Regulation of the cytosolic free Ca\(^{2+}\) concentration by nerve growth factor was investigated in C6–2B glioma cells newly expressing the high affinity nerve growth factor receptor trkA, using Fura-2 fluorescence ratio imaging. In these cells, nerve growth factor (50 ng/ml) evoked a novel ~3-fold increase in cytosolic free Ca\(^{2+}\) concentration, while no measurable Ca\(^{2+}\) response was observed in wild type or mock-transfected cells lacking a functional trkA receptor. K-252a, a tyrosine kinase inhibitor which prevents nerve growth factor-mediated responses in C6–2B cells expressing trkA, also blocked the rise in cytosolic free Ca\(^{2+}\) concentration by nerve growth factor. Moreover, basic fibroblast growth factor, which in these cells elicits biochemical changes similar to nerve growth factor, failed to affect cytosolic free Ca\(^{2+}\) concentration, further supporting the specificity of nerve growth factor/trkA receptor in mediating a Ca\(^{2+}\) response. While insensitive to chelation of extracellular Ca\(^{2+}\), the response was abolished following depletion of Ca\(^{2+}\) stores or blockade of intracellular Ca\(^{2+}\) release, providing strong evidence that intracellular Ca\(^{2+}\) is the main source for nerve growth factor-evoked cytosolic free Ca\(^{2+}\) concentration increase. Nerve growth factor increased the cytosolic free Ca\(^{2+}\) concentration also in NIH3T3 cells overexpressing trkA but devoid of p75 nerve growth factor receptor. Our data suggest that trkA but not p75 is required for nerve growth factor-evoked Ca\(^{2+}\) signaling.

Neurotrophic factors are required for the development, survival, and maintenance of distinct populations of neurons. Among various growth factors which have been proposed to function as neurotrophic agents in the central nervous system is nerve growth factor (NGF)\(^{1}\) (1), a prototype member of the neurotrophin family of growth factors which includes brain-derived growth factor, neurotrophin-3, and neurotrophin-4/5 (reviewed in Ref. 2). NGF is required for the differentiation and survival of sympathetic and some sensory neurons in the peripheral nervous system and provides trophic support for the cholinergic neurons of the basal forebrain (reviewed in Ref. 3).

NGF exerts its neurotrophic activity by binding to a receptor complex comprised of a low affinity component, p75\(\text{NGFR}^{1}\) (4, 5), which has been postulated to interact with G-proteins (6, 7) and an high affinity component, trkA (8–11), which contains a cytoplasmic domain with tyrosine kinase activity (12). trkA, the product of trkA protooncogene (13), undergoes autophosphorylation upon NGF binding (12), and it has been shown to be essential for the biological activity of NGF (14, 15).

A great deal of information is now available on the various biological responses elicited by NGF. However, at present, the role played by either receptor component in the NGF signal transduction mechanism(s) as well as the second messenger(s) leading to specific NGF responses are still under investigation. Controversial results have been reported showing that a number of second messengers are affected by NGF, such as cAMP (7, 16, 17), cGMP (18), phosphoinositides (7, 19), arachidonic acid (20, 21), and glycosylphosphatidylinositol metabolites (22). The role of Ca\(^{2+}\) as a putative mediator of NGF responses has also been investigated; however, the data gathered so far are highly contradictory. The original report that NGF caused a small Ca\(^{2+}\) efflux (23) was not supported by subsequent studies (24). Likewise, the more recent findings (25, 26) that NGF evokes a small and rapid increase in cytosolic free Ca\(^{2+}\) concentration (\([\text{Ca}^{2+}]_{\text{i}}\)) have not been confirmed (27). In addition, NGF has been proposed to increase Ca\(^{2+}\) uptake, possibly through a unique Ca\(^{2+}\) channel (28, 29).

All of the above studies have been performed in PC12 cells (a pheochromocytoma cell line widely used as a model for studying NGF-induced responses) which express both low and high affinity NGF receptor components (30). C6–2B cells, a rat glioma cell line expressing p75\(\text{NGFR}^{1}\) (31) but devoid of the high affinity receptor trkA (32), are unresponsive to NGF. Upon stable transfection with trkA rat cDNA, C6–2B cells (C6trk\(^{+}\)) exhibit novel NGF-mediated biochemical responses (c-fos induction and phosphorylation of trkA) and morphological changes (increased length of process extension) (32). NIH3T3 cells, devoid of p75\(\text{NGFR}^{1}\) (9), display a novel responsiveness to NGF when expressing trkA (trkA NIH3T3) (33). Therefore, we reasoned that C6trk\(^{+}\) and trkA NIH3T3 cell lines NGF increases \([\text{Ca}^{2+}]_{\text{i}}\) by mobilizing intracellular Ca\(^{2+}\), and (ii) trkA, but not p75\(\text{NGFR}^{1}\), is essential for the NGF-evoked Ca\(^{2+}\) response.

**MATERIALS AND METHODS**

Cell Culture—C6–2B glioma cells (34) were grown as monolayer cultures in Ham's F-10 nutrient mixture (Life Technologies, Inc.), sup-
transfected (mock transfected) and C6trk- cells. Fura 2-AM loaded C6–2B mock transfected (open squares) and C6trk- cells (solid squares) were challenged with NGF (50 ng/ml) and [Ca^{2+}]i changes were measured as described under "Materials and Methods." ATP (100 μM) was then added to mock transfected cells (open squares). Data are the population mean of the [Ca^{2+}]i responses from one cover-slip per each culture and are representative of 5 coverslips (mock transfected cells) and 32 coverslips (C6trk- cells) with 10–50 cells/cover-slip imaged in a single field.

Digital Fluorescence Microscopy—Single cell fluorescence Ca^{2+} imaging was performed as described previously (35) using an Axiovert Fluovision digital fluorescence microscopy system (Axion Instruments Inc., Roddickville, MD). Briefly, cells were grown on 25-mm round, 1-mm thick glass coverslips and loaded with 10 μM cell-permeable acetoxy-methylester of Fura-2 (Fura-2AM, Molecular Probes, Eugene, OR) for 20 min at 37 °C in serum-free Ham’s F-10 medium buffered with 20 mM Hepes, pH 7.4. Cells were washed, given 10 min to allow Fura-2 deesthification, and imaged in the same buffer either at room temperature or at 30–32 °C with a Zeiss Axiocvert TV 135 microscope and a 40× achroplan objective (Plan-apochromat oil immersion objective). Fura-2 was excited at 334 and 380 nm with its emission monitored at 510–530 nm. The 334/380 nm excitation ratio increases as a function of the [Ca^{2+}]i responses comparable to those previously described were observed when these cells were challenged with either ATP, a purinergic receptor agonist or dantrolene (Sigma), ionomycin (Calbiochem), thapsigargin (Research Biochemicals Inc., Natick, MA), NGF (Collaborative Research Inc., Bedford, MA), human recombinant basic FGF (Farmitalia Carlo Erba s.p.a., Milan, Italy), or the Ca^{2+} ionophore ionomycin (data not shown). Three independent C6trk- cell clones were tested (nos. 41, 43, and 44) and found to display a novel [Ca^{2+}]i increase upon exposure to NGF. Shown in Fig. 1 (solid squares) is the Ca^{2+} response to NGF in cells from clone no. 43. Single cell fluorescence Ca^{2+} imaging revealed heterogeneity in the Ca^{2+} response such that, upon NGF treatment, 80% of the cells from clone no. 43, while only 20% of the cells from clone no. 41, displayed a measurable [Ca^{2+}]i increase. For this

Effect of NGF on [Ca^{2+}]i in C6–2B Cells—Treatment with NGF (1–100 ng/ml) failed to elicit a measurable [Ca^{2+}]i increase in either wild type (data not shown) or mock-transfected C6–2B cells (Fig. 1, open squares) expressing p75NGFR but lacking trkA (32). However, Ca^{2+} responses comparable to those previously described were observed when these cells were challenged with either ATP, a purinergic receptor agonist which, in C6–2B cells, has been shown to mobilize intracellular Ca^{2+} (36) (Fig. 1, open squares), or the Ca^{2+} ionophore, ionomycin (data not shown). Single cell fluorescence Ca^{2+} imaging revealed heterogeneity in the Ca^{2+} response such that, upon NGF treatment, 80% of the cells from clone no. 43, while only 20% of the cells from clone no. 41, displayed a measurable [Ca^{2+}]i increase. For this

RESULTS

Effect of NGF on [Ca^{2+}]i in C6–2B Cells—Treatment with NGF (1–100 ng/ml) failed to elicit a measurable [Ca^{2+}]i increase in either wild type (data not shown) or mock-transfected C6–2B cells (Fig. 1, open squares) expressing p75NGFR but lacking trkA (32). However, Ca^{2+} responses comparable to those previously described were observed when these cells were challenged with either ATP, a purinergic receptor agonist which, in C6–2B cells, has been shown to mobilize intracellular Ca^{2+} (36) (Fig. 1, open squares), or the Ca^{2+} ionophore, ionomycin (data not shown). Three independent C6trk- cell clones were tested (nos. 41, 43, and 44) and found to display a novel [Ca^{2+}]i increase upon exposure to NGF. Shown in Fig. 1 (solid squares) is the Ca^{2+} response to NGF in cells from clone no. 43. Single cell fluorescence Ca^{2+} imaging revealed heterogeneity in the Ca^{2+} response such that, upon NGF treatment, 80% of the cells from clone no. 43, while only 20% of the cells from clone no. 41, displayed a measurable [Ca^{2+}]i increase. For this
reason, clone no. 43 was used in the majority of the experiments described in this study. The degree of NGF responsiveness among the three C6trk+ clones correlated with the degree of trkA mRNA expression in each clone, with clone no. 43 exhibiting higher levels of trkA mRNA than clone no. 41 (32). Therefore, the heterogeneity of Ca2+ responses is likely the result of the differential expression of trkA receptor in each clone. In line with this interpretation, it was also found that C6trk+ clone no. 43 responded to NGF with a greater induction of tyrosine phosphorylation of trkA and Erk, an NGF target protein (37), than clone no. 41 (Fig. 2).

Characterization of [Ca2+]i. Increase by NGF in C6trk+ Cells—Upon exposure to NGF (50 ng/ml), C6trk+ cells displayed a 3.0 ± 0.6-fold increase in [Ca2+]i (Fig. 1). [Ca2+]i rise was delayed, showing a lag phase of about 30 s, relatively slow (t1/2 for NGF-induced peak of [Ca2+]i increase was 52.8 ± 9.1 s), and long lasting, with [Ca2+]i remaining elevated above resting levels (basal [Ca2+]i = 85 ± 18.6 nM) for at least 8 min. The lowest dose of NGF that yielded a measurable [Ca2+]i rise, although in a very exiguous number of cells (<2%), was 1 ng/ml (38 pm) (Fig. 3). The extent of the [Ca2+]i response evoked in these cells by 1 ng/ml NGF was about 58% of the maximal response elicited by 50 ng/ml NGF. The response reached a plateau at NGF doses of greater than 5 ng/ml (0.2 nM). Unless otherwise indicated, NGF working concentration used in all experiments was 50 ng/ml (2 nM).

Mechanism(s) of [Ca2+]i. Rise by NGF—To investigate whether the NGF-evoked [Ca2+]i increase was of intracellular or extracellular origin, C6trk+ cells were challenged with NGF either in the absence or presence of EGTA. When the cells were pretreated with 2 mM EGTA for 30–90 s and then exposed to NGF always in the presence of EGTA, the peak [Ca2+]i, was minimally affected (from an average of 250 ± 39 nM in the absence to 216 ± 48 nM in the presence of EGTA, Fig. 4). These data indicate that the NGF-evoked [Ca2+]i rise primary involves the mobilization of Ca2+ from intracellular stores rather than Ca2+ influx. However, while the rate of onset of the Ca2+ response was comparable in the absence and in presence of EGTA, the decay kinetics of [Ca2+]i, were much faster in Ca2+-depleted medium than in Ca2+-containing medium with an apparent t1/2 for [Ca2+]i, to return to resting levels of 85 ± 20 s and >181 s, respectively. This is consistent with the notion that a large component of the decay phase of the [Ca2+]i, rise involves a capacitative entry of external Ca2+ triggered by the depletion of intracellular stores (38, 39).

Further support for the hypothesis that intracellular Ca2+ is the main source for the peak response to NGF was provided by experiments in which either the internal stores were depleted of Ca2+ with the microsomal Ca2+-ATPase inhibitor thapsigargin (TG) (40) or the release of Ca2+ from the stores was blocked with dantrolene, a muscle relaxant widely used as an inhibitor of intracellular [Ca2+]i mobilization (36, 41, 42). Exposure of C6trk+ cells to 100 nM TG elicited a [Ca2+]i increase similar in magnitude and kinetics to that previously described in wild type C6–2B cells (36, 42). When TG-pretreated cells were challenged with NGF (50–100 ng/ml), no increase in [Ca2+]i, was observed (Fig. 5). Because the response to the Ca2+ ionophore ionomycin (5 µM) was unaffected by TG (Fig. 5), these data provide strong evidence that NGF increases [Ca2+]i by mobilizing the cation from intracellular stores. In line with the above results, when C6trk+ cells were exposed to NGF first and subsequently to ATP, which in C6–2B cells induces inositol 1,4,5-trisphosphate (IP3) formation and releases Ca2+ from internal stores (36), the Ca2+ response to ATP was reduced by
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About 50% in those cells which had responded to NGF, compared to NGF-unresponsive cells (Fig. 6). Likewise, TG evoked a 2.6 ± 0.1-fold increase in [Ca\textsuperscript{2+}] in cells which previously responded to NGF, while it elicited a 5.7 ± 0.8-fold increase in NGF-unresponsive cells (n = 3). The results obtained with ATP and TG indicate that NGF evokes Ca\textsuperscript{2+} release from intracellular stores which are included in the IP\textsubscript{3} and TG-sensitive Ca\textsuperscript{2+} pools. This is consistent with previous findings showing that, in PC12 cells, NGF induces hydrolysis of phosphatidylinositol (7, 19, 43) with consequent production of IP\textsubscript{3} which is released from intracellular stores (39). Last, dantrolene, which in C6–2B cells prevents the [Ca\textsuperscript{2+}] increase evoked by either agonists of receptors coupled to phosphatidylinositol hydrolysis or TG (36, 42), abolished NGF-induced Ca\textsuperscript{2+} transient in C6trk\textsuperscript{+} cells without appreciably affecting the ability of ionomycin to elicit a Ca\textsuperscript{2+} entry response (Fig. 7).

Effect of K-252a on the Ca\textsuperscript{2+} Response to NGF—K-252a, an alkaloid-like protein kinase inhibitor (44), has been shown to specifically inhibit many of the actions of NGF in PC12 cells, including IP\textsubscript{3} formation, increase in intracellular Ca\textsuperscript{2+}, and neurite outgrowth (26, 45, 46). In C6trk\textsuperscript{+} cells, inhibition of trkA tyrosine phosphorylation activity by K-252a blocked NGF-elicited responses (32), such as c-fos induction and phosphorylation of trkA and suc-associated neurotrophin factor-induced tyrosine-phosphorylated target (SNT) (47). The [Ca\textsuperscript{2+}] increase evoked by NGF in C6trk\textsuperscript{+} cells appears to be mediated by the newly expressed trkA receptor, as neither wild type nor mock-transfected C6–2B cells (expressing p75NGFR but lacking trkA) respond to NGF with a [Ca\textsuperscript{2+}] rise. Therefore, we investigated whether the inhibition of the kinase activity associated with trkA, could affect the Ca\textsuperscript{2+} response to NGF. As shown in Fig. 8, pretreatment of C6trk\textsuperscript{+} cells with 200 nM K-252a for 20 min virtually abolished NGF-evoked [Ca\textsuperscript{2+}] rise. In K-252a-treated cells, TG did induce [Ca\textsuperscript{2+}] increase (Fig. 8), although to a lesser extent than in K-252a-untreated cells (see Figs. 5 and 9 for comparison). A recent report shows that, in human fibroblasts, tyrosine kinase inhibitors affect the Ca\textsuperscript{2+} entry triggered by the depletion of intracellular Ca\textsuperscript{2+} stores with TG (48). A similar effect by K-252a could account for the reduced TG response observed also in C6trk\textsuperscript{+} cells.

Effect of Basic FGF (bFGF) on [Ca\textsuperscript{2+}] in C6–2B and C6trk\textsuperscript{+} Cells—bFGF is a well known trophic factor which exerts its biological effects by binding to a tyrosine kinase receptor (49). In C6trk\textsuperscript{+} cells, bFGF, similarly to NGF, elicits c-fos expression (32) and induces phosphorylation of various target proteins, such as SNT and Erk2 kinase (50), suggesting that these two trophic factors trigger a similar transduction pathway. In the attempt to characterize whether Ca\textsuperscript{2+} signaling, rather than being a common step in tyrosine kinase receptor activation, is unique for NGF, we investigated the effect of bFGF on the [Ca\textsuperscript{2+}] dynamics was evaluated in another cell line, trkA NIH3T3 Cells—Because C6trk\textsuperscript{+} cells also express p75NGFR (32), the possibility exists that the low affinity receptor component might play a necessary or permissive role in the NGF-evoked trkA-mediated Ca\textsuperscript{2+} signaling. Therefore, the effect of NGF on the intracellular Ca\textsuperscript{2+} dynamics was evaluated in another cell line, trkA

![Figure 5](http://www.jbc.org/content/early/1999/04/26/jbc.274.30145.0163F5)

**Fig. 5.** Thapsigargin prevents [Ca\textsuperscript{2+}], increase induced by NGF in C6trk\textsuperscript{+} cells. Cells were exposed to thapsigargin (TG, 100 nM; open squares) or vehicle (0.001% dimethyl sulfoxide; solid squares), washed, and challenged with NGF (50 ng/ml). Ionomycin (5 μM) was also added to TG-pretreated cells. The [Ca\textsuperscript{2+}], profiles shown are the population means from one coverslip and are representative of two other experiments (10–50 cells/coverslip in a single field).

![Figure 6](http://www.jbc.org/content/early/1999/04/26/jbc.274.30145.0163F6)

**Fig. 6.** Effect of NGF on intracellular Ca\textsuperscript{2+} mobilization by ATP. C6trk\textsuperscript{+} cells were exposed to NGF (50 ng/ml) and subsequently to ATP (100 μM). Data are the mean [Ca\textsuperscript{2+}], responses recorded from five cells which responded (open squares) and five cells which did not respond (solid squares) to NGF. Cells are from the same field and were imaged simultaneously. Similar results were obtained from several (>10) other coverslips (30–50 cells/coverslip in a single field).
NIH3T3 cells which overexpress trkA but are devoid of p75NGFR (9, 33). trkA NIH3T3 cells responded to NGF with a 3-fold increase in 
\[Ca^{2+}]_i\] (Fig. 10). The 
\[Ca^{2+}]_i\] response was similar in magnitude and kinetics to that observed in C6trk+ cells. Also, NGF increased 
\[Ca^{2+}]_i\] even in the absence of extracellular Ca\(^{2+}\) (Fig. 10A) while it failed to do so when internal Ca\(^{2+}\) stores were depleted with TG (Fig. 10B), suggesting that NGF mobilizes intracellular Ca\(^{2+}\) in trkA NIH3T3 cells. These results, taken together, provide evidence that p75NGFR is not required for NGF-induced Ca\(^{2+}\) signaling. Similar to C6trk+ cells, the Ca\(^{2+}\) response appears to be a specific NGF-induced event rather than a result of generalized tyrosine kinase pathway activation because bFGF failed to increase [Ca\(^{2+}\)] in trkA NIH3T3 cells (data not shown).

**DISCUSSION**

We have previously shown that C6–2B cells express only the low affinity component (p75NGFR) of the NGF receptor complex (32). Consistent with the notion that trkA is required for the biological activity of NGF (14, 15), C6–2B cells are unresponsive to NGF, as demonstrated by the lack of induction of c-fos, phosphorylation of NGF target proteins, and morphological changes, following NGF treatment (32). All of these responses, which are among NGF-mediated biological effects, can be observed in PC12 cells, an NGF responsive cell line expressing both trkA and p75NGFR receptors (30). In this report we have shown that wild type and mock-transfected C6–2B cells expressing p75NGFR but lacking a functional trkA receptor (32) failed to display a measurable Ca\(^{2+}\) response upon NGF treatment. However, upon exposure to ATP, which in C6–2B cells mobilizes Ca\(^{2+}\) from internal stores (36), or ionomycin, which induces Ca\(^{2+}\) entry, Ca\(^{2+}\) responses comparable to those previously described (36) were observed, implying that the lack of changes in the [Ca\(^{2+}\)] following NGF cannot be ascribed to inefficiency of the mechanisms involved in either intracellular Ca\(^{2+}\) release or Ca\(^{2+}\) influx.

The novel expression of trkA in C6–2B cells induces NGF responsiveness (32). C6trk+ cells undergo a transient [Ca\(^{2+}\)] increase when exposed to NGF. Although the kinetics of the [Ca\(^{2+}\)] rise by NGF are similar to those previously described in
PC12 cells (25), both the potency and the efficiency of NGF in increasing $[\text{Ca}^{2+}]_i$ were greater in C6trk$^- \times$ cells (+200% at ≤0.2 mM) than in PC12 cells (+50% at >3 mM). In C6trk$^- \times$ cells, the expression of p75NGFR mRNA is nearly equivalent to that found in PC12 cells (30–32), while trkA mRNA levels are at least 5–10-fold higher than in PC12 cells (32). Thus, the overexpression of the newly synthesized high affinity receptor in C6trk$^- \times$ cells could explain the greater Ca$^{2+}$ response evoked by NGF in these cells compared to PC12 cells.

Previous findings in PC12 cells showed that the small and transient increase in $[\text{Ca}^{2+}]_i$ evoked by NGF was mainly due to Ca$^{2+}$ entry (25, 28), possibly through a unique Ca$^{2+}$ channel (29). Our study shows that NGF evokes an increase in $[\text{Ca}^{2+}]_i$ also in Ca$^{2+}$-depleted medium, but it fails to do so when intracellular Ca$^{2+}$ cannot be mobilized due to depletion of the stores with TG or blockade of the release from the stores with dantrolene. We therefore propose that, in C6trk$^- \times$ cells, NGF elicits $[\text{Ca}^{2+}]_i$ rise by causing intracellular Ca$^{2+}$ mobilization rather than extracellular Ca$^{2+}$ influx. However, we cannot rule out the involvement of Ca$^{2+}$$^{2+}$ channels in the NGF-mediated $[\text{Ca}^{2+}]_i$ rise in other systems because C6–2B cells do not express functional voltage-dependent Ca$^{2+}$ channels as KCl de-polarization failed to change $[\text{Ca}^{2+}]_i$.

The exact mechanism utilized by NGF in evoking Ca$^{2+}$ response does not appear to be easily definable. In C6trk$^- \times$ cells, K-252a, an inhibitor of the tyrosine kinase activity associated with trkA (12, 51), blocked NGF-evoked $[\text{Ca}^{2+}]_i$ rise. Therefore, it is reasonable to infer that the novel Ca$^{2+}$ response to NGF observed in C6trk$^- \times$ cells is mediated by trkA. NGF stimulates phosphorylation of phospholipase C$_{\gamma}$ on tyrosine as well as serine residues (52) by a kinase activity associated with trkA (53). Activated phospholipase C$_{\gamma}$ is known to lead to phosphatidylinositol hydrolysis with accumulation of diacylglycerol and IP$_3$. The latter, acting at receptors located on the endoplasmic reticulum, induces release of Ca$^{2+}$ (39), suggesting that phospholipase C$_{\gamma}$ signaling might mediate the NGF-evoked $[\text{Ca}^{2+}]_i$ rise. However, bFGF, which, similarly to NGF, activates phospholipase C$_{\gamma}$ in C6trk$^- \times$ and trk NIH3T3 cells (data not shown), failed to induce an appreciable Ca$^{2+}$ response in these same cells. Thus, it appears that [Ca$^{2+}]_i$ rise is a unique response to trkA activation. Interestingly enough, in C6trk$^- \times$ cells, NGF elicits morphological changes and is a weaker mimic than bFGF (32). Thus, although only speculative at present, the hypothesis that [Ca$^{2+}]_i$ rise elicited by NGF could be a crucial player in triggering the cascade of molecular events leading to cell differentiation is appealing.

Recent data have shown that, although p75NGFR is not required for NGF biological activity (54), the low affinity receptor appears to be involved in the binding of NGF and signal transduction of trkA (55–59). From our results, trkA appears to be solely responsible and sufficient for NGF-evoked $[\text{Ca}^{2+}]_i$, increase since Ca$^{2+}$-signaling triggering by NGF occurs also in trkA NIH3T3 cells lacking p75NGFR. In a recent report (7), p75NGFR has been proposed to mediate, through a pertussis toxin sensitive G-protein, the increase in CAMP accumulation caused by NGF in PC12 cells. p75NGFR is also involved in the NGF-mediated activation of sphingomyelin cycle (60). Thus, p75NGFR might be involved in signal transduction pathways other than Ca$^{2+}$, while trkA in signaling other than CAMP. Interestingly, and in agreement with our interpretation, K-252a blocked NGF-induced IP$_3$ accumulation (7, 45), while it did not affect the NGF-elicted CAMP formation (7).

In conclusion, we have demonstrated that (i) NGF is able to largely modulate $[\text{Ca}^{2+}]_i$ by a mechanism which involves mobilization of intracellular Ca$^{2+}$, and (ii) trkA and not p75NGFR is required and sufficient to mediate this response. Important questions which could be addressed using C6trk$^- \times$ cells as a model include the relationship (if any) between Ca$^{2+}$ signal and morphological changes, as well as cell differentiation.

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