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Stem Cell Factor Is a Neurotrophic Factor for Neural Crest–Derived Chick Sensory Neurons

Josette F. Carnahan, Darshna R. Patel, and James A. Miller

Amgen Center, Neurobiology Department, Thousand Oaks, California 91320

We have found that stem cell factor (SCF) selectively enhances the survival of cultured embryonic chick dorsal root ganglia (DRG) neurons. Neurons grown in the presence of SCF expressed both neurofilament 150 kDa subunit and calcitonin-gene related peptide. SCF does not, however, enhance the survival of parasympathetic, placode-derived sensory or sympathetic neurons in culture. Combining SCF with brain-derived neurotrophic factor or neurotrophin-3, but not with NGF, maintains more neurons than either factor alone, suggesting that these factors have partially overlapping activities. SCF preferentially rescues small neurons from the DRG. Labeling studies with bromodeoxyuridine indicate that the neurons sustained by SCF are not differentiating from a dividing progenitor.

[Key words: stem cell factor, sensory neurons, neurotrophic factor, development, differentiation, chick, hematopoiesis]

Autonomic neurons and melanocytes are derived from a transient embryonic structure, the neural crest (Le Douarin, 1982). The phenotypic diversity generated by crest progenitors arises along and at the end of various migratory pathways. For example, trunk crest cells migrating under the ectoderm differentiate into melanocytes, while those undergoing ventral migration between the neural tube and somites populate the dorsal root ganglion (DRG), the sympathetic ganglia, and the adrenal primordium. Even after crest cells have migrated and coalesced into spinal ganglia, both pigment cells and neurons can be generated from undifferentiated precursors still present in the forming ganglia (Duff et al., 1991). In fact, both *in vivo* lineage tracing experiments (Le Lievre et al., 1980; Bronner-Fraser and Fraser, 1989; Frank and Sanes, 1991) and clonal analysis *in vitro* (Sieber-Blum and Cohen, 1980; Baroffio et al., 1988) have demonstrated the existence of pluripotent progenitors both in the neural crest and in the migrating crest populations. In the chick embryo, by day 4 of gestation (E4), trunkal crest cells have reached both dorsal root sensory and sympathetic ganglia (Weston, 1963). Between E4 and E7, chick DRG contain progenitors whose initial survival is independent of NGF (Ernsberger and Rohrer, 1988). As development proceeds, sensory neuron survival can be enhanced by NGF, brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT3) (Levi-Montalcini and Angeletti, 1968; Barde et al., 1980; Lindsay et al., 1985; Rohrer et al., 1985; Ernfors et al., 1990; Hohn et al., 1990; Maisonpierre et al., 1990).

Stem cell factor (SCF) was recently purified from a rat liver cell line–conditioned medium (Zsebo et al., 1990). Under different names, the same factor was characterized from other sources (Huang et al., 1990; Williams et al., 1990). SCF is the ligand for the receptor (c-*kit*) encoded by the proto-oncogene W (Huang et al., 1990). Mouse mutants lacking functional SCF (Sl) and c-kit (W) share very similar phenotypic defects in three cell lineages (Mayer and Green, 1968; Witte, 1990): germ cell, hematopoiesis, and melanogenesis (Besmer, 1991). Both c-*kit* and SCF are expressed in the nervous system (Matsui et al., 1990; Orr-Urtreger et al., 1990; Keshet et al., 1991; Motro et al., 1991; Morii et al., 1992), raising the possibility that SCF may also act on neurons. Chicken SCF was recently cloned from a chicken brain cDNA library and shown to have 57% homology at the amino acid level with human SCF (F. Martin, personal communication). Here we demonstrate a neurotrophic activity for chicken SCF on chicken DRG neurons *in vitro*.

Materials and Methods

Cell culture. Ganglia were dissected from White Leghorn chick embryos at various ages, cleaned from surrounding tissue, trypsinized, and dissociated as described (Barde et al., 1980); two sequential preplating steps were used to enrich for neuronal cells. Cells were then plated at 1000–2000 cells (high density) or 100–200 cells (low density) per well on eight-well tissue culture chamber slides (Nunc, Inc., Naperville, IL) coated with polyornithine/laminin (Sigma, St. Louis, MO) (Lindsay et al., 1985). Cultures were maintained in F-14 medium (Barde et al., 1980), supplemented with 10% heat-inactivated horse serum (GIBCO, Grand Island, NY). The factors used were mouse 2.5S NGF (Boehringer Mannheim, Indianapolis, IN) used at 10 ng/ml, and human brain-derived neurotrophic factor (BDNF), human NT3, and chicken SCF used at 10–20 ng/ml, which were recombinant proteins generously provided by Bob Rosenfeld (BDNF), Dr. Jane Talvenheimo and Ling Cai (NT3), and Dr. Keith Langley and Beth Mendiaz (SCF) (Amgen Center, CA).

Quantitation of neuronal survival. Quantitation of neuronal survival was based on morphological observations using light microscopy. Neurons were counted under 250× magnification, once at 4 hr postplating (where neurons were defined as phase bright with round cell bodies, with short or no neurite) and again at 48 hr postplating. Only phase-bright cells with neurites at least four times larger than the cell diameter were scored as surviving neurons. The percentage of survival is calculated as the ratio of these two counts for each individual well. Each experimental condition had four identical wells, and each experiment was repeated three to five times.

Image analysis of neuronal size distribution. Neurons were plated as described and photographed live at 48 hr postplating using light microscopy with a 25× phase-contrast objective. We determined neuronal perimeter from the photograph using an image analyzer (Cambridge

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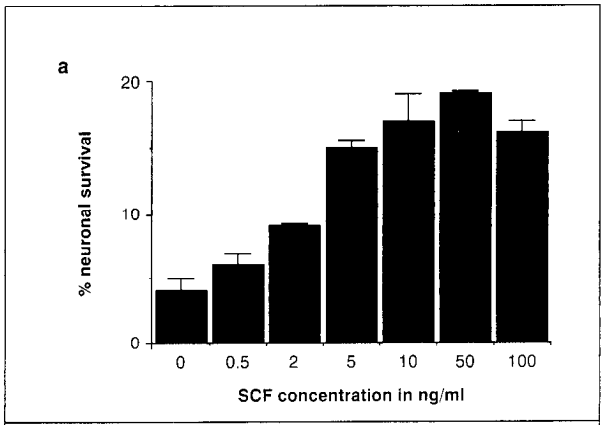
Correspondence should be addressed to Dr. Josette F. Carnahan, Amgen Center, Neurobiology Department, B5, 1900 Oak Terrace Lane, Thousand Oaks, CA 91320.



b

	0	NGF	BDNF	CNTF	SCF
SG	0.9	94	0	nd	0
N	5	0	57	0	5
CG	0.6	0	0	60	0.2
DRG	4	70	30	0	20

Figure 1. SCF rescues in a dose-dependent manner (*a*) and specifically neural crest–derived sensory neurons (*b*). *a*, E8 DRG neurons were grown for 48 hr and their survival was determined for concentrations of SCF ranging from 0.5 ng/ml to 100 ng/ml, compared with survival without any added growth factor. *b*, Neurons from selected peripheral ganglia (*SG*, sympathetic ganglia; *CG*, parasympathetic ciliary ganglia; *N*, placode-derived sensory neurons from nodose ganglia; and neural crest–derived sensory neurons from DRG) were plated onto polyornithine/laminin-coated multichamber slides at a final density of 50–100 neurons per well. Sampled wells were scored 5 hr postplating for attached neuron-like cells (round, phase bright, often already neurite bearing) and after 48 hr neurite-bearing cells (neurite longer than 3 soma diameters) were scored again in each well. Neuronal survival is expressed in percentages, as the ratio of count 2 to count 1, using murine NGF (10 ng/ml), recombinant human BDNF (20 ng/ml), ciliary neurotrophic factor (*CNTF*) prepared from chick embryo eye extract used at a concentration subsaturating for survival of ciliary neurons dissociated culture, recombinant chick stem cell factor (*SCF*; 10 ng/ml), or no growth factor (*0*). These results were obtained using E8 chick embryos but the same qualitative specificity was observed at E6 and E12. For each ganglion and developmental stage the experiment was repeated using 50 ng/ml of purified growth factors with the same qualitative results.

Instruments, Cambridge, UK) equipped with an Epson LX 810 computer.

Immunofluorescent staining of cultures. Cultures were briefly rinsed with phosphate-buffered saline solution (PBS) (GIBCO, Grand Island, NY). Cells were fixed for 10 min on ice using freshly prepared 4% paraformaldehyde, and incubated in a blocking solution of 10% normal goat serum (NGS) (GIBCO, Grand Island, NY) in PBS containing 0.1% Tween 20 for 30 min. Primary antibody incubation was performed at room temperature (RT) for 1 hr followed by four washes in PBS with 1% NGS. The blocking step was then repeated before incubation in secondary antibody (high fluorescein-conjugated, goat anti-mouse IgG; Antibodies Inc., Davis, CA). For double staining, high fluorescein goat anti-mouse was used to detect the monoclonal antibody and a biotinylated goat anti-rabbit antibody was used to detect the rabbit polyclonal antiserum; these were incubated simultaneously for 1 hr at RT. After washing as described above, the cells were rinsed twice with PBS without serum and incubated with streptavidin–Texas red (Amersham, Arlington Heights, IL), diluted 1:200 for 30 min at RT. After final washings, the wells were carefully detached from the multichamber slides and the slides mounted using a glycerol solution containing phenylenediamine (Sigma, St Louis, MO) to prevent fading (Johnson and de Nogueira Araujo, 1981).

The sections were examined using a Zeiss Axioskop fluorescence microscope equipped with a prism allowing the filters for fluorescein isothiocyanate (FITC) and rhodamine to be used simultaneously.

The antibodies used were rabbit antisera: against tyrosine hydroxylase (TH), 1:200 (Eugene Inc., Ramsey, NJ); calcitonin gene–related peptide (CGRP), 1:100 (Amersham); neurofilament 150 kDa, 1:200 (Cambridge Research Biochemicals, Wilmington, DE).

BrdU incorporation. DRG from E5.5 chick embryos were dissociated as described above and preplated in 60 mm tissue culture petri dishes (Falcon, Lincoln Park, NJ). Simultaneously cells were incubated with 10 nM (+5)-bromo-2′-deoxyuridine (BrdU) (Aldrich Chemicals, Milwaukee, WI) prepared fresh, added to the culture medium (Gratzner, 1982). After 3 hr cells were flushed with a pipette and collected for plating in medium containing both each neurotrophic factor and BrdU; 48 hr later cells were immunostained with an anti-BrdU monoclonal antibody at 1:500 (Caltag Laboratories, San Francisco, CA) and an antiserum against the 150 kDA neurofilament subunit (Cambridge Research Biochemicals, Wilmington, DE).

Results

SCF is neurotrophic factor for neural crest–derived sensory neurons in vitro

We dissociated E8 chick DRG, preplated the cells twice to enrich for neurons, and cultured at a density of 1 neuron per mm^2. We observed that recombinant chick SCF is a neurotrophic factor in the absence of any other added factor, and determined the dose–response of this effect. Survival activity plateaus at 10 ng ml (Fig. 1*a*). This concentration was subsequently used for the rest of studies. Quantitation of neurotrophic activity was performed at low plating density to eliminate potential indirect effects due to the induction of a second factor secreted by the neurons themselves or by contaminating non-neuronal cells. In the initial experiments, SCF was added after neurons had attached, in order to eliminate the possibility that the observed SCF activity might be due to an effect on cell adhesion to the surface. However, we found that the percentage survival observed in the presence of SCF did not change when neurons were plated directly in medium containing the factor, indicating that the neurotrophic activity of SCF is not due to increased adhesion of selected neurons.

SCF was then tested for the ability to maintain survival of neurons from selected peripheral ganglia for at least 48 hr of culture using known neurotrophic factors for each neuronal type as standards of comparison (Fig. 1*b*). We found that in the absence of any other growth factor, SCF promotes the survival of DRG sensory neurons but is ineffective in supporting neurons from nodose, sympathetic, or ciliary ganglia. Although survival was quantified after 48 hr, we found that SCF can maintain sensory neurons for at least 2 weeks in culture (data not shown). The response of these various ganglia to SCF was unchanged over the developmental period E6–E12. The results described above were obtained in medium containing serum; SCF has qualitative (Fig. 2*C*) and quantitative similar effects in serum-free medium.

Neurons surviving in the presence of SCF are sensory neurons

Progenitors able to differentiate into sympathetic as well as sensory neurons are present in avian DRG up to midgestation (Rohrer et al., 1985; Xue et al., 1985, 1987). The neurons supported by SCF are not differentiating along the sympathetic pathway based on our observations that they express neurofilaments and CGRP, lack immunoreactivity for TH (Fig. 2*A,B,D*), and are frequently observed to have a T-shaped morphology. These characteristics are common to sensory ganglionic neurons *in vivo* as well as in culture in the presence of NGF or BDNF. However, not all sensory neurons are SCF responsive at E8;

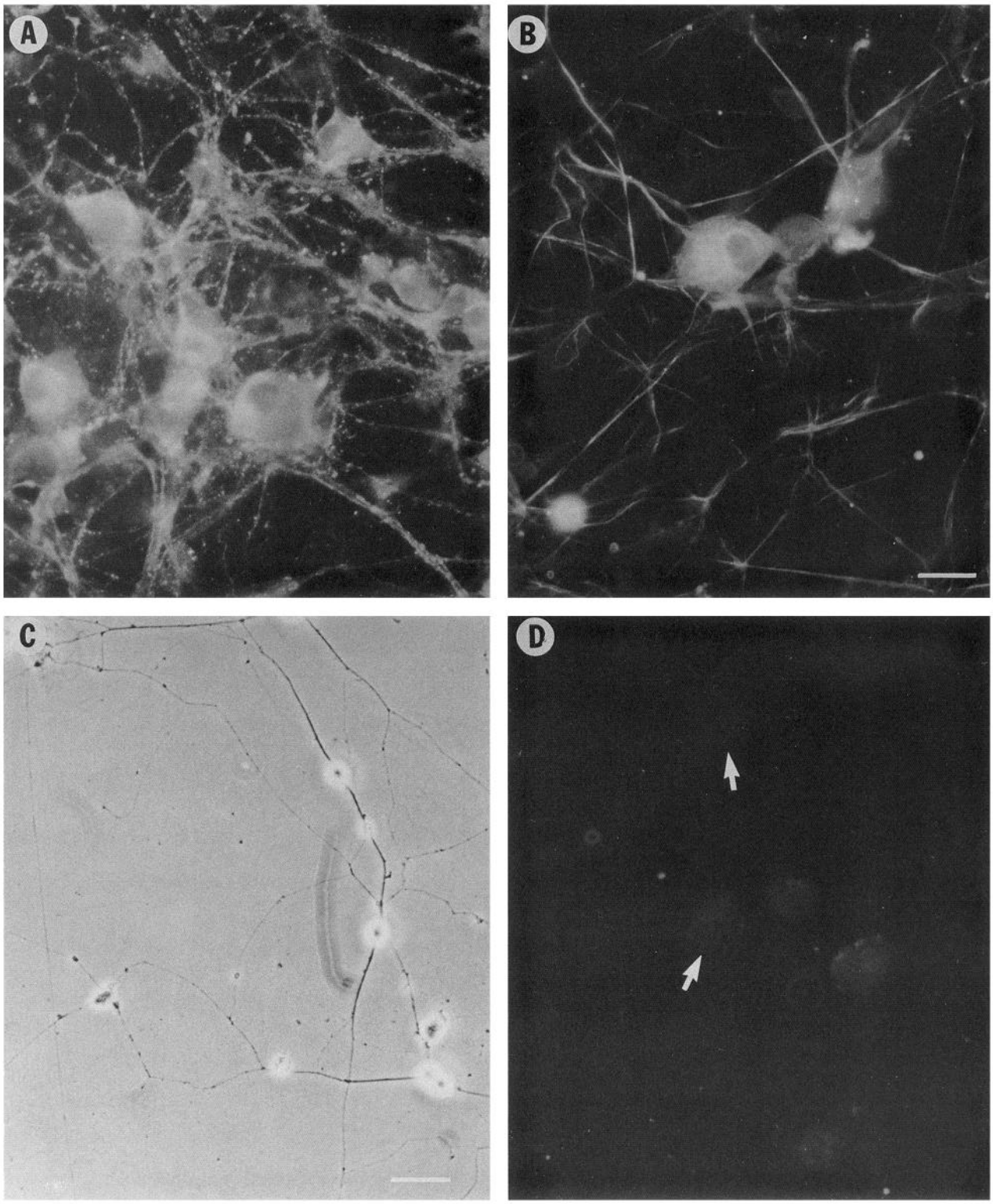


Figure 2. Neurons grown in SCF are sensory neurons. E8 DRG were plated at high density and after 4 d in culture immunostained using polyclonal antisera against CGRP (*A*), neurofilament 150 kDa subunit (*B*), and TH (*D*) as described. *C* shows a picture of live neurons in phase contrast in a sister culture grown in serum-free medium containing SCF. In *D*, the *arrows* indicate two examples of neurons grown in the presence of SCF, lacking TH immunostaining. Scale bar: 22 μm for *A*, *B*, and *D*; 72 μm for *C*.

typically 20% of the total number of neurons plated are supported by SCF (Fig. 1*a*). The percentage of neurons that survive in SCF does not change when DRG from various rostrocaudal levels are cultured (data not shown), suggesting either that there is no correlation between SCF dependence and target innervation, or that all surviving neurons have a common target, such as the skin. We also observed that the neuropeptide immunoreactivities for substance P, CGRP (Fig. 2*A*), and somatostatin

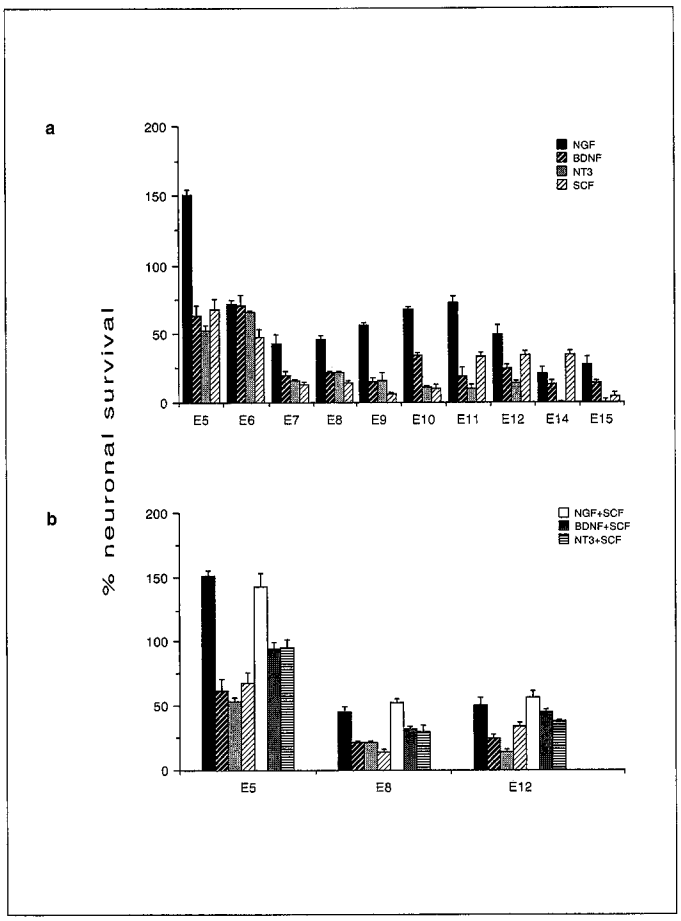


Figure 3. SCF neurotrophic activity is developmentally regulated and overlaps totally with NGF but not with BDNF or NT3. *a*, Survival at low-density plating was determined after 48 hr in presence of SCF and compared with individual neurotrophic factors at various stages of development. *b*, Survival in presence of individual neurotrophic factors was compared to survival in presence of combinations of two factors, at three developmental stages. In this figure the percentage survival in absence of any growth factor was subtracted for clarity but did not exceed 6% before E12 and was on the order of 14% thereafter.

showed comparable heterogeneity between sister cultures grown in SCF, NGF, BDNF, or NT3, consistent with the known heterogeneity of spinal ganglia.

SCF responsiveness is developmentally regulated

Neuronal survival in culture using individual neurotrophic factors. The developmental time course of SCF responsiveness was determined in parallel with that of NGF, BDNF, and NT3 (Fig. 3*a*). For clarity, the background percentage survival in control cultures, which lack added growth factors, has been subtracted at each time point shown in this figure; up to E12 the growth factor–independent survival was less than 6%, rising by E15 to 14%. We observed a similar, biphasic course of responsiveness for NGF, BDNF, and SCF. NT3 survival activity did not demonstrate a pronounced biphasic nature, and its ability to maintain survival ceased between E12 and E14. At the low plating densities used in these experiments (100 neurons per well), the percentage of neurons surviving with BDNF was much lower than previously reported (Lindsay et al., 1985). We therefore repeated the experiment using high-density plating (2000 neurons per well), conditions more comparable to earlier reports. Survival under these conditions was considerably higher, comparable to the prior reports. This may indicate an autocrine or paracrine effect, with the neurons or auxiliary cells secreting factors supporting their own survival *in vitro*.

Survival effect of SCF in combination with other neurotrophins. Since SCF did not maintain all DRG neurons in culture, we examined the survival effects of adding SCF in combination with either NGF, BDNF, or NT3 (Fig. 3*b*), all factors being used at saturating concentration. We found no increase survival percentages when SCF was combined to NGF, implying that the neurons supported by SCF are a subpopulation of the total number of NGF-responsive neurons. However, combination of SCF with either BDNF or NT3 produced higher survival levels when compared to either factor alone. Therefore, some of the DRG neurons can respond to SCF, BDNF, or NT3.

SCF, NGF, and BDNF rescue different neuronal populations

Sensory neurons grown in culture exhibit the same size heterogeneity observed *in vivo* (Fig. 4). We noticed that neurons surviving in the presence of SCF tended to be smaller than those grown with NGF; this observation was quantified using optical microscopy and image analysis. Figure 4 presents the results of such an analysis of E7 cells (these data are comparable to those obtained at E12, not shown). Neurons grown in the presence of SCF tend to have smaller cell bodies, especially when compared with those grown in the presence of BDNF, supporting the notion that SCF rescues only a subpopulation of neurons.

SCF acts on postmitotic neurons

SCF was initially characterized as a proliferation-inducing factor for murine bone marrow hematopoietic stem cells and a mast cell line (Huang et al., 1990; Nocka et al., 1990; Williams et al., 1990; Zsebo et al., 1990). Thus, SCF could be enhancing proliferation of neuronal progenitors in the DRG cultures. BrdU labeling reveals that neurons in the cultures treated with SCF are, in fact, postmitotic (Fig. 5*a,b*, arrows). This was also the case for the vast majority of neurons grown in NGF, BDNF, or NT3, although we did observe a small but significant number (estimated to be between 2% and 5%) of neurons (neurite-bearing) double labeled for BrdU and neurofilaments in such cultures (Fig. 5*c*, in BDNF; 5*d* in NGF). Interestingly, we also observed in the SCF cultures occasional cells that were flat and neurite free but expressed neurofilaments and were BrdU labeled (Fig. 5*a*, curved arrow). These cells, although rare in the SCF cultures, were not found during extensive observations of sister cultures that had been grown with NGF, BDNF, or NT3. In these experiments, we examined 4000–5000 neurons per set of culture conditions and found these cells only in SCF cultures.

Discussion

SCF, a new neurotrophic factor

We report here our finding of a new component in the spectrum of activities known for SCF, namely, its ability to act as a neurotrophic factor, and we compare this activity with those of the well-established neurotrophins NGF, BDNF, and NT3. SCF is active at a saturating concentration comparable to that of other neurotrophins (10 ng/ml), with a half-maximum concentration of 2 ng/ml (4×10^{-8} M). In our survival assays, of the peripheral neurons tested, only DRG sensory neurons were responsive to SCF. This is in agreement with the reported expression of both SCF and its receptor c-*kit* in murine DRG (Orr-Urtreger et al., 1990; Keshet et al., 1991; Motro et al., 1991). In addition, the

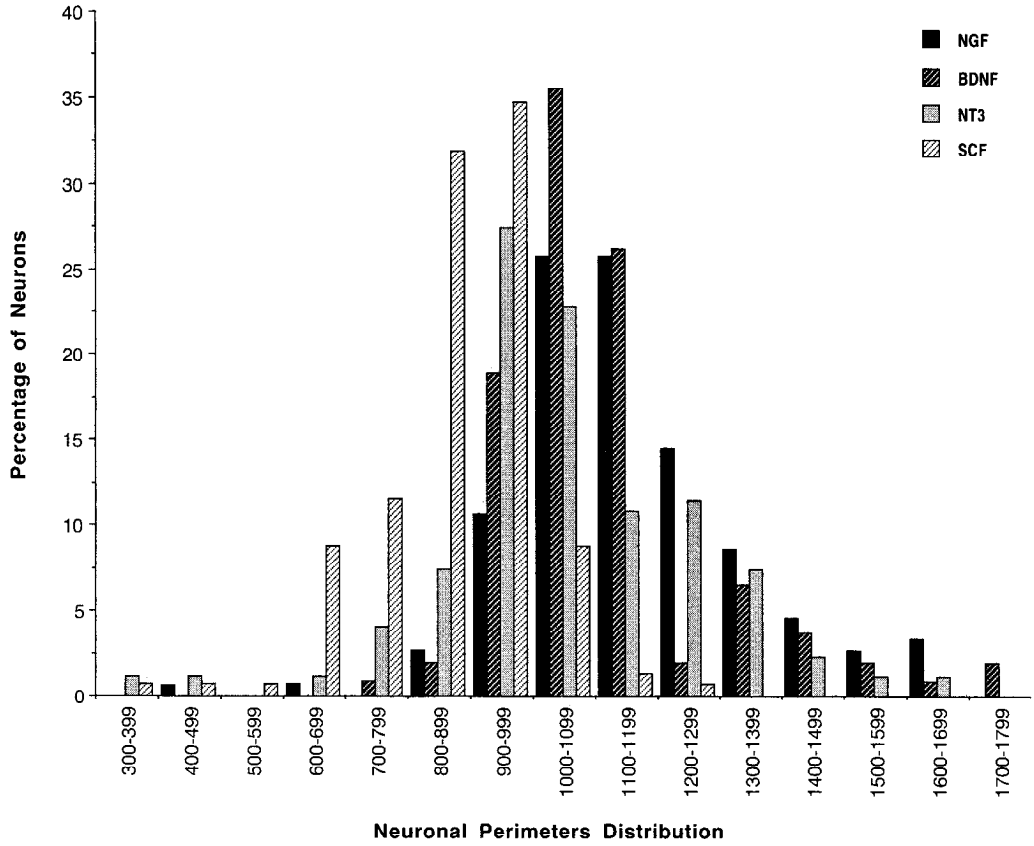


Figure 4. SCF preferentially rescues small neurons. We measured soma perimeters (in micrometers) of neurons dissected from E7 DRG embryos and grown for 24 hr in the presence of individual neurotrophic factors. For each set of cultures, between 150 and 200 neurons were measured and the numbers were arbitrarily divided into categories spanning 100 μm each. Each bar represent the percentage of neurons that fall into each category. The *solid bars* represent the perimeter distribution of neurons grown with NGF, the *dark hatched bars* represent BDNF, the *light hatched bars* represent SCF, and the *stippled bars* represent NT3.

overlapping expression of SCF and its receptor in the DRG suggests that SCF may not be a classical, target-derived factor but rather a factor acting in a paracrine or autocrine fashion.

A subpopulation of neurons taken from E5–E15 DRG can be maintained in SCF alone. This broad age of responsiveness parallels closely that of NGF and BDNF (Fig. 2*A*). Neuronal responsiveness to SCF seems to show two maxima near E5 and E10, which are also similar to those found for NGF and BDNF (Lindsay et al., 1985). These maxima correspond to the peak times for the birth of large and small neurons, respectively (McMillan and Simpson, 1978).

Although our findings with NGF, BDNF, and NT3 agree with previous reports, it was surprising that cultures treated with NGF at E5 had a 150% survival rate (Fig. 2*A*). It is possible that at this early stage, in spite of our efforts to enrich for neurons, the cultures contained contaminating progenitors that were not scored as neurons, but divided and differentiated during the 48 hr culture period. Alternatively, cells that were scored as neurons in the initial counting might actually be dividing in culture; this would, however, be inconsistent with reports that, unlike sympathetic neurons, sensory neurons do not divide (Anderson and Axel, 1986; Rohrer and Thoenen, 1987). In the initial scoring, we defined as neurons cells whose cell body was rounded and phase bright with either short or no neurites. It is our experience that upon neurofilament staining, all such cells are immunoreactive for neurofilament (data not shown). In addition, a significant number of neurons grown from E5.5 DRG in the presence of NGF, BDNF, or NT3 did show BrdU incorporation, supporting the possibility that some sensory neurons are still mitotic at this stage of development.

Although we found survival with BDNF to be affected by neuronal density, SCF supported the same percentage of neurons regardless of the plating density. This suggests a direct action on the neurons rather than an induction of another factor. In addition, we tested survival of sensory DRG neurons in response to SCF in the presence of blocking antiserum to NGF or to BDNF. Neither antiserum had any effect on neuronal survival (data not shown). Therefore, the neurotrophic activity of SCF is unlikely to be mediated by secretion of either NGF or BDNF.

SCF is acting on a subpopulation of sensory neurons

In the age range we examined (E5.5–E15), chicken SCF supports fewer neurons than does NGF. The SCF-supported neurons were apparently a subpopulation of the NGF-responsive neurons. Our attempts to correlate this subpopulation with a particular neuropeptide phenotype or a location along the rostrocaudal axis were unsuccessful. However, we did observe that smaller neuronal size (Fig. 4) and frequently a bipolar shape (data not shown) were characteristic features of the neurons responding to SCF, especially when compared to neurons grown in BDNF or NT3. The small size of neurons cultured in the presence of SCF might reflect the specificity of SCF in rescuing small sensory neurons, which would be consistent with their expression of CGRP, and also with the hypothesis that the neu-

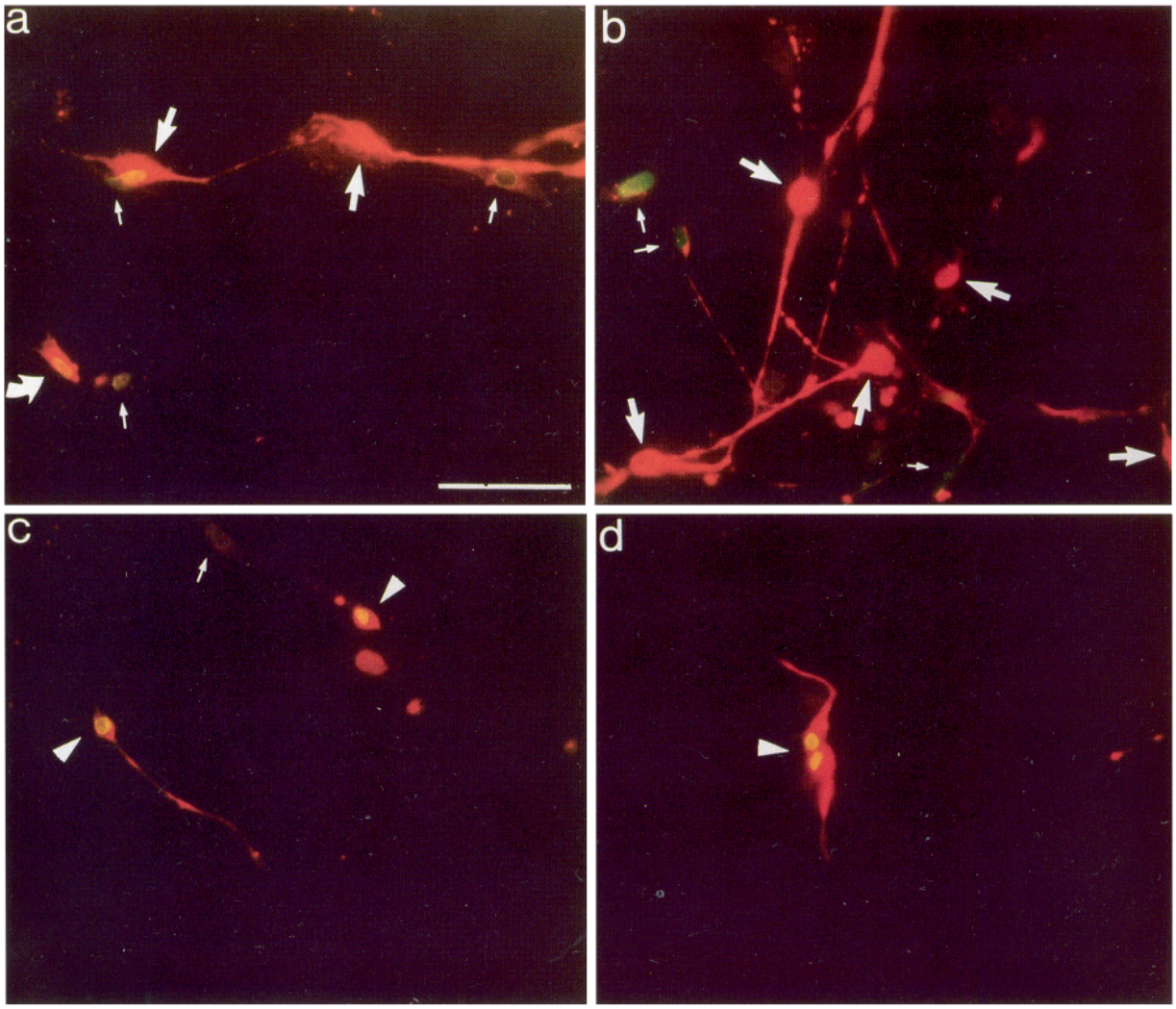


Figure 5. SCF is a trophic factor for postmitotic neurons. E5.5 DRG neurons were preincubated with BrdU prior to and during plating with various growth factors. They were then doubly immunostained for BrdU (*green*) and neurofilament (*red*). *a–d* show representative fields of cultures grown in the presence of SCF (*a* and *b*), BDNF (*c*), and NGF (*d*). The majority of neurons in all cultures were only labeled with neurofilament antibodies (*thick arrows*) and the BrdU incorporation was seen mainly in neurofilament-negative cells (*thin arrows*). *c* and *d* are selected fields from BDNF- and NGF-treated cultures, respectively, showing incorporation of BrdU in neurofilament-positive cells bearing neurites (*arrowheads*). Such cells could not be found in SCF treated cultures. In *a* the *curved arrow* points to the only type of cells that showed double staining for neurofilament and BrdU. These cells were typically small, flat, and without neurites. They were rare in the SCF cultured wells (less than 2% of the neurofilament-positive cells) but were not at all found in NGF-, BDNF-, or NT3-treated cultures. Scale bar, 50 μm.

rons have skin as a target organ. Although the peak time for the birth of small sensory neurons is around E10 (McMillan and Simpson, 1978), small, CGRP-containing sensory neurons are present in the DRG as early as E5 (New and Mudge, 1986). It is possible these are the neurons responding to SCF in our cultures.

An alternative explanation is that neurons surviving in the presence of SCF might preferentially rescue younger neurons at all stages of development. Experiments are in progress to address these questions.

SCF rescues postmitotic neurons

SCF has been reported to stimulate proliferation of hematopoietic progenitors (Berstein et al., 1991; de Vries et al., 1991), which prompted us to examine its effects at early stages of neuronal development. In contrast to previously reported activities on hematopoietic cells, SCF primarily rescued postmitotic neurons, as shown by the lack of incorporation of BrdU in neurite-bearing, neurofilament-stained cells (Fig. 5*a,b*). This is in contrast with the surprising finding of BrdU-labeled neurons in the cultures treated with BDNF, NGF, or NT3. Neurons cultured with BDNF, NGF, or NT3 were also found immunonegative for TH, suggesting they are not from the pool of sympathetic progenitors. The BrdU labeling found in these latter cultures could be due to the presence of neurons that initially were growth factor independent and mitotic (Ernsberger and Rohrer, 1988), as discussed above. Although BDNF and NT3, but not NGF, have been reported to accelerate sensory neuronal maturation (Wright et al., 1992), we observed the same proportion of BrdU-labeled neurons in these three conditions. These labeled neurons may have initially been from the 5–6% of young neurons that are growth factor independent. However, the fact that we could not find labeled neurons in the cultures treated with SCF suggests that SCF might actually prevent neuronal maturation (neurite outgrowth and cell body growth). This hypothesis is reinforced by the presence in SCF cultures of small flat cells expressing

both neurofilament and BrdU immunoreactivity but which are neurite free. These cells remain to be further characterized, but they could represent progenitors (sensory or sympathetic) arrested in their maturation. One could speculate that *in vivo*, SCF is maintaining a pool of progenitors present in the DRG.

Significance of neurotrophic activity for SCF

In addition to presumptive migrating melanoblasts, expression of c-*kit* has been reported both in ventrally migrating neural crest cells and in sensory neurons in mouse DRG at E15 (Keshet et al., 1991) and E12.5 (Manova and Bachvarova, 1991). In the mouse, mRNA for SCF is detectable in the DRG after E14 (B. Hogan, personal communication). The pattern of expression of SCF and its receptor in the mammalian nervous system is therefore consistent with the evidence of its neurotrophic activity on chicken neurons. We have preliminary evidence of a similar pattern of c-*kit* expression in avian tissues, including in the DRG, suggesting parallel roles for SCF in the two systems. Expression of c-*kit* seems restricted on sensory neurons (Keshet et al., 1991) but that of SCF spread over the entire ganglion. This suggests an autocrine/paracrine mechanism for SCF.

There is clear evidence that SCF is an early-acting factor in neural crest development *in vivo*. Injection of a blocking antibody to the SCF receptor into mouse embryos affects melanogenesis (Nishikawa et al., 1991). It will also be of interest to explore the relationship of SCF+BDNF, especially in the context of aggregation of neural crest cells to form DRG *in vivo* (Kalcheim et al., 1987). In addition, BDNF has been shown to drive clonal neural crest cells toward neuronal differentiation (Sieber-Blum, 1991); thus, it might be of interest to investigate the effects of SCF upon the decision branch of neuronal or glial versus pigment lineage (Stocker et al., 1991).

So far we have not been able to demonstrate *in vitro* any neurotrophic activity for SCF in a mammalian species. Sequence analysis demonstrates that chicken SCF is quite divergent from any mammalian SCF studied to date (Martin, personal communication) with 57% homology in amino acid sequence. Though there is no cross-reactivity for ligand binding between the mammalian and avian nervous system, chicken SCF has actions consistent with those known for mammalian SCF when tested in a chicken hematopoietic assay (J. Lipsick, personal communication). Although no obvious sensory deficits have been reported for SCF or c-*kit* mutations, it is possible that deficits caused by these mutations are sufficiently subtle that they are as yet undetected. Given the relatively late appearance of SCF in developing mouse DRG, it is possible that in mammals SCF might not be an early neurotrophic factor but rather might be involved in a later step of neuronal maturation.

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