Emerging role of neuregulin as a modulator of muscle metabolism

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Gumà A, Martínez-Redondo V, López-Soldado I, Cantó C, Zorzano A. Emerging role of neuregulin as a modulator of muscle metabolism. Am J Physiol Endocrinol Metab 298: E742–E750, 2010. First published December 22, 2009; doi:10.1152/ajpendo.00541.2009.—Neuregulin was described initially as a neurotrophic factor involved in the formation of the neuromuscular junction in skeletal muscle. However, in recent years, neuregulin has been reported to be a myokine that exerts relevant effects on myogenesis and the regulation of muscle metabolism. In this new context, the rapid and chronic metabolic effects of neuregulin appear to be related to muscle contraction. Indeed, the effects of neuregulin resemble those of exercise, which are accompanied by an improvement in insulin sensitivity. In this review, we challenge the classical role assigned to neuregulin in muscle and propound the emerging concept of its involvement in the regulation of energetic metabolism and insulin responsiveness.

ErbB receptors; exercise; glucose transport; insulin; mitochondrial biogenesis
activated as homodimer. NRG differential splicing generates variants of the EGF-like domain, thereby resulting in $\text{NRG-1}^{-}$, $\text{NRG-2}^{-}$, and $\text{NRG-3}^{-}$-isoforms that display distinct affinity for NRG receptors (37, 63, 78). Phosphorylation of tyrosine residues in the cytoplasmic domains of ErbB receptors gives rise to binding sites for several intracellular signaling molecules, which initiate a diverse array of downstream signaling events (7, 85), notably the mitogenic MAP kinase (MAPK) cascade, the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB)/Akt pathway, and the stress-inducible p38 MAPK pathway (85, 90).

In adult skeletal muscle, data on the expression of ErbB2, ErbB3, and ErbB4 receptors have accumulated over the last 10 years. Cultured myotubes predominantly express ErbB2 and ErbB3 but rarely express ErbB4 (57, 89). Adult skeletal muscle fibers express ErbB2 and ErbB4 receptors (8), but there is some controversy in regard to ErbB3 expression levels in this tissue. All ErbB receptors accumulate at the neuromuscular junction (NMJ). ErbB2 and ErbB4 are found specifically at the muscular postsynaptic site, in contrast to ErbB3, which is concentrated at presynaptic terminal Schwann cells (74). Muscle ErbB receptors are not only confined to the NMJ but also abundant in the transverse tubular (T-tubules) system (60, 75). In cardiac myocytes, ErbB4 is located in caveolin-associated microdomains (87), whereas in neurons it is abundant in lipid rafts (48). In T47D cells, ErbB receptors translocate to lipid raft microdomains in the plasma membrane in response to NRG (84). This observation points to these sites being hotspots in NRG signaling.

**Effects of Neuregulin: Lessons from Transgenic Mice**

Many things can be learned about the in vivo action and functions of NRG from the transgenic mouse models of NRG-1 and its ErbB receptors. NRG functions can be classified on the basis of a number of common phenotypes shown by these models (Table 1). First, observations from most transgenic lines highlight a critical role for NRG in cardiac morphogenesis (62). Massive cardiac developmental malformations are found in pan-NRG-1$^{-}$ mice, in which the common EGF-like domain present in all NRG isoforms is disrupted (52), as well as in Ig-NRG-1$^{-}$ (39), ErbB2$^{-}$ (42), ErbB3$^{-}$ (14), and ErbB4$^{-}$ (23) mice. Embryos from these genotypes die in utero as a result of cardiac development failure during the switching from maternal to their own circulation. The pan-NRG-1$^{-}$, Ig-NRG-1$^{-}$, ErbB2$^{-}$, and ErbB4-null mice lack heart ventricular trabeculation and die at embryonic day 10.5. Although they develop normal trabeculation, ErbB3$^{-}$ mice are unable to maintain a proper cardiac function due to an abnormal endocardial cushion development, which is involved in the formation of cardiac valves, causing blood reflux and...
leading to death at embryonic day 13.5. This malformation is less severe, and some pups even survive to term. This latter cardiac malformation is also shown by pan-NRG-1/−/− and some ErbB2/−/− mice. An overt cardiac phenotype has also been reported in mice with a targeted mutation on the cytoplasm tail of NRGs, which disrupts their traffic to the plasma membrane.

No cardiac defects are observed in the mice lacking CRD-NRG (81), which is an isoform highly expressed by motor neurons. This finding indicates that all the effects of NRG on cardiac development are related to the action of Ig-NRG isoforms. Despite normal heart function, CRD-NRG1−/− mice manifest a lethal phenotype and die soon after birth as a result of their inability to breathe. This alteration affects the development of presynaptic components, neurons, and glial cells and compromises neuromuscular synapse formation. These synaptic defects have been described globally as a “presynaptic phenotype” that is characterized by the failure of motor nerve terminals to localize the end-plate zone and hence, lacking functional synapses. This phenotype also displays a marked reduction of Schwann cells and a 50% reduction of spinal motor and sensory neurons because of increased neuron death. This phenotype is not present in the pan-NRG-1−/− mice because they die as a result of the aforementioned heart failure, before the development of the first neuromuscular synapses. Surprisingly, CRD-NRG-1−/− mice do not show a reduction in AChRs in synapses. Heterozygous Ig-NRG-1+/− mice (67) are viable and fertile and do not show cardiac alterations, which contrasts with homozygous Ig-NRG-1−/− mice. However, in contrast to CRD-NRG-1−/− mice, heterozygous Ig-NRG-1+/− mice have affected the synaptic end plate in skeletal muscle fibers, with a 50% reduction in the content of AChRs. This observation suggests that NRG-1 isoforms containing the Ig-like domain are involved in the induction of AChR expression.

More clarifying conclusions were derived from the study of tissue-specific knockouts. The results from the neuron-specific knockout of NRG-1 (83) resembled those found in the CRD-NRG-1−/− mice, thereby indicating the existence of a presynaptic phenotype with no impact on AChR expression in the muscle end plate. A presynaptic phenotype is also observed in the formation of the NMJ in ErbB2−/− mice once they are rescued by inducing heart-selective ErbB2 expression (ErbB2 heart rescued knockout) (80). Despite the severe presynaptic phenotype, AChR synapse-specific transcription was only moderately affected, thus challenging the view that NRG-1 is a critical regulator of AChR transcription in synapses. Muscle-specific ErbB2−/− mice show a modest 20% reduction in AChR expression in the NMJ region (43), whereas ErbB4−/− with cardiac rescue show no abnormalities in neuromuscular development (73). Similarly, the muscle-specific knockout mice for ErbB2 and ErbB4 display only a 10% reduction in AChR protein density and a 30% reduction in synaptic mRNA for AChR. These observations suggest that, although NRG activates AChR expression (2, 17), other mechanisms also contribute to the maintenance of the differentiated NMJ (15).

Multiple Effects of Neuregulin on Muscle Development

In addition to NRG involvement in cardiac morphogenesis and NMJ formation and maintenance, NRG plays a crucial role in myogenesis in skeletal muscle. It has long been observed that nerves have the capacity to stimulate skeletal muscle growth and maintenance in an electrical stimuli-independent fashion but are dependent on some myotrophic agents (29). GGF2, one of the NRG isoforms generated by neurons, was identified as a myogenic factor in L6A1 rat fibroblasts. This growth factor acts in an additive manner with insulin-like growth factor I (IGF-I) (19). Studies in L6 myocytes showed that NRG is released by these cells at the initial stage of myogenesis, and, acting in an autocrine manner through ErbB3, they are essential for myogenic differentiation (38). A differentiation state-dependent effect of NRG on skeletal myogenesis (20) by which NRG can differentially promote mitogenic or myogenic effects depending on its concentration and the culture differentiation stage has been reported. As a myogenic factor, NRG-1 promotes myogenin expression, thereby prompting myoblasts to withdraw from the cell cycle and differentiate, thus inducing fusion to multinucleated myotubes. NRG also induces muscle spindle formation. NRG-1 plays a positive role in intrafusal fiber development by increasing nuclear bag fiber formation (36, 65).
NRG also plays a relevant role in muscle regeneration. It has antiapoptotic effects in skeletal muscle satellite cells activated from quiescence to proliferate after myotrauma is induced. This effect is dependent on ErbB2 activity (3). NRG-1 content also increases in motor neurons in the spinal cord, thereby innervating and helping to regenerate the muscle (32). In addition, NRG-1 increases protein synthesis by an average of 30% in C2C12 myotubes and rat diaphragm (31). Therefore, NRG-1 could have therapeutic applications in myodystrophies and muscle regeneration after injury. In this regard, NRG-1 stimulates the phosphorylation of peroxisome proliferator-activated receptor (PPAR)γ coactivator-1α (PGC-1α) and GA-binding protein, thereby allowing the recruitment of PGC-1α to the GA-binding protein complex (human homolog known as nuclear respiratory factor-2) and thus enhancing the transcription of a broad number of genes encoding proteins clustered at the NMJ (30). PGC-1α transgenic overexpression in mdx mice, dystrophin-deficient mice that show a very similar phenotype to Duchenne muscular dystrophy (DMD), ameliorates some parameters characteristically affected in DMD, including muscle histology, running performance, and plasma creatine kinase levels. PGC-1α also inhibits FoxO3-induced muscle atrophy gene transcription (66). Thus, NRG-1 activation of PGC-1α could provide a new therapeutic target to treat DMD.

From a Myogenic Factor to a Metabolic Regulator Involved in Contraction Events

In addition to the major roles as a myogenic and neurotrophic factor initially assigned to NRG in muscle, in recent years NRG has emerged as a modulator of muscle metabolism. Earlier studies indicate that NRG stimulates muscle glucose uptake in both an acute and a chronic manner (71). NRG induces glucose uptake in skeletal muscle by recuiting glucose transporters at cell surface membranes. This effect is additive to the insulin action, thereby pointing to independent signaling pathways between these two factors. It is important to note that NRG receptors do not transactivate insulin receptors (10). However, studies in L6E9 myotubes show that wortmannin, a highly specific inhibitor of PI3K class IA, which is involved in insulin action (86), blocks NRG action on glucose uptake (71). The common presence of PI3K in the initial steps of the insulin and NRG signaling cascades makes it difficult to elucidate their additive effects, especially when analysis of the PI3K activity shows no additive activation. NRG activates multiple signaling cascades, such as the PKB/mTOR/p70S6K pathway, the stress-associated p38 MAPK pathway, and the mitogenic p42/44 MAPK pathway (1, 2, 45, 72), in multiple cell types. In accordance with studies based on the use of specific inhibitors and dominant negative models in L6E9 muscle cells, the action of NRG on glucose uptake is reduced 22, 27, and 59% by PKB, p38 MAPK, and p42/44 MAPK inhibition, respectively. These observations indicate that, although PKB and p38 MAPK are essentially not involved in the NRG stimulation of glucose transport, p42/44 MAPK may participate to some extent in this action (10). The finding that PKB is not involved in NRG action, in contrast to insulin action, partially differentiates these two pathways. In this regard, whereas insulin induces PKB phosphorylation at sites Thr308 and Ser473, NRG preferentially exerts this action at the first site, thereby indicating that, unlike insulin, NRG derives its effects from the activation of 3-phosphoinositide-dependent protein kinase-1 (PKD1), which phosphorylates Thr308, and to a lesser extent, if any, through PKD2, which phosphorylates Ser473 (10). To date, there is no explanation for the differences in the PKB activation level between the insulin and NRG pathways. Finally, atypical protein kinase Cζ (PKCζ), which is also involved in the insulin pathway to stimulate glucose uptake (5), is required for NRG action (10), but again, no additive effect is observed for insulin- and NRG-induced PKCζ activity. In summary, NRG requires a PI3K/PDK1/PKCζ pathway to induce GLUT4 translocation. Since insulin and NRG do not induce additive activities on these kinases, there may be other parallel NRG signaling cascades or differences distal to PKCζ.

To further investigate the bases for NRG additivity on insulin action, the muscle contraction pathway is reviewed here since it leads to insulin-additive effects on glucose transport (13, 22). Muscle contraction signals its effects on glucose transport through the second messenger calcium ion and the energetic state sensitizer 5′-AMP, which activate calcium/calcmodulin-dependent protein kinase II (CaMK II) and 5′-AMP-dependent protein kinase (AMPK), respectively (82). These pathways, apparently unconnected, maintain a relationship since AMPK is also activated by CaMK kinase-induced phosphorylation, among others (79). Given the additive effects of NRG and insulin, it is reasonable to postulate that NRG contributes to muscle contraction events. During contraction, NRG is proteolyzed in a calcium/metalloprotease-dependent manner, thereby inducing NRG release and ErbB receptor activation, in both glycolytic and oxidative muscle fibers (8, 40). NRG operates in parallel to the calcium effects on CaMK II and the energy sensor AMP on AMPK activation to stimulate glucose uptake since none of these kinases are directly activated by NRG (8, 10) (Fig. 2). Therefore, is NRG release a collateral contraction effect with no physiological impact on the regulation of glucose uptake? When ErbB4 receptors are blocked with specific monoclonal antibodies, the in vivo contraction effect on glucose uptake is highly impaired in oxidative (~70%) and glycolytic (~40%) fibers, with a dramatic impact on muscle glycogen, ATP, and creatine phosphate levels (8). These observations indicate the essential role of NRG signaling in these events. The beneficial effect of acute NRG action during muscle contraction on glycogen content is also clearly observed in incubated rat soleus muscles subjected to electrical stimulation for 10 min in vitro. In these conditions, contraction reduces glycogen content by 50%, but the addition of a high NRG concentration (5 nM) in the incubation medium prevents this effect (Cantó C, Chibalin A, Barnes BR, Grond S, Zierath JR, and Gumà A, unpublished observations).

The NRG signaling pathway that drives the induction of glucose uptake under contraction events is not clear. Although CaMK II is not activated by NRG, this kinase interacts to some extent with the NRG signaling pathway since its inhibition impairs NRG effects on glucose uptake (8). Given that PKCζ is involved in the NRG activation of glucose uptake, this kinase is a good candidate for the NRG signaling cascade during muscle contraction since it also participates in contraction events that induce glucose uptake (11). Special attention should be paid to the initial signal that leads to PKCζ stimulation and PI3K dependence on it. Whereas insulin and NRG act on PKCζ activation in a PI3K-dependent manner in a...
noncontraction context (10), muscle contraction induces PKCζ in a p42/44 MAPK-dependent manner (11). MAPKs are involved in the AMPK-dependent pathway that leads to the synthesis of phosphatidic acid, an activator of atypical PKCs (11). Interestingly, NRG is a potent activator of p42/44 MAPK in muscle cells (21). Thus the question arises as to whether the calcium-NRG pathway also activates a p42/44 MAPK/PKCζ pathway in muscle contraction as an alternative to the previously described AMPK/PKCζ pathway to induce GLUT4 translocation. Future studies should address this issue.

In summary, the demonstration that NRG action is involved in muscle contraction events improves our understanding of the bases of the additive effects between NRG and insulin. Although we are far from delineating the specific mechanisms that contribute to the additive effects with insulin, it is clear that all events that induce GLUT4 translocation cooperate and partially overlap each other to guarantee the glucose homeostasis and the maintenance of muscle energy status (Fig. 2).

Is Neuregulin Involved in the Cellular Adaptations to Chronic Exercise?

Since NRG is involved in the effects of muscle contraction, it is relevant to analyze whether chronic NRG effects resemble muscle adaptation to exercise training, which is characterized by enhanced oxidative capacity and improved insulin sensitivity.

Expression of skeletal muscle NRG is not increased by progressive resistance training (41). Nonetheless, chronic treatment with NRG promotes gene expression reprogramming, which leads to the increased expression of many respiratory chain- and β-oxidation-related genes in cardiomyocytes (24). Moreover, and consistent with the notion that NRG regulates mitochondrial gene expression, the inhibition of ErbB2 causes mitochondrial dysfunction, thereby generating a marked decrease in oxidative capacity and the development of dilated cardiomyopathy (28, 60). In this regard, it has been proposed that attenuation of NRG-ErbB signaling in cardiomyocytes is a causal factor for mitochondrial dysfunction and apoptotic cardiomyocyte loss in aging myocardium but also in failing myocardium (64). Chronic treatment with NRG, at picomolar concentrations, induces oxidative capacity by increasing mitochondrial biogenesis and the GLUT4 content in muscle cells (Fig. 3) (9). At such low concentrations, NRG has no effect on myogenesis, which requires a range of nM concentrations for its induction (71).

The transcriptional coactivator PGC-1α is the main orchestrator in the regulation of mitochondrial biogenesis. PGC-1α achieves this effect by coactivating transcription factors such as PPARα and PPARγ, both of which are involved in the increase of oxidative capacity (44). Adaptation of skeletal muscle to exercise is driven by increased expression of PGC-1α and PPARγ, whereas PPARα and -γ are undetectable in these cultured cells despite the fact that skeletal muscle tissue expresses relatively high levels of PPARα. The NRG-dependent increase in mitochondrial activity is abrogated in a PPARγ-dominant negative model but unaltered by PPARα inhibition (9). PPARγ participates in muscle adaptation to training by increasing endurance capacity as a result of its effects on oxidative capacity, respiratory complex content, and PGC-1α expression (68, 76). Accordingly, chronic effects of NRG resemble those of muscle training and induce a switch toward oxidative metabolism similar to that of type I oxidative fibers. Oxidative slow-twitch muscle fibers are more sensitive to insulin (35). Consistently, oxidative fibers display a higher content of insulin-signaling effectors (70), and in exercised muscle there is an increase in insulin receptors (12). Conditions in which muscle develops insulin resistance are characterized by a decrease in the expression of PGC-1α as well as in the components of the oxidative phos-
phorylation pathway (55, 56, 61). Interestingly, PPARγ-knockout mice develop diabetes (68). Taking into consideration all these data, it renders a link between oxidative capacity and insulin sensitivity. NRG increases insulin sensitivity, by one order of magnitude, on its action inducing glucose uptake through GLUT4 translocation. Moreover, NRG increases the expression of multiple components of the insulin-signaling cascade in a PPARδ-dependent manner (Fig. 3) (9). Given the similarities between the insulin and IGF-I signaling pathways, it would also be relevant to study whether NRG potentiates the IGF-I cascade. In all, NRG is postulated as a strong candidate second, NRG induces a rapid effect, providing glucose during a short period of time, from some minutes to 1 day. This release may serve as an emergency factor in the event of muscle contraction through ErbB4 activation in adult muscle. A baseline release could occur as a consequence of muscular tone and may contribute to the development of the fiber type metabolic profile based on muscle cell oxidative capacity and insulin signaling. A baseline release could occur as a consequence of muscular tone and may contribute to the development of the fiber type metabolic profile based on muscle cell oxidative capacity and insulin signaling.

**Views and Perspectives**

In summary, NRG is involved in the differentiation and metabolic regulation of skeletal muscle, and its release is induced by the muscle contraction. A baseline release could occur as a consequence of muscular tone and may contribute to the development of the fiber type metabolic profile based on chronic NRG action at the picomolar concentration. Further studies will be required to determine the precise contribution of NRG to muscle adaptation in response to training.

Muscle NRG release reaching nanomolar concentrations appears to be a transient phenomenon that lasts for a relatively short period of time, from some minutes to 1 day. This release has a dual role in muscle. First, a NRG autocrine effect is required to induce myogenesis through ErbB3 activation, and second, NRG induces a rapid effect, providing glucose during muscle contraction through ErbB4 activation in adult muscle. This view of NRG action suggests that a transient and massive NRG release may serve as an emergency factor in the event of muscle damage to induce myogenic regeneration or to respond to metabolic stress, such as that acutely induced by exercise, to maintain cell energy status.

An excessively prolonged high NRG concentration could be dangerous for the cell. Overexpression of NRG and/or ErbB2 and ErbB3 receptors in several cell types is associated with the deregulation of cell growth control and with the manifestation of cancerous pathologies (53). These findings indicate the relevance of the fine regulation of NRG availability and ErbB receptor expression/activation. Furthermore, McGuire et al. (50) recently reported that persistent activation of ErbB2 contributes to the progression of the diabetic peripheral neuropathy in caveolin-1-knockout mice.

Given the relationship between insulin and NRG action in the regulation of muscle glucose uptake, future studies should focus on establishing whether defects in NRG action contribute to the development of the hyperglycemia associated with the insulin resistance states that characterize the metabolic syndrome. They should also address the question of whether the NRG-associated mechanisms involved in the regulation of glucose uptake provide a therapeutical target to ameliorate these physiopathological alterations.

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**Fig. 3.** Chronic effects of neuregulin in improving muscle cell oxidative capacity and insulin signaling on glucose transport. Cultured muscle cells, containing mainly ErbB2 and ErbB3 receptors, respond to chronic treatment with a recombinant neuregulin, containing the EGF domain, by inducing peroxisome proliferator-activated receptor (PPARγ) coactivator-1α (PGC-1α) and PPARα expression. These gene expression regulators are involved in mitochondrial biogenesis, thereby generating an increase in the cell oxidative capacity by neuregulin. NRG also improves insulin sensitivity-enhancing insulin stimulation of glucose uptake by a mechanism that could be partially sustained through the increase in the oxidative capacity but also through the increase in GLUT4 and insulin-signaling mediator content. 

† Increase in protein cellular content. IR, insulin receptor; PDK, 3-phosphoinositide-dependent protein kinase; P13K, phosphatidylinositol 3-kinase. Hatched section in ErbB3 indicates protein kinase inactive.
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DISCLOSURES
No conflicts of interest are declared by the author(s).

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