Molecular Neurobiology

ISSN0893-7648/02/25(1): 67–77/$12.75

Neuregulin Signaling via ErbB Receptor Assemblies in the Nervous System

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Abstract

Neuregulins (NRG) play important roles in the development, maintenance, and repair of the nervous system, with influences on neuronal migration, synaptogenesis, receptor subunit composition, and the proliferation/survival of oligodendrocytes and Schwann cells. However, the precise detail of how the NRGs signal through ErbB receptors, particularly at central synapses, is incomplete. The receptor kinase domain provides sites for association with adaptor proteins. In addition, evidence from recent reports suggests that ErbB2/4 receptors, through their C-terminal amino acids, can form specific associations with scaffolding proteins. The existence of such assemblies expands the range of signaling cascades available to the NRGs.

Index Entries: PDZ domain; nitric oxide; PSD proteins; erbin; nitric oxide synthase-1; synaptogenesis; ErbB receptors; neuregulins.

Introduction

The neuregulins (NRGs) are members of the superfamily of epidermal growth factor (EGF)-like polypeptide growth factors. This ligand superfamily, which includes EGF, transforming growth factor alpha (TGF-α), betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), epiregulin (ERG), and amphiregulin (AR), is characterized by the presence of an "EGF domain" in the protein structure *(1,2).* The EGF domain, which is required for receptor activation, contains six cysteine residues in a consensus sequence that predicts three disulfide bridges. The NRG products of four distinct genes (NRG 1–4) are primarily produced as transmembrane peptides, with a single membrane-spanning domain. Soluble NRG is produced by proteolysis of the membrane-

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bound protein, an event that may be regulated by the cytoplasmic tail *(3),* or by RNA splice variation that produces a secreted form *(1).* Alternate splicing of the NRG-1 gene generates neu differentiation factor (NDF; *4*), glial growth factor (GGF; *5*), acetylcholine receptor-inducing activity (ARIA; *6*), sensory and motor neuron-derived factor (SMDF; *7*), and heregulin (HER; *8*). Further splicing events can produce three alternate cytoplasmic tails (a,b,c), various extracellular juxtamembrane regions, an α- or β-EGF-like domain, and amino terminal domains consisting of an immunoglobulin-like domain with (Type 1), or without (Type 2) a carbohydrate-spacer region, or cysteine-rich region (Type 3) that contains neither Type 1 nor Type 2 motifs *(1,9).* Each of these NRG-1 isoforms is thought to serve a distinct function, and they are developmentally, as well as cell- and tissue-specifically, regulated *(10–13).*

The NRGs signal via members of the ErbB family of receptor tyrosine kinases. The basic structure of the prototype EGF receptor (ErbB1/HER1) has an extracellular amino terminus, two cysteine-rich domains that function in ligand binding, a transmembrane domain, a short intracellular juxtamembrane region, a kinase domain, and a carboxy terminal tail *(1,14).* Both ErbB3 and ErbB4 receptors bind NRG-1, but not ErbB1 or ErbB2 *(15,16).* Theoretically, all four of the ErbB receptors may participate in NRG-1 signaling via ligand-induced heterodimer formation. However, ErbB2 is the preferred heterodimerization partner, and the association of distinct dimer pairs may be driven by the distinct isoform of NRG-1 that serves as the ligand *(17,18).* Furthermore, the constituent pair within an ErbB receptor regulates ligand affinity *(19,20),* the nature of the signaling pathways recruited *(21,22),* and the subsequent cellular response *(23,24).* Homodimers of ErbB4 are signaling-competent *(15,16);* however, the ErbB3 receptor is kinase-deficient, and therefore homodimers cannot signal *(25).* Ligand-induced dimerization activates receptor kinase activity, and the phosphorylation of tyrosine residues in the C-terminal tail. These phosphorylated tyrosines, as part of a consensus motif, serve as binding sites for signaling molecules containing Src homology (SH) or phosphotyrosine binding (PTB) domains. Therefore, depending on the constituents of the ErbB dimer, HRG can activate p38 MAPK, the ERKs, PI 3-kinase, and JNK/SAPK *(15,16).*

Buonanno and Fischbach *(26)* have produced a concise review of the recent literature. Here, we focus on the association between ErbB receptors and specific submembranous proteins. This newly found association has profound implications for NRG signaling between cells of the nervous system.

Distribution of NRG and ErbB Receptors

Products of the NRG-1 gene are expressed widely in the central nervous system (CNS) and peripheral nervous system (PNS). Clearly, their role is essential in mid-gestation, as indicated by aberrant cardiac development in NRG-deficient mice at around d 10 postfertilization and in utero lethality *(27).* Using *in situ* hybridization, the presence of NRG-1 transcripts can be detected by E9 in mouse *(12);* both mesenchymal and neuronal cell types express NRG-1 during embryogenesis and into perinatal development. Localization of NRG-1 is to recently differentiated motor neurons in the spinal cord, preceding the onset of neuromuscular junction (NMJ) formation, and also to cells in crest-derived dorsal-root ganglia. In the intact brain, immunocytochemistry for NRG-1 reveals neuronal cell bodies *(28–30).* In addition, cultured neonatal Schwann cells, astrocytes, and oligodendocytes, as well as adult human oligodendrocytes, secrete NRG *(32–36).* By comparison, NRG-2 is expressed in the heart and brain, with the highest concentration reported in the cerebellum *(37–39).* The pattern of NRG-2 expression is distinct from that of NRG-1, and despite activating identical receptors, the biological events activated by the two gene products are distinct *(40).* The expression of NRG-3 is limited to the developing and

adult CNS, and has been shown to activate the ErbB4 receptor in vitro *(41).* Harari et al. *(42)* reported NRG-4 mRNA expression in human pancreas and muscle. The expressed protein was demonstrated to activate the ErbB4 receptor, but to have a much reduced binding affinity when compared to NRG-1.

Expression of the ErbB receptors is regulated, through development into adulthood, in a cell- and tissue-specific manner *(15,16,31,43,44).* Aberrant development of the heart and nervous system, and the lethal phenotype observed in ErbB2 *(45),* ErbB3 *(46),* and ErbB4 *(47)* knockout mice, similar to that reported for NRG-1 *(27),* underscore the vital importance of NRG-ErbB signaling. Although a ligand for ErbB2 has not been reported *(4),* this receptor is the preferred heterodimer partner among the ErbB family *(20,22,48).* The observed enhancement of signaling via ErbB2 heterodimers may be due to differential endocytic routing of ligand-bound receptors, and their rapid recycling to the cell surface *(49,50).* Normal physiologic functions for ErbB2 include the development and maintenance of neuromuscular synapses *(51),* and of Schwann cells in the periphery *(52,53).* The ErbB2 receptors are on neuronal cell bodies in cerebral cortex and hippocampus *(36),* but on astrocytes in the hypothalamus *(54).* No ErbB2 receptors are present in the cerebellum at any time during development *(31,55,56).* Cultures of human oligodendrocytes also express ErbB2 *(34).* The ErbB2 receptor has been widely studied with respect to its role in the development of cancers *(57).* Medulloblastoma, an embryonal tumor of the cerebellum, is believed to arise from the external granule-cell layer, and ErbB2/ErbB4 heterodimerization occurs in >50% of cases *(31).* The ErbB3 receptor is expressed by glia in the postnatal cerebellum *(31,58),* and also found in cultured oligodendrocytes *(30,34).* Transcripts for ErbB4 are found in rat CNS germinal zones as early as E12, but are restricted to forebrain germinal zones and the external granule-cell layer of the cerebellum by PO *(43).* In addition, hypothalamic astrocytes *(54)* and cultured oligodendrocytes express ErbB4 receptors *(30,34).*

Neuregulins and Myelinating Cells

Survival and proliferation of Schwann cells depend upon neuregulins *(59),* and precursors fail to develop in mice with a deletion of the NRG-1 gene *(27).* Mature Schwann cells express ErbB2 and ErbB3 receptors that complex as dimers, and recruit the small adaptor protein Grb2 *(60).* Proliferating cells migrate along axons and extend processes that gradually segregate them into progressively smaller bundles. This continues until each myelinated axon is wrapped by a single myelinating Schwann cell. Migration is mediated, at least in part, by the MAPK pathway, which might be responsible for malignant transformation of Schwann cells into an invasive phenotype *(61).* In fact, addition of NRG-1 (GGF) to cell cultures in which myelination has taken place causes Schwann cells to demyelinate and start to proliferate *(62).*

The early stages of oligodendrocyte development are also controlled by NRG-1 *(63),* and precursors fail to develop in mice with a deletion of the NRG-1 gene *(53).* It is proposed that NRG-1 maintains the growth of early oligodendrocyte precursors, but that the subsequent release of a soluble receptor (ErbB4?) inhibits mitogenesis and allows differentiation to occur *(64).* Certainly, NRG-1 will promote remyelination and retard progression of experimental autoimmune encephalomyelitis, suggesting a useful addition to few currently available therapies for multiple sclerosis *(65).*

Association of ErbB Receptors with Scaffold Proteins

Organization of cellular activities is achieved via protein compartmentalization. One mechanism is to package specific proteins into 'caveolae,' or 'caveolar-like membranes.'

Frenzel and Falls *(66)* have shown recently that the NRG-1 proprotein and C-terminal fragment, together with the ErbB4 receptor, are components of lipid rafts in brain. Another

protein that accumulates in lipid rafts is PSD-95 *(67),* and its association with NRG signaling is now clear.

ErbB4-PSD

Among the ErbB receptors, ErbB4 is promiscuous in its affinity for ligands *(16),* and it co-localizes with NMDA receptors in the brain *(68,69).* These two recent reports reveal that ErbB4 forms a complex with postsynaptic density proteins, such as PSD-95, via specific interaction with two of the latter's three PDZ domains. A consensus binding site (T-V-V) in the carboxy-terminal end of ErbB4 directly interacts with PDZ domains 1 or 2. In fact, PSD-95 promotes ErbB4 dimerization, and inhibition of PSD-95 expression reduces NRG signaling. There is some association between ErbB2 and PSD-95, but the affinity is 20-fold less than ErbB4. However, there is no association between ErbB3 and PSD proteins.

ErbB2/Erbin/PSD

The ErbB2 receptor has an important role in interactions between migrating neurons and radial glia in the developing cerebral cortex, and accumulates at synaptic junctions *(70).* Akin to the association of PSD-95 with ErbB-4, Erbin (ErbB-2 interacting protein) interacts via its single PDZ domain with the C-terminus (V-P-V) of ErbB2, but not with ErbB3/4 *(71,72).* Furthermore, Erbin interacts with the first two PDZ domains of PSD-95, but not through the Erbin PDZ domain. This suggests that Erbin could interact simultaneously with ErbB2 and PSD-95 *(73).*

Other Associated Proteins?

The cytoplasmic tails of ErbB2/4 receptors possess an array of binding sites for adaptor proteins, such as grb2, shc, chk, and p85, that initiate distinct signaling cascades *(15).* In addition, a few terminal amino acid residues impart associations of ErbB2/ErbB4 with proteins, such as PSD-95 and Erbin, that serve as scaffolds for protein assemblies. For example, PSD-95 interacts with components of the cytoskeleton and with cell-adhesion molecules *(67).* As ErbB receptors are expressed in extrasynaptic locations in the nervous system, such as glia, it will be intriguing to discover whether receptors at these sites form similar associations with submembranous proteins.

Nitric oxide synthase (NOS)-1 is another component of this synaptic assembly. The protein has long been known to interact with the middle PDZ domain of PSD-95 *(74),* and this appears to involve a region immediately following the NOS-1 PDZ domain *(75).* The protein itself displays a single, interactive PDZ domain in the N-terminal region, which permits the direct targeting of NOS-1 to membrane receptors, such as serotonin 2B *(76).* While ErbB receptors do not appear to interact directly with the PDZ domain of NOS-1 *(69),* an association with NOS-1 is possible via PSD-95.

Although there is no physical evidence for this association, NOS-1 could form part of the NRG signaling cascade (*see* Fig. 1). In terms of their effects on the developing nervous system, NRG and NO display many similarities *(26,77).* Furthermore, recent evidence suggests that NRG can transiently increase NOS-1 expression in neurons via activation of the ErbB4 receptor *(55,56).* This effect is independent of a change in NOS-1 mRNA expression, and suggests either a decrease in ubiquitination of NOS-1 *(78),* or that NOS-1 is subject to translational control *(79).* Exposure of granule neurons to HRG increases the amount of phosphorylated ErbB4 receptor, and stimulates MAPK and PI 3-K activities. The increase in NOS-1 expression is partially reversed by a MAPK inhibitor, but unaffected by inhibition of PI 3-K, suggesting a role for other kinases. In response to HRG, MAPK can activate ras, which has been shown to cross-activate the JNK/SAPK pathway *(80).* Also of interest is the association of receptor tyrosine kinases with the activation of Janus kinases (Jaks) and the 'signal transducer and activator of tran-

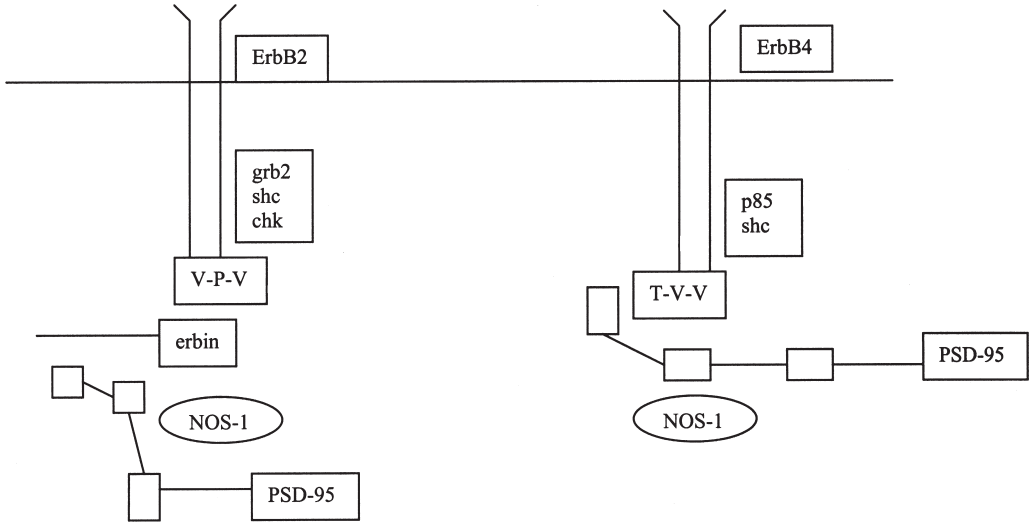

Fig. 1. Association of synaptic ErbB receptors with other proteins. The terminal amino acid residues allow ErbB4 to associate with the first two PDZ domains of PSD-95, and ErbB2 to associate with the single PDZ domain of Erbin. In turn, a site adjacent to its PDZ domain allows Erbin to interact with the first two PDZ domains of PSD-95. Based on its association with the second PDZ domain of PSD-95, NOS-1 is depicted as part of a hypothetical complex. The cytoplasmic tails of the ErbB receptors can also recruit various adaptor molecules.

scription' (Stat) family of transcription factors *(81).* Activation of Stat5 by the ErbB4 receptor has been reported in mammary development *(19),* and studies using transfected cells have shown that neuregulin can activate Stat5 through ErbB2/ErbB4 heterodimers, independent of the MAPK pathway *(79,82).*

Parallels Between NRG and NO Actions in the Developing Nervous System

Neuregulins are important in regulating developmental events in the cerebellum. Radial glial fibers guide the migration of granule neurons into the internal granule layer via NRG-1 signaling *(83).* Ozaki et al. *(84)* suggest that the formation of mossy-fiber synapses on granule neurons is dependent on NRG-1 expression. During the same period of cerebellar developmental, Ozaki et al. *(58)* observed a NRG-1 induced switch in granule neuron NMDA receptor subunit expression, from NR2B to NR2C. In a separate model system, Rieff et al. *(85)* report that NRG-1 stimulates expression of the $GABA_A$ receptor β2 subunit, and also neurite outgrowth in granule neurons. Likewise, Villegas et al. *(86)* and Vaskovsky et al. *(87)* showed that NRG induces neurite outgrowth in PC12 cells by phosphorylating the ErbB4 receptor, with associated increase in the growth-associated protein GAP-43.

The NOS-1 protein has also been implicated in postnatal development of cerebellar granule cells *(88),* and in regulating the growth and differentiation effects of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) *(89,90).* During migration, granule cells display periodic fluctuations of cytoplasmic calcium. At comparable stages of cerebellar development, a similar permissive role

towards migration from the external to the internal granular layer is played by NO. Using in vitro slice cultures, it was demonstrated that a NOS inhibitor significantly decreased the migratory index of granule cells *(91).* NO can stimulate the growth and branching of dendrites *(77),* and neurite extension in PC12 cells *(92).* Through an ability to nitrosylate, and so modify proteins such as synaptotagmin and SNAP-25 involved in exocytosis *(93),* NO can induce the release of transmitters from vesicular stores *(94).* Inhibition of NOS-1 activity during postnatal development also results in disturbance of layer formation in the cerebellum *(95).* However, Klocker et al. *(96,97)* found that chronic BDNF treatment upregulates NOS-1 expression in axotomized retinal ganglion cells, which then counteracts the neuroprotective effects of BDNF.

Whether or not there is a physical association between ErbB4 and NOS-1, it will be important to affirm a functional link through experiments designed to reveal whether NO mediates (some of) the reported effects of NRGs on neurons. For example, does NOS-1 inhibition affect the transcriptional changes in receptor subunit expression provoked by NRG? How is neuritic outgrowth influenced? Are the developmental changes in axon extension and synapse-associated proteins, such as GAP-43 *(98–101)* and synapsin I *(102–103),* regulated by NRG acting through NOS-1?

In conclusion, the discovery of specific associations between ErbB2/4 receptors and proteins such as PSD-95 and Erbin adds new potential pathways to what is already known about NRG signaling in the nervous system. Based on the observation that NRG upregulates NOS-1 expression, we suggest that one of these pathways involves NO.

Acknowledgments

We are grateful to the USPHS-NIH for training grant support (RK) and to the Biotechnology and Biological Sciences Research Council (BBSRC, UK) for a project grant award (SM).

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