Involvement of ras p21 in Neurotrophin-induced Response of Sensory, but Not Sympathetic Neurons

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Abstract. Little is known about the signal transduction mechanisms involved in the response to neurotrophins and other neurotrophic factors in neurons, beyond the activation of the tyrosine kinase activity of the neurotrophin receptors belonging to the *trk* family. We have previously shown that the introduction of the oncogene product ras p21 into the cytoplasm of chick embryonic neurons can reproduce the survival and neurite-outgrowth promoting effects of the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), and of ciliary neurotrophic factor (CNTF). To assess the potential signal-transducing role of endogenous ras p21, we introduced function-blocking anti-ras antibodies or their Fab fragments into cultured chick embryonic neurons. The BDNF-induced neurite outgrowth in E12 nodose ganglion neurons was reduced to below control levels, and the NGF-induced survival of E9 dorsal root ganglion (DRG) neurons was inhibited in a specific and dose-dependent fashion. Both effects could be reversed by saturating the epitope-binding sites with biologically inactive ras p21 before microinjection. Surprisingly, ras p21 did not promote the survival of NGF-dependent E12 chick sympathetic neurons, and the NGF-induced survival in these cells was not inhibited by the Fab-fragments. The survival effect of CNTF on ras-responsive ciliary neurons could not be blocked by anti-ras Fab fragments. These results indicate an involvement of ras p21 in the signal transduction of neurotrophic factors in sensory, but not sympathetic or ciliary neurons, pointing to the existence of different signaling pathways not only in CNTF-responsive, but also in neurotrophin-responsive neuronal populations.

NEUROTROPHIC factors are proteins regulating the survival of neurons during normal vertebrate development, and the maintenance of differentiated neuronal functions in the adult. Recently, it has become apparent that some of these proteins – the neurotrophins – are structurally related. The in vivo administration of either NGF or of the related brain-derived neurotrophic factor (BDNF)[1] prevents normally occurring neuronal death in different, though somewhat overlapping neuronal populations (e.g., NGF prevents cell death in peripheral sympathetic ganglia [Oppenheim et al., 1982], while BDNF increases neuronal numbers in placode-derived sensory ganglia [Hofer and Barde, 1988]). High-affinity receptors have been described on NGF- and BDNF-responsive neurons (Sutter et al., 1979; Rodríguez-Tébar and Barde, 1988), and these receptors have been demonstrated to clearly distinguish between NGF and BDNF (Rodríguez-Tébar et al., 1990). Recently, evidence has been presented that these high-affinity neurotrophin receptors consist (at least in part) of tyrosine kinase membrane proteins expressed in neurons and are members of a gene family related to a previously identified oncogene, *trk* (Pulciani et al., 1982). Thus, NGF and BDNF have been demonstrated to bind to *trk* and *trk*B, respectively, with substantial affinities, and this binding leads to the activation of the tyrosine kinase domains of the *trk*s (Hempstead et al., 1991; Klein et al., 1991; Soppet et al., 1991; Squinto et al., 1991). A few other characterized proteins, unrelated to the neurotrophins, have been shown to be able to rescue neurons in vitro and in vivo. One of them, ciliary neurotrophic factor (CNTF) (Manthorpe et al., 1982; Stöckli et al., 1989; Lin et al., 1989), has been shown to promote the in vitro survival of parasympathetic ciliary neurons (Barbin et al., 1984), and of spinal motoneurons both in vitro and in vivo (Arakawa et al., 1990; Sendtner et al., 1990). In contrast to the neurotrophin receptors, the receptor for CNTF consists of membrane proteins related to cytokine receptors, but not to tyrosine kinase receptors (Davis et al., 1991).

Most of the information available concerning NGF signal transduction has come from studies with rat pheochromocy-

1. *Abbreviations used in this paper*: BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; DRG, dorsal root ganglia.

toma cells (PC12 cells; Greene and Tischler, 1976), which stop dividing and produce fibers in response to NGF. Studies with these cells have suggested a role for the ras oncogene proteins in NGF signal transduction. The mammalian oncogenes Ha-*ras*, Ki-*ras*, and N-*ras*, extensively studied for their role in cellular proliferation (for review, see Barbacid, 1987), encode three highly homologous proteins of the same molecular weight (21 kD), sharing structural and biochemical properties with the α subunits of guanine nucleotide binding regulatory proteins (G proteins), in particular the binding and hydrolysis of GTP. Like NGF added extracellularly, cytoplasmic microinjection of Ha-ras p21 protein induced fiber outgrowth in PC12 cells (Bar-Sagi and Feramisco, 1985). In addition, microinjected function-blocking anti-ras antibodies were able to prevent NGF-induced fiber outgrowth in PC12 cells (Hagag et al., 1986). Recently, results have been obtained with dominant-negative mutants of ras that suggest a role of ras p21 in the signal transduction not only of NGF, but also of EGF and FGF in PC12 cells (for review, see Chao, 1992).

Little information is yet available as to the nature of the signal transduction mechanism for neurotrophins in primary neurons. We have previously reported that ras p21 protein, when introduced into the cytoplasm of chick embryonic neurons from the nodose, ciliary, and dorsal root ganglia (DRG), can promote their survival and fiber outgrowth, mimicking the in vitro effects of NGF, BDNF, and CNTF added extracellularly (Borasio et al., 1989). We have further shown that, unexpectedly, the protein kinase inhibitor K252a, which specifically blocks NGF action on PC12 cells by inhibiting the tyrosine kinase activity of the *trk*-receptor (Ohmichi et al., 1992; Tapley et al., 1992), promotes on its own the survival of chick sensory neurons, while it inhibits the NGF-induced survival of chick sympathetic neurons (Borasio, 1990). To elucidate the role of ras p21 in neurotrophic signal transduction, we have blocked the endogenous ras activity by introducing function-blocking anti-ras antibodies (or their Fab fragments) into cultured chick embryonic neurons. The results obtained suggest that ras p21 is essential for neurotrophic signal transduction in sensory, but not sympathetic or ciliary neurons, pointing to the existence of multiple intracellular signaling pathways.

Materials and Methods

Materials

NGF and BDNF were purified as described (Hofer and Barde, 1988) and CNTF was purified from adult rat sciatic nerve as in Stöckli et al. (1989). Plastic dishes for pre-plating were from Nunc (Wiesbaden, Germany). Petriperm dishes for final plating were from Heraeus, F14 medium was from GIBCO BRL (Gaithersburg, MD), horse serum from Boehringer Mannheim (Mannheim, Germany), laminin from BRL. All other reagents were from Sigma Chemie (München, Germany). ras p21 was produced in E. coli and purified as described (Tucker et al., 1986).

Cell Culture and Microscopy

Chick embryonic DRG neurons (E9), sympathetic neurons (E12), ciliary ganglion neurons (E8), and nodose ganglion neurons (E12) were isolated from the corresponding ganglia at the indicated ages and cultured using previously described methods (Lindsay et al., 1985; Hughes et al., 1988). After trypsinization and dissociation (see below), the cell suspension was preplated as described (Lindsay et al., 1985). The neuron-enriched cell suspensions were plated on Heraeus Petriperm dishes with Flexiperm divisions as described (Borasio et al., 1989). The cell density was ~5,000 cells/1.7-cm well.

Immediately after plating, survival factors were added to the appropriate wells at the following final concentrations: NGF 10 ng/ml; BDNF 10 ng/ml; and CNTF 5 ng/ml. Culture conditions and survival determinations were as described (Borasio et al., 1989). All data shown represent mean ±SD unless otherwise indicated. Each experiment was performed at least three times with essentially identical results (for details see figure legends). Cell counting was done on an inverted microscope (model ICM 405; Carl Zeiss, Oberkochen, Germany) using phase contrast objectives with high numerical opening, at a magnification of 320.

Trituration and Microinjection

Microinjection was performed as described (Graessmann et al., 1980) using an Eppendorf/Zeiss microinjection system with pre-pulled capillaries. Trituration of the Fab fragments into the neuronal cytoplasm was performed as described (Borasio et al., 1989) with the following modification: instead of flushing the trypsinized ganglia through a siliconized, fire-polished Pasteur pipette, we used a Gilson yellow tip connected by an adapter to a manual pipettor (10 ml volume, Bel-Art Products, Pequannock, NJ). The yellow tip was pressed against the bottom of the Eppendorf tube during the trituration procedure. This improved the reproducibility of the method, due to the constant diameter of the yellow tips' opening. Efficiency and cell survival were unchanged.

Preparation and Affinity Purification of Anti-ras Fab Fragments

Y13-259 and YA6-172 rat hybridoma cells (Furth et al., 1982) were cultured at 37°C and 10% CO_2 in DME containing 4.5 g/l glucose, 15% FCS, 1 mM sodium pyruvate, and 4 mM L-glutamine. Confluent cultures were washed three times with PBS and cultured in serum-free medium for 4 d. The conditioned medium was then harvested and the antibodies were partially purified and concentrated by consecutive 25 and 50% ammonium sulphate precipitation. The antibody pellet was dialyzed against PBS and diluted to 10 mg/ml. Protein concentration was measured by the Bradford method (Bradford, 1976) using rat IgG as a standard. For papain digestion (Porter, 1959) 1 mM EDTA, 28 mM 2-mercaptoethanol, and 10 μg papain (Fluka Chemie AG, Switzerland)/mg protein were added. The reaction mixture was incubated at 37°C for 60 min and then stopped by addition of 30 mM iodoacetamide. A ras–Sepharose affinity column was prepared by coupling Ha-ras protein to CH-Sepharose (Pharmacia, Freiburg, Germany) following the manufacturer's instructions. 5 mg protein were coupled per 1-ml matrix. The digest was loaded onto the ras–Sepharose column and the Fab fragments were eluted with 100 mM glycine, pH 2.7. The eluate was concentrated and the buffer changed to PBS with Centricon-10 concentrators (Amicon, Beverly, MA) in a Sorvall RC-5B centrifuge. The purity of the preparation as checked by HPLC gel filtration using a 300 × 7.5 Bio-Sil TSK 125 column (Bio Rad Laboratories, Hercules, CA) was found to be >90%.

Biological Activity or Fab Fragments

Y13-259 antibodies and their Fab fragments were introduced into cultured fibroblasts, where ras p21 is known to mediate serum-stimulated DNA synthesis (Mulcahy et al., 1985). Both the antibodies and the Fab fragments blocked serum-induced DNA synthesis in a dose-dependent fashion. This effect could be reversed by addition of COOH-terminally truncated (i.e., biologically inactive) ras p21 immediately before injection, thus saturating the epitope-binding sites (Roden, R., and J. Metcalfe, personal communication). In PC12 cells, microinjection of anti-ras antibodies or Fab fragments blocked NGF-induced neurite outgrowth, in accordance with previous results (Hagag et al., 1986) (data not shown).

Results

Cytoplasmic Delivery of ras p21 and Anti-ras Antibodies and Fab Fragments

The uncleaved anti-ras antibodies were introduced into E12 nodose ganglion neurons by injection via glass microcapillaries. Injection efficiency was monitored by coinjection of

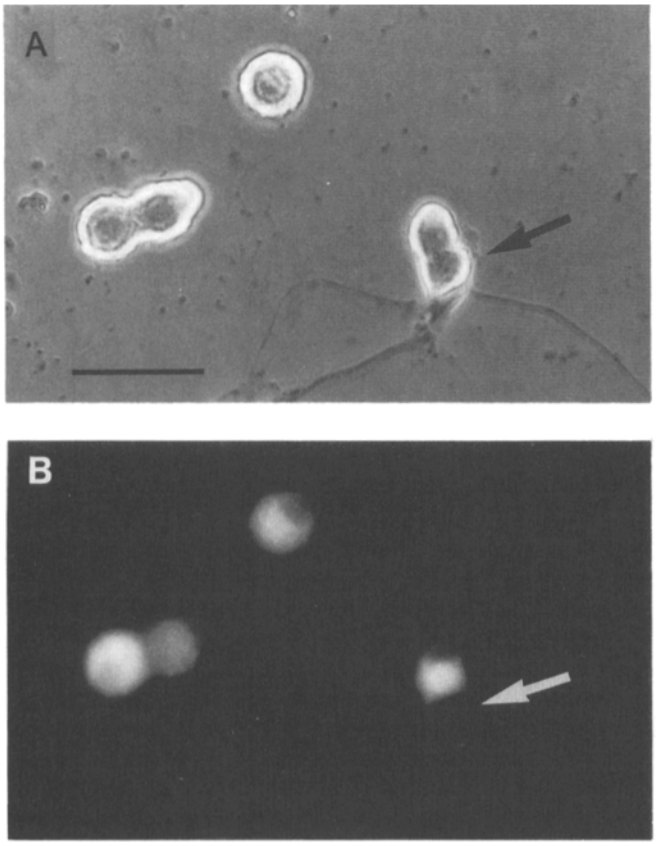


Figure 1. Nodose ganglion neurons after microinjection. E12 nodose ganglion neurons are shown 18 h after microinjection with Y13-259 antibodies and rhodamine-coupled dextrane (70 kD). (*A*) Phase contrast; (*B*) fluorescence micrographs. Note that the only neuron with neurite outgrowth is the noninjected one (*arrow*). Nuclear sparing indicates successful cytoplasmic injection and good cell viability. Bar, 50 μm.

fluorescent dextrane (70 kD), as shown in Fig. 1. To conveniently introduce antibodies into a large number of neurons, we then turned to the trituration method, which had already been successfully used with these neurons (Borasio et al., 1989). The method makes use of the mechanical damage to the neuronal and axonal membranes which arises during trituration of ganglia to obtain single cells. The efficiency of the trituration method is dependent on the size of the molecule to be introduced. While ras p21 (21 kD) entered almost 100% of the cells (Borasio et al., 1989), only very low efficiences (~5%) could be obtained with IgG antibodies (150 kD). Fab fragments (50 kD) showed a trituration efficiency of ~90% (data not shown). Their intracellular localization was confirmed using confocal microscopy (Fig. 2). Thus, Fab fragments can be easily and reliably introduced into a large number of cultured neurons using the trituration method.

Inhibition of BDNF Activity by Anti-ras Antibodies in E12 Nodose Ganglion Neurons

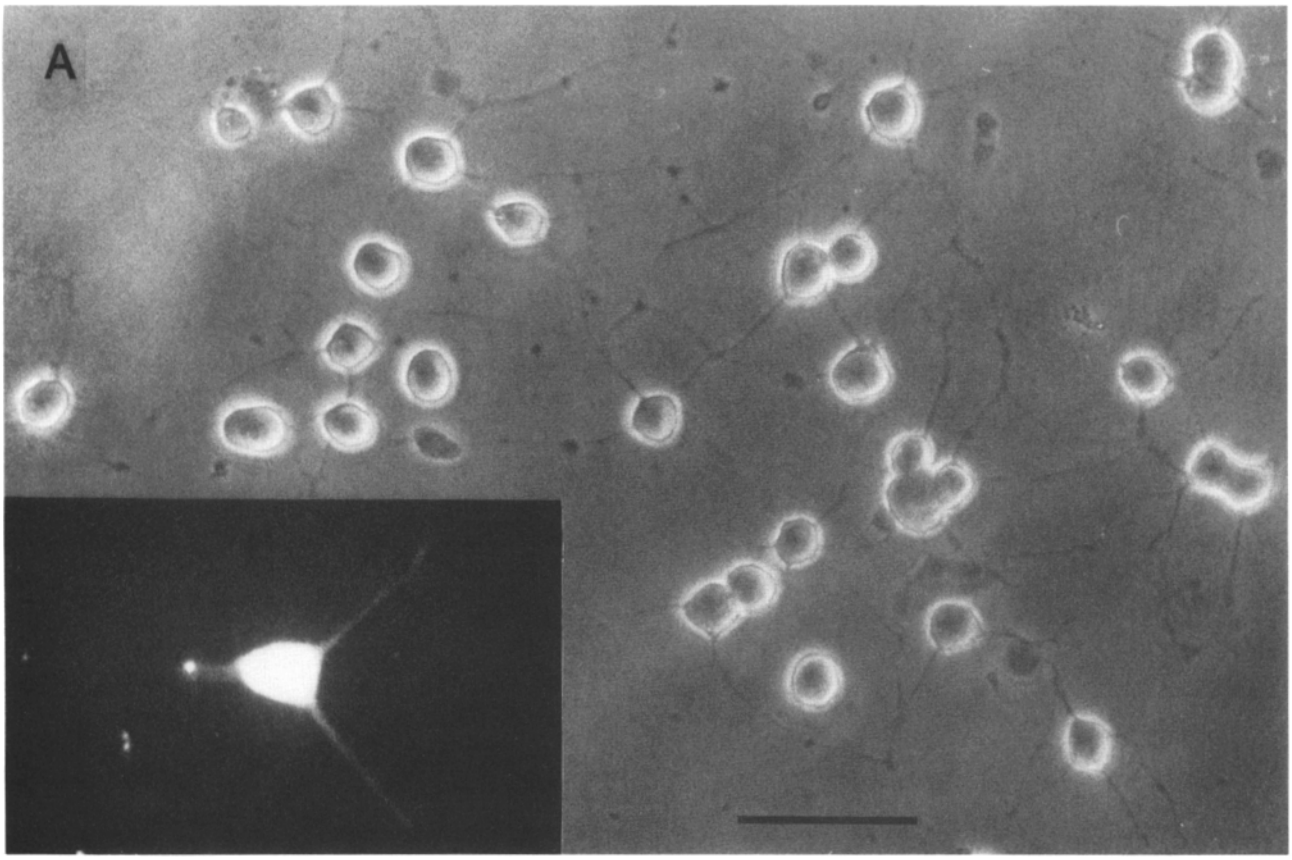
When cultured at low density, E12 nodose ganglion neurons of the chick embryo are dependent on BDNF for fiber outgrowth but not survival in vitro (Lindsay et al., 1985). The neurons were cultured as described (Borasio et al., 1989) and plated on laminin-coated dishes. 1 h after plating, the neurons were microinjected with Y13-259 anti-ras mAb solution at different concentrations using glass microcapillaries as described (Graessmann et al., 1980). To visualize the injected cells, rhodamine-labeled dextrane (70 kD) was co-injected. Only neurons which survived for >54 h after the injection were counted (Fig. 3). The results show a reduction of the BDNF-induced neurite outgrowth to levels slightly below negative control 18 h after microinjection of Y13-259 antibodies at 30 mg/ml. Reduction of the antibody concentration to 10 mg/ml yielded a markedly reduced inhibition. Saturation of the epitope-binding sites by addition of double equimolar amounts of biologically inactive, COOH-terminally truncated ras p21 (termed T′-ras) before injection completely reversed the inhibitory effect of the anti-ras antibodies. The antibody-mediated inhibition of neurite outgrowth was reversible with time, probably due to intracellular proteolysis, and was undetectable 54 h after plating. Naive rat IgG had no effect in this system (data not shown). These results show that microinjection of anti-ras antibodies into E12 nodose ganglion neurons temporarily blocks BDNF-induced neurite outgrowth in these cells.

Inhibition of NGF Activity by Anti-ras Fab Fragments in E9 DRG Neurons

Neural crest-derived sensory neurons from the DRG of chick embryos survive and extend processes in culture in the presence of NGF, whereas they die rapidly in its absence (Levi-Montalcini and Angeletti, 1963). We have previously shown a dose-dependent survival effect of oncogenic and proto-oncogenic ras p21 in these neurons (Borasio et al., 1989). To determine whether the endogenous ras p21 is part of the NGF signal transduction cascade, we triturated affinity-purified, function-blocking anti-ras Fab fragments at various concentrations into E9 DRG neurons. As shown in Fig. 4, the Fab fragments inhibited NGF-induced survival in a dose-dependent fashion. However, ~30% of the DRG neurons survived even after trituration with very high Fab concentrations (50 mg/ml). The Fab effect could be reversed by addition of equimolar amounts of truncated ras p21 before trituration. The survival effect of the protein kinase inhibitor K252a (Borasio, 1990) was not affected by the Fab fragments. Affinity-purified rat IgG and anti-ras YA6-172 Fab fragments showed no significant reduction in neuronal survival. The rat monoclonal antibody YA6-172 binds to ras p21 (Furth et al., 1982), but does not block its biological activity (Kung et al., 1986). Thus, anti-ras Fab fragments show a specific and dose-dependent reduction of NGF-induced survival in E9 DRG neurons.

No Effect of ras p21 or Anti-ras Fab Fragments in E12 Sympathetic Neurons

E12 sympathetic neurons of the chick embryo are fully dependent on NGF for survival, both in vitro and in vivo (Cohen, 1960; Levi-Montalcini and Angeletti, 1963). We first attempted to induce NGF-independent survival in sympathetic neurons by trituration with oncogenic ras p21. Surprisingly, no survival effect could be detected even at p21 concentrations as high as 30 mg/ml (Fig. 5) or 60 mg/ml (not shown). Conversely and in line with these results, anti-ras Fab fragments were not able to block survival induced by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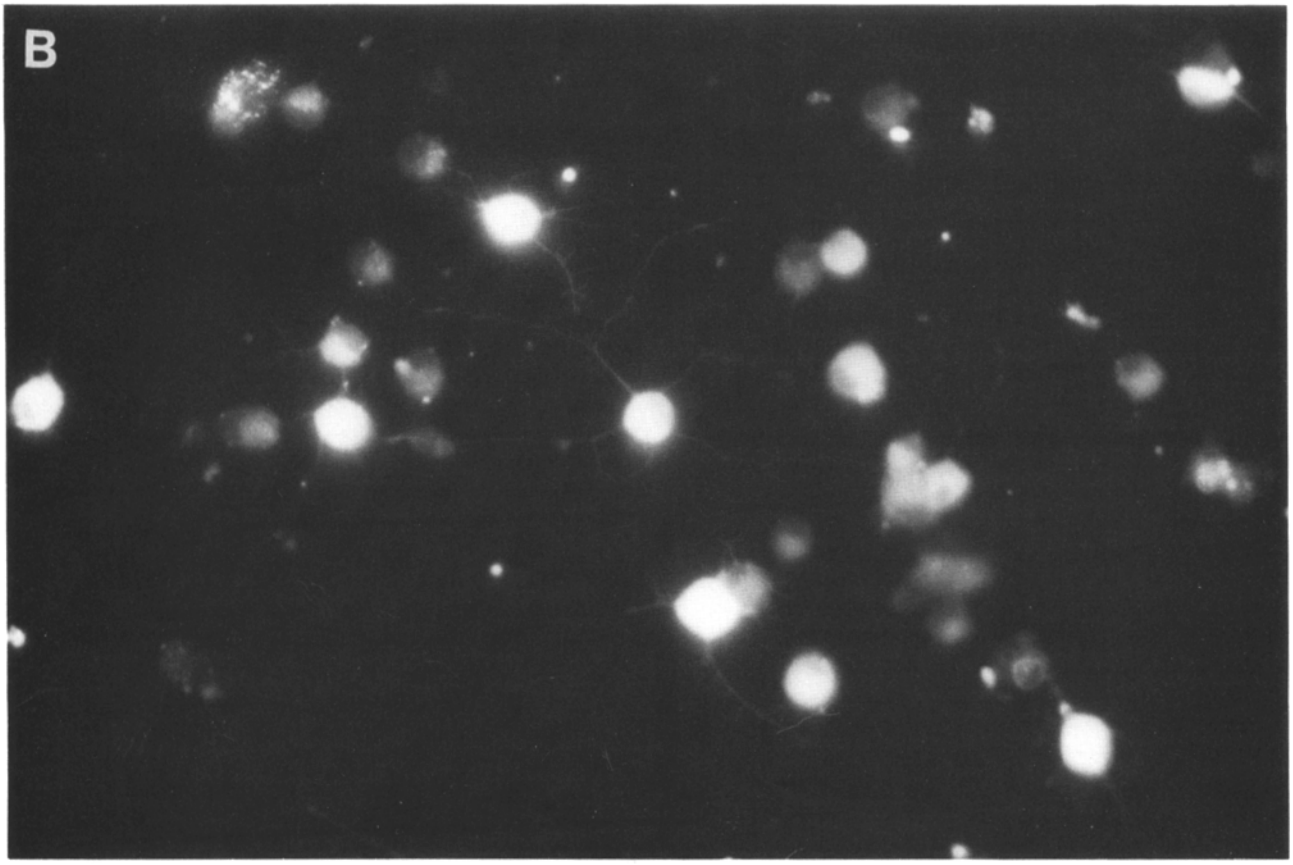
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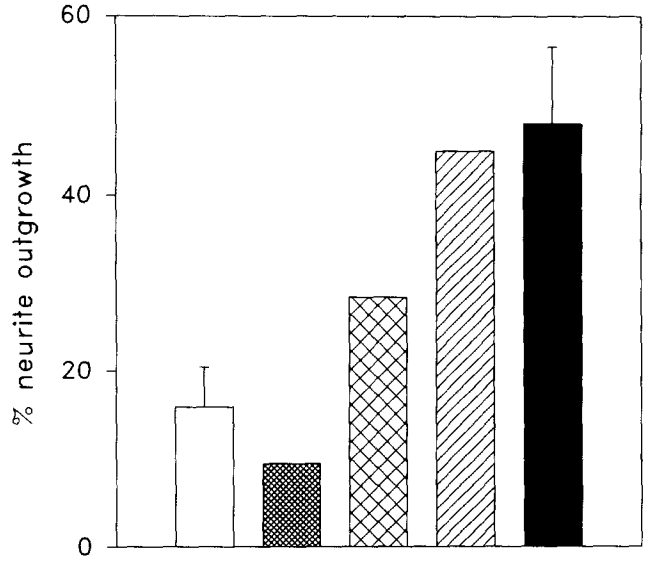

Figure 3. Antibody microinjection in nodose ganglion neurons. E12 nodose ganglion neurons were cultured on laminin-coated dishes as described in Materials and Methods in the absence (□) or presence (all other bars) of BDNF (10 ng/ml). Each experimental bar represents a group of at least 100 cells which were microinjected 1 h after plating with the following substances: function-blocking anti-ras antibody Y13-259 at 30 mg/ml (▩) the same antibody at 10 mg/ml (▨); and the same antibody at 10 mg/ml plus an equimolar amount of biologically inactive, COOH-terminally truncated ras p21, termed T' (▨). Neurite outgrowth (>4× cell diameter) was determined after 18 h in culture. Values for the control bars represent mean ± SD of triplicate determinations. Shown is one of four experiments. The antibody effect was reversible after 54 h (not shown).

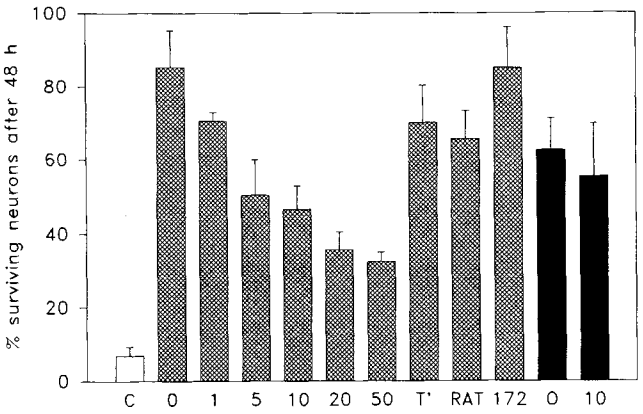

Figure 4. Fab trituration in DRG neurons. E9 DRG neurons were cultured on laminin-coated dishes in the absence (□) or presence (▨) of 10 ng/ml NGF, or 1 μm K252a (■). Trituration was performed with the following substances, as indicated below the bars: *C* and *0*, buffer only; *1* to *50*, function-blocking anti-ras Fab fragments at the corresponding concentration (mg/ml); *T'*, function-blocking anti-ras Fab fragments (10 mg/ml) plus an equimolar amount of biologically inactive, COOH-terminally truncated ras p21 (T'-ras) to saturate the epitope-binding sites; *RAT*, nonspecific Fab fragments from rat IgG (26 mg/ml); and *172*; nonfunction-blocking anti-ras Fab fragments (17 mg/ml). Neuronal survival was determined after 48 h in culture as described in Materials and Methods. The data shown are pooled from seven separate experiments. Survival values are given as mean ± SD. The number of experiments (e) and independent determinations (n) for each assay condition are as follows (e/n): *C*, e = 3/n = 7; NGF-treated cells. *0*, 5/19; *1*, 2/7; *5*, 2/6; *10*, 6/22; *20*, 2/6; *50*, 3/9; *T'*, 3/11; *RAT*, 2/8; *172*, 1/4; K252a-treated cells: *0*, 3/12; *10*, 2/9.

NGF in these cells (Fig. 6). This was not due to a lack of trituration efficiency, since experiments with rhodamine-labeled ras p21 and anti-ras Fab fragments showed that >99% of the neurons were loaded with ras p21 and 90% with Fab fragments after trituration (not shown). In a parallel experiment, DRG neurons triturated with ras p21 showed the known survival response (Fig. 5; Borasio et al., 1989). Taken together, these results show an unexpected and impressive difference in the response of sympathetic compared with DRG neurons to ras p21 as well as in the inhibition of NGF-induced survival by anti-ras Fab fragments.

No Effect of Anti-ras Fab Fragments on E8 Ciliary Neurons

E8 ciliary neurons do not respond to NGF or BDNF, but can be kept alive in culture by CNTF (Barbin et al., 1984). Survival induced in these cells by trituration with ras p21 was indistinguishable from the CNTF-induced one (Borasio et al., 1989). Interestingly, trituration with anti-ras Fab fragments at 20 mg/ml resulted in no appreciable reduction of CNTF-induced survival (Fig. 7). Thus, intracellular ras activity does not seem to be essential for CNTF signal transduction in vitro.

Discussion

The mechanisms transducing the survival effects of neurotrophic factors such as NGF on their physiological target cells, namely neurons, remain largely unknown. In a previous study, we showed that ras p21 can mimic the effects of BDNF, NGF, and CNTF on their respective target neurons in vitro (Borasio et al., 1989). In the present study, we tested the hypothesis that endogenous ras p21 protein, which is expressed at high levels throughout the peripheral and central nervous system (Furth et al., 1987; Klinz, 1989), is required for signal transduction by neurotrophic factors. This appeared all the more interesting since membrane tyrosine kinases are components of the neurotrophin receptors and are likely to be involved in neurotrophin-mediated neuronal survival. Recently, receptor tyrosine kinases have been demonstrated to be linked to biological events other than cell divi-

Figure 2. Chick embryonic neurons after Fab trituration. E9 DRG neurons were loaded by trituration with rhodamine-coupled anti-ras Fab fragments and cultured on laminin-coated dishes as described in Materials and Methods. The trituration efficiency is ~90%. (*A*) Phase contrast; (*B* and *A*, *inset*) fluorescence micrographs. (*A*, *inset*) Fab-loaded neuron as seen with a Bio-Rad confocal laser scanning microscope. The intracellular location of the Fab fragments is confirmed, since the thickness of the optical section visualized here is <1 μm. Bar: (*A* and *B*) 50 μm; (*A*, *inset*) 25 μm.

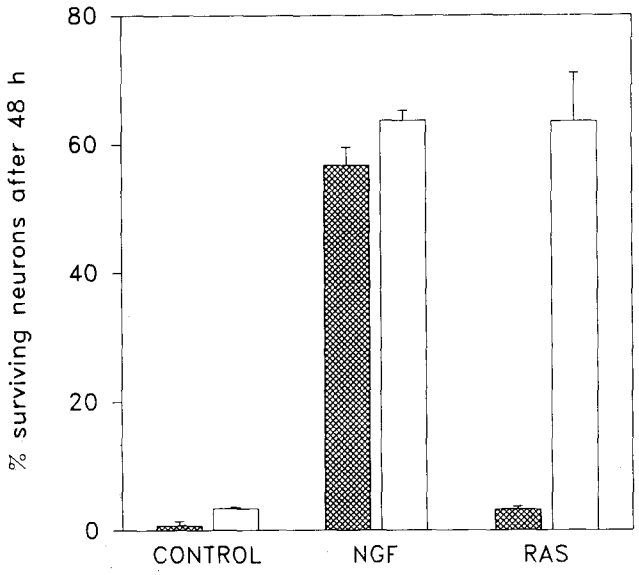

Figure 5. Differential effects of ras p21 on DRG vs. sympathetic neurons. E12 sympathetic and E9 DRG neurons were triturated in the presence of ras p21 (30 mg/ml) as described (Borasio et al., 1989) and cultured on laminin-coated dishes. Neuronal survival was determined after 48 h in culture. Survival values are given as mean ± SD of triplicate determinations. In contrast to DRG neurons, no survival effect could be detected with sympathetic neurons, even with ras p21 concentrations as high as 60 mg/ml (not shown). Shown is one of three experiments. (▩) SG; (□) DRG.

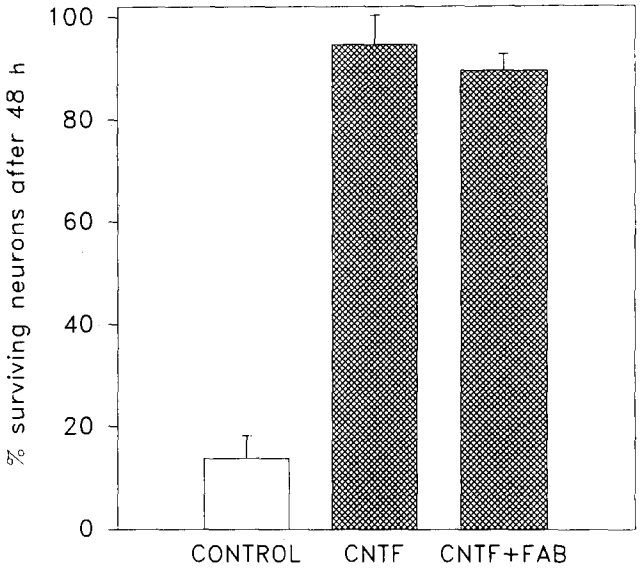

Figure 7. No effect of anti-ras Fab fragments on ciliary neurons. E8 ciliary ganglion neurons were cultured on laminin-coated dishes in the absence (□) or presence (▩) of 5 ng/ml CNTF. Neuronal survival was determined after 48 h in culture. Although ciliary neurons can be kept alive by ras p21, no reduction in CNTF-induced survival was observed after trituration with 20 mg/ml anti-ras Fab fragments. Values represent mean ± SD of quadruplicate determinations. Shown is one of three experiments.

sion. One of the clearest demonstrations of this has been with the *Drosophila* gene *sevenless* that codes for a membrane protein tyrosine kinase, Sev (Hafen et al., 1987). The inactivation of this gene leads to the transformation of a photoreceptor (R7) precursor cell into a cone cell. Relevant to the present work is the observation that reduction of the activity of the *Drosophila* ras equivalent, termed Ras1, impairs Sev-mediated signaling (Simon et al., 1992), and that activated Ras1 allows R7 precursors to become R7 photoreceptors in *sev* null mutants (Fortini et al., 1992). The results obtained in the present study strongly support the idea of a necessary involvement of ras in BDNF-mediated fiber outgrowth using sensory neurons isolated from the ganglion nodosum, as well as in NGF-mediated survival using sensory neurons isolated from E9 DRG. The purified, ras-inactivating Fab fragments introduced into the latter cells showed a specific and dose-dependent reduction in NGF-induced survival. However, we note that ~30% of the neurons survived even at very high Fab concentrations. This might be due to incomplete trituration efficiency, a possibility that is supported by the variation in the fluorescence intensity between cells in the control experiments (Fig. 2). The possibility also exists of a ras-independent pathway, which can be activated only in a subpopulation of NGF-responsive DRG neurons. While the existence of both a ras-dependent and -independent pathway for NGF signal transduction has been postulated for PC12 cells (Qiu et al., 1991), various lines of evidence using anti-ras antibodies (Hagag et al., 1986) or a dominant inhibitory ras mutant (Szeberényi et al., 1990; Thomas et al., 1992) argue in favor of an essential role for ras in NGF signal transduction in PC12 cells. While these somewhat contradictory results might reflect differences between the PC12 sublines studied, the present study demonstrates that different pathways exist in different neuronal populations. Much to our surprise, we found that sympathetic neurons, long known to be NGF dependent for survival in vitro (see Levi-Montalcini and Angeletii, 1963), cannot be rescued by ras, nor can the survival effects medi-

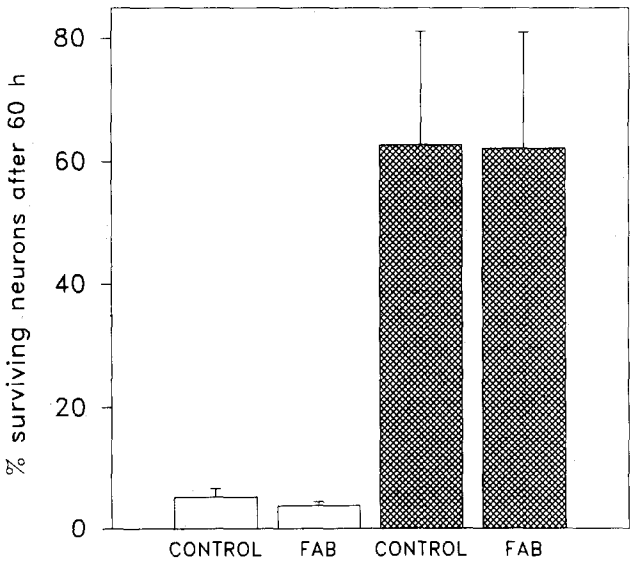

Figure 6. No effect of anti-ras Fab fragments on sympathetic neurons. Trituration of E12 sympathetic neurons with anti-ras Fab fragments at 20 mg/ml yielded no appreciable change in survival after 60 h in culture in the absence (□, n = 3) or presence (▩, *n* = 8) of 10 ng/ml NGF. The survival values shown are pooled from two separate experiments and represent mean ± SD. Survival induced by high K^+ was also unaffected by the Fab fragments (not shown).

ated by NGF be blocked by antibodies to ras. This points to some major differences in the signaling pathways operating in these neurons compared with sensory neurons. In fact, in previous reports, indications of such differences can be found. For instance, sympathetic but not sensory neurons can be kept alive in culture by high K^+ concentrations (Wakade et al., 1983) and the protein kinase inhibitor K252a (shown to block NGF action on PC12 cells by inhibiting *trk* tyrosine kinase) (Ohmichi et al., 1991; Tapley et al., 1992), inhibits NGF-induced survival of sympathetic neurons, but promotes survival of DRG neurons in culture (Borasio, 1990). With regard to the differences in the involvement of ras between sensory and sympathetic neurons, it is interesting to note that the targeted disruption of the low affinity NGF receptor gene in transgenic mice profoundly affects the development of sensory, but not that of sympathetic neurons (Lee et al., 1992). Possibly, a link exists between the low affinity NGF receptor and ras that is functionally more significant or critical in sensory than in sympathetic neurons.

The problem of specificity in signal transduction, with two or more factors leading to divergent cellular reactions (e.g., proliferation vs. differentiation) by eliciting very similar cellular responses, has recently been discussed in detail with regard to PC12 cells (Chao, 1992). Our results demonstrate that one single factor, NGF, can activate different pathways in different physiological target cells to elicit the same cellular reaction, i.e., neuronal survival.

Ciliary neurons do not respond in vitro to any known member of the neurotrophin family. Accordingly, a recently characterized receptor subunit for CNTF bears resemblance to certain types of cytokine receptors (IL-6 in particular) but not to the high or low affinity neurotrophin receptors (Davis et al., 1991). Since anti-ras Fab fragments do not block CNTF-induced survival in ciliary neurons, a ras-independent pathway has to be postulated for the action of CNTF on these cells. However, since ras itself has been shown to promote survival of ciliary neurons, the existence of an intracellular ras-responsive pathway can be envisaged in these cells. Whether this pathway is of physiological significance or merely exists as a redundant "salvage" pathway is unclear at present.

In summary, ras p21 is involved in the transduction of neurotrophin-induced responses in sensory, but not sympathetic neurons, suggesting the existence of multiple pathways for neurotrophic signal transduction in neurons. Future experiments using primary cells and in vivo systems should provide new insights into the complex array of intracellular responses elicited by neurotrophic factors in their physiological target cells.

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