Nerve Growth Factor Signals through TrkA, Phosphatidylinositol 3-Kinase, and Rac1 to Inactivate RhoA during the Initiation of Neuronal Differentiation of PC12 Cells*

Received for publication, April 15, 2002, and in revised form, July 19, 2002
Published, JBC Papers in Press, July 19, 2002, DOI 10.1074/jbc.M203617200

Nóra Nusser‡§, Elvira Gosmanovar®, Yi Zheng™, and Gabor Tigyit¶**

From the Departments of Physiology and Molecular Sciences, University of Tennessee Health Science Center, Memphis, Tennessee 38163 and the Department of Biology, Medical School, University of Pecs, 7643, Hungary

In PC12 rat pheochromocytoma cells, nerve growth factor (NGF)-induced neuronal differentiation is blocked by constitutively active dominant mutants of RhoA but augmented by negative ones, suggesting a not yet elucidated inhibitory signaling link between NGF receptors and RhoA. Here we show that NGF treatment rapidly translocates RhoA from the plasma membrane to the cytosol and simultaneously decreases RhoA affinity to its target Rho-associated kinase (ROK), a key mediator of neurite outgrowth. This effect was transient, because after 2 days of NGF treatment, RhoA relocated from the cytosol to the plasma membrane, and its GTP loading returned to a level found in undifferentiated cells. Inhibition of RhoA is mediated by activation of the TrkA receptor, because NGF failed to induce RhoA translocation and inhibition of ROK binding in nnr5 cells that lack TrkA, whereas the inhibition was reconstituted in receptor add-back B5 cells. In MM17-26 cells, which do not express dominant negative Ras, NGF-stimulated transient RhoA inhibition was unaffected. The inhibitory pathway from TrkA to RhoA involves phosphatidylinositol-3-kinase (PI3K), because the inhibitors LY294002 or wortmannin prevented NGF-induced RhoA translocation and increased RhoA association with ROK. Furthermore, inhibition of PI3K significantly reduced NGF-mediated Rac1 activation, whereas dominant negative Rac1 abolished the inhibitory signaling to RhoA. Taken together, these data indicate that NGF-mediated activation of TrkA receptor stimulates PI3K, which in turn increases Rac1 activity to induce transient RhoA inactivation during the initial phase of neurite outgrowth.

The small GTPases RhoA and Rac1 are members of the Rho subfamily within the Ras superfamily of GTPases. RhoA plays an important role in the organization of the actin cytoskeleton, gene transcription, cell cycle progression, cell transformation, and membrane trafficking (for reviews, see Refs. 1–3). RhoA cycles between the GDP-bound inactive and active forms.

Three classes of molecules are known to interact and regulate GDP/GTP cycling of RhoA: guanine nucleotide exchange factors (GEFs) catalyze the exchange of GDP for GTP, GTPase-activating proteins (GAPs) stimulate the intrinsic GTPase activity of RhoA, and guanine nucleotide dissociation inhibitors (GDI) inhibit the exchange of GDP for GTP and also stabilize cytosolic RhoA form binding GTP (2). For RhoA to transmit signals, two criteria must be satisfied: first, it must in a GTP-loaded form; second, RhoA, which is geranylgeranylated, must be in the right signaling compartment attached to the plasma membrane, where it can interact with its regulators and targets. Thus, RhoA in its GTP-bound active form associates with the inner surface of the plasma membrane (4), whereas GDI-mediated inactivation of RhoA translocates it to the cytoplasm and prevents it from binding to GTP.

The role of RhoA during neuronal differentiation was first suggested by studies using the Rho-specific ADP-ribosyltransferase C3 toxin (Clostridium botulinum C3 Rho-ADP-ribosylating exoenzyme), which induces neurite outgrowth in naive (not treated with NGF) PC12 cells (5). Furthermore, RhoA has been shown to be involved in the regulation of neurite outgrowth (6–10). In our earlier studies (11), we found that expression of activated V14RhoA mutant prevented NGF-induced neurite outgrowth. In contrast, dominant negative RhoA (N19RhoA) expression led to an increase in neurite initiation and branching. Furthermore, RhoA was shown to have a dual role during neuronal differentiation. Inactivation of RhoA appears necessary for the initiation of neuronal differentiation, although during later stages of neurite elongation, introduction of N19RhoA causes the formation of short neurites (11). Although these findings suggest an important role of RhoA in NGF-induced neuronal morphogenesis, little is known about the signal transduction pathways that couple NGF signaling to RhoA.

In their active forms, RhoA proteins interact with and modulate the activity of effector proteins that include the serine/threonine protein kinase n N120, PKN), p160 RhoA-associated kinase ROKα (ROK or ROCK-I), p150 RhoA-binding kinase ROKβ (ROCK-I), citron-K, and nonkinases rhophilin, rhotekin, citron-N, and p140mDia (12, 13). Among these, ROK has been shown to mediate the formation of stress fibers, focal adhesions, regulation of myosin phosphorylation, and c-fos expression (for review, see Ref. 14). Moreover, activation of ROK is
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NaCl, and 0.05% Tween 20 (TBST) containing 5% nonfat dry milk (Bio-Rad). After blocking, the membranes were probed for 2 h with the primary antibody diluted in TBST and washed three times with TBST before incubation for 45 min with either peroxidase-conjugated secondary anti-mouse antibody (Sigma, 1:5,000) or donkey anti-rabbit antibody (Jackson Immunoresearch, West Grove, PA; 1:7,500). The SuperSignal West Femto Maxi (Pierce) was used to visualize antibody binding. The following primary antibodies were used: anti-RhoA monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA; diluted 1:500), anti-Rac1 monoclonal antibody (Santa Cruz Biotechnology; diluted 1:500), anti-phospho-Akt Ser473 antibody (New England Biolabs, Beverly, MA; diluted 1:1,000), and anti-Akt antibody (New England Biolabs; diluted 1:1,000).

RESULTS

NGF Treatment Induces a Rapid and Transient Translocation and Inactivation of RhoA during Neurite Initiation Phase—RhoA was detected in the HMF of control naive PC12 cells (Fig. 1A). A brief 20-min treatment with 100 ng/ml NGF caused a translocation of RhoA from the membrane to the cytosol, and the membrane-associated fraction remained low up to 2 h (Fig. 1, A and B). As a positive control, we used C3 toxin, which inactivates RhoA by ADP-ribosylating it on Asn42 and blocks its ability to activate downstream targets. C3 treatment almost completely removed RhoA from the HMF (Fig. 1A) and diminished its binding to ROK both in the membrane fraction (Fig. 1A) and in whole cell lysates (data not shown). The RhoA blots were stripped and reprobed with an antibody to Rac1 (Fig. 1B). In contrast to RhoA, the amount of Rac1 increased slightly in the same HMF samples. RhoA showed a slight increase in the cytoplasm, whereas when the same blot was probed for Rac1 after stripping, a modest decrease was observed. To quantify the amount of RhoA detected by Western blotting, the area and intensity of bands were integrated and normalized to the untreated control sample (Fig. 1A–C). In contrast to Rac1, the amount of Rac1 increased significantly different at p < 0.05.

MATERIALS AND METHODS

Cell Culture—Wild-type PC12 cells and the M-M17-26 clone, which stably expresses dominant negative Ras mutant, were kindly provided by Dr. G. Cooper (Boston University, Boston, MA). The TrkA-deficient nnr5 clone was provided by Dr. L. A. Green (Columbia University, New York). B5 cells derived from nnr5 overexpressing TrkA were a gift from Dr. Susan Meakin (Robarts Research Institute, Ontario, Canada). All of these clones were grown in RPMI medium supplemented with 10% horse serum, 5% fetal bovine serum, and 1% glutamine (normal medium). These clones were grown in RPMI medium supplemented with 10% horse serum, 5% fetal bovine serum, and 1% glutamine (normal medium). The B5 cells derived from nnr5 overexpressing TrkA were kindly provided by Dr. Susan Meakin (Robarts Research Institute, Ontario, Canada).

Statistical Methods—Student's t test for paired variables was used to test for differences elicited by NGF treatment, and data were considered significantly different at p < 0.05.
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Expression of the Dominant Negative N17Ras Mutant Does Not Inhibit the Effect of NGF on RhoA Translocation and Inactivation—Because Ras activation is a necessary and sufficient signaling event for neuronal differentiation, we tested NGF-induced inactivation of RhoA in M-M17-26 cells, which stably express high amounts of the dominant negative mutant N17-Ras. In M-M17-26 cells, a brief 20-min NGF treatment led to a 48 ± 7.8% decrease in membrane-bound RhoA. Furthermore, 2 h of NGF treatment reduced the amount of RhoA in the HMF to 27 ± 14.2% of that in untreated control cells (Fig. 3). This effect of NGF did not differ significantly from that found in wild-type PC12 cells (Fig. 1). The amount of ROK-associated RhoA also decreased during the initial phase of NGF-induced inactivation of RhoA.

NGF Increases RhoA Activation during Neurite Elongation—Wild-type PC12 cells were exposed to either NGF or vehicle for 2 days. The PC12 cells used in the present study responded rapidly to NGF; after 1 day, the cells began extending neurites that reached the diameter of a cell body on the second day of NGF treatment. Thus, after 2 days of NGF exposure, these cells had initiated neurite elongation. NGF increases RhoA expression but not Rac1 activity during neurite elongation.

TrkA Receptor Is Required for RhoA Activation and Translocation—NGF activates TrkA and p75 receptors in PC12 cells. To assess the role of the TrkA receptor in the regulation of RhoA, we tested whether overexpression of TrkA in nnr5 cells restored NGF-induced inactivation of RhoA. In contrast to wild-type PC12 cells, NGF exposure did not significantly change the amount of membrane-associated RhoA in nnr5 cells (Fig. 3). NGF treatment caused no detectable change in the amount of ROK-bound RhoA, whereas Rac1 immunoreactivity increased in both whole cell lysate and the HMF, whereas no changes were detected in its level of association with ROK. B, quantification of RhoA in whole cell lysate, in the HMF, or available to bind ROK after a 2-day NGF treatment. Band intensity was normalized to that of the untreated control sample; data are the means (±S.E.) of three experiments (*, p < 0.05).

The nnr5 cell line, a clone of the PC12 cell line, lacks functional TrkA, whereas the B5 cell line is an nnr5 clone that stably overexpresses TrkA. These two clones were used to assess the role of the TrkA receptor in the regulation of RhoA. In contrast to wild-type PC12 cells, NGF exposure did not significantly change the amount of membrane-associated RhoA in nnr5 cells (Fig. 3). NGF treatment caused no detectable change in the amount of ROK-bound RhoA, whereas Rac1 immunoreactivity increased in both whole cell lysate and the HMF, whereas no changes were detected in its level of association with ROK. B, quantification of RhoA in whole cell lysate, in the HMF, or available to bind ROK after a 2-day NGF treatment. Band intensity was normalized to that of the untreated control sample; data are the means (±S.E.) of three experiments (*, p < 0.05).

NGF-induced changes in membrane-associated RhoA and RhoA activity during neurite elongation. A, whole cell lysate (WCL, upper panel) and the HMF (middle panel) were isolated from untreated (control) or differentiating cells treated with NGF (100 ng/ml) for 2 days. Protein (20 μg/lane) was analyzed by Western blotting with an anti-RhoA antibody. HMF protein (100 μg) was used in the pull-down assay with GST-ROK (lower panel). The amount of RhoA immunoreactivity increased in both whole cell lysate and the HMF, whereas no changes were detected in its level of association with ROK. B, quantification of RhoA in whole cell lysate, in the HMF, or available to bind ROK after a 2-day NGF treatment. Band intensity was normalized to that of the untreated control sample; data are the means (±S.E.) of three experiments (*, p < 0.05).

NGF induces translocation and inactivation of RhoA during neurite initiation. A, the HMF was isolated from untreated controls or cells treated with either NGF (100 ng/ml) or C3 (10 μg/ml) for 20 min. Protein (20 μg per sample) was analyzed by Western blotting with an anti-RhoA antibody. For the pull-down assay with GST-ROK, 100 μg of HMF protein was used. For controls, a 10 μM concentration of either GDP or GTP-S was added to the HMF for 20 min prior to incubation with GST-ROK. NGF and C3 treatment of the membranes decreased the amount of membrane-associated RhoA and its ability to bind to ROK. B–D, PC12 cells were treated with NGF (100 ng/ml) for the times indicated, and the HMF was isolated and analyzed by Western blotting or GST-ROK pull-down assay. For Rac1 determination, blots were stripped of the anti-RhoA antibody and reprobed with an anti-Rac1 antibody. The intensity of the bands was measured and normalized to an untreated control sample. Data shown are the mean values (±S.E.) from at least three separate experiments (*, p < 0.05). Note in B and C that the amount of RhoA decreased in the HMF, whereas Rac1 showed a modest increase (B). Concomitant but opposite changes were seen in the RhoA and Rac1 immunoreactivity in the cytosol. The decrease in the membrane-associated RhoA was accompanied by diminished binding to ROK-GST (D).
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Fig. 3. Functional TrkA but not Ras is required for NGF-induced RhoA translocation and decreased ROK binding. Top, nrr5 cells lacking a functional TrkA receptor show no translocation of RhoA and have decreased association with ROK. Middle, B5 cells are nrr5 cells that overexpress the TrkA receptor. They show restored responses, as seen in wild-type cells. Bottom, M-M17-26 cells expressing dominant negative Ras show translocation of RhoA and decreased ROK binding after NGF treatment, as seen in wild-type cells. Protein (20 μg/lane) was analyzed by Western blotting with an anti-RhoA antibody (left panels). HMF protein (100 μg) was used in the pull-down assay with GST-ROK, followed by Western blotting with anti-RhoA antibody (right panels). Results shown are representative of three experiments.

induced differentiation.

Expression of Dominant Negative Rac1 (N17Rac1) Inhibits the Effect of NGF on RhoA Activity—The Rac1 GTPase has been found capable of regulating RhoA activity in fibroblasts (19) and neuroblastoma cells (8). To test the role of Rac1, the dominant negative N17Rac mutant was transiently expressed in PC12 cells using a tandem vector that also expresses GFP. An empty vector plasmid expressing GFP only was used as a negative control. Expression of N17Rac, but not of GFP, inhibited neuronal differentiation in NGF-treated cells, as these cells developed no neurites (Fig. 4A). To further control the effect of transient expression of N17Rac protein, NGF-induced activation of Rac1 was measured using a PAK pull-down assay (Fig. 4B). NGF treatment caused rapid activation of Rac1 in vector-transfected cells but did not cause a detectable activation of Rac1 in N17Rac1-expressing cells (Fig. 4B). These experiments confirmed that the expression of N17Rac1 inhibited the function of Rac1 in these cells. In cells expressing N17Rac1, NGF did not induce RhoA translocation from the membrane to the cytosol (Fig. 4C). Moreover, the amount of ROK-associated RhoA showed no detectable decrease after NGF treatment. As in wild-type PC12 cells, NGF decreased the amount of both membrane- and ROK-associated RhoA in vector-transfected cells (Fig. 4, B and C), suggesting that Rac1 mediates an inhibitory signal from TrkA to RhoA.

Yamaguchi et al. (37) reported recently that expression of constitutively active V14RhoA blocked NGF-induced activation of Rac1. However, these authors used an 18-h-long serum starvation, which in our hands induces apoptosis. We have tried to reproduce these findings but with a shorter 4-h serum starvation in PC12 cells transiently transfected with V14RhoA. As shown in Fig. 4D, under these conditions, activation of Rac1 following a 10-min exposure to NGF measured by PAK pull-down was not attenuated in PC12 cells transfected with V14RhoA as compared with vector-transfected cells.

PI3K Inhibitors, Wortmannin and LY294002 Block NGF-induced Inactivation of RhoA—Signals from the TrkA-receptor are transmitted in part by PI3K, which in other cell types has been linked to the regulation of Rac1 (20). To test the role of PI3K in the NGF-induced inactivation of RhoA, a brief 2-h LY294002 treatment was applied before exposure to NGF. Pretreatment of the cells with 30 μM LY294002 alone did not change the amount of membrane- or ROK-associated RhoA (data not shown). In cells pretreated with LY294002, there was no detectable change in the amount of RhoA in the HMF after NGF treatment (Fig. 5). Furthermore, using the ROK pull-down assay, we found that the amount of ROK-associated RhoA also remained unchanged after NGF treatment (Fig. 5). Similar results were obtained when the same experiments were carried out in cells pretreated with another PI3K inhibitor, wortmannin (Fig. 5). These results indicate that PI3K activity is required for the NGF-induced regulation of RhoA.

In PC12 Cells PI3K Acts Upstream from Rac1 Mediating the Inactivation of RhoA—We have also investigated the hierarchical relationship between Rac1 and PI3K in signaling events leading to the inactivation of RhoA. First, we inhibited PI3K with LY294002 and then measured the NGF-induced activation of Rac1 by using the PAK pull-down assay. In the control cells, without LY294002 pretreatment, NGF increased the amount of activated Rac1 4-fold (Fig. 6A). In contrast, in PC12 cells pretreated with 30 μM LY294002 for 2 h prior to 5 min NGF treatment, PAK activation was reduced to 2-fold but not abolished (Fig. 6A). Next, to test whether Rac1 mediates PI3K activation, a dominant negative N17Rac1 mutant was transiently transfected in PC12 cells. To monitor the inhibitory effect of N17Rac1, the GST-PAK pull-down assay was used (see Fig. 4B). To determine whether N17Rac1 inhibited the activation of PI3K, we measured the phosphorylation of Akt by Western blot using anti-phospho-Akt antibody. There was no detectable phosphorylated Akt in serum-starved PC12 cells (Fig. 6B). NGF caused a rapid increase in the phosphorylation of Akt in vector- and N17Rac1-transfected PC12 cells (Fig. 6B). Using anti-Akt antibody, we found that the amount of Akt was same in every sample (Fig. 6B). Thus, our data indicate that NGF-induced activation of PI3K in PC12 cells was independent of Rac1.

DISCUSSION

In the present report, we sought to establish the mechanism underlying our earlier observations using constitutively active and dominant negative RhoA mutants (11), which provided circumstantial evidence for a dual role of RhoA in NGF-induced differentiation of PC12 cells. Activated RhoA prevented the withdrawal of the cell from the cell cycle and abolished neurite outgrowth, whereas dominant negative RhoA enhanced differentiation. This observation implied that NGF signaling should negatively couple to RhoA to promote the early events of differentiation, which include the cessation of cell proliferation and the initiation of neurite outgrowth. In contrast to naive PC12 cells, in differentiated cells, we found that the expression of activated RhoA accelerated the rate of neurite elongation, whereas dominant negative RhoA reduced it. In the present study, we first investigated the effect of NGF signaling on RhoA by measuring its translocation and GTP-loading as measured by the affinity to its target, ROK.

Here we provide evidence that a brief treatment with NGF leads to RhoA inactivation, as shown by its rapid translocation from the membrane to the cytosol and a decrease in its ability to associate with its downstream target, ROK (Fig. 1). These results provide a mechanism for our earlier phenomenological observation that NGF somehow inactivated RhoA-ROK signaling during the initiation phase of neuronal differentiation. We extended our study to determine not only the localization but also the activation state of RhoA during neurite elongation. Again, in agreement with the previous study using RhoA mutants, we found that a prolonged 2-day NGF treatment increased RhoA expression and its association with the plasma membrane during the neurite elongation phase. Although prolonged NGF treatment caused no elevation in the GTP-bound
state of RhoA because the amount of ROK-bound RhoA returned to the a level seen in naive cells, this change represents a 70% increase as compared with the initiation phase (compare Fig. 1 and Fig. 2). Taken together, these results support our dual-role model proposed earlier: NGF treatment rapidly inactivates RhoA during the initiation phase, but it returns to the plasma membrane compartment with a basal level of GTP loading during the elongation phase of neuronal differentiation.

Which NGF receptor mediates the inhibitory effect to RhoA? Using nnr5 cells deficient in functional TrkA receptor and a receptor add-back clone the B5 cells, we found evidence that TrkA is necessary for the inhibitory coupling to RhoA. We next turned our focus to TrkA-linked signaling pathways, specifically the Ras pathway because it has a central role in mediating NGF-induced neuronal differentiation. Settleman et al. (21) reported a cross-talk between Ras and Rho by showing that p190Rho-GAP was tyrosine-phosphorylated and formed a complex with the SH2 domain of p120Ras-GAP, suggesting that the latter may act as a Ras effector negatively regulating the activity of RhoA. Booden et al. (22) found that expression of constitutively activated Ras elicited neurite outgrowth that was prevented by the co-expression of inhibitory Rho only in state of RhoA because the amount of ROK-bound RhoA returned to the a level seen in naive cells, this change represents a 70% increase as compared with the initiation phase (compare Fig. 1 and Fig. 2). Taken together, these results support our dual-role model proposed earlier: NGF treatment rapidly inactivates RhoA during the initiation phase, but it returns to the plasma membrane compartment with a basal level of GTP loading during the elongation phase of neuronal differentiation.

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FIG. 5. Wortmannin and LY294002, inhibitors of PI3K, block NGF-induced inactivation of RhoA. A, PC12 cells were treated with either 30 μM LY294002 compound or 500 nM wortmannin for 2 h prior to treatment with 100 ng/ml NGF for the times indicated. HMF protein (20 μg/lane) was analyzed by Western blotting with an anti-RhoA antibody (left panels). HMF protein (100 μg) was used in the pull-down assay with GST-ROK, followed by Western blotting with anti-RhoA antibody (right panels). Wortmannin and LY294002 abolished NGF-induced translocation and decreased GST-ROK binding of RhoA. B, quantification of RhoA content in the HMF and association with ROK relative to vehicle-treated control. Data represent the means (±S.E.) of at three experiments (*, p < 0.05). Both inhibitors abrogated the changes seen in vehicle-treated controls and even caused a modest rise in the level of activated RhoA associated with ROK.

GST-PAK, and the amount of activated Rac1 was monitored by an anti-Rac-1 antibody by Western blotting. NGF-elicited Rac1 activation was indistinguishable in vector-transfected and V14Rho-expressing cells.
the presence of increased amounts of inhibitory Rac1. These results suggest that RhoA and Rac1 were coupled to Ras; however, these authors did not elicit differentiation using NGF, but rather with an activated Ras mutant. We reported that activation of RhoA during neurite retraction caused by lysophosphatidic acid was independent of Ras (23), suggesting the possibility of Ras-independent signaling from NGF toward RhoA. Recently, Boglari and Szeberenyi (24) showed that a TrkA-independent Ras pathway exists that is sufficient to mediate neurite outgrowth. Using a PC12 subclone, the M-M17-26 line that expresses a dominant negative N17Ras (25), we found no significant difference between the effect of NGF on the inactivation of RhoA in wild-type and N17Ras-expressing cells. The M-M17-26 cell line has been well characterized, and there is no evidence for NGF-induced ERK1/2 activation in these cells (data not shown), whereas NGF activates PI3K and consequently Akt (26). Taken together, our results provide further evidence for a dichotomous signaling originating from TrkA: a Ras-dependent branch that activates the MAPK cascade and a Ras-independent one that mediates the inactivation of RhoA and is linked to the PI3K-Akt pathway.

The lipid kinase PI3K can mediate signals from Ras to Rac1 required for the oncogenic transformation of Rat-1 cells (27). In vitro data suggest that Rac1 specifically interacts with phosphatidylinositol 3,4,5-trisphosphate, a product of PI3K (28). RhoA also displays significant, although much weaker, binding to this lipid (28). Expression of an activated Ras mutant caused increased Rac1 GTP binding and PI3K activity in membranes of PC12 cells (29).

A hierarchical cascade linking CDC42, Rac1, and RhoA was first described in fibroblasts (19). According to this hypothesis based on studies conducted in fibroblasts, activation of Cdc42 induces activation of Rac1, which in turn leads to activation of Rho (20, for review, see Refs. 2 and 30). To extend this model, functional studies established PI3K as an effector of Ras and an upstream modulator of Rac1 activity (20). However, in Swiss 3T3 fibroblast cells, wortmannin does not interfere with Ras-mediated actin remodeling (31). Hence, a secondary pathway is likely to exist (receptor tyrosine kinase → PI3K → Rac1) that is Ras-independent and turned on by the activation of receptor tyrosine kinases, including TrkA. The Ras-independent pathway for Rac1 regulation is supported by the phosphorylation-dependent direct association of PI3K with the Tyr 

FIG. 6. PI3K regulates Rac1 in the NGF-activated inhibitory signal transduction pathway to RhoA. A, PC12 cells were pretreated with 30 µM LY294002 compound for 2 h prior to exposure to 100 ng/ml NGF for the times indicated. Whole cell lysate (100 µg) was used in the pull-down assay with GST-PAK, followed by Western blotting with anti-Rac1 antibody (upper panels). The intensity of the bands was measured and normalized to the control. Data shown are the mean ± S.E.) from at least three separate experiments (*, p < 0.05). Although LY294002 substantially reduced Rac1 activation, it did not abolish it. B, PC12 cells were transiently transfected with either pMX-IRES-GFP vector or pN17Rac1-IRES-GFP plasmid expressing the dominant negative N17Rac1. Three days after transfection, cells were treated with NGF for the times indicated, and whole cell lysate was isolated. Protein (20 µg/lane) was analyzed by Western blotting with either an anti-phospho-Akt (Ser 

FIG. 7. A scheme of the signaling pathways linking TrkA to the inhibition of RhoA. TrkA, independently of Ras, activates PI3K. PI3K activates Rac1, and active Rac1 induces the translocation of RhoA to the cytoplasm, where it may complex with Rho-GDIs and also inhibits its ability to bind to ROK.
the assay. In our hands an 18-h-long serum withdrawal has adverse effect of the adherence of the cells, and cells are beginning to undergo apoptosis (data not shown).

Taken together, our results in naive PC12 cells support a Ras-independent signal transduction pathway linking TrkA to Rac1 during the early stages of neuronal differentiation (Fig. 7). The signaling complexes involved in coupling these molecules is subject of ongoing studies.

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