Neuregulin-1/ErbB4 signaling regulates Kv4.2-mediated transient outward K+ current through the Akt/mTOR pathway

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Submitted 6 February 2013; accepted in final form 13 May 2013

Yao JJ, Sun J, Zhao QR, Wang CY, Me YA. Neuregulin-1/ErbB4 signaling regulates Kv4.2-mediated transient outward K+ current through the Akt/mTOR pathway. Am J Physiol Cell Physiol 305: C000–C000, 2013. First published May 22, 2013; doi:10.1152/ajpcell.00041.2013.—Neuregulin-1 (NRG-1) is a member of a family of neurotrophic factors that is required for the differentiation, migration, and development of neurons. NRG-1 signaling is thought to contribute to both neuronal development and the neuropathology of schizophrenia, which is believed to be a neurodevelopmental disorder. However, few studies have investigated the role of NRG-1 on voltage-gated ion channels. In this study, we report that NRG-1 specifically increases the density of transient outward K+ currents (IA) in rat cerebellar granule neurons (CGNs) in a time-dependent manner without modifying the activation or inactivation properties of IA channels. The increase in IA density is mediated by increased protein expression of Kv4.2, the main α-subunit of the IA channel, most likely by upregulation of translation. The effect of NRG-1 on IA density and Kv4.2 expression was only significant in immature neurons. Mechanistically, both Akt and mammalian target of rapamycin (mTOR) signaling pathways are required for the increased NRG-1-induced IA density and expression of Kv4.2. Moreover, pharmacological blockade of the ErbB4 receptor reduced the effect of NRG-1 on IA density and Kv4.2 induction. Our data reveal, for the first time, that stimulation of ErbB4 signaling by NRG-1 upregulates the expression of K+ channel proteins via activation of the Akt/mTOR signaling pathway and plays an important role in neuronal development and maturation. NRG1 does not acutely change IA and delayed-rectifier outward (IK) of rat CGNs, suggesting that it may not alter excitability of immature neurons by altering potassium channel property.

Cerebellar granule neurons (CGNs) are small glutamatergic cells and constitute the largest homogeneous neuronal population in the mammalian brain. Due to their postnatal generation and well-defined developmental pathway in vitro, cultures of primary CGNs have been established as a model for studying neuronal maturation, differentiation, and synaptic plasticity (9, 39). Previous studies have indicated that growth and differentiation factors can either stimulate or inhibit CGN development and maturation by regulating multiple signaling pathways (41, 45). In addition, NRG-1 selectively induces the expression of the GABA_A receptor β2-subunit in CGNs by binding to the ErbB4 receptor and recruiting PSD-95 (40, 41).

One characteristic of CGN development and maturation is regulation of the expression of K+ channels (28, 35). Primary culture CGNs display several voltage-activated outward K+ currents (26). We previously reported that the enhancement of delayed-rectifier outward (IK) and fast transient outward (IA) potassium currents was associated with CGN apoptosis (17, 20). In addition, IK also plays a role in promoting CGN migration and maturation (24, 45). Recently, our study indicated that neurtin, a new neurotrophic factor that is involved in activity-dependent synaptic plasticity, activates the insulin receptor pathway to upregulate Kv4.2-mediated IA in rat cerebellar granule neurons (43), indicating that neurotrophic factors may alter the status and function of CGNs by modulation of the expression of different potassium channels.

The goal of this study was to examine whether NRG-1 modulates K+ channels as part of its role in synaptic activity and neurodevelopment. To test this hypothesis, we examined the effect of NRG-1 on K+ channels in rat CGNs. We found that NRG-1 increases IA density by regulating the expression of Kv4.2 potassium channel α-subunits and that activation of Akt/mTOR signaling by NRG-1-ErbB4 is required.

EXPERIMENTAL PROCEDURES

Chemicals. Recombinant human NRG-1 was purchased from Pepro Tech (Rocky Hill, NJ). Triethanolamine (TEA), tetrodotoxin (TTX), 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB), cycloheximide, AG1478, rapamycin, LY294002, and poly-L-lysine were purchased from Sigma (St. Louis, MO). Fetal calf serum, DMEM, and antibiotic-antimycotic solution were purchased from GIBCO Life Technologies (Grand Island, NY).

Cell culture. All experimental procedures were carried out in accordance with the European guidelines for the care and use of laboratory animals (Council Directive 86/609/EEC). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Fudan University. Cells were derived from the cerebella of 7-day-old Sprague-Dawley rat pups as described previously (29). Isolated cells were plated at a density of 10^6 cells/ml onto 35 mm Petri dishes coated with poly-L-lysine (1 μg/ml). Cultured cells were incubated at 37°C in 5% CO2 in DMEM supplemented with 10% fetal...
calf serum, insulin (5 μg/ml), KCl (25 mM), and 1% antibiotic-

antimycotic solution. After culture for 24 h, cytoseine β-2,3-arabinofuran-

oside (5 μM) was added to the culture medium to inhibit the

proliferation of nonneuronal cells. Cells were used for experiments after

2–7 days in culture unless otherwise indicated.

**Patch-clamp recordings.** Whole-cell currents of granule neurons were recorded using conventional patch-clamp techniques. Before IA

current recording, the culture medium was replaced with a bath

solution containing the following (in mM): 125 NaCl, 2.5 KCl, 10

HEPES, 1 MgCl2, 0.001 TTX, 20 TEA, and 10 glucose (pH adjusted
to 7.4 with NaOH). Soft-glass recording pipettes were filled with an

internal solution containing the following (in mM): 135 potassium

gluconate, 10 KCl, 10 HEPES, 1 CaCl2, 1 MgCl2, 10 EGTA, 1 ATP,

and 0.1 GTP (with pH adjusted to 7.3 using KOH). The pipette

resistance was 4–6 MΩ after filling with an internal solution. The

cultured granule cells selected for electrophysiological recording

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was half-activated at \(-8.5 \pm 1.3\) mV (\(n = 27\)) and \(-7.3 \pm 0.8\) mV (\(n = 25\)) for the control and NRG-1-treated groups, respectively (\(P > 0.05\)). These data suggest that NRG-1 treatment does not alter the steady-state activation of the voltage dependence of \(I_A\).

To study steady-state inactivation of \(I_A\), currents were elicited using 1 s conditioning prepulses from \(-110\) to 0 mV before a 200-ms test pulse of +50 mV. After normalizing each current amplitude to the maximal current, the amplitude obtained from the \(-110\) mV prepulse was used as a function of the conditioning prepulse potential and fitted to the function \(I_A/I_{A\text{-max}} = 1/[1 + \exp((V_m/2 - V_{50})/k)]\). Hence, we obtained an inactivation curve of the \(I_A\) and calculated the \(V_{50}\) (the voltage at which the current amplitude was half-inactivated). Similarly, the inactivation curve of the \(I_A\) was not significantly shifted after incubation with 10 nM NRG-1 for 24 h (Fig. 2, C and D). The half-maximal inactivation voltage for the control and NRG-1-treated groups was \(-73.0 \pm 4.7\) mV (\(n = 20\)) and \(-76.7 \pm 2.4\) mV (\(n = 22\)), respectively (\(P > 0.05\)). These data indicate that NRG-1 does not affect \(I_A\) inactivation kinetics. Together, the electrophysiological recordings demonstrate that the NRG-1-mediated enhancement of \(I_A\) amplitude is not associated with the modification of \(I_A\) activation or inactivation properties.
NRG-1 upregulates the expression of Kv4.2 by promoting translation of Kv4.2 mRNA. Next, we hypothesized that the NRG-1-induced $I_{\text{A}}$ density might be due to upregulation of potassium channel expression levels. The key α-subunits accounting for the $I_{\text{A}}$ in CGNs include Kv4.2, Kv4.3, and Kv1.1 (18). Because our previous study indicated that Kv4.2 was the main α-subunit that is sensitive to neurotrophic factors, we focused our investigation on Kv4.2. Antibodies to Kv4.2,
Kv4.3, and Kv1.1 α-subunit were used to measure their protein expression levels after incubation of CGNs with NRG-1. Western blotting indicated that protein levels of Kv4.2 were significantly enhanced following incubation of CGNs with NRG-1 for 24 h. There was, however, no significant change in the protein expression levels of Kv4.3 and Kv1.1 (Fig. 3A; n = 5; P > 0.05). Kinetic of Kv4.2 induction was in parallel to the increase in I_A amplitudes upon NRG-1 treatment (Fig. 3, B and C). These data indicate that NRG-1 increases I_A density by upregulating the expression of Kv4.2.

The Kv 4.2 gene expression change in response to neuritin is thought to occur at the transcriptional level (43). Therefore, we used a transcription inhibitor, DRB, to test whether Kv4.2 α-subunit mRNA levels were increased by NRG-1. However, coincubation of CGNs with NRG-1 (10 nM) and DRB (10 μM) failed to abolish the NRG-1-induced increase in I_A density and the increased expression of Kv4.2 (Fig. 4, A and B). DRB itself reduced I_A density by 34.4 ± 1.7%, but incubation with NRG-1 in the presence of DRB upregulated the I_A density by 38.9 ± 0.6% vs. CGNs incubated with DRB alone (P < 0.05), an increase comparable to that observed in cells treated with NRG-1 alone. These results suggested that ongoing transcription is not required for the NRG-1-dependent increase in I_A density and upregulation of the expression of Kv4.2.

We then addressed whether NRG-1 upregulates the levels of Kv4.2 by promoting the translational efficiency of Kv4.2 mRNA. To test this hypothesis, CGNs were incubated with NRG-1 in the presence or absence of the translation inhibitor cycloheximide (10 μM), and the I_A density and Kv4.2 expression levels were determined. As shown in Fig. 5, A and B, incubation with cycloheximide completely abolished the effects of NRG-1 on both the I_A density and the expression of

Fig. 4. Inhibition of transcription fails to abolish the NRG-1-induced increase in the I_A density and the expression of Kv4.2. A: effects of the transcriptional inhibitor 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB), on the NRG-1-induced increase in I_A density. *P < 0.05, compared with the vehicle control (medium without NRG-1) by the unpaired t-test. B: effects of DRB on the NRG-1-induced up-regulation of the expression of Kv4.2 measured by Western blot. Data obtained from 5 independent experiments are shown as the means ± SE. *P < 0.05, compared with the vehicle control (medium without NRG-1) by the unpaired t-test.

Fig. 5. Inhibition of translation abolished the NRG-1-induced increase in the I_A density and the expression of Kv4.2. A: effects of the translation inhibitor cycloheximide (Cyc) on the NRG-1-induced increase in I_A density. Data are shown as the means ± SE. *P < 0.05, compared with the vehicle control (medium without NRG-1) by the unpaired t-test. B: Western blot analysis of the effect of Cyc on NRG-1-induced upregulation of the expression of Kv4.2. Data obtained from five independent experiments are shown as the means ± SE. *P < 0.05, compared with the vehicle control (medium without NRG-1) by the unpaired t-test.
Kv4.2. Together these results argue that NRG-1 acts at the translational level to enhance Kv4.2 channel protein expression and $I_A$ density. NRG-1-induced increases in $I_A$ density and expression of Kv4.2 are sensitive to inhibitors of the ErbB4 receptor and the AKT/mTOR pathway. Previous observations suggested that NRG-1 signals through the ErbB4 receptor (27). Next, we asked whether blockade of the ErbB4 pathway would affect the NRG-1-induced increase in $I_A$ density and Kv4.2 protein level. To address this question, we used AG 1478, the EGF receptor tyrosine kinase inhibitor that also inhibits the ErbB4 receptor Tyr kinase. Blockade of ErbB4 activity by AG 1478 reduced NRG-1-mediated increases in both the $I_A$ density and the Kv4.2 protein level (Fig. 6, A and B). In the presence of 5 μM AG 1478, the $I_A$ density increased by NRG-1 was significantly reduced from 28.6 ± 3.6 to −6.3 ± 1.7% ($n = 25$, $P < 0.05$).

Similarly, the induction of the Kv4.2 α-subunit was decreased to $−9 \pm 0.1\%$. We also tested the effect of AST-1306, a novel irreversible inhibitor of the epidermal growth factor receptor 1 and 2, which has a much lower IC50 for the ErbB4 than ErbB2 receptor (42). Our study of its effects on neuregulin-induced $I_A$ density and Kv4.2 expression, presented in Fig. 6, C and D, indicated that in the presence of 2 nM AST1306, the $I_A$ density and Kv4.2 α-subunit expression increased by NRG-1 were significantly reduced to $−3.56 \pm 8.0\%$ ($n = 24$; $P < 0.05$) and $−5.6 \pm 7.9\%$ ($n = 5$; $P < 0.05$), respectively. These data indicate that NRG-1 may activate ErbB4 receptor pathway to increase the $I_A$ density and Kv4.2 protein level.

Phosphatidylinositol 3-kinase (PI3K)-Akt-S6K pathways are frequently activated by NRG-1-induced stimulation of ErbB receptor homo- or heterodimers (27). In addition to ErbB4 activity, we also determined whether the Akt/mTOR

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![Graph A](image1.png)

**A** NRG-1

Vehicle

AG1478

$+\text{NRG-1}$

-100 mV

60 mV

500 pA

50 ms

Normalized $I_A$ densities

![Graph B](image2.png)

**B** NRG-1

Control

AG1478

$\text{Vehicle}$

$\text{NRG-1}$

$\text{GAPDH}$

Normalized Kv4.2/GAPDH

![Graph C](image3.png)

**C** NRG-1

Vehicle

AST-1306

$+\text{NRG-1}$

-100 mV

60 mV

500 pA

50 ms

Normalized $I_A$ densities

![Graph D](image4.png)

**D** NRG-1

Control

AST-1306

$\text{Vehicle}$

$\text{NRG-1}$

$\text{GAPDH}$

Normalized Kv4.2/GAPDH

Fig. 6. Activation of the ErbB4 receptor is needed for the NRG-1-induced increase in $I_A$ density and increased Kv4.2 α-subunit expression in CGNs. A, AG 1478, the blocker of the ErbB4 receptor, reduced the NRG-1-induced increase in $I_A$ density. Data are shown as the means ± SE. *$P < 0.05$, compared with the vehicle control (medium without NRG-1) by unpaired $t$-test. B: Western blot analysis of the effect of 5 μM AG 1478 on NRG-1-induced upregulation of expression of Kv4.2. Data obtained from 5 independent experiments are shown as the means ± SE. *$P < 0.05$, compared with the vehicle control (medium without NRG-1) by unpaired $t$-test. C: AST-1306, another blocker of the ErbB4 receptor, reduced the NRG-1-induced increase in $I_A$ density. Data are shown as the means ± SE. *$P < 0.05$, compared with the vehicle control (medium without NRG-1) by unpaired $t$-test. D: Western blot analysis of the effect of 2 nM AST-1306 on NRG-1-induced upregulation of expression of Kv4.2. Data obtained from 5 independent experiments are shown as the means ± SE. *$P < 0.05$, compared with the vehicle control (medium without NRG-1) with an unpaired $t$-test.
pathway, which was reported recently to affect protein synthesis and is relevant to synaptic plasticity (15), is activated by NRG-1. After NRG-1 treatment, the phosphorylation levels of Akt (pAkt) and mTOR (pmTOR) were significantly increased to 74.4 ± 10.3 and 63.8 ± 13.2%, respectively (n = 5) at 24 h (Fig. 7, B and C). Activation of Akt/mTOR by NRG-1 was confirmed using pharmacological inhibitors. Blockade of Akt activity by LY294002 or mTOR activity by rapamycin prevented the NRG-1-mediated increase in pAkt and pmTOR levels, respectively (Fig. 7, A–C). Moreover, in the presence of 20 μM LY294002, the increase in the pmTOR level induced by NRG-1 was significantly inhibited. These data indicate that the Akt/mTOR pathway is activated by NRG-1 and NRG-1 activates mTOR via the Akt kinase.

We then determined whether the Akt/mTOR pathway is required for the NRG-1-induced upregulation of the IA density and Kv4.2 α-subunit expression. Figure 7, D and E, shows that blockade of Akt/mTOR activity by 20 μM LY294002 or 50 nM rapamycin prevented the NRG-1-mediated increase in Kv4.2 protein expression and the increased IA density. In the presence of LY294002 or rapamycin, the increase in IA density induced by NRG-1 was reduced (Fig. 7E) from 28.6 ± 3.6 to 12.5 ± 18.3 and −4.1 ± 8.8% (n = 4 and 5; P > 0.05). Similarly, the expression levels of the Kv4.2 α-subunit protein (Fig. 7D) were decreased to 5.2 ± 13.9% (n = 5) and 0.6 ± 12.1% (n = 5), respectively. These data indicate that the mTOR pathway is also required for the NRG-1-stimulated upregulation of the IA density and increase in Kv4.2 α-subunit expression.

Because our previous experiments revealed the existence of K+ channel-dependent migration in granule cells (22), the effects of neuregulin on the migration of cerebellar granule cells were then tested using the Transwell migration assay, which is an in vitro model for invasive migration. In this assay, purified granule cells are plated on one side of a porous membrane and migration through the membrane into a second compartment is quantified. Control medium, neuregulin, AST-1306, and 4-aminopyridine (4-AP), which is a specific blocker of IA channels, were placed in the lower compartment, and granule cells were seeded into the top wells and allowed to migrate toward the lower surface of the separating membrane for 12 h. Figure 8A shows that a directional granule cell movement across the Transwell filter under vehicle control (medium without NRG-1) and addition of 10 nM neuregulin significantly increased granule cell migration by 51.3 ± 17.04% (P < 0.05). These neuregulin-induced migration effects could be modified by the addition of AST-1306, which decreased the number of granule cells migrating through the filter by 40.0 ± 8.35%. Moreover, this increase in migration effects induced by neuregulin was eliminated by cotreatment with 4-AP. Preincubation of cells with 4-AP, followed by treatment with neuregulin, only increased cell migration by 7.07 ± 6.32 or 8.09 ± 12.41% (Fig. 8B).

**DISCUSSION**

CGNs contain two major voltage-dependent outward K+ currents: a delayed rectifier potassium current (IK) and a fast transient potassium current (IKA). NRG-1 treatment for 24 h failed to modulate IK in CGNs. We thus examined the effect of neuregulin on IKA in this study. The IKA has been described in neurons from many regions of the central nervous system (16, 37). Short-term modulation of the IKA density could arise from a rapid mechanism due to changes in voltage-gating properties.
Kv1.1 are the main target of neurotrophins in developing neurons. We have shown previously that Kv4.2, Kv4.3, and Kv4.1 subunits (27) expressed in rat CGNs (18). Upon NRG-1 treatment, only Kv4.2 mRNA and protein expression could be involved (30). We observed that NRG-1 applied acutely to the bath solution does not affect the $I_A$ amplitude. NRG-1, however, significantly increased the $I_A$ density without modification of $I_A$ activation and inactivation properties, indicating that the mechanism of action of NRG-1 involves long-term effects.

Recent studies have suggested that the Kv4 shal family forms the major component of the $I_A$ in the central nervous system (32). Expression of the Kv4 family was found in CGNs, pyramidal neurons of the hippocampus and cortical neurons (30, 34). We have shown previously that Kv4.2, Kv4.3, and Kv4.1 are the main $\alpha$-subunits expressed in rat CGNs (18). Upon NRG-1 treatment, only Kv4.2 $\alpha$-subunit mRNA and protein levels were significantly increased. More importantly, the kinetics of Kv4.2 induction paralleled the increase in $I_A$ upon NRG-1 treatment. These data indicate that NRG-1 specifically increases the expression of Kv4.2. Interestingly, our recent studies indicated that neuritin, an important neurotrophin that plays multiple roles in the process of neural development, synaptic plasticity, and synaptic maturation, could activate the IR and ERK/mTOR signaling pathways to increase the $I_A$ amplitude and the expression of Kv4.2 in CGNs (43). Together, the results suggested that Kv4.2 might be the main target of neurotrophins in developing neurons.

Previous studies using cultured CGNs showed that the subcellular redistribution of Kv4.2 from the soma to the dendrites and synapses is induced by synapse formation. The regulation of Kv4.2 can be further influenced by synaptic activity (33). Indeed, the expression of Kv4.2 in CGNs was increased with the duration of the micro explant culture period (35) and CGNs in culture (43), in agreement with our findings showing a concomitant increase in the $I_A$ and Kv4.2 from 3 to 5 DIC. Notably, after NRG-1 treatment, the levels of $I_A$ and Kv4.2 in 3 DIC CGNs were similar to those in 5 DIC CGNs. Moreover, the NRG-1-induced increase in $I_A$ density and Kv4.2 expression only occurs in 3 DIC and 5 DIC CGNs. These observations suggest that the effect of NRG-1 on Kv4.2 expression may be developmentally regulated and associated with neuronal maturation. Our result is consistent with the findings of Ting et al. (38), which demonstrated that NRG-1 increases the number of excitatory synapses in GABAergic interneurons, and this effect only occurs in developing but not mature neurons. Meanwhile, our primary data also showed that NRG-1 promoted migrations of CGNs as measured by Transwell analysis and that incubation of CGNs with 4-AP (a blocker of $I_A$) suppressed the effect of NRG-1 on CGNs migration, suggesting that the 4-AP-sensitive $I_A$ current is connected to neuronal migration of granule cells. Although neuronal migration is the one of the signs of differentiation and mutation of CGNs, the mechanisms involved in the NRG-1-induced increase of $I_A$ on CGNs migration remain to be tested.

It has been reported that stimulation of NRG-1/ErbB signaling upregulates the expression of NMDA and GABA$_\alpha$ receptors in cultured cortical GABAergic interneurons and rat CGNs in vivo (1, 41), although Gajendran et al. (13) recently found that the developmental expression patterns of the mRNAs encoding the NMDA and GABA$_\alpha$-R is normal in mice lacking the NRG receptors ErbB2 and ErbB4. In addition to ligand-gated ion channels, reports have shown that the expression of voltage-gated ion channels is also induced by NRG-1. In developing chick ciliary ganglion neurons, NRG-1 regulates the functional expression of large-conductance Ca$^{2+}$-activated K$^+$ channels (BK) in an acute and sustained manner by the trafficking of channel proteins (4, 6, 10). Recently, the laboratory of Li et al. (22) reported that NRG-1-ErbB4 signaling increased the excitability of parvalbumin interneurons through downregulation of Kv1.1 which is as a risk factor for epilepsy. However, their data demonstrated that there was no significant change in the expression level of Kv1.1 after NRG-1 treatment but the level of tyrosine-phosphorylation of the Kv1.1 channel protein in the membrane was regulated by NRG-1 signaling (22), which is a mechanism different from NRG-1-induced upregulation of Kv4.2. This difference may be caused by neuroregulin activation of a different signal transduction pathway involving different neuron types, different developmental stage, and the different animal used.

Although NRG-1 is known to contain EGF-like repeats and mediates its effects by binding to members of the ErbB receptor family including ErbB2, ErbB3, and ErbB4 (5, 11), ErbB4 homodimers bind NRG-1 and are activated in the CNS. NRG-1 may function as the ligand for ErbB4 forward signaling (27). Evidence obtained from cultured postnatal hippocampal slices showed that NRG-1-ErbB4 signaling is essential for synapse maturation and plasticity (21). Coincidentally, previous studies in CGNs demonstrated that both ErbB3 and
ErbB4 are present in cultured CGNs, but ErbB2 was virtually undetectable and that ErbB4 receptor activation is required for NRG-1-induced GABA_A receptor β2/3-subunit expression (41). Considering the fact that ErbB3 must heterodimerize with ErbB2 to be activated and that ErbB4 receptors can function as homodimers (5), we focused on NRG-1-induced activation of ErbB4. Our studies indicate that selective blockade of ErbB4 receptor activation inhibits the effects of NRG-1 on the expression of Kv4.2. This is consistent with the earlier findings of Xie and colleagues (40, 41) in CGNs and demonstrates that the ErbB4 receptor is responsible for regulating Kv4.2 expression and the IA density in cultured CGNs.

In canonical forward signaling, NRG-1-induced ErbB dimerization activates the ErbB kinase domain, resulting in auto- and trans-phosphorylation of the intracellular domains. The phosphorylated tyrosine residues serve as docking sites for phosphopeptide-binding adaptor proteins or enzymes (27). The phosphorylated Akt level when added to the culture media is undetectable and that ErbB4 receptor activation is required for the upregulation of INK-4A density in cultured CGNs. This result is in agreement with previous studies from Xie et al. (7) in which rapamycin was found to regulate Akt phosphorylation through the mTORC1 and mTORC2 signaling pathways.

However, we noted that NRG-1-induced regulation of GABA_A receptor β2/3-subunit expression in CGNs reported by Xie et al. (41) requires at least three signaling pathways, including MAPK, P3K, and CDK5, and their data suggest that signals from these three pathways converge, possibly at the level of GABA_A receptor β2/3-subunit gene transcription. In this study, we showed that both LY294002 and rapamycin completely reduced the NRG-1-induced upregulation of Kv4.2 and IA, and in studies with nuclear factor of activated T cells transcription factor knockout mice or with a calcineurin calcineurin inhibitor, there was no effect on the actions of NRG-1 (data not shown). Moreover, NRG-1-induced upregulation of Kv4.2 subunit expression was at level of Kv4.2 gene translation rather than gene transcription. All together, these data suggest that the NRG-1-induced upregulation of Kv4.2 subunit expression in CGNs occurs mainly through the Akt/mTOR pathway. Interestingly, neurtin also modulated Kv4.2 expression and the IA density in CGNs by upregulation of Kv4.2 subunit expression at the transcriptional and translational levels by activating both ERK and mTOR signaling (43). Together, these studies indicated that the same NRG-1-ErbB4 signaling pathway may regulate the expression of different proteins by activating different downstream signaling pathways; alternatively, the same gene expression could be regulated by different neurotransphins through different mechanisms.

In summary, our studies provide insight into the mechanism used by NRG-1 to regulate Kv4.2 subunit expression in CGNs. These studies suggest that proteins known to play important roles in neuronal development and maturation also participate in NRG-induced signaling. How these signaling pathways interact and activate downstream signaling pathways leading to translation remains to be determined.

REFERENCES


