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Rapid Communication

Members of Several Gene Families Influence Survival of Rat Motoneurons In Vitro and In Vivo

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The survival and functional maintenance of spinal motoneurons, both during the period of developmental cell death and in adulthood, have been shown to be dependent on trophic factors. In vitro experiments have previously been used to identify several survival factors for motoneurons, including CNTF, LIF, and members of the neurotrophin, FGF, and IGF gene families. Some of these factors have also been shown to be active in vivo, either on chick motoneurons during embryonic development or on lesioned facial and spinal motoneurons of the newborn rat. Here we demonstrate that lesioned newborn rat facial motoneurons can be rescued by NT-4/5, IGF-I, and LIF. Furthermore, in contrast to chick motoneurons, the survival of isolated embryonic rat motoneurons can be maintained by the neurotrophins BDNF, NT-3, and NT-4/5. IGF-I and FGF-5 were also active in this system, each supporting more than 50% of the originally plated neurons. The responsiveness of motoneurons to multiple factors in vitro and in vivo suggests that motoneuron survival and function are regulated by the coordinated actions of members of different gene families. © 1993 Wiley-Liss, Inc.

Key words: immunopanning, facial nerve transection, neurotrophin, fibroblast growth factor, insulin-like growth factor

INTRODUCTION

Naturally occurring cell death during embryonic development is a feature of many cell types (for review, see Raff, 1992). This phenomenon has been particularly well characterized for a variety of neuronal populations (for review, see Oppenheim, 1991). In a number of instances, the survival of developing neurons during this critical period has been shown to be dependent on specific factors supplied by target tissues (for review, see Barde, 1989). The same or other factors might be involved in the regulation of additional parameters of neuronal function, such as modulation of transmitter and synaptic plasticity (for reviews, see Rao and Landis, 1993; Patterson and Nawa, 1993), and promotion of regeneration after nerve damage (Heumann et al., 1987; Caroni and Grandes, 1990; Meyer et al., 1992; Gurney et al., 1992; Sendtner et al., 1992a; Ip et al., 1993a). The molecular identification of the factors responsible for these processes can be expected not only to yield valuable information regarding the physiology of neuronal survival, but also to pave the way for novel therapeutic methods for the treatment of degenerative diseases of the nervous system.

Spinal motoneurons have long been a focus of attention in neurobiology. In particular, the role of the motoneuron target (i.e., skeletal muscle) in maintaining the survival of these neurons during development is well established (Hamburger, 1958; Hollyday and Hamburger, 1976). However, spinal motoneurons also come into contact with different cell types, including other central nervous system (CNS) neurons, peripheral nervous system (PNS) neurons, and both central and peripheral glial cells. In the last decade, it has become apparent that these other cell types, in particular glial cells, are also capable of exerting trophic effects on motoneurons (Eagleson et al., 1985; Eagleson and Bennett, 1986). Because motoneurons are potentially subject to complex "social controls" (Raff, 1992) from several distinct cellular sources, the precise trophic requirements of spinal motoneurons in vivo are only beginning to be understood on a molecular level.

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In an attempt to define factors involved in the trophic support of motoneurons, we investigated the responses of rat motoneurons to a variety of known trophic and growth factors in vitro and in vivo. Like chick motoneurons (Arakawa et al., 1990; Hughes et al., 1993), the survival of cultured motoneurons prepared by an immunopanning technique was found to be supported by basic FGF (bFGF), FGF-5, and IFG-I. Notably, IGF-I was considerably more effective in promoting rat motoneuron survival in serum free medium than has been reported for cultured chick neurons. Furthermore, the neurotrophins BDNF, and to a lesser extent NT-3 and NT-4, supported the survival of cultured rat motoneurons, even though chick motoneurons were known not to respond to these factors. Like CNTF and BDNF (Sendtner et al., 1990, 1992a), IGF-I, NT-4, and leukemia inhibitory factor (LIF) were also active in reducing the loss of facial motoneurons in the newborn rat following nerve transection in vivo, whereas under the same experimental conditions, bFGF and FGF-5 were inactive.

MATERIALS AND METHODS

Materials

MC192 hybridoma cells, producing monoclonal antibody to the low affinity nerve growth factor receptor, were obtained from M. Hosang, then of Basel, Switzerland. Recombinant human FGF-5, rat bFGF, and rat LIF were kind gifts from M. Goldfarb, New York, USA; W. Risau, Martinsried, Germany; and M. Wrann, Sandoz, Vienna, Austria, respectively. Recombinant rat BDNF, rat NT-3 and *Xenopus* NT-4 were generously supplied by R. Kolbeck, J. Stubbusch, and Y.-A. Barde, Martinsried, Germany. Recombinant rat CNTF was purified from *E. coli* inclusion bodies as described (Hughes et al., 1993). NGF purified from mouse salivary glands was obtained from D. Lindholm, Martinsried, Germany. Sheep anti-mouse affinity purified IgG and recombinant mouse IGF-I were gifts from K. Naujoks, Boehringer Mannheim, Germany. Leibovitz's L-15 medium was obtained from Gibco BRL, Germany. Gel foam (Spongostan) was a kind gift from K. Unsicker, Marburg, Germany. Other reagents used were obtained from Sigma, Deisenhofen, Germany.

Embryonic Rat Motoneuron Cultures

Cultures of E15 rat motoneurons were prepared by an immunopanning technique similar to that described by Camu and Henderson (1992). Briefly, ventral parts of E15 rat spinal cords were dissected, treated with trypsin, and triturated, and the resultant single cell suspension transferred to a polystyrene petri dish. The dish had previously been coated with affinity purified, sheep-anti-mouse IgG (1 μg/ml) and subsequently MC192 hybridoma supernatant (diluted 1:5 with PBS). After 1 hr, non-adherent cells were removed by washing the plate, and a motoneuron-enriched cell fraction eluted by adding excess supernatant. Motoneurons prepared in this way were plated at a density of 2,000–3,000 cells per well in a serum free medium (bicarbonate buffered L-15 medium supplemented with transferrin, 100 μg/ml, progesterone 20 nM, putrescine 100 μM, sodium selenite 30 nM, and insulin 5 μg/ml (Bottenstein and Sato, 1979), in 4-well tissue culture dishes coated with poly-ornithine and laminin as described previously (Arakawa et al., 1990). After incubation for 1 hr at 37°C in an atmosphere containing 5% CO_2, neurotrophic and growth factors were added. Culture medium and factors were renewed after 24 hr in cultures and thereafter every 48 hr. Total number of cells plated per well was determined four hours after plating of the neurons. Surviving neurons in cultures were counted after 5 days in culture in predetermined fields corresponding to a total of 23% of the area of each well. Results are presented as a percentage of the originally plated cell number.

Assay of Choline Acetyltransferase (ChAT) Activity

Aliquots (between 1×10^4 and 8×10^5 cells) of total cells from dissociated ventral spinal cord and cells obtained by immunopanning as described above were assayed for ChAT activity as described (Fonnum, 1975). Relative ChAT activity was standardized for 1×10^6 cells.

Facial Nerve Lesion Studies

In vivo effects of putative trophic factors for motoneurons were assayed in the newborn rat facial nerve lesion model as described (Sendtner et al., 1990). Briefly, newborn rats were anaesthetized by hypothermia, and the right facial nerve exposed, freed of connective tissue and transected. Gel foam soaked in 30 μl of PBS containing the factor to be analysed was inserted at the site of lesion, and the skin sutured. After 7 days, the rats were killed by ether overdose, and perfused transcardially with 4% paraformaldehyde (50 ml). Brainstems were dissected and prepared for serial sections (7 μm). The sections were stained with cresyl violet, and facial motoneurons with a clearly identifiable nucleus and nucleolus were counted on both sides at a magnification of 125× in every fifth section as previously described (Sendtner et al., 1990). The counts were not corrected for split nucleoli.

RESULTS

Culture of Embryonic Rat Motoneurons

Cultures of E15 rat spinal motoneurons were prepared with an immunopanning technique first described

TABLE I. Recovery and ChAT Activity of E15 Rat Spinal Cord Neurons Before and After Panning With Anti-LNGFR Monoclonal Antibody*

	Proportion of total cells obtained(%)	ChAT activity (cpm × 10^{-6} cells)
Before panning		
Ventral spinal cord	100	$5.7 \pm 1.5 \times 10^5$
After panning		
Adherent cells	19 ± 4	$1.2 \pm 0.2 \times 10^6$

*Results are means ±SD of two observations from two independent experiments.

TABLE II. Maximal Effects of Putative Growth and Trophic Factors on the Survival of Cultured Rat Motoneurons Purified by Panning*

Factor	Concentration (ng/ml)	Survival (%)
IGF-I	100	52 ± 5
NGF	100	9 ± 2
BDNF	1	65 ± 4
NT-3	10	36 ± 2
NT-4/5	10	40 ± 3
bFGF	50	40 ± 2
FGF-5	10	53 ± 4
CNTF	100	14 ± 3
LIF	100	11 ± 2
Control	—	10 ± 4

*Results are means ±SD from at least eight independent observations of surviving neurons counted after 5 days in culture.

by Henderson and colleagues (Camu and Henderson, 1992; Henderson et al., 1993). In this technique, motoneurons are selected from dissociated ventral spinal cord with an immobilized monoclonal antibody (MC192) (Chandler et al., 1984) to the low affinity nerve growth factor receptor (p75). The enrichment of motoneuron content following this procedure was assessed by assaying lysates of cell suspensions from E15 rat spinal cord for choline acetyl transferase (ChAT) activity. Putative motoneurons isolated by panning, (19% of cells recovered from the ventral spinal cord), exhibited a 2-fold increase in specific ChAT activity over total ventral spinal cord (Table I). Cells purified by panning were used directly to establish primary cultures of motoneurons.

Effects of Single Factors in Promoting Motoneuron Survival In Vitro and In Vivo

IGF-I. In cultures of E15 rat motoneurons prepared by panning, recombinant mouse IGF-I was found to support maximally 52% of the originally plated neurons after 5 days in culture (Table II), with half maximal survival effects at 500 pg/ml (Fig. 1A). Control cultures incubated without additional factors typically showed 10% background survival after the same time.

The ability of IGF-I to promote motoneuron survival in vivo was determined in the newborn rat facial nerve lesion model (Sendtner et al., 1990). As previously reported (Sendtner et al., 1990, 1992a), control treatments with BSA led to a loss of 81% of motoneurons in the facial nucleus seven days after lesion, compared to the unlesioned side (Table III). In contrast, 29% of motoneurons in the facial nucleus could be rescued by treatment with 10 μg of IGF-I (a dose comparable to that used previously for CNTF, BDNF, and NT-3), representing a significant survival effect ($P = 0.018$) in preventing lesion-induced death in newborn rats.

Neurotrophins. We had previously demonstrated a lack of response of cultured chick spinal motoneurons to members of the neurotrophin gene family (Arakawa et al., 1990). In contrast to this observation, we found that the neurotrophins BDNF, NT-3, and NT-4/5 were effective in promoting embryonic rat motoneuron survival in vitro, supporting maximally 65%, 36%, and 40% of the originally plated neurons, respectively, with half-maximal survival effects at approximately 20 pg/ml (Table II; Fig. 1B). NGF, even at high concentrations (100 ng/ml), failed to increase motoneuron survival above control levels (Table II).

Like BDNF and NT-3 (Sendtner et al., 1992a), NT-4 was also found to support motoneuron survival in vivo. Ten micrograms of recombinant NT-4 applied to the lesioned facial nerve of newborn rats rescued 46% of motoneurons in the facial nucleus (Table III).

Members of the FGF gene family. bFGF and FGF-5 exhibited marked survival effects on cultured rat motoneurons, supporting maximally 40% and 52%, respectively, of the initially plated cells after five days in culture (Table II), with half maximal survival responses being obtained at 50 pg/ml and 30 pg/ml, respectively (Fig. 1C). To ascertain the ability of bFGF and FGF-5 to support motoneuron survival in vivo, the effects of 10 μg of either bFGF or FGF-5 (the latter in combination with 10 μg heparin) were examined in newborn rats after facial nerve lesion. However, despite their effectiveness in vitro, neither bFGF (19% motoneuron survival seven days after lesion) nor FGF-5 (23% survival) were significantly better in preventing the loss of facial motoneuron cell bodies after lesion than control treatment with BSA (19%) (Table III).

CNTF and LIF. Neither CNTF nor the related polyfunctional cytokine LIF exhibited survival effects significantly above control levels in cultures of E15 rat motoneurons (Table II), even though both compounds have been reported to promote motoneuron survival in several different experimental systems (Sendtner et al.,

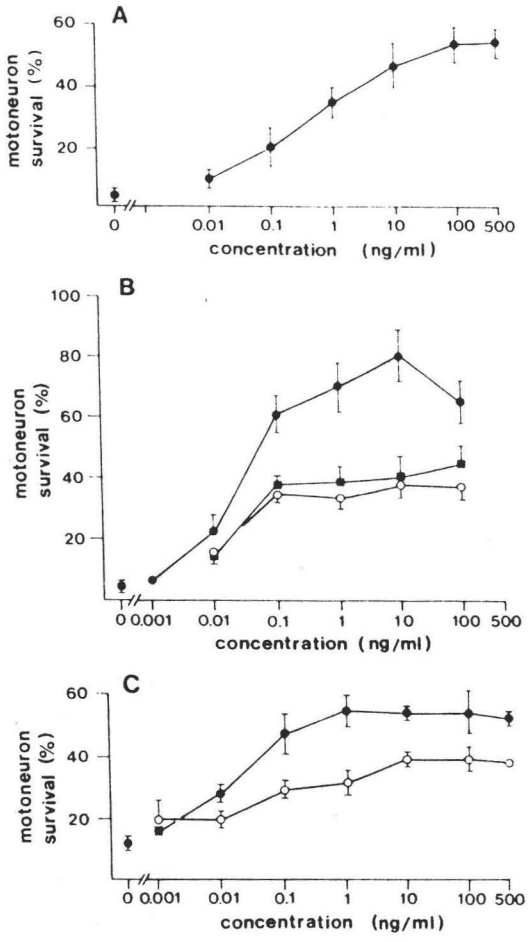

Fig. 1. Effects of recombinant trophic factors on survival of cultured E15 rat spinal neurons. **A:** IGF-I. **B:** The neurotrophins BDNF (closed circles), NT-3 (open circles), and NT-4/5 (closed squares). **C:** Members of the FGF gene family FGF-5 (closed circles) and bFGF (open circles). FGF-5 was assayed in the presence of heparin (500 ng/ml) as described (Hughes et al., 1993). Surviving neurons were counted after five days in culture and are expressed as percentages of the original number of plated neurons. Results are means ±SD of at least four determinations.

1990, 1992b; Arakawa et al., 1990; Oppenheim et al., 1991; Martinou et al., 1992; Henderson et al., 1993). In contrast, recombinant mouse LIF, like CNTF (Sendtner et al., 1990), was able to significantly reduce motoneuron death after lesion in vivo. Ten micrograms of LIF applied to the lesioned facial nerve of neonatal rats maintained the survival of 35% of motoneurons in the facial nucleus compared to the control, unlesioned side (Table III).

Effects of Combinations of Survival Factors for Motoneurons In Vitro

NGF selectively inhibits the survival effect of BDNF. We had previously noted that administration of NGF to newborn rats following facial nerve lesion led to a small increase in motoneuron loss compared to control animals treated with BSA (Sendtner et al., 1992a). To determine whether this effect may be due to the specific interference of NGF with another member of the NGF gene family, we examined the effects of increasing NGF concentrations on rat motoneuron survival in the presence of a fixed concentration of BDNF (Fig. 2). At equimolar concentrations (1 ng/ml), the effect of BDNF on motoneuron survival was unchanged. However, at higher concentrations of NGF (10 ng/ml and 100 ng/ml), the survival effect of BDNF after five days in culture was reduced, significantly ($P = 0.02$) with 100 ng/ml of NGF. This negative effect of NGF on the motoneuron survival activity of BDNF is not due to a general toxic effect of NGF, as NGF alone did not affect control survival levels, nor did it reduce the survival effect of IGF-I (100 ng/ml) (Fig. 2) or FGF-5 (data not shown).

Effects of BDNF, NT-3 and NT-4 on motoneuron survival in vitro are non-additive. Combinations of the three neurotrophins tested with survival activity on cultured motoneurons, BDNF, NT-3, and NT-4, were examined. Singly, these factors supported maximally 66%, 39%, and 45% survival, respectively, after 5 days in culture. Combinations of supramaximal concentrations of pairs of these factors led to motoneuron survival not significantly greater in extent than the most effective member of the pair (Table IV).

The motoneuron survival effect of IGF-I is partially additive to that of BDNF and FGF-5. The effect

TABLE III. Effects of Putative Motoneuron Survival Factors on the Prevention of Motoneuron Cell Death After Facial Nerve Lesion in the Newborn Rat†

Treatment	Number of surviving neurons	
	Unlesioned	Lesioned
BSA n = 7	5,011 ± 128	952 ± 134
IGF-I (10 μg) n = 4	5,331 ± 284	1,549 ± 137*
NT-4/5 (10 μg) n = 5	4,703 ± 293	2,157 ± 352**
bFGF (10 μg) n = 6	4,366 ± 260	851 ± 164
FGF-5 (10 μg) + heparin (10 μg) (n = 5)	5,025 ± 251	1,163 ± 167
LIF (10 μg) n = 4	4,849 ± 145	1,714 ± 180***

†Results are presented as the mean No. of neurons±SEM counted in serial sections of the facial nucleus 7 days after lesion either with the treatment indicated or in the unlesioned (contralateral) side. Significantly different (Student's T-test) from BSA treatment: *$P<0.02$; **$P<0.005$; ***$P<0.01$.

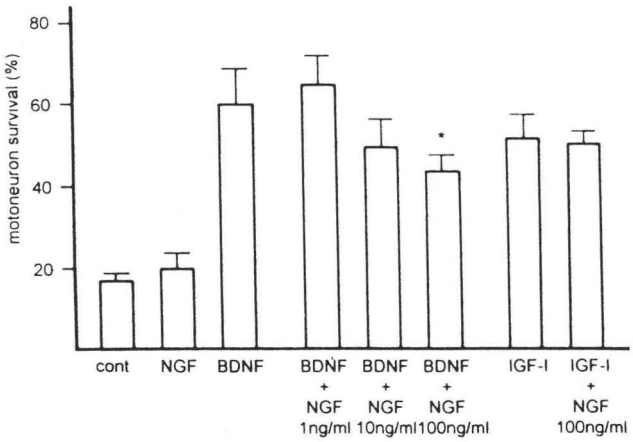

Fig. 2. Effect of NGF on BDNF and IGF-I mediated survival of cultured E15 rat spinal motoneurons. Surviving neurons were counted after 5 days in culture and are expressed as percentages of the original number of plated neurons. Results are means ±SD of at least four determinations. Concentrations used: BDNF, 1 ng/ml; IGF-I, 100 ng/ml; NGF, 100 ng/ml alone, in combination with other factors as indicated. *Significantly different from BDNF alone, $P = 0.02$.

TABLE IV. Effects of Combinations of the Neurotrophins BDNF (1 ng/ml), NT-3 (10 ng/ml), and NT-4/5 (10 ng/ml) on the Survival of E15 Rat Motoneurons After 5 Days in Culture†

Factor	Survival (%)
BDNF	66 ± 2
NT-3	39 ± 2
NT-4/5	45 ± 2
BDNF + NT-3	63 ± 2*
BDNF + NT-4/5	63 ± 2*
NT-3 + NT-4/5	46 ± 4**

†Results were obtained from at least 5 observations from two independent experiments.
*Not significantly different (Student's t-test) from BDNF alone.
**Not significantly different from NT-4/5 alone.

of IGF-I on E15 rat motoneuron survival in vitro was found to be partially additive with the other muscle-derived factors BDNF and FGF-5 (Table V). Thus, the survival effect of BDNF (1 ng/ml) was increased significantly ($P = 0.04$) from 52% to 65% in the presence of IGF-I (100 ng/ml). Similarly, motoneuron survival in vitro in cultures containing FGF-5 was increased ($P = 0.004$) from 52% in the absence of IGF-I to 63% in its presence. The combination of BDNF and FGF-5 failed to elicit a survival response greater than either factor alone (Table V).

TABLE V. Effects of Combinations of BDNF (1 ng/ml), FGF-5 (10 ng/ml), and IGF (100 ng/ml) on the Survival of E15 Rat Motoneurons After 5 Days in Culture†

Factors	Survival (%)
BDNF	52 ± 4
FGF-5	52 ± 2
IGF-I	44 ± 2
BDNF + FGF-5	57 ± 2
BDNF + IGF-I	65 ± 4*
FGF-5 + IGF-I	63 ± 2**

†Results were obtained from at least 6 observations from three independent experiments.
*Significantly different (Student's t-test) from BDNF alone, $P<0.05$.
**Significantly different from FGF-5 alone, $P<0.005$.

DISCUSSION

IGF-I is a polypeptide hormone exerting an array of proliferation and differentiation effects on cells through both autocrine and paracrine mechanisms (for review, see Froesch et al., 1985). The presence of IGF-I in developing muscle (Ralphs et al., 1990), and the ability of IGF-I and the related molecule IGF-II to support the survival of cultured chick motoneurons (Arakawa et al., 1990) and induce sprouting in adult intramuscular nerves (Caroni and Grandes, 1990) raises the possibility that members of the IGF family are specifically involved in the development and maintenance of motor innervation. This hypothesis is supported by our observations that IGF-I is an effective survival factor for cultured embryonic rat motoneurons, and that it prevents lesion-mediated motoneuron death in vivo.

We had previously reported that IGF-I is only a weak survival factor for cultured chick motoneurons, supporting only 15% of the originally plated motoneurons after three days in culture, at a concentration of 1 μg/ml (Arakawa et al., 1990). The reason for the difference to the results described here could lie in the culture media used. Unlike rat motoneurons, which were kept in serum-free medium, chick motoneurons were optimally cultured in the presence of horse serum (10%). The latter has been shown to contain significant amounts of IGF binding proteins (IGFBPs) (Prosser and McLaren, 1992) which can inhibit the biological effects of IGF-I (DeMellow and Baxter, 1988). Although the physiological role of IGFBPs is yet to be established, the coordinated expression of IGFs and IGFBPs in the retina and cerebellum suggests that IGFBPs may act to spatially restrict IGF actions (Lee et al., 1992).

We found that the survival effect of IGF-I on embryonic rat motoneurons in vitro is partially additive to the effects of BDNF and FGF-5, and had previously shown that IGF-I is able to enhance the survival effects of CNTF and bFGF on cultured chick motoneurons (Arakawa et al., 1990). The ability of IGF-I to enhance the survival activity of other factors in vitro suggests that this factor may play a key role in modulating motoneuron survival in vivo. The system resembles the oligodendro-

cyte cell lineage, in which IGF in combination with a member of the neurotrophin family (NT-3) and CNTF (or LIF) has been shown to be necessary for long term oligodendrocyte survival (Barres et al., 1992, 1993).

In comparison to the motoneuron rescue effects of CNTF and BDNF (Sendtner et al., 1990, 1992a), the effect of recombinant IGF-I following facial nerve lesion was small. However, IGF-I has been shown to bind to high affinity receptors on cultured fetal rat brain neurons with a K_D of 1×10^{-9} M (Nielsen et al., 1991), making the affinity of IGF-I for its high affinity binding sites at least an order of magnitude weaker than that of CNTF ($1–10 \times 10^{-11}$ M, Hall and Rao, 1992) and BDNF (1.7×10^{-11} M, Rodríguez-Tébar and Barde, 1988) for their respective high affinity receptors. Thus, the potential maximal effect of IGF-I in preventing motoneuron death after lesion may be greater than that observed.

Our observations that BDNF, NT-3 and NT4/5 support the survival of embryonic rat motoneurons in culture confirm recent studies showing that members of the neurotrophin gene family promote motoneuron survival both in vitro (Henderson et al., 1993) and in vivo (Yan et al., 1992; Oppenheim et al., 1992a; Sendtner et al., 1992a; Koliatsos et al., 1993). mRNA species for BDNF, NT-3, and NT-4/5 have been shown to be present in rat muscle or limb (Hohn et al., 1990; Maisonpierre et al., 1990; Henderson et al., 1993), consistent with a physiological role for these factors in modulating motoneuron survival. Furthermore, NT-3 mRNA has also been detected in spinal motoneurons (Ernfors and Persson, 1991; Schecterson and Bothwell, 1992). Whether BDNF, NT-3, and NT-4/5 each play a specific and concerted role in the support of motoneuron survival and function, or whether they represent a redundant mechanism safeguarding the integrity of a vital system will need to be investigated by other means, such as gene ablation studies.

BDNF and NT-4 are thought to mediate their biological responses by interacting with a common receptor, the product of the proto-oncogene *trk*B (Klein et al., 1991; Soppet, 1991; Squinto et al., 1991; Berkemeier et al., 1991; Klein et al., 1992; Ip et al., 1993b). In contrast, NT-3 is believed to elicit its effects on responsive cells by binding with high affinity to the receptor tyrosine kinase trkC (Lamballe et al., 1991; Ip et al., 1993b). Indeed, mRNA for trkB and trkC has been shown by in situ hybridization studies to be expressed by developing rat motoneurons (Ernfors et al., 1993; Henderson et al., 1993). In an elegant experiment by Henderson and colleagues (1993), retrograde labelling of motoneurons in adult rat combined with in situ hybridisation of spinal cord with specific probes for trkB and trkC showed that the populations of spinal motoneurons expressing trkB and trkC mRNA are almost fully coincident. Thus, the lack of additivity of the survival effect of NT-3 with that of BDNF and NT-4/5 in vitro probably arises because the receptors for BDNF/NT-4/5 and NT-3 are expressed on the same cells, rather than all three factors acting via a common receptor. Competitive binding studies of neurotrophins to their receptors on motoneurons are needed to confirm this hypothesis.

Recently, it was demonstrated that the ability of BDNF and NT-4/5 to enhance the ChAT activity of cultured rat spinal cord neurons enriched for motoneurons by density centrifugation is additive to the ChAT-inducing effect of NT-3 (Wong et al., 1993). These data suggest that the mechanisms by which activation of trkB and trkC lead to ChAT induction in rat motoneurons differ. Whether there is also divergence in the mechanisms of motoneuron survival following activation of trkB and trkC receptors remains to be established.

Both bFGF and FGF-5 have been shown to support the survival of cultured embryonic chick motoneurons (Arakawa et al., 1990; Hughes et al., 1993). bFGF, although present in rat skeletal muscle (Gonzalez et al., 1990), is apparently a cytosolic molecule (Abraham et al., 1986), thus making it unlikely that bFGF is involved in the physiological regulation of cell death. In contrast, the secretory nature of FGF-5 (Zhan et al., 1988; Bates et al., 1991), its major contribution to the survival activity present in extracts of developing and adult skeletal muscle (Hughes et al., 1993), and its effectiveness in supporting embryonic rat motoneuron survival in vitro are consistent with a role for FGF-5 as a target-derived trophic factor for motoneurons.

Paradoxically, neither bFGF nor FGF-5 could prevent lesion-induced facial motoneuron death in newborn rat. It might be that exogenously administered bFGF and FGF-5 are trapped by heparin-like molecules of the extracellular matrix in the vicinity of the lesion site, or that FGFs require the presence of specific, muscle derived, heparan sulfate proteoglycans (HSPGs) to mediate a survival response. Indeed, HSPGs necessary for FGF action and with specificity for either aFGF or bFGF have been shown to be differentially regulated during murine embryonic development (Nurcombe et al., 1993). Such HSPGs might also act to restrict the field of FGF action, in a manner comparable to that proposed for IGFBPs and IGFs (Lee et al., 1992), and explain why the administration of aFGF and bFGF to the chorioallantoic membrane of developing chicks fails to elicit survival effects on neurons (Nurcombe et al., 1991; Oppenheim et al., 1992b).

It is puzzling that CNTF did not support rat motoneuron survival in culture above control levels. Although originally purified to homogeneity from sciatic nerve and cloned on the basis of its ability to support the survival of embryonic chick ciliary neurons in culture (Stöckli et al., 1989; Lin et al., 1989), it has since been

firmly established that CNTF is a survival factor in vitro for a variety of other embryonic *chick* neurons, including sympathetic, nodose and motoneurons (for review, see Sendtner et al., 1991). Nonetheless, a lack of effect of CNTF on *rodent* neurons has precedents: CNTF fails to support the survival of newborn mouse nodose neurons (Barbin et al., 1984), whereas it can support more than 40% of E8 chick nodose neurons for 2 days or longer in culture (Sendtner et al., 1991); and CNTF can only delay the death of newborn rat sympathetic neurons (Sadaat et al., 1989), although virtually 100% of the corresponding neurons from E10 chick can be maintained in culture for extended periods in the presence of CNTF (Manthorpe et al., 1986). Interestingly, this lack of effect of CNTF cannot be explained by the absence of functional CNTF receptors, as cultures of rat sympathetic neurons show an induction of ChAT following CNTF treatment (Sadaat et al., 1989).

Like CNTF, the polyfunctional cytokine LIF also induces ChAT activity in cultured rat sympathetic neurons without supporting their survival (Fukada, 1985; Yamamori et al., 1989; Rao et al., 1990). However, in contrast to our observations, both CNTF and LIF have previously been shown to support embryonic rat motoneuron survival in vitro, in cultures of embryonic rat motoneurons enriched by either by a fluorescence-assisted cell sorting (FACS) method (Martinou et al., 1992) or by a combination of panning and gradient centrifugation (Henderson et al., 1993). These methods would be expected to enrich for specific sub-populations of motoneurons and thus exclude others. Furthermore, presumably due to low overall cell viability, the absolute effects of CNTF and LIF on rat motoneuron survival in cultures prepared by these methods are very low (e.g., LIF supports only 17% of the initially plated motoneurons after FACS purification). The differences in composition and viability of the cultures may explain the absence of a detectable survival effect of CNTF and LIF in our in vitro system.

The effectiveness of CNTF and LIF in preventing motoneuron cell death in vivo suggests that co-factors potentiating the effects of these factors might be present in vivo. Indeed, CNTF has been proposed to support the survival and induce ChAT expression on motoneurons in mixed cultures of E14 rat spinal cord (Magal et al., 1991), where a specific co-factor could be provided by other cells in culture. The ability of LIF to reduce motoneuron loss after lesion in the newborn rat (35% of control) is less than that previously observed for CNTF (76%, Sendtner et al., 1990), in concordance with the lower affinity of LIF for its high affinity receptor (K_D of 400 pM) compared to CNTF (K_D of 10–100 pM) (Hall and Rao, 1992). Unfortunately, the use of greater amounts of recombinant LIF was precluded in this study because of the manifestation of marked toxic effects. A similar phenomenon has been described following systemic administration of LIF (Metcalf and Gearing, 1989).

We had observed that the administration of NGF gives rise to enhanced motoneuron cell death following facial nerve axotomy (Sendtner et al., 1992a). Similarly, Miyata et al. (1986) had reported a significant increase in the loss of motoneuron cell bodies after crush lesion of the medial gastrocnemius nerve of newborn rats after treatment with NGF (28% death after lesion in the absence of NGF, increased to 42% in NGF treated animals). At the concentration of NGF with which we observe a reduction in BDNF-mediated motoneuron survival in vitro, NGF has been shown to begin to compete with BDNF binding to chicken trkB (Dechant et al., 1993). Thus NGF might reduce BDNF mediated motoneuron survival in vitro and in vivo by interfering with the binding of BDNF to trkB. That a neurotrophin might act as a competitive antagonist of other neurotrophins may have consequences in the therapeutic administration of neurotrophins in humans.

The list of factors known to promote motoneuron survival includes proteins derived from muscle (e.g., BDNF, FGF-5, and LIF), Schwann cells (CNTF) and neurons (NT-3), as well as factors present generally in the circulation (e.g., IGF-I). Local fine-tuning of the availability of factors to motoneurons might be controlled by their binding to other molecules, as exemplified by the modulation of IGF-I activity by IGFBPs, and the association of members of the FGF family to specific HSPGs. The observation that combinations of factors act synergistically in vitro supports the notion that interplay between factors, rather than functional redundancy, is a hallmark of the maintenance of motoneuron survival in vivo. Indeed, coordination of the effects of factors may be a general means by which survival and function of cells in the nervous system are regulated.

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