TrkC Isoforms with Inserts in the Kinase Domain Show Impaired Signaling Responses*

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The genetic locus for the TrkC/neurotrophin 3 (NT-3) receptor tyrosine kinase encodes multiple isoforms including receptors with inserts in the catalytic domain. This study examines the signaling capabilities of TrkC and related kinase insert isoforms TrkC14 and TrkC25. We show that in PC12 cells expressing both TrkC and TrkA/nerve growth factor (NGF) receptors, different morphological changes occur upon addition of NGF or NT-3. NT-3-treated cells exhibit longer neurites and larger cell bodies as compared to NGF-treated cells. Both TrkC and TrkA mediate qualitatively similar increases in the tyrosine phosphorylation of phospholipase C (PLC)-γ1, Shc, SNT, and MAPK and the transcription of the c-fos, c-jun, NGFI-A, and NGFI-B immediate early genes. However, the TrkC kinase insert forms fail to stimulate these events. Furthermore, TrkC14 and TrkC25 have only a low intrinsic tyrosine kinase activity, and insertion of the TrkC14 kinase insert into TrkA at an equivalent position results in a dramatic reduction of the kinase activity and signaling capabilities of TrkA. The TrkC14 and -25 isoforms may fail to transmit signals due to their low intrinsic kinase activity and failure to activate and/or tyrosine phosphorylation targets shown to be involved in neurotrophin signal transduction pathways.

Many developmental processes are controlled by growth factors that activate receptor tyrosine kinases (1). For example, during the development of the nervous system, the trk gene family that includes the TrkA, TrkB, and TrkC receptor tyrosine kinases, play prominent regulatory roles (2). In vivo analysis of Trk and their ligands, the neurotrophins, has verified their importance for normal development and survival of the majority of sensory and sympathetic neurons (2). For example, mice lacking expression of TrkC or its ligand, NT-3, exhibit loss of specific subsets of sensory neurons (3−6).

Another useful system for studying Trk function is the PC12 pheochromocytoma tumor cell line (7). Nerve growth factor (NGF), the major ligand for TrkA (8−10), promotes the differentiation of the PC12 cell line into cells resembling sympathetic neurons (7). NGF exerts its effects by binding to the second immunoglobulin-like domain of TrkA (11, 12). The kinase domain is rapidly transphosphorylated on specific tyrosine residues in the catalytic and noncatalytic intracellular domains of the receptor (13). For example, the phosphorylated residue Tyr-490 has been shown to bind and mediate the tyrosine phosphorylation of the Src proteins, that in turn associate with the small adaptor protein, Grb2 (13, 14). The coupling of TrkA to Shc stimulates the activity of Ras and the serine/threonine kinases of the Ras pathway that include B-Raf, MEK, MAPK, and p90Rsk (15). The other major autophosphorylation site in the noncatalytic domain, Tyr-785, interacts with PLC-γ1 (16, 55). This site has been shown to participate in a variety of effects on NGF-treated PC12 cells, including the stimulation of peripherin transcription and the activation of MAPK (16). Site-directed mutagenesis of Tyr-490 and Tyr-785 has established these residues as primary effectors of NGF-dependent neurite outgrowth and survival responses in PC12 cells (13, 16, 17). Expression of dominant inhibitory or activated alleles or overexpression of Ras, Raf, Shc, MeK, and MAPK in PC12 cells can mimic some but not all of NGF-induced differentiation events (18−21), indicating the importance of these proteins in the differentiation process while suggesting that alternative Ras-independent signaling pathways mediate some of the effects of NGF (15). One such pathway, defined by a deletion in TrkA at amino acids 441−443 (ΔJFG), may involve the nuclear protein SNT (24). TrkA encoding this deletion is defective in mediating the NGF-induced initiation of neurite outgrowth, somatic hypertrophy, and cessation of cell proliferation (24). A third pathway involving phosphatidylinositol 1,4,5-trisphosphate kinase may modulate NGF-induced neurite outgrowth and survival responses (22, 23). Taken together, these results suggest that several signaling pathways cooperate to regulate the establishment and maintenance of the neuronal phenotype of PC12 cells.

The PC12 cell system has also been useful for studying the function of TrkB and TrkC. Brain-derived neurotrophic factor, a member of the NGF family of growth factors, can activate TrkB (receptor tyrosine kinase) and induce neurite outgrowth in PC12 cells transfected with this receptor (25). Similarly, NT-3 will stimulate neurite outgrowth in PC12 cells transfected with TrkC (26). The TrkC locus encodes at least eight isoforms including forms without the kinase domain or with kinase insertions adjacent to the major autophosphorylation site (26, 27). These forms arise by alternative splicing events and are expressed in different tissues and cell types (26−29). We and others have shown that the mammalian kinase insert forms of TrkC, TrkC14, and TrkC25 do not have the capability to activate TrkB, Shc, or other pathways that mediate PC12 cell responses.

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1 The abbreviations used are: NGF, nerve growth factor; Tridine, N-tris(hydroxymethyl)methylglycine; PLC, phospholipase C; MAPK, mitogen-activated protein kinase; SNT, Suc1-associated neurotrophic factor target.
to induce PC12 cells transiently expressing these receptors to differentiate in response to NT-3 (26–28). The basis of the lack of signaling potential of the TrkC kinase insert isoforms in neuronal cells has not been investigated. Here, we analyze the signal transduction capabilities of TrkC and its related kinase insert isoforms relative to TrkA in the same cellular background, PC12 cells. We show that TrkC uses the same known signal transduction pathways as TrkA, although the morphological changes in PC12 cells induced by these two receptors are distinct. We show that the TrkC kinase insert isoforms fail to transduce signals to known substrates, induce immediate early gene transcription, or efficiently autophosphorylate in vitro. Placement of the TrkC14 insert at a similar position in the kinase domain of the TrkA receptor results in a dramatic reduction of the tyrosine kinase activity of TrkA and its signaling capabilities.

**EXPERIMENTAL PROCEDURES**

Materials—Wheat germ agglutinin-Sepharose 6B and Protein A-Sepharose 4B were from Pharmacia Biotech Inc. The antibodies used included anti-pan Trk antisera (43) (29 or 203) (31), anti-Trk anti-serum 656 (11), anti-PLC-γ1 (Upstate Biotechnology Corp. or a gift of G. G. Rhee, NIH), anti-Src (Upstate Biotechnology Corp. or anti-Tyr(P) monoclonal 4G10 (Upstate Biotechnology Corp. or a gift of D. Morrison, NCI-FCRDC), and anti-ERK14 (Santa Cruz Biotechnology). p135crk, agarose was a gift of N. Michaud (NCI-FCRDC). Purified recombinant human NGF and NT-3 were supplied by Genentech (South San Francisco, CA).

**Cell Culture, Plasmids, Constructs, Transfection, and Selection**—PC12 cells stably transfected with rat TrkC, TrkC14, TrkC25 (26) were generated as described (26) and grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum and 5% heat-inactivated fetal bovine serum. The construction of TrkC25 cDNA into the pMEXneo vector was performed as described previously (26). PC12 and NIH3T3 cells were transfected by lipofection (DOTAP Liposomal Transfection Reagent, Boehringer Mannheim) with the pMEXneo- TrkC25 plasmid. Four days later, selection was initiated with 500 mg/ml Geneticin (Life Technologies, Inc.) in culture medium. Resistant colonies were detected by wheat germ agglutinin-Sepharose precipitation and Western transfer as described previously (9). Neurite outgrowth was assayed at different days as described previously (26) by quantitating cells with processes of lengths of two or more cell bodies in diameter. Somatic size was assayed in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum, with or without 100 ng/ml NGF or NT-3. Forty cells were randomly chosen and their cell body diameters were determined.

Human TrkA containing the TrkC14 kinase insert (TrkA14) was generated by conventional mutagenesis (BioRad) using human trkA as template. The residues corresponding to the TrkC14 insert were inserted into position 606 and confirmed by sequencing. The PLC-γ1 baculovirus was a gift of V. Cleghorn and D. Morrison (NCI-FCRDC). TrkA14, TrkA (16), and PLC-γ1 baculoviruses were used as described (16). For recombinant protein production, 2 × 10^6 S9 cells were co-infected with PLC-γ1 and either TrkA or TrkA14 at a multiplicity of infection of 5 and lysed at 26 h postinfection.

Immunoprecipitation, Western Immunoblotting, and In Vitro Kinase Assay—Cell lysis and immunoprecipitations were performed as described (15). SNT precipitations were as described previously (53). Immunoprecipitates were electrophoresed on 7.5% SDS-polyacrylamide gels, proteins were transferred to nitrocellulose filters, and the filters were probed with anti-Tyr(P). Shown is an autoradiograph of a Western blot developed using enhanced chemiluminescence.

**RESULTS**

NT-3 and NGF Induce the Tyrosine Phosphorylation of Their Receptors in PC12 Cells Transfected with TrkC Isoforms—PC12 cells were stably transfected with TrkC, TrkC14, and TrkC25. At least two independent cell lines for each construct were analyzed in subsequent experiments. It was shown previously that NT-3 is a weak mitogen for NIH-3T3 cells expressing TrkA, and that the expression of elevated levels of TrkA in PC12 cells can accelerate the rate of neurite outgrowth after NGF treatment (30, 31). We therefore asked whether NT-3 can interact with endogenous TrkA in PC12 cells and whether the levels of transfected TrkC were similar to TrkA. Parental PC12 cells and PC12 cells expressing TrkC (PC12TRKC) were treated with NT-3 and NGF. TrkC was immunoprecipitated from cell lysates using anti-pan Trk or anti-TrkC (11) and assessed for TrkC tyrosine phosphorylation by probing the Trk proteins with anti-phosphotyrosine (Tyr(P)). In the parental PC12 cells, NGF induced the tyrosine phosphorylation of a 140-kDa protein corresponding to the TrkA receptor (Fig. 1). The intensity of tyrosine phosphorylation of TrkC (NT-3 lane) was half the intensity of TrkA (NGF lane). The faster migrating TrkA was not phosphorylated in response to NT-3. In addition, we could not detect complexes of TrkA and TrkC following immunoprecipitation of NT-3-treated cells with anti-TrkC and probing Western blots of the immunoprecipitated protein with a specific anti-TrkA antibody (data not shown). Thus, under our experimental conditions, there is a lack of complex formation between activated forms of TrkA and TrkC. Furthermore, the levels of ligand-induced TrkA and TrkC tyrosine phosphorylation were similar in the cell lines examined.

TrkC14 and TrkC25 Isoforms Have Impaired NT-3-induced Tyrosine Autophosphorylation—Our previous studies with TrkC and TrkC14 receptors expressed in PC12 cells demonstrated that TrkC was tyrosine-phosphorylated to a much greater extent than TrkC14 in response to NT-3 (26). We therefore compared the differences between tyrosine phosphorylation of TrkC and that of TrkC14 and TrkC25 in the different cell lines (PC12TRKC14, PC12TRKC25) expressing these receptors treated with increasing concentrations of NT-3 (Fig. 2). Maximal levels of tyrosine phosphorylation for all receptors was achieved at 100 ng/ml of NT-3. However, at the different concentrations tested, the TrkC kinase insert isoforms were
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ERK/MAPK Tyrosine Phosphorylation in Cells Expressing TrkC Isoforms—Erk1 and Erk2 (mitogen-activated protein (MAP) kinase (p42\textsuperscript{mapk} and p44\textsuperscript{mapk})) are serine/threonine kinases known to act downstream of Ras in neurotrophin-mediated signal transduction pathways (15). MAPK activation is dependent upon the Trk-induced association and tyrosine phosphorylation of Shc or PLC-\(\gamma\) (13, 14, 16). Furthermore, sustained tyrosine phosphorylation and activation of MAPK correlates with rapid acceleration of neurite outgrowth in ligand-stimulated PC12 cells overexpressing TrkA, epidermal growth factor, or insulin receptors (31–33). We therefore examined the tyrosine phosphorylation of Erk1 and Erk2 (an indicator of the activation state of these proteins) in NT-3 or NGF-stimulated PC12TRKC cells, SNT phosphorylation was higher in the NT-3-treated PC12TrkC cells than in those treated with NGF (Fig. 4C). Thus, both TrkA and TrkC stimulate the tyrosine phosphorylation of the same targets examined, although quantitative differences were observed. In addition, TrkC14 and TrkC25 failed to induce the tyrosine phosphorylation of TrkC targets.

ERK1 and Erk2 were immunoprecipitated with anti-Erk and probed in Western blots with anti-Tyr(P). NGF and NT-3 induced the tyrosine phosphorylation of the SNT protein. SNT tyrosine phosphorylation was observed after stimulation with NT-3 in PC12TRKC cells and not in PC12TrkC14 and PC12TrkC25 cells (Fig. 4C). NGF induced the tyrosine phosphorylation of SNT in all cell lines including those expressing the TrkC kinase insert isoforms (Fig. 4C). In contrast to the reduced tyrosine phosphorylation of Shc and PLC-\(\gamma\) in NT-3 versus NGF-stimulated PC12TRKC cells, SNT phosphorylation was higher in the NT-3-treated PC12TrkC cells than in those treated with NGF (Fig. 4C). Thus, both TrkA and TrkC stimulate the tyrosine phosphorylation of the same targets examined, although quantitative differences were observed. In addition, TrkC14 and TrkC25 failed to induce the tyrosine phosphorylation of TrkC targets.

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These results suggest that TrkC can stimulate the MAP kinases with kinetics similar to TrkA. In contrast, TrkC14 and TrkC25, although tyrosine-phosphorylated in response to NT-3, fail to activate signaling pathways that stimulate MAPK tyrosine phosphorylation. Furthermore, the acceleration of neurite outgrowth by NT-3 in PC12TRKC cells does not correlate with a more sustained phosphorylation of Erk1 and Erk2, as compared to NGF.

Immediate Early Gene Responses in Cells Expressing TrkC Isoforms—In PC12 cells, NGF stimulates a variety of immediate early response genes within minutes (34). We next compared, by Northern blot analysis, the immediate early gene inductions induced by NGF or NT-3 treatment using two independent cell lines for each TrkC isoform treated with 100 ng/ml NGF and NT-3. The genes analyzed were c-fos (35), c-jun (36), NGFI-A/TIS8 (37, 38), and nur77/NGFI-B/TIS1 (39–41). The RNA samples were probed with the four different cDNAs probes, and the blots were reprobed with a cDNA for cyclophilin (42) to control for variability in RNA loading and transfer (Fig. 6). The timing and extent of the gene inductions were similar in the NGF-treated samples in the PC12TRKC, PC12TrkC14, and PC12TrkC25 cell lines (Fig. 6, A–D, and data not shown). However, NT-3 induced the same immediate early genes as NGF only in PC12TRKC, as compared to NGF.

Impaired in Vitro Kinase Activity of TrkC14 and TrkC25—We next determined whether the reduced NT-3-mediated tyrosine autophosphorylation of TrkC14 and TrkC25 as compared to TrkC was due to a defect in receptor protein kinase activity. PC12 cells expressing TrkC, TrkC14, or TrkC25 were treated with 100 ng/ml NGF or NT-3, cell lysates were immunoprecipitated with anti-pan Trk, and Trk proteins were assessed for in vitro kinase activity using as a substrate a peptide encoding amino acids 664–681 of human TrkA (peptide 1251). This peptide contains three of the major tyrosine autophosphorylation sites conserved in all Trk family members and is efficiently phosphorylated on tyrosine by NGF-activated TrkA in vitro (43). Ligand-activated TrkA or TrkC immunoprecipitated from PC12TrkC cells phosphorylated peptide 1251, while TrkC14 and TrkC25 were 10-fold less efficient in phosphorylating this peptide (Fig. 7). Thus, the TrkC encoding kinase inserts are defective in catalyzing the phosphorylation of an exogenous substrate in vitro.

TrkA Encoding the Kinase Insert from TrkC14 Is Defective in NGF-induced Tyrosine Autophosphorylation and PLC-γ Tyrosine Phosphorylation in Vivo—To explore whether the kinase insert of TrkC could suppress the activity of other Trk family members, the 14-amino acid insert from rat TrkC (amino acids 711–724) was inserted into the equivalent position of TrkA (following amino acid 676). We then used the baculovirus expression system to assess the tyrosine autophosphorylation of TrkA (TrkA14) and the Trk-induced tyrosine phosphorylation of a TrkA substrate, PLC-γ1 (15). Sf9 cells were co-infected with PLC-γ1 and either TrkA or TrkA14. The cells were treated
The amounts of immunoprecipitated PLC-γ1 in phenotype, including changes in cell shape and hypertrophy, for 10 min with 100 ng/ml NGF, and Trk and PLC-γ1 were autophosphorylated with anti-PLC-γ1. Lysates were immunoprecipitated with specific antibodies. The tyrosine phosphorylation of Trk and PLC-γ1 was then assessed in Western blots using anti-Tyr(P). Both TrkA and TrkA14 were autophosphorylated in response to NGF, although TrkA14 tyrosine phosphorylation was much reduced as compared to TrkA (Fig. 8A). TrkA14 was incapable of mediating the tyrosine phosphorylation of PLC-γ1, consistent with the results obtained with PC12 cells expressing TrkC14 (Fig. 8C). Similar levels of TrkA, TrkA14 (Fig. 8B), and PLC-γ1 (Fig. 8D) were expressed in all experiments. The TrkC14 kinase insert therefore markedly reduces the ability of TrkA to autophosphorylate on tyrosine or to stimulate PLC-γ1 tyrosine phosphorylation.

**DISCUSSION**

In response to NGF, PC12 cells undergo dramatic alterations in phenotype, including changes in cell shape and hypertrophy, extension of long neurites, and the acquisition of electrical excitability (44). Recent studies have identified correlations between biological responses and the activation of selective intracellular signaling pathways mediated by NGF-activated TrkA in PC12 cells (13, 15–17, 24). We likewise used the PC12 cell system to explore and compare the cellular modifications and signaling pathways utilized by TrkC and related kinase insert isoforms to those used by TrkA. Our results indicate that TrkA and TrkC show quantitative and qualitative differences in their abilities to induce neuronal differentiation, and that the consequence of inserts in TrkC is to impair biological and biochemical signaling capacities.

To compare the signaling potentials of TrkA and TrkC, these receptors were expressed such that they exhibited similar levels of ligand-induced tyrosine phosphorylation in PC12 cells. When bound by ligand, these receptors did not appear to form heterodimeric complexes, since immunoprecipitates of NT-3-activated TrkC did not contain TrkA. Neurite outgrowth and cell hypertrophy were more pronounced in cells exposed to NT-3 than those exposed to NGF. Thus, TrkC and TrkA, when autophosphorylated to similar extents, have distinct morphological effects in PC12 cells. One explanation for more rapid responses mediated by TrkC is that this receptor causes a prolonged activation of the proteins of the Ras signaling pathway. Sustained signaling through Ras has been suggested to be responsible for the ability of normally mitogenic receptors such as the insulin and epidermal growth factor receptors to elicit neurites when overexpressed in PC12 cells (32, 33, 45, 46).

However, the tyrosine phosphorylation of Shc and PLC-γ1 was reduced in NT-3-treated cells as compared to NGF, and the strength and duration of Erk tyrosine phosphorylation and the induction of the c-fos, c-jun, NGFI-A, and NGFI-B genes were similar. The differences in biological responses elicited by NGF and NT-3, therefore, cannot be correlated with the duration and strength of signaling through targets of Ras such as Erk. Quantitative differences in the tyrosine phosphorylation of proteins such as SNT could account for differential biological response. However, the precise molecular nature of SNT is not yet known, and other cellular proteins that are activated or phosphorylated by neurotrophin treatment such as phosphoinositide 3-kinase (14, 47, 48), SH-PTP1 (49), and Nck (50) may be responsible for the differences in TrkA and TrkC signaling.

We also show that the inability of TrkC14 and TrkC25 to induce phenotypic changes correlates with their inability to stimulate the tyrosine phosphorylation of Shc, PLC-γ1, Erk, and SNT or to mediate immediate early gene induction events. This is in accordance with other studies showing that TrkC14 and TrkC25 fail to activate PLC-γ1 and phosphoinositide 3-kinase and induce biological responses in NIH-3T3 cells (28, 56). We suggest that the inability of TrkC14 and TrkC25 to stimulate morphological and signaling responses is most likely due to their inefficiency at ligand-induced autophosphorylation and kinase activity (Figs. 7 and 8).

It is not known what structural constraints the kinase inserts confer to the kinase domain that prevent proper activation of the receptor and stimulation of primary effectors. The crystal structure of the catalytic domain for the human insulin receptor has been determined, and a general mechanism for the activation of receptor tyrosine kinases has been proposed, known as the cis-inhibition and trans-activation model (51). The activation loop in the insulin receptor contains three tyrosines at positions 1157, 1162, and 1163 (YETDYY) that become autophosphorylated upon receptor activation. However, in the unphosphorylated state, one of these residues, Tyr-1162, is engaged in the active site with the catalytic base of Asp-1132. This engagement prevents the accessibility of exogenous sub-

**Fig. 4. Tyrosine phosphorylation of PLC-γ1, Shc, and SNT in PC12 cells expressing TrkC and TrkC kinase insert isoforms.** Lysates were prepared from mock-treated (0), NGF-treated (100 ng/ml for 5 min), or NT-3-treated (100 ng/ml for 5 min) cells expressing the TrkC, TrkC14, and TrkC25 receptors and equalized for cell protein. A, tyrosine phosphorylation of PLC-γ1. Lysates were immunoprecipitated with anti-PLC-γ1 and probed with anti-Tyr(P) (top panel). To verify that the amounts of immunoprecipitated PLC-γ1 were similar in each lane, the blot in the top panel was stripped of antibody and reprobed with anti-PLC-γ1. The positions of PLC-γ1 and Trk proteins are indicated by arrows. B, tyrosine phosphorylation of Shc. Lysates were immunoprecipitated with anti-Shc and probed with anti-Tyr(P) (top panel) followed by reprobing with anti-Shc (bottom panel). The positions of Shc are indicated by arrows. C, tyrosine phosphorylation of SNT. SNT proteins were precipitated with p130crk-agarose and probed with anti-Tyr(P).
strate and ATP binding, thus repressing kinase activity. When insulin binds the receptor, Tyr-1162 is trans-autophosphorylated and disengaged from the active site, and ATP is bound. Consequently, the activation loop is stabilized in a new conformation. These intermolecular changes result in increased ATP and substrate accessibility and greatly enhanced kinase activity. As in the insulin receptor, the Trk receptors contain three tyrosines, YXXDDY, located in a similar position in a potential activation loop. In TrkA, these three tyrosines have been found to be major targets of autophosphorylation (13). The 14- and 25-amino acid inserts in the kinase domain of TrkC are found within two amino acids from the carboxyl-terminal of the tyrosine residues. The presence of additional sequences directly following the activation loop and their proximity to the tyrosines that become autophosphorylated during receptor activation may adversely affect the disengagement of the tyrosine from the active site or access to substrate. Such a mechanism could reduce the efficiency of the trans-autophosphorylation that alters the final conformation of the intracellular domains of TrkC that would result in a lack of interaction or access of this receptor to its substrates. Our data are consistent with such a model. The autophosphorylation of TrkC insert isoforms was reduced substantially in PC12 cells, and the intrinsic kinase activity of these receptors in vitro was reduced greatly.
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TrkC insert isoforms are expressed in the central nervous system by the formation of heterodimers with TrkC or by co-expression of classes of receptors in the same cell. In neuronal cell populations such as TrkC, it remains unclear whether both TrkC insert isoforms have a low signaling capability in neuronal cells. While TrkC kinase inserts are expressed in the same cells as TrkC, it is not clear whether the impaired tyrosine kinase activity of TrkC14 is not diminished. We conclude that the TrkC kinase inserts confer functional constraints to other Trk family members, indicating that the impaired tyrosine kinase activity of TrkC14 is specific to TrkC.

Furthermore, when the TrkC14 insert form was inserted in a similar position in TrkA, tyrosine kinase activity was greatly diminished. We conclude that the TrkC kinase inserts confer functional constraints to other Trk family members, indicating that the impaired tyrosine kinase activity of TrkC14 is specific to TrkC.

In summary, our observations support the concept that the TrkC insert isoforms have a low signaling capability in neuronal cells. While TrkC kinase inserts are expressed in the same cell populations as TrkC, it remains unclear whether both classes of receptors are co-expressed in the same cell. In neurons, TrkC insert isoforms may modulate signal transduction either by the formation of heterodimers with TrkC or by competitive binding of available ligand. We have observed that TrkC insert isoforms are expressed in the central nervous system during postnatal and adult life, which could explain the low level of NT-3-induced tyrosine phosphorylation of TrkC in isolated adult brain tissues. However, since the TrkC insert isoforms have a low signaling capability when overexpressed in a non-neuronal background (28, 56), it is possible that these receptors might have some functional capability in vivo.

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