

Signal Transduction of Neurotrophin Receptors: Basic Concepts and Available Pharmacological Tools

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*** In memoriam:** David Rasouly was tragically killed in an automobile accident since submission of this article. David was 23 years old and was working on an M.D./Ph.D. degree. He was an enthusiastic researcher and had a deep scientific curiosity. His death is a great loss for his friends, colleagues and the larger scientific community.

The neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), are a family of related polypeptides which regulate the survival and differentiation of discrete, and sometimes overlapping, neuronal populations (1-4). High affinity receptors expressed on the surface of responsive neurons mediate the biological activity to these factors (5). The past five years have seen major progress in unraveling the identities of the neurotrophin receptors and their mechanisms of signal transduction.

Trk signal transduction

In a series of publications from the laboratories of Kaplan, Parada, Chao and Barbacid, NGF was reported to bind and activate the receptor tyrosine kinase, Trk (6-8). The Trk family is composed of related transmembrane tyrosine receptor kinases that specifically bind neurotrophins: Trk (also known as TrkA) binds NGF; TrkB binds BDNF, NT-3 and NT-4/5; and TrkC binds NT-3 (5). Activation of Trk is now known to be followed by receptor oligomerization and trans-autophosphorylation of five tyrosine residues on Trk, Tyr490, Tyr785, Tyr670, Tyr674 and Tyr675 (9-11). These phosphotyrosine residues act as docking sites for downstream effector substrates by specifically binding these intracellular substrates through a non-catalytic region of 100-110 amino acid residues called *src* homology 2 (SH2) domains (12). Four intracellular proteins are known to bind to activated Trk: PLC-gamma-1, SHC, PI-3 kinase and Erk 1 (13, see Figure 1). The binding of substrates to Trk molecules enhances their specific activity either through receptor-induced tyrosine phosphorylation or alterations of protein conformation, thereby initiating a cascade of signaling events that ultimately leads to neuronal differentiation.

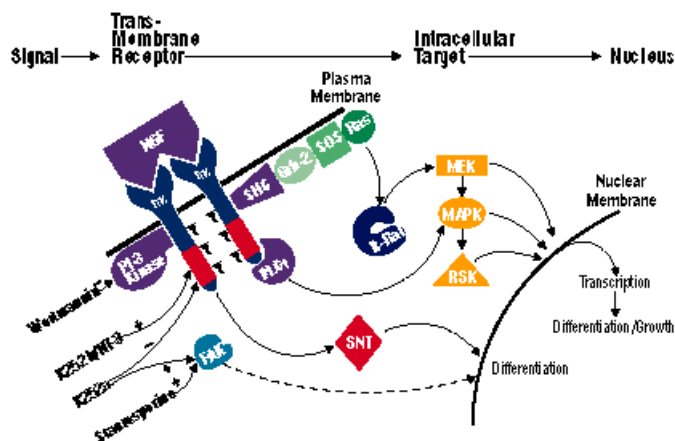


Figure 1. Schematic of Trk signal transduction pathways. The binding of NGF to Trk stimulates rapid tyrosine phosphorylation and activation of many signal-transducing proteins. Activated Trk receptors bind to and induce the tyrosine phosphorylation and activation of the signaling molecules PLC-gamma-1, PI-3 kinase and SHC. The latter stimulates Ras activity by binding to Grb-2 and the SOS guanine nucleotide exchange proteins. (Ras is positively regulated by Grb/SOS; it may be negatively regulated by rasGAP.) Subsequently, Ras sequentially activates a series of serine/threonine kinases, including B-Raf,

MAPK kinase (MEK), Erk (MAPK) 1 and 2 and p90Rsk. Erk and p90Rsk may play a role in activating transcriptional events. PLC-gamma-1 also is involved in the activation of MAPK. All of the proteins detailed in this pathway become activated in cells following treatment with NGF or with the mitogen epidermal growth factor.

Trk also mediates the phosphorylation of tyrosine residues on SNT, which localizes to the nucleus. SNT is a specific target for neurotrophic factors, but not mitogens for neurons and PC12 cells, and it is in a signal transduction pathway that is independent of Ras. PI-3 kinase activity may be blocked by the fungal metabolite wortmannin, while Trk activity may be selectively suppressed by K252a. However, K252b potentiates Trk activity. In addition, K252a and staurosporine have neurotrophic effects on certain neuronal cells, apparently by a Trk-independent mechanism that involves focal adhesion kinase (FAK). Abbreviations: Y indicates tyrosine residues; + indicates stimulation; - indicates inhibition; dashed line indicates putative involvement.

Post-receptor targets associated with Trk signaling

Tyrosine phosphorylated SHC binds to Grb2, an adaptor protein that bridges SHC to SOS (13,14). SOS, a member of the guanine nucleotide releasing proteins (GNRP), enhances the rate of GDP-GTP exchange on Ras, leading to Ras activation. Activated Ras binds to B-Raf (14), which in turn, phosphorylates and activates MEK (MAPKK; 15,16). MEK is a dual specificity kinase that phosphorylates the mitogen activated proteins (MAPKs) on tyrosine and threonine residues, leading to their activation. MAP kinase (also known as extracellular relay kinase, or Erk) phosphorylates and activates p90Rsk (14), and both enzymes translocate into the nucleus to participate in the activation of transcription factors that may regulate the expression of NGF-inducible genes. Studies with dominant negative and constitutively activated forms of Ras and MEK indicate that this pathway is essential and sufficient for neurite outgrowth in PC12 cells (17-20), a cellular model to study NGF actions (21). Other evidence for the importance of the Ras pathway was provided by a Trk mutant that is defective in inducing MAP kinase activity in PC12 cells (10). This site-directed mutant, which lacks the ability to bind PLC-gamma-1 and SHC, is unable to extend and maintain neurites (10).

Recent studies have revealed another novel target of NGF-mediated signaling events in PC12 cells - the SNT protein (*suc*-associated neurotrophic factor-induced tyrosine-phosphorylated target; 22). In neuronal cells, this protein undergoes rapid tyrosine phosphorylation upon treatment with NGF and fibroblast growth factor (22). SNT phosphorylation is independent of any of the known signaling cascades initiated by Trk, and its tyrosine phosphorylated form localizes to the nuclear fractions. Currently, it is the only known target that is specifically activated by factors that induce differentiation but not proliferation of neuronal cells (22).

Pharmacological tools to study Trk signaling

In addition to molecular manipulation of Trk, pharmacological and neuroscience approaches also have been exploited to modulate NGF signal transduction pathways. For example, using a pharmacological approach, Kimura *et al.* recently described a role for PI-3 kinase in Trk signaling (23). This group identified the fungal metabolite wortmannin, which binds to the catalytic subunit of PI-3 kinase and thereby selectively inhibits PI-3 kinase activity *in vitro* and *in vivo*. Wortmannin appears to block the elongation of neurites from NGF-treated PC12 cells.

K252a, another pharmacological tool available to study Trk signaling, is a fungus-derived kinase inhibitor (24) which inhibits the activity of Trk (25-28) but not other tyrosine receptor kinases. K252a and its derivatives, K252b, KT5720, KT5823 and staurosporine block the biological effects of NGF in PC12 cells (29-31). While the K252a family of compounds generally are Trk inhibitors, the more hydrophilic derivative, K252b, can potentiate the neurotrophic and TrkA-stimulatory activity of NT-3 in PC12 and primary neurons (28). In addition, K252a and staurosporine have neurotrophic activities in some cells without apparently altering Trk activity or stimulating known Trk substrates (31-34). In human neuroblastoma cells, K252a induces neurite outgrowth and the tyrosine phosphorylation of p125FAK (focal adhesion kinase, also known as FAK) (34). FAK, a tyrosine kinase that resides in focal adhesion plaques, is a major intracellular target of pp60src-induced tyrosine kinase activity (35). Staurosporine, another member of this family of fungal alkaloids, also is a very potent protein kinase C inhibitor *in vitro* (36). Staurosporine rapidly induces neurite outgrowth in PC12 and SY5Y neuroblastoma cells (31,33). The neurotrophic effects in PC12 cells appear to occur through signal transduction pathways that are independent from those used by Trk (32) and may involve *src* family members and a novel 145kDa tyrosine phosphorylated protein (32). K252a, K252b and staurosporine also promote or potentiate the differentiation or survival of rat spinal cord, sensory and CNS cholinergic neurons (34,37).

K252a and its derivatives provide pharmacological tools for basic research on neuronal signal transduction cascades in both primary and immortalized neuronal cell culture systems. Furthermore, the results obtained with primary neurons strongly suggest that these relatively specific compounds may prove to be useful for the treatment of neurodegenerative diseases. The potent neurotrophic effects of K252a and staurosporine in neuroblastoma cells and the possibility that these compounds may suppress Trk activity in colon carcinoma, where Trk activation has been observed (38), also suggest roles for these agents in the clinical management of neoplasia.

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Ordering Information

Product	Size	Cat. #
NGF, 2.5S, Murine	100µg	G5141
NGF, 7S, Murine	100µg	G5151
Anti-Human NGF mAb	50µg	G1131
Anti-Human NGF pAb, Affinity Purified	100µg	G1541