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N-Cadherin Signaling in Synapse Formation and Neuronal Physiology

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Abstract

Neural cadherin (N-cadherin) is an adhesion receptor that is localized in abundance at neuron-to-neuron synapses. N-cadherin contains an extracellular domain that binds to other cadherins on juxtaposed cell membranes, a single-pass transmembrane region, and a cytoplasmic tail that interacts with various proteins, including catenins, kinases, phosphatases, and presenilin 1. N-cadherin contributes to the structural and functional organization of the synaptic complex by ensuring the adhesion between synaptic membranes and organizing the underlying actin cytoskeleton. Additionally, recent findings have shown that N-cadherin may participate in synaptic physiology by regulating calcium influx through voltage-activated calcium currents. The diverse activities of N-cadherin stem from its ability to operate as both an adhesion molecule that links cytoskeletons across cell membranes and a ligand-activated homophilic receptor capable of initiating intracellular signaling. An important mechanism of cadherin signaling is the regulation of small Rho guanosine triphosphatase activity that affects cytoskeleton dynamics and calcium influx. Because both the regulation of cadherin adhesive activity and cadherin-mediated signaling are affected by the binding of molecules to the intracellular domain, changes in the composition of the N-cadherin complex are central to the regulation of cadherin-mediated functions. This article focuses on the roles that N-cadherin might play at the level of the synapse through its effect on adhesion and signaling in the proximity of the synaptic junction.

Index Entries: N-cadherin; synapse formation; Rho GTPases; calcium current; catenins; juxtamembrane domain; actin cytoskeleton.

Introduction

Neural cadherin (N-cadherin) was isolated during the search for the neural member of the calcium-dependent adhesion system. This molecular system is involved in cell-type-specific segregation during embryogenesis *(1,2)*. The hypothesis that cadherins may contribute to the formation of tissue structures is derived from the finding that cells express different types of cadherins during particular morphogenic events *(3,4)*. Because cadherin bind-

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ing is homophilic and exerts an adhesive force between cells, cadherins can provide both cell recognition and adhesion, which are required for the separation of cells into distinct embryonic structures *(5)*.

When cadherins were identified at synaptic contacts *(6,7)*, researchers suggested that they may play a similar role in the formation of neuronal connections by contributing to the recognition of appropriate targets and establishing an adhesive bond between synaptic membranes. N-cadherin is now considered as potentially the most important adhesive component of the synaptic junctional complex. Additionally, classical cadherins (types I and II; ref. *8*) could participate in establishment of specific interneuronal connections between certain brain structures *(9–13)*. However, the number of molecules in this group of proteins is too small compared with the complexity of the code believed to be required to establish the large variety of connections formed between neurons in the nervous system *(14)*. Therefore, attention is now focused on a different, but related, class of proteins, the protocadherins *(14a)*. The genetic structure of such proteins predicts the assembly of combinatorial forms, which would lead to the expression of a large number of different molecules that could serve as a code for establishing specific neuronal contacts *(15,16)*. Although key functional domains in protocadherins, such as the cytoplasmic tail, differ from those found in classical cadherins, the extracellular portion is composed of cadherin motifs, suggesting that this protein domain could participate in establishing specific interneuronal links, as researchers originally suggested *(17)*.

Since the identification of N-cadherin as a synaptic component, various new signaling and regulatory properties have been attributed to cadherins. Indeed, classical cadherins, which were originally believed to contribute to the structural organization of cells through recognition and adhesion mechanisms, are now considered to have a more dynamic role as ligand-activated adhesion receptors capable of transducing signals that regulate cell physiology *(18–21)*. Some of these functions may affect the cytoskeleton and calcium influx by regulating the activity of small Rho guanosine triphosphatases (GTPases) in the vicinity of the junction *(22,23)*, whereas others influence more distant physiological processes, including gene transcription and cell differentiation *(24,25)*. This article focuses on the roles that N-cadherin could play in synapse formation and neuronal physiology through its effect on membrane adhesion and signaling in the close proximity of the synaptic contact.

The N-Cadherin Complex

N-cadherin is a single-pass transmembrane adhesion receptor that binds to a homologous protein on the juxtaposed cell membrane *(4,26,27)*. N-cadherin is composed of an ectodomain that contains five cadherin motifs, a transmembrane region, and a cytoplasmic tail *(26,28)*. Although the extracellular portion homophilically binds to another cadherin, the intracellular domain is essential for the association with actin filaments, creating a bridge between the cytoskeletons across the cell membranes *(5,29,30)*.

The extracellular domain (565 amino acids) is the largest portion of the mature N-cadherin polypeptide (747 amino acids), suggesting that the bulk of cadherin activity is performed by this part of the molecule *(26)*. However, very few regulatory mechanisms affecting N-cadherin function have been identified in the ectodomain *(31)*. The most important mechanism is the modification in calcium concentration, which appreciably affects the structure of the ectodomain and cadherin-mediated cell adhesion *(32)*. Therefore, this parameter is frequently exploited as an experimental perturbation. Despite the requirement of calcium for N-cadherin structure and function *(33)*, its contribution to the physiological regulation of N-cadherin activity has remained elusive because of the overall homeostatic calcium concentration in the extracellular milieu. Nevertheless, it has been suggested that changes in calcium

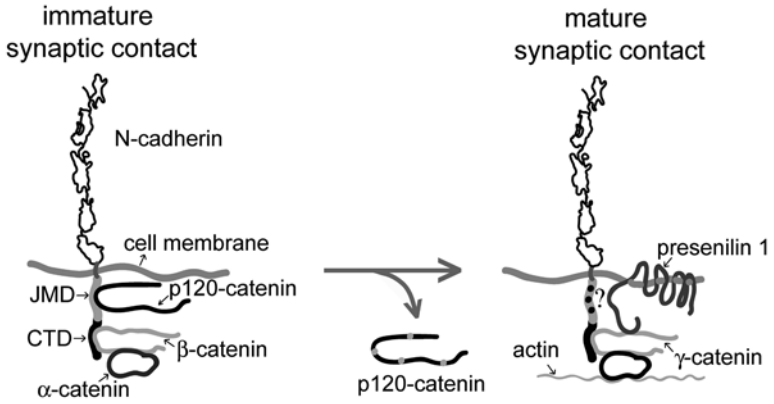

Fig. 1. Schematic representation of the changes in molecular composition of the N-cadherin complex associated with synapse maturation. At immature synaptic contacts, N-cadherin JMD is associated with p120-catenin, whereas the C-terminal domain binds β-catenin. Conversely, at mature contacts, the JMD and/or p120-catenin have been modified, leading to the uncoupling of p120-catenin. The JMD may be now associated with preseniliin 1, which favors the incorporation of γ-catenin into the complex. These changes in the composition of the N-cadherin complex suggest that N-cadherin exists in at least two states with distinct functional properties. The switch between these two N-cadherin functional states that occur during development may also occur throughout the life of a synapse in response to changes in synaptic physiological or to the incorporation of proteins into the N-cadherin complex. Molecules are not drawn to scale. JMD, juxtamembrane domain; CTD, C-terminal binding domain.

concentrations within micro-environments of the extracellular space, such as the synaptic cleft, may affect the adhesive activity of N-cadherin (ref. *34*; *see* Regulation of N-Cadherin Activity by Neuronal Depolarization: The Missing Link section).

Conversely, the shorter cytoplasmic domain (159 amino acids) binds to various cytosolic and membrane proteins, including catenins *(35–37)*, kinases *(38,39)*, phosphatases *(40,41)*, heterotrimeric G proteins *(42)*, E3 ubiquitin-ligase *(43)*, adaptor proteins *(44)*, and presenilin 1 *(45)*. It contains two main binding regions: the C-terminal domain and the juxtamembrane domain (JMD). The C-terminal domain mainly binds to β- and γ-catenin and anchors the protein to the actin cytoskeleton via αN-catenin, *(37,46,47)*. The JMD interacts with p120-catenin family members (p120-catenin, δ-catenin, armadillo-repeat gene deleted in velocardiofacial syndrome [ARVCF], and p0071), hakai, and presenilin 1 *(43,45,48,49)* and participates in the regulation of cell–cell adhesion and cell physiology by affecting cadherin clustering, cytoskeleton dynamics, and calcium influx (refs. *23* and *49–53*; Fig. 1).

In addition to their role within the cadherin complex, both β- and p120-catenin subfamilies contribute to signaling mechanisms when uncoupled from cadherins, such as the *wnt* signaling pathway *(54)* and the regulation of Rho GTPase activity *(52,55)*. These catenin functions are affected by their binding to the cadherin cytoplasmic tail *(52,53,56–58)*;therefore, cadherin and catenin signaling can be regulated by cadherin–catenin interactions.

Signaling Through Cadherin JMD

The JMD has emerged as an important regulatory region within the cadherin cytoplasmic tail because it is involved in cellular functions that require changes in cell adhesion and/or the cytoskeleton, including cadherin clustering *(49,50)*, cell migration *(59,60)*, neurite outgrowth *(61)*, and calcium influx *(23)*. The role of the JMD in these cellular events is believed

to stem at least partly from its ability to bind to p120-catenin *(48)*. Notably, the JMD and its binding partners affect the activity of small GTPases *(55)*. This is important because substantial evidence indicates that cadherin homophilic ligation can initiate changes in cell physiology by modulating the activity of small GTPases of the Rho family (Rac1, Cdc42, and RhoA; refs. *19, 22,* and *62*). Additionally, activation of Rho GTPases affects cadherin functions *(63–66)*, and therefore, the JMD is potentially involved in both outside-in and inside-out signaling mechanisms *(18)*.

Several lines of evidence support the role of Rho GTPases as mediators in cadherin signaling. Rho GTPases localize at cadherin-mediated cell–cell contacts *(67)*; their recruitment to the cadherin complex is rapidly initiated by the formation of intercellular junctions *(68)*; and their level of activity is affected by cadherin homophilic binding *(69–73)*. However, the effect of cadherins on each Rho GTPase varies depending on the cellular context *(64)*. Additionally, Rho GTPases can affect the activity of other Rho GTPases *(74–76)*. Therefore, it has been difficult to determine which Rho GTPase is directly influenced by cadherin homophilic binding. Nevertheless, the most well characterized responses triggered by cadherin ligation are the activation of Rac1 *(19,68,69,71)* and the inhibition of RhoA *(70)*, which occur within minutes and hours of cadherin binding, respectively.

Rac1 activation in response to cadherin binding has been suggested to derive from two mechanisms that are not mutually exclusive. The first involves activation of the phosphoinositide 3 (PI3) kinase and generation of phosphatidylinositol phosphate-3 *(39,71,77)*. The PH domain of phosphatidylinositol phosphate-3 interacts with various GTPase-exchange factors (GEFs), including Tiam1, which specifically activates Rac1 *(74,78)*. Therefore, the recruitment and activation of PI3 kinase, in response to cadherin ligation *(71)*, can lead to the membrane localization of Tiam1 and to the activation of Rac1 in the vicinity of the cadherin complex *(79)*. Although the precise region involved in Rac1 activation has not been mapped, the cadherin cytoplasmic tail appears to be essential *(70)*. On the other hand, Rac1 can be activated by vav2, a GEF for the Rho family of GTPases *(80,81)* that binds cytosolic p120-catenin. Overexpression of p120-catenin causes changes in the actin cytoskeleton that can be abolished by inhibition of Rac1, suggesting that cytosolic p120-catenin can activate Rac1 through its interaction with vav2 *(53)*. Additionally, it has been suggested that binding of p120-catenin to the cadherin JMD affects Rac1 activity in vivo *(82)*. Finally, activation of Rac1 can regulate actin polymerization by recruiting the actin nucleator complex Arp2/3 to cell contacts *(83,84)* and can stabilize the cadherin complex by inhibiting interactions between IQGAP1 and β-catenin. The latter enhances the association of β- with α-catenin and links the complex to the cytoskeleton *(85–87)*.

Conversely to Rac1, there is no evidence for a direct enzymatic link between cadherin homophilic binding and the inhibition of RhoA activity. However, cadherin-unbound p120-catenin binds RhoA *(88)* and inhibits its activation by either preventing the binding to a Rho GEF or acting as a guanosine diphosphate dissociation inhibitor *(52)*. These p120-catenin activities are modulated by its interaction with the JMD. Indeed, expression of cadherin JMD reverts the RhoA-mediated cell branching phenotype caused by cytosolic p120-catenin *(52,53)*, and this effect is abolished by point mutations that suppress p120-catenin–JMD interactions *(52)*. Additionally, infusion of a soluble JMD into neurons causes a RhoA-mediated inhibition of voltage-activated calcium currents, suggesting that the JMD can affect RhoA activity by interacting with a regulator of Rho GTPases *(23)*. Although these studies indicate that the JMD affects RhoA activity (most likely through its binding with p120-catenin), regulation of RhoA by interactions of the JMD with other molecular components cannot be ruled out.

The fact that p120-catenin-mediated regulation of RhoA may depend on binding to the JMD suggests that p120-catenin may exist in

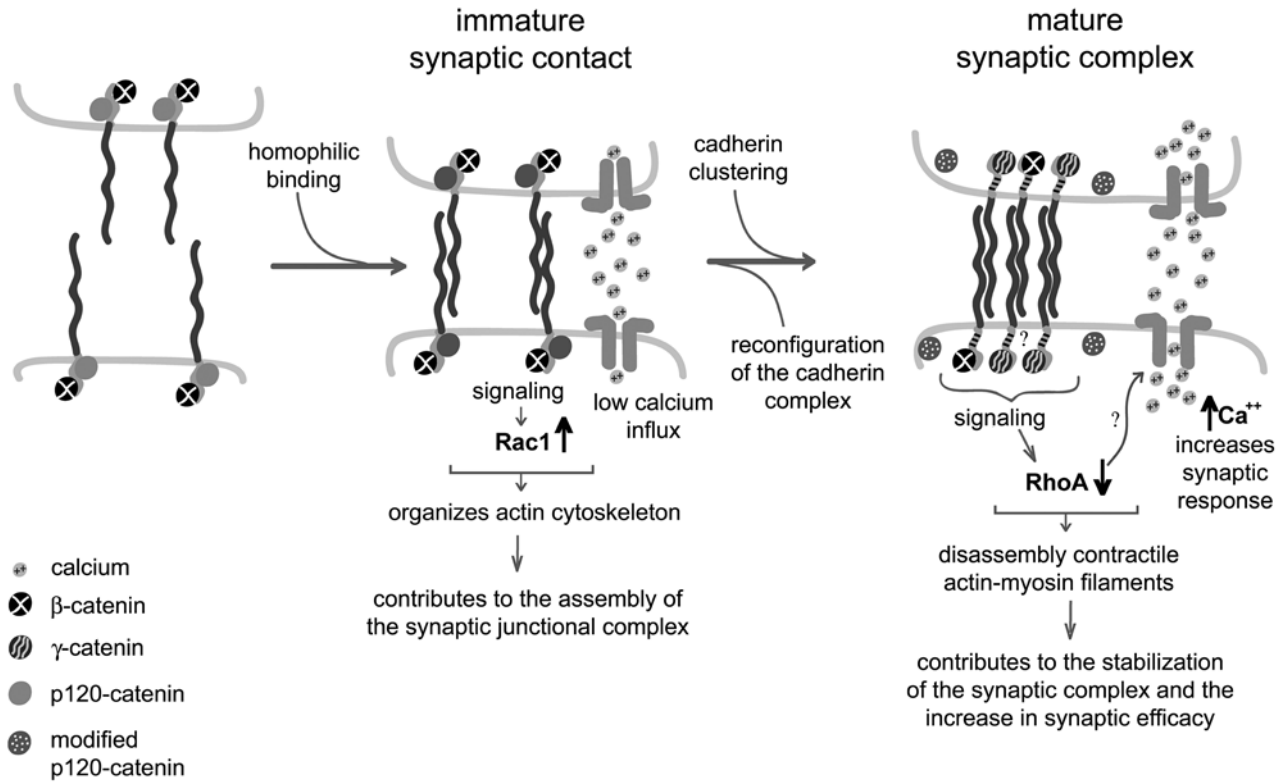

Fig. 2. Hypothetical changes that occur in the N-cadherin complex during synaptic maturation and their consequences on signaling and synaptic physiology. At immature synaptic contacts with limited synaptic transmission, N-cadherin is present in small clusters with restricted adhesive activity. During synaptic maturation, N-cadherin clusters into larger puncta, which exert strong adhesive bonds between synaptic terminals. This transition may be triggered by N-cadherin homophilic binding or by electrical activity. The redistribution and the modifications in the composition of the N-cadherin complex may initiate intracellular signaling, represented by the activation of Rac1 and inactivation of RhoA. These changes in the activity of Rho GTPases organize the actin cytoskeleton and promote the stabilization of the synaptic complex. Additionally, inhibition of RhoA may increase calcium influx by enhancing the activity of voltage-activated calcium channels.

two distinct pools inside the cell: one in which p120-catenin is bound to cadherins and cannot affect Rho GTPase activity and another in which unbound p120-catenin physically interacts with RhoA and prevents its activation. The shift between these two states would provide a mechanism for the regulation of both cytoskeleton dynamics in the vicinity of the junction and neuronal physiology by affecting calcium influx through the regulation of Rho GTPases. This mechanism can involve any regulator of Rho GTPases whose activity is influenced by the binding to the JMD.

The key question in the latter model is whether cadherin homophilic ligation can affect the binding of p120-catenin to the cadherin JMD. Although no rapid changes in the composition of the cadherin complex have been reported as a consequence of cadherin ligation *(89–92)*, the clustering of N-cadherin observed during synaptic maturation in vivo is associated with changes in the composition of the N-cadherin complex, including uncoupling of p120-catenin *(93)*. Additionally, membrane-associated and cytosolic pools of p120-catenin have been observed in the developing central

nervous system *(94)*, suggesting that the N-cadherin complex can be reconfigured as a result of homophilic ligation and clustering and that such modifications can have downstream effects, including cytoskeleton re-organization and calcium influx through the modulation of Rho GTPase activity (Fig. 2).

N-Cadherin and Synapse Formation

N-cadherin is localized at synaptic contacts in various neural structures, including the rodent hippocampus, cerebellum and cerebral cortex *(6,95–97)*, and the avian midbrain *(7)*, optic tectum *(98)*, and ciliary ganglion *(93,99)*. N-cadherin is found at presumptive synaptic sites from the onset of synapse formation, and synapse maturation occurs in parallel to the clustering of N-cadherin at the site of contact, both in vivo *(13,93,95)* and in vitro *(34,100,101)*. Because cadherin clustering enhances cell adhesion *(49,50)*, the development of N-cadherin puncta is believed to contribute to the adhesive strengthening between synaptic membranes and to the increase in synaptic efficacy observed during synaptic maturation *(102–104)*. Moreover, the cadherin–catenin adhesion system is crucial for the development of the adhesive structures that form the adhesive complex between epithelial cells *(105–107)*. Therefore, the N-cadherin-mediated junctions that develop adjacently to the active zone may contribute to the formation of the adhesive components of the synaptic junctional complex *(7,108–110)*.

The redistribution of N-cadherin, which is observed during synaptic maturation, is associated with changes in the composition of the N-cadherin complex (Fig. 1). The small N-cadherin clusters that appear during the initial phases of synapse formation are primarily associated with p120-catenin and β-catenin. Conversely, the larger N-cadherin puncta localized within matured synaptic junctions are mostly depleted of p120-catenin, contain a reduced amount of β-catenin, and are associated with both γ-catenin and presenilin 1 *(93,94,111)*. Because the binding of p120-catenin to the JMD can reduce N-cadherin adhesive activity *(112,113)*, the uncoupling of p120-catenin may increase adhesion between synaptic membranes and, therefore, may stabilize the synaptic contact. Additionally, both γ-catenin and presenilin 1 enhance the linkage of cadherins to the cytoskeleton *(45,114)*, suggesting that their increasing association with N-cadherin puncta may further contribute to stabilizing the synaptic complex.

N-cadherin may also participate in synapse formation by organizing the actin cytoskeleton underlying the synaptic junction *(115)*. These cytoskeletal modifications may stem from the regulation of Rho GTPase activity that is triggered by cadherin homophilic ligation. In epithelial cells, homophilic epithelial cadherin (E-cadherin) binding induces actin assembly, which generates a protrusive force between membranes that is necessary for establishing a lasting intercellular contact *(116)*. The first step in this process appears to be the recruitment of PI3 kinase to the cadherin complex *(39,71)*, which leads to Rac1 and the assembly of actin filaments through the incorporation of the actin nucleator complex Arp 2/3 *(83)*. If this sequence of events also occurs during the binding of N-cadherin between synaptic membranes, then the incipient formation of an N-cadherin-mediated contact may induce the activation of Rac1 and the assembly of the underlying actin cytoskeleton, which is known to be essential for synapse development *(115)*.

Conversely to Rac1, RhoA appears to be inhibited by the formation of cadherin-mediated junctions. RhoA inhibition does not occur immediately after cadherin binding; rather, it correlates with junctional development *(70)*. RhoA is known to induce the assembly of stress fibers and promote actin-myosin contraction *(117)*. These effects are mediated by downstream effectors such as Rho-associated kinase (ROCK), which inhibits the myosin light-chain phosphatase, thereby maintaining the myosin head in a phosphorylated state that is required for its binding to polymerized actin *(118)*. Therefore, inhibition of RhoA and ROCK may stabilize the actin cytoskeleton in the proxim-

ity of the synaptic junction by disassembling contractile actin-myosin fibers *(119)*. Although the mechanism by which cadherin homophilic binding inhibits RhoA is not known, maturation of the synaptic complex involves the uncoupling of p120-catenin from N-cadherin *(93,94)*. As uncoupled p120-catenin inhibits RhoA *(52)*, the changes that occur in the composition of the N-cadherin JMD during synapse development may contribute to the inactivation of RhoA and to the stabilization of the actin-cytoskeleton.

N-cadherin JMD may also play a role in the functional coupling between pre- and postsynaptic compartments by affecting Rho GTPase activity. Indeed, both the small GTPases and the cytoskeleton have been implicated in the regulation of voltage-activated calcium currents *(120–122)*. Therefore, the ability of the JMD to regulate Rho activity may lead to modifications in calcium influx and neuronal physiology. In fact, infusion of soluble N-cadherin JMD into neurons causes an immediate and substantial suppression of voltage-activated calcium current *(23)*. Moreover, the suppression of this current is significantly reverted by the inhibition of RhoA and its downstream effector ROCK, suggesting that the RhoA–ROCK pathway is involved in mediating the effect of the JMD on calcium channel activity *(23)*. This mechanism may involve a direct effect of ROCK on the phosphorylation state of a channel subunit or may affect the activity of the channel by regulating actin-myosin contractility *(23)*. Because synaptic maturation is paralleled by an increase in voltage-activated calcium influx *(123)*, these studies suggest that inhibition of RhoA during synaptic maturation may cause an increase in the activity of voltage-gated calcium channels, thereby enhancing synaptic transmission.

Despite the importance of N-cadherin in synapse formation, N-cadherin alone does not appear to be sufficient to trigger the assembly of the presynaptic compartment *(124)*. The linkage of N-cadherin to the cytoskeleton via β-catenin and αN-catenin is not essential for the localization of synaptic vesicles and active zone proteins at presumptive synaptic sites *(125,126)*. Additionally, blockade of N-cadherin binding through the application of function-blocking antibodies does not prevent synaptogenesis in vitro *(127)*, and genetic deletion of N-cadherin does not affect presynaptic development in vivo *(127a)*, indicating that the initiation of synapse formation is determined by molecules other than N-cadherin.

Nevertheless, the binding of β-catenin to the N-cadherin cytoplasmic tail contributes to the recruitment of synaptic vesicles to the terminal through direct interaction between the β-catenin PDZ-binding domain and synaptic vesicle proteins *(125,128)*. These interactions participate in maintenance of a pool of vesicles required for efficient synaptic transmission *(125)*. Additionally, the association of αN-catenin with N-cadherin is crucial for the stabilization of dendritic spines, which leads to the formation of a synaptic contact *(126,129,130)*. Moreover, N-cadherin is transported in a vesicle that contains components of the active zone *(131)*, and it is required for maturation of the presynaptic compartment. Indeed, both gene ablation and the blockade of functions of N-cadherin result in smaller synaptic contacts with undifferentiated cytoplasmic structures and decreased recycling of synaptic vesicles *(127,132)*. Therefore, N-cadherin may not be sufficient to initiate the formation of a synaptic contact but is necessary for the proper structural assembly and functional maturation of the synapse.

Regulation of N-Cadherin Activity by Neuronal Depolarization: The Missing Link

Once the synaptic complex has matured, N-cadherin remains associated with the synapse, suggesting that it may play an active role in regulating synaptic physiology. Indeed, the application of function-blocking antibodies or peptides directed against the extracellular domain of N-cadherin on hippocampal slices reduces long-term potentiation (LTP) without

affecting the basal properties of synaptic transmission *(133)*. This suggests that neuronal electrical stimulation causes changes in N-cadherin homophilic binding that are required for LTP. Moreover, neuronal depolarization induces N-cadherin dimerization and the appearance of synaptic puncta, which are indications of increased cell adhesion *(111)*. Therefore, the adhesive activity of N-cadherin at synaptic contacts is regulated by neuronal firing and is required to increase synaptic efficacy.

Although the mechanism that links synaptic activity with N-cadherin adhesion is not completely understood, it has been suggested that changes in calcium concentration in the extracellular space triggered by neuronal firing may affect N-cadherin adhesive activity *(34)*. Indeed, computational models have predicted that high-frequency synaptic stimulation can decrease calcium concentrations at the synaptic cleft *(134)*. Additionally, experimental measurements in the hippocampus and cerebral cortex have shown extracellular changes in calcium concentration induced by neural activity *(135,136)*. However, analysis of calcium depletion and replenishment in the synaptic cleft between individual neurons has shown that calcium concentration is much more resistant to synaptic activity than originally believed, according to calculations based on predicted cleft volume *(137)*. Therefore, under physiological synaptic activity, calcium changes in the extracellular space are not expected to appreciably affect N-cadherin adhesive activity.

Nevertheless, raising calcium concentration abolishes the inhibitory effect of N-cadherin-blocking antibodies on LTP *(133)*, suggesting that higher calcium counteracts the blockade caused by the antibody. However, calcium can affect LTP independently of the strength of N-cadherin homophilic binding. For example, higher extracellular calcium raises calcium in the synaptic compartments and influences transmitter release and synaptic potentiation *(138)*. Remarkably, N-cadherin homophilic binding increases intracellular calcium in neurons *(139)*, and N-cadherin JMD affects voltage-activated calcium currents *(23)*, suggesting that cadherin ligation could regulate calcium influx during membrane depolarization. Therefore, it is conceivable that the compensatory effect on LTP, following the increase in extracellular calcium, is associated with the maintenance of high calcium levels at synaptic terminals, even when cadherin homophilic binding is blocked by antibodies.

In addition to the sensitivity to calcium concentrations, the intensity and specificity of cadherin-mediated cell adhesion can be regulated at the level of both gene transcription and post-translational modifications *(51,140,141)*. In hippocampal neurons, repetitive firing induces the formation of N-cadherin puncta, which can be blocked by the application of protein synthesis inhibitors *(101)*. This suggests that the level of N-cadherin expression and activity can be modified by neuronal stimulation.

Additionally, rapid changes in N-cadherin activity can occur within minutes of repetitive neuronal firing, indicating that the regulation of N-cadherin activity can involve pre-existing molecules *(34,111)*. This rapid regulation includes conformational modifications and/or changes in the binding of proteins that compose the cadherin complex. For example, conformational alterations of cadherins lead to *cis*-dimer formation and clustering on the plane of the membrane *(28,33,50,142,143)*. The fact that neuronal depolarization triggers dimer formation suggests that synaptic activity may induce N-cadherin dimerization and increase the binding between synaptic membranes, thereby enhancing synaptic transmission *(111)*.

Despite the various mechanisms that have been proposed to explain the link between synaptic activity and N-cadherin-mediated adhesion, it is still unclear how neuronal depolarization affects N-cadherin. Structurally, cadherins differ from proteins that are capable of sensing membrane voltage, and changes in N-cadherin activity arise as a consequence of repetitive neuronal depolarization or chemical activation of cyclic adenosine monophosphate *(101,111)*. Therefore, the regulation of N-cadherin should occur in response to the activation of an upstream event initiated by neuronal

firing. For example, neuronal depolarization affects the activity of kinases, phosphatases, and Rho GTPases *(76,144–147)*, and the phosphorylation state of the cadherin cytoplasmic tail and/or catenins affect their interaction and regulate the activities of both proteins *(34,91,112, 148–151)*. Therefore, changes in the composition of the N-cadherin complex may occur in response to neuronal electrical stimulation by affecting the affinity of N-cadherin for its binding partners *(34)*. These changes may represent a plausible control mechanism of N-cadherin activity and may, in turn, affect synaptic function.

Conclusions

This article supports the view that N-cadherin contributes to both synapse development and synaptic function through two different, but probably related, mechanisms. N-cadherin acts as an organizer of the synaptic complex by providing a linkage across synaptic membranes and by recruiting synaptic vesicles to the site of contact. This N-cadherin linkage is based on homophilic binding between ectodomains and the anchorage of the C-terminal domain to both the actin cytoskeleton and synaptic vesicle proteins via β-catenin. N-cadherin ligation could also organize the underlying actin cytoskeleton by modulation the activity of small Rho-GTPases.

Therefore, N-cadherin can be considered a provider of structural support that helps to integrate components of the synaptic complex and contributes to the efficacy of synaptic transmission. In addition to its structural role, N-cadherin-mediated contact may affect synaptic physiology by regulating calcium levels. Indeed, N-cadherin homophilic binding increases cytosolic calcium *(152)*, and changes in the N-cadherin JMD may affect voltage-activated calcium channels by inhibiting RhoA *(23)*. In this case, N-cadherin ligation enhances synaptic efficacy by increasing calcium levels in the vicinity of the junction.

How can the regulation of calcium influx by N-cadherin contribute to neural function? Activity-dependent synaptic competition is an important mechanism for both the fine-tuning of the neuronal circuitry and neural plasticity. The competition between synaptic contacts largely depends on the magnitude of the postsynaptic response elicited by the activation of each presynaptic terminal *(153,154)*. Because both transmitter release and postsynaptic response are regulated by calcium, it is tempting to speculate that the development of an N-cadherin-mediated junction between pre- and postsynaptic terminals results in an enhanced postsynaptic response through the increase of calcium concentration in both synaptic compartments. This increase in calcium would enhance synaptic efficacy and activate downstream mechanisms involved in long-term changes of synaptic plasticity that contribute to the stabilization and prevalence of the synaptic contact. Because cadherin activity is regulated by interactions with cytosolic and membrane proteins, this scenario suggests that the molecular events leading to the reconfiguration of the N-cadherin complex are important for synapse formation and synaptic physiology.

Acknowledgments

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