptor complex.

In vitro experiments suggest that Nrxns and NLs regulate synapse formation bidirectionally. Nrxns expressed in nonneuronal cells or coated on beads induced

dendritic clustering of proteins involved in excitatory and inhibitory synaptic transmission in contacting dendrites (55). The NLs, in contrast, induced presynaptic differentiation to recruit presynaptic proteins (56–58). Knockout mouse of α-Nrxns or NLs showed serious functional but no apparent structural deficits. Triple knockout of NL1–3 reduced the frequencies of both miniature excitatory and inhibitory postsynaptic currents (mEPSCs and mIPSCs). Since spine densities and mean amplitudes of the miniature postsynaptic currents were normal in the triple knockout compared with wild-type mice, the decreased frequencies of mEPSCs and mIPSCs reflect reduced presynaptic release probability (59).

Recently it has been reported that postsynaptic NL1 is implicated in the modulation of presynaptic release probability by cooperating with postsynaptic PSD-95 and presynaptic Nrxn. Futai et al (60) showed that manipulating postsynaptic expression levels of PSD-95 and NL1 altered presynaptic release probability in organotypic hippocampal slice culture, suggesting that PSD-95-NL1 interaction retrogradely regulates presynaptic release probability. Paired pre- and postsynaptic recordings of two CA3 pyramidal neurons indicate that presynaptic overexpression of a dominant-negative form of β-Nrxn reduced the release probability. Therefore, the effect is most likely through the interaction between presynaptic β-Nrxn and postsynaptic NL. However, presynaptic overexpression of β-Nrxn did not mimic the effect of postsynaptic overexpression of PSD-95 or NLG, suggesting that β-Nrxn exists in redundancy and postsynaptic PSD-95–NLG complex has an instructive role for this transsynaptic mechanism. Since α-Nrxn modulates presynaptic calcium channel, it might be possible that α-Nrxn is involved in some way. However, NLG1 used in this study does not bind to α-Nrxn (61), but still fully exerts its effects. Therefore, the effects of NLG1 observed in our assays do not require direct interaction with presynaptic α-Nrxn, though we do not rule out indirect involvement.

Ephrin Receptor-Ephrin Ligand Mediated Transsynaptic Signaling

Both Eph receptors, which are tyrosine kinase receptors, and their ligands are divided into to subclasses: A and B. The ephrinA ligands are tethered to the membrane through glycosylphosphatidylinositol (GPI)-linkage anchors, and specifically bind to EphA receptors, while the ephrinB ligands associate with the plasma membrane through a transmembrane domain, and preferentially bind to EphB receptors. The intracellular carboxy-terminal tail of Eph receptors contains the tyrosine kinase domain, a SAM protein interaction domain, and a consensus motif for binding to PDZ domain-containing proteins. Interestingly, several Eph receptors bind synaptic PDZ domain proteins such as the glutamate receptor interacting protein GRIP1, the protein kinase C–interacting protein PICK1, the syndecan-binding protein syntenin, and the Ras-binding protein AF-6 (62,63). The ephrinB ligands also have PDZ domain-binding motifs in the carboxy terminal region, which can mediate association with syntenin, PICK1, GRIP1, and GRIP2 (63–65). Thus, the Eph receptors

and the ephrinB ligands may be linked to the synaptic scaffold through PDZ-mediated protein interactions. Both EphA and EphB receptors have been detected mainly in postsynaptic sites (62,66,67), but some of the Eph receptors are also expressed in presynaptic terminals (63). In contrast, there is little evidence for the synaptic localization of ephrin ligands, and the pattern of expression is different among different subtypes. In the adult hippocampus, for example, ephrin-B2 is expressed mainly in CA1 pyramidal cells, and is more abundant at the postsynaptic side (69–71), whereas ephrin-B3 is expressed in dentate gyrus granule cells and is targeted to mossy fiber axons and terminals (69,71,72). It has been reported that transsynaptic retrograde signaling from postsynaptic EphB receptors to presynaptic ephrinB ligands contributes to the induction of an NMDA receptor (NMDAR)-independent LTP between hippocampal mossy fibers and CA3 pyramidal neurons. Interfering with EphB/ephrinB transsynaptic signaling by the intracellular application of the carboxyl-terminal peptide of EphB2 receptor blocked mossy fiber LTP, while expression of a dominant-negative form of ephrinB3 ligand reduced LTP (72,73). On the other hand, extracellular application of soluble EphB2 receptor and ephrinB1 ligand to activate EphB/ephrinB transsynaptic signaling occluded LTP. Interestingly, ephrinB3 knockout mice exhibited normal mossy fiber LTP (72). This lack of effect may be due to redundant functions of other ephrinBs.

Transsynaptic Signaling Mediated by Other Candidate Molecules

Neuronal specific immunoglobulin superfamily protein, SynCAM, works as a homophilic cell adhesion molecule at the synapse. The intracellular domain of SynCAM binds to PDZ-domain proteins such as CASK (74). Expression of SynCAM in HEK293 cells that were cocultured with hippocampal neurons induced synaptogenesis in these nonneuronal cells, while postsynaptic overexpression of SynCAM in hippocampal neurons increased the frequency of mEPSCs without changing the number of synapse, indicative of presynaptic site of modification (75). Also postsynaptic overexpression of SAP97 and Shank1 increased staining intensity of presynaptic sites with an FM dye and increased the frequency of mEPSC (76,77), suggesting a retrograde regulation of the release machinery by these molecules. These proteins are localized intracellularly, however, and the actual mechanism that transmits signaling is unknown.

[Au4]

How Do Cell-Adhesion Molecules Change the Presynaptic Functionality?

Recent findings show that dendritic spines expand rapidly and persistently after LTP induction, which is accompanied by synaptic translocation of other molecules such as AMPA receptor, CaMKIIα, β-catenin, and actin (10,35,78,79).

These observations suggest that LTP might be accompanied by an increase in synaptic components in general. Therefore, it is likely that the cell adhesion molecules are translocated to the synapse as part of a process of rebuilding larger postsynaptic structures. The increased number of postsynaptic cell adhesion molecules will then recruit more presynaptic counterparts, which may stabilize synaptic structure by further recruiting synaptic components. This may lead to an increased number of synaptic vesicles as well as active zone components, thereby increasing the number of synaptic vesicles released per action potential. The presynaptic binding partner of postsynaptic neuroligin, β-Nrxn, binds to the PDZ domain of CASK through its intracellular carboxyl terminus. N-cadherin also binds to CASK indirectly through the interaction with β-catenin and LIN-7/Veli/Mals, which makes a complex with CASK (80). CASK then links β-Nrxn and N-cadherin to synaptic vesicle trafficking via binding with Mint1 (X11), which directly interacts with Munc18, a functional regulator of neurotransmitter release. Mutations in CAMGUK, the *Drosophila* CASK homologue, caused a serious presynaptic functional deficit (81). Knockout mice of CASK showed a change in the frequency of spontaneous release events with no structural abnormalities (82).

In the hippocampal CA1 synapse, a multivesicular release has been recently proposed, as opposed to the monovesicular release, which was originally proposed for this synapse (83,84). Therefore, it is reasonable to assume that the presynaptic terminus has the capacity to regulate the number of released vesicles by changing the number of active zones rather than by increasing the probability of release per vesicle without changing the total number of vesicles. An increased number of vesicles released per action potential can explain the observed increase in cleft glutamate concentration (60). Because AMPA receptors at the synapse are not saturated with glutamate (85), the change in glutamate concentration can change postsynaptic response properties. In this way, a qualitative change in the synaptic vesicles can change the temporal pattern of synaptic transmission, which has been measured as presynaptic release probability. Unlike originally proposed retrograde messengers, which are presumed to activate signaling cascades, changing the number of synaptic cell adhesion molecules can provide a way to change the efficacy of synaptic transmission. The effect will persist as long as a constant number of molecules exist, and does not require a mechanism to persistently alter biochemical signaling.

[Au5]

In the future, it is highly desired to elucidate the constructive process of synapse modification after LTP induction, from changes in synaptic cell adhesion molecules to rearrangements of presynaptic structures and vesicular release machineries. With such information in hand, we would then be able to truly understand pre- and postsynaptic roles in LTP.

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Author Queries:

[Au1]: Does "Yoshiwara et al" refer to reference 27? If so, cite "(27)" after et al, and align the spelling of Yoshiwara in text with Yoshihara in reference.
[Au2]: Define the "i" in RNAi.
[Au3]: Define PDZ.
[Au4]: Define FM.
[Au5]: Define RIKEN.