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Fibroblast growth factor and its implications for developing and regenerating neurons

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ABSTRACT **FGF is a multifunctional heparin-binding protein which was characterized by its mitogenic and angiogenic action outside the nervous system. Recent data confirm this multifunctionality also with regard to the nervous system. The distribution of FGF and its receptors seems not to be in agreement with the idea of a single function for one population but argues for a more complex action, which might be dependent on the development stage and cell type. FGF and its receptors are widely distributed in the nervous system. In brainstem and spinal cord motoneurons and in sensory ganglia the FGF-2 staining pattern is developmentally regulated suggesting a functional change during embryonic and postnatal development. In addition, after nerve lesion the FGF-2 expression is altered in sensory and motoneurons. Administration of FGF-2 reveals trophic effects on survival and transmitter metabolism *in vivo* and *in vitro*. According to a more general neurotrophic factor concept, a physiological role of FGF for distinct neuron populations during development is likely. In the motor system, for example, FGF could act synergistically with certain neurotrophins, CNTF, or other non-identified co-factors. In the sensory system, a possible non-neurotrophic role for at least postnatal and adult sensory neurons has to be further addressed in the future. In order to further define and characterize the actions of the FGFs a mapping of the different family members and their respective receptor molecules during development and in the adult has to be done.**

KEY WORDS: *FGF, motoneurons, sensory neurons, neurotrophic factor*

Introduction – The changing neurotrophic factor concept

Ontogenesis of the vertebrate nervous system is characterized by two successive phases. During the initial progressive phase, cells of the nervous system proliferate and migrate to their final destinations where they aggregate to form functional entities. After differentiation of the precursor cells to neurons and glial cells there is directed neurite outgrowth and synapse formation. Shortly after the onset of target innervation, the regressive phase begins. At this time a variable portion of neurons, depending on their particular neuron population, dies by apoptosis. This naturally occurring neuronal death was thought to be highly regulated by target-derived peptide growth factors, the neurotrophic factors (NTFs; Cowan, 1982; Purves, 1986; Oppenheim, 1991). Results from studies with nerve growth factor (NGF) led to the formulation of the neurotrophic factor hypothesis (Barde, 1988; Oppenheim, 1989). According to his theory, an NTF is thought to be produced and secreted in limiting amounts by the target tissue of certain neuron populations in a developmentally regulated manner. Competition for the limiting amount of trophic factor, which is taken up by neurons via high-affinity receptors and retrogradely transported to the cell body, regulates the number of neurons that survive this sensitive period. The fact that NGF addressed only a limited number of neuron populations (sympathetic neurons, neural crest-derived sensory neurons, and cholinergic neurons of the basal forebrain; Levi-Montalcini, 1987; Thoenen *et al.*, 1987) suggested that the interaction between the neurotrophic factor and its target cells is highly specific and stimulated the search for other molecules involved in the regulation of neuron death.

Identification of brain-derived neutrophic factor (BDNF), neurotrophin-3 (NT-3), Xenopus NT-4, and mammalian NT-4/5 showed that NGF belongs to a family of homodimeric proteins, the neurotrophins (Eide *et al.*, 1993). The discovery of the different neurotrophins satisfied the need for new molecules but did not provide the complete solution. On the contrary, a more detailed characterization of BDNF, NT-3, NT-4/5 distribution and receptor binding revealed that the concept which was based mainly on NGF was no longer sufficient to explain the new data.

Abbreviations used in this paper: BDNF, brain-derived neurotrophic factor; ChAT, choline acetyltransferase; CNTF, ciliary neurotrophic factor; DRG, dorsal root ganglion; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; NGF, nerve growth factor; NT, neurotrophin; NTF, neurotrophic factor.

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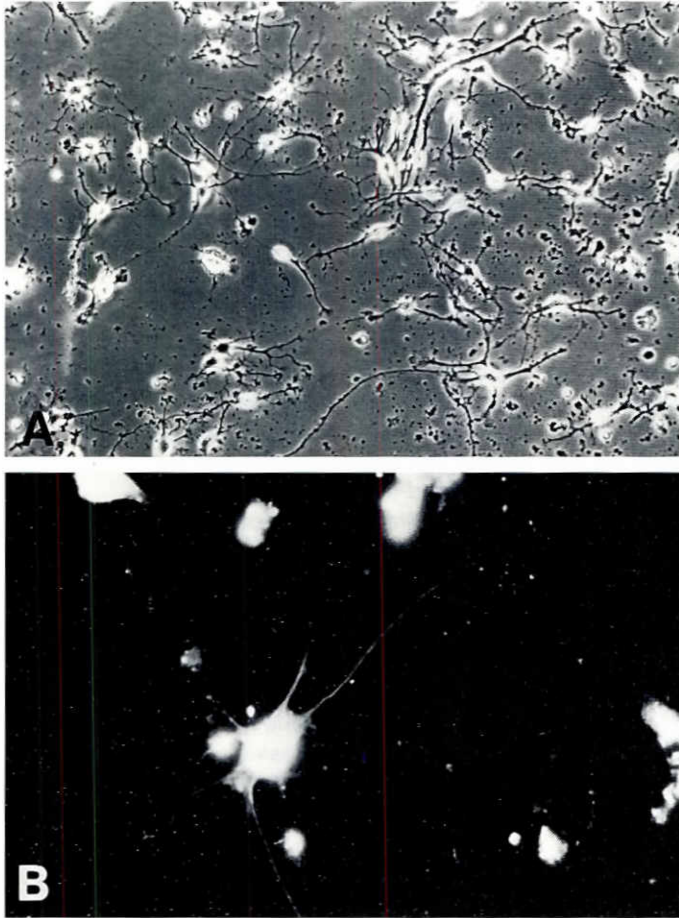

Fig. 1. Culture of hypoglossal neurons of 7-day-old rats treated with FGF-2 after 4 days. *No survival is found in control of in NGF- or CNTF-treated cultures (not shown).* **(B)** *Hypoglossal culture of 7-day-old rats after 4 days in the presence of FGF-2. Neurons are identified by anti-neurofilament antibodies. Magnification: A, 130x; B, 970x.*

Local or autocrine modes of action, for example, are now conceivable because some neurons do not only express the trophic molecule itself but also the corresponding receptor (Schecterson and Bothwell, 1992; Wright *et al.,* 1992). It appears that, similar to the immune system, there is a certain degree of redundancy because some neurons are able to respond to different trophic molecules (listed in Korsching, 1993). Furthermore, the expression of trophic factors does not always correspond to the critical phase of neuron death. The expression of NTFs or receptors can persist into adulthood suggesting additional roles of NTFs for maintenance and repair of the adult nervous system (e.g. Hefti *et al.,* 1989; Maisonpierre *et al.,* 1990). Finally, the expression of neurotrophic molecules was found to be not restricted to the nervous system implying functional significance for non-neuronal cells (Sariola *et al.,* 1991; Wheeler and Bothwell, 1992).

These results demonstrate that the neurotrophic factor hypothesis has to be redefined. A more general hypothesis will also include molecules, known to be trophic for neurons, but which are not homological to the neurotrophins and do not fulfil all of the NGF-based criteria. This sheds new light on non-neurotrophin molecules, for example ciliary neurotrophic factor (CNTF) and the fibroblast growth factors (FGFs) and their putative neurobiological function. CNTF was originally extracted from embryonic chick eye tissue on the basis of its trophic activity for cultured parasympathetic ciliary ganglion neurons (Barbin *et al.,* 1984). Sequencing of the molecule showed that it lacks a signal peptide, typical for secretory proteins, and is not homologous to any other known protein (Stöckli *et al.,* 1989; Lin *et al.,* 1989). Subsequently, it was shown that CNTF not only addresses a variety of neuronal cell population, but also acts on glial cells (Unsicker *et al.,* 1992a; Louis *et al.,* 1993).

During the last few years evidence was accumulated that members of another peptide growth family, the FGFs, act specifically on central and peripheral neurons during development and regeneration (Unsicker *et al.,* 1993). The FGFs were initially identified as mitogenic and angiogenic proteins. They were found to regulate a variety of cellular processes, including stimulation of proliferation, differentiation, and angiogenesis and promotion of cell maintenance, chemotaxis, and repair (Böhlen, 1989). FGF-1 (acidic) and FGF-2 (basic) are the most prominent members of a family that currently consists of nine molecules. Besides the sequence homologies the other FGF family members display further distinct molecular and biological properties (Delli Bovi *et al.,* 1987; Dickson and Peters, 1987; Zhan *et al.,* 1988; Finch *et al.,* 1989; Marics *et al.,* 1989; Tanaka *et al.,* 1992; Miyamoto *et al.,* 1993). Heterogeneity of the FGFs is paralleled by a diversity of their high- and low-affinity receptors (see below).

In the following review we survey the pharmacological effects of FGF in the nervous system. Since most of the data currently available are from studies with FGF-1, FGF-2 and FGF-5, we will focus on the actions of these FGF family members on neurons *in vivo* and *in vitro.* Since information concerning the distribution of the FGF receptors is a prerequisite for the understanding of the biological role of FGF, we will summarize data concerning spatial regulation of endogenous FGF and its high-affinity receptor during development and regeneration. Although FGF-2 influences several neuronal and glial cell types (Westermann *et al.,* 1990) we will primarily deal with sensory and motoneurons. Finally, we will discuss the physiological role of FGF in relation to the neurotrophins and CNTF.

Pharmacological effects of FGF on neurons *in vitro* and *in vivo*

It is well established that members of the FGF family influence several key functions of cultured neurons. FGF-2, for example, influences survival and/or transmitter metabolism of a variety of different central neuron populations (Unsicker *et al.,* 1993). The survival-promoting effect of FGF-2 on chick ciliary neurons (Unsicker *et al.,* 1987) was further characterized using single neuron cultures. Results from these experiments demonstrated that FGF-2 affects its target cell via a direct mechanism suggesting that ciliary neurons *in vitro* possess functional FGF binding sites (Unsicker *et al.,* 1992b). FGF-2 displays survival-promoting capacity in dissociated cultures of the hypoglossal nucleus (Fig. 1; Grothe and Unsicker, 1992) and is able to stimulate choline acetyltransferase (ChAT)-activity and survival of cultured neurons of the spinal cord (McManaman *et al.,* 1989; Grothe *et al.,* 1991a). Controversy exists about the rescue effect of FGF-2 in cultures of highly purified motoneurons. Whereas

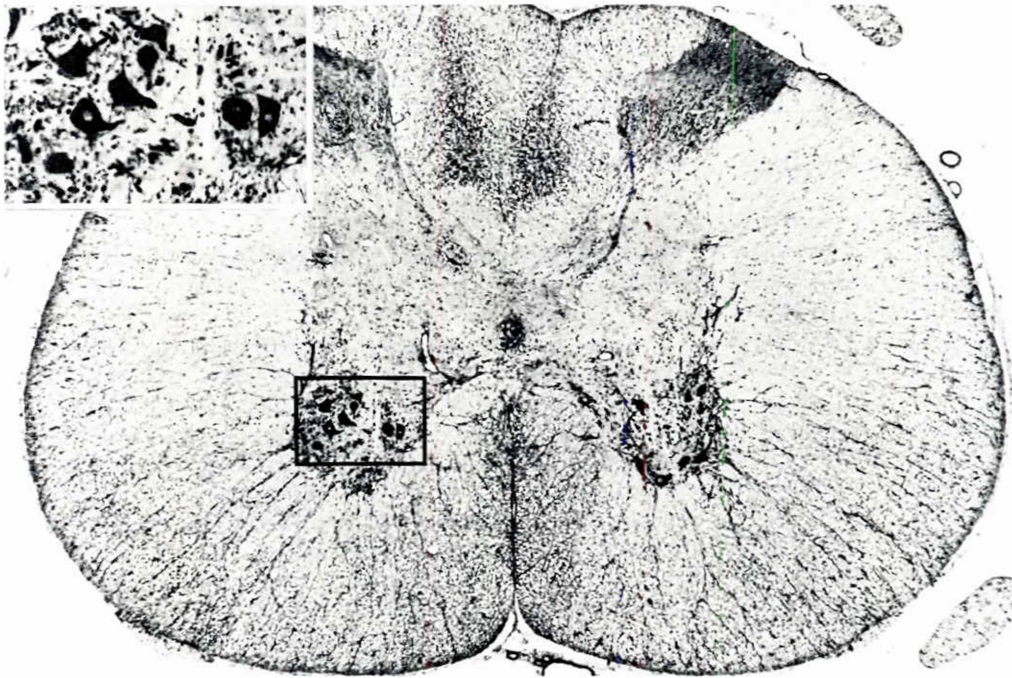

Fig. 2. Localization of FGF-2 IR in the spinal cord of the adult rat. *FGF-2 IR is strongly expressed in motoneurons. Insert shows a magnification of the ventral horn. Magnification: 67x.*

Hughes *et al.* (1993b) and Arakawa *et al.* (1990) demonstrated activity of FGF-2 on chick and rat spinal motoneurons, Henderson *et al.* (1993) only found small effects of FGF in cultures of purified rat spinal motoneurons. Since Henderson *et al.* (1993) and Hughes *et al.* (1993b) both used the immunopanning technique to enrich for motoneurons, this difference might be due to minor variations of the culture conditions. Very recently, it was shown that another member of the FGF family, FGF-5, also displays neurotrophic activity in cultures of purified chick and rat spinal motoneurons (Hughes *et al.*, 1993a,b). Since this protein possesses a signal sequence and is present in skeletal muscle it was suggested that the protein may act as a target-derived neurotrophic factor (Hughes *et al.*, 1993a).

Several reports documented *in vivo* activity of exogenously applied FGF (Unsicker *et al.*, 1992a). Administration of FGF-2 *in ovo* during the phase of the physiological motoneuron death resulted in a significant stimulation of ChAT-activity, as measured in extracts of total spinal cord, but the number of motoneurons surviving this period was not affected (Grothe *et al.*, 1991a; Oppenheim *et al.*, 1992a). The rescue effect of FGF-2 for hypoglossal motoneurons from lesion-induced cell death is age-dependent, since an increasing effectiveness of FGF-2 during postnatal development was found. However, the lesion-induced decrease of ChAT-activity in the adult was not prevented by FGF-2 (Grothe and Unsicker, 1992). Neither FGF-2 nor FGF-5 rescued facial motoneurons from cell death after nerve lesion in the newborn rat (Hughes *et al.*, 1993b). These results could indicate that FGF regulates ChAT-activity during embryonic development and rescues a motoneuron subpopulation after nerve lesion in the postnatal rat. On the other hand, these data could also indicate that FGF might need a co-factor to develop optimal efficiency, as we will discuss later on. Application of FGF-2 to the transectioned sciatic nerve showed that FGF-2 does also have a neurotrophic capacity to rescue sensory neurons from cell death (Otto *et al.*, 1987).

Regulation of endogenous FGF and FGF-receptors during development and regeneration

The broad expression of FGF-1 and FGF-2 as well as FGF receptor type 1-3 in the nervous system indicates important functions for these molecules (Baird, 1994). For characterization of the biological function of FGF-2, a comprehensive analysis of the distribution and regulation of the molecule and its receptors is necessary. At present the FGF receptor (FGFR) family consists of four members displaying high-affinity binding properties. The tyrosine kinase transmembrane receptors belong to the immunoglobulin (Ig) superfamily, and differential splicing of these genes leads to a vast variety of different isoforms (Givol and Yayon, 1992; Johnson and Williams, 1993). It is clear that the binding capacities of the FGFRs are different for the diverse FGFs, FGF-1 and FGF-2 bind FGFR-1 *(flg)* and -2 *(bek)* with similar affinity, whereas FGFR-3 shows a 20-fold tighter binding for FGF-1 than for FGF-2 (Thomas, 1993). In addition to high-affinity binding sites, mediation of the FGF signal apparently requires low-affinity receptors, which were characterized as cell surface heparan sulfate proteoglycans, e.g. the integral membrane proteoglycan syndecan (Klagsbrun and Baird, 1991; Bernfield *et al.*, 1992).

Expression of FGF and FGFR in the motor system

FGF-1 mRNA was found to be localized in cranial nerve and spinal cord motoneurons (Bean *et al.*, 1991; Elde *et al.*, 1991). Antibodies against FGF-2 label almost all motoneurons of the embryonic spinal cord and of cranial nerve nuclei, including, for example, the trigeminal and the facial motor nuclei (Weise *et al.*, 1993). In postnatal and adult cranial motor nuclei and spinal cord motor columns, however, FGF-2 expression is switched to a more restricted distribution, according to specific antibodies which label neuronal subpopulations (Fig. 2; Bean *et al.*, 1991; Despres *et al.*, 1991; Grothe *et al.*, 1991b; Gomez-Pinilla *et al.*, 1992; Weise *et al.*, 1993). Northern blot analysis demonstrates FGF-2 mRNA in the embryonic and adult rat spinal cord and adult brainstem (Weise *et al.*, 1993; Grothe and Janet, 1995). The cellular source of FGF-2 mRNA, however, remains to be identified. Polymerase chain reaction analysis of extracts of the hypoglossal nucleus revealed the absence of a FGF-2 transcript, suggesting that other brainstem nuclei (e.g. red nucleus, medial

nucleus of the trapezoid body) synthesize FGF-2 (Grothe and Janet, 1995). Skeletal muscle, the target tissue of motoneurons, contains both FGF-2 and FGF-5 during development and in the adult (Kardami *et al.,* 1985; Joseph-Silverstein *et al.,* 1989; McManaman *et al.,* 1989; Hughes *et al.,* 1993a).

FGFR-1 is distributed in a time-dependent manner. Whereas no protein or mRNA is detectable in embryonic spinal cord motoneurons, the receptor can be found in the newborn and adult (Wanaka *et al.,* 1990, 1991; Weise *et al.,* 1993). As shown by Wanaka *et al.* (1990) and Grothe and Janet (1995), cranial nerve nuclei neurons of the adult express FGFR-1. FGFR-2, which also binds FGF-2 with high-affinity, is present in the developing brain but little is known about its distribution in spinal cord (Jaye *et al.,* 1992).

The regulation of FGF-2 in the hypoglossal system after lesion was studied in detail. FGF-2 immunoreactivity (IR) is detectable in the tongue, the target tissue of hypoglossal motoneurons (Grothe and Unsicker, 1992). FGF-2 shows a high-affinity receptor-mediated retrograde transport in hypoglossal motoneurons after injection of iodinated FGF-2 (Grothe and Unsicker, 1992). In line with this observation, FGF-2 IR is greatly reduced in the hypoglossal nucleus after peripheral nerve transection (Grothe and Unsicker, 1992 and in the facial nucleus, Grothe, unpublished observations). Colchicine, which is known to block fast axonal transport, eliminates FGF-2 IR in the hypoglossal nucleus (Grothe and Janet, 1995).

Expression of FGF and FGFR in the sensory system

FGF-1 mRNA can be detected in embryonic chick and adult rat dorsal root ganglion (DRG) neurons (Elde *et al.,* 1991; Schnürch and Risau, 1991). Similar to the expression of FGF-2 protein in the motor system, FGF-2 shows a more restricted distribution during postnatal development and in the adult where the protein is strictly co-localized with the neuropeptide somatostatin (Weise *et al.,* 1992, 1993). In embryonic DRGs almost all neurons contain FGF-2 IR (Weise *et al.,* 1993). Northern blot analysis of adult rat DRGs reveals the FGF-2 transcript (Grothe and Janet, 1993, 1995). FGFR-1 IR and mRNA are weakly expressed in almost all sensory neurons of the embryonic rat (Wanaka *et al.,* 1991; Weise *et al.,* 1993). During postnatal development and in the adult FGFR-1 IR is co-localized to the FGF-2/somatostatin subpopulation (Fig. 3; Grothe and Janet, 1993).

Ligation of the sciatic nerve does not lead to proximal or distal accumulation of FGF-2 IR (Grothe and Janet, 1993). Injection of iodinated FGF-2 into the food pads does also not result in a specific accumulation of radioactivity distal to the ligation (Ferguson *et al.,* 1990). Axotomy of the sciatic nerve, however, increases the percentage of FGF-2 and FGFR-1 immunoreactive DRG neurons in both postnatal and adult rats (Fig. 3; Grothe and Janet, 1993). Crush of the sciatic nerve results in an increase of the FGF-2 mRNA in DRGs and in the proximal and distal nerve stump (Grothe and Janet, 1993; Grothe *et al.,* submitted).

Low levels of FGFR-1 mRNA are found in the intact sciatic nerve. After sciatic nerve crush FGFR-1 mRNA level displays a time-dependent increase in the distal and in the proximal nerve stump (Grothe and Janet, 1993; Grothe *et al.,* submitted). This indicates that FGF is necessary during the regenerating process, e.g. Schwann cell proliferation or fiber outgrowth. In this context it is interesting to note that FGF-2 can strongly suppress the forskolin-mediated induction of the *Po* gene in cultured Schwann cells (Morgan *et al.,* 1994). The expression of Po coincides with the onset of myelination (Mirsky *et al.,* 1980). This suggests that FGF is involved in the regulation of myelination during axon regrowth.

The new neurotrophic factor concept and the role of FGF

Studies on the spatiotemporal distribution of FGF and its receptors as well as functional data indicate important significance of the molecule during development, maintenance, and repair of the nervous system. FGF has been characterized as a multifunctional protein predominantly by its mitogenic and angiogenic action outside the nervous system (Baird and Klagsbrun, 1991). The recent data confirm this multifunctionality with regard to the nervous system. The distribution of FGF and its receptors seems to be not in agreement with the idea of a single function for one neuronal population but argues for a more complex action, which might be dependent on the developmental stage and cell type. The wide distribution of FGF-2 in embryonic brain, spinal cord, and in sensory ganglia, as determined by immunocytochemistry and RNase protection assay, could imply that the peptide serves more general trophic functions in neuronal maturation (Weise *et al.,* 1993; Grothe and Meisinger, 1995). The more dynamic and patterned distribution of FGF-2 IR in distinct neuronal subpopulations in postnatal and adult motor nuclei and sensory ganglia argues for a more specific action and suggests that the function of the protein undergoes changes during development (Weise *et al.,* 1992, 1993; Grothe *et al.,* 1991b). A time-dependent expression is also detectable at the receptor level: whereas FGFR-1 is absent from embryonic spinal cord motoneurons, the receptor is found in the newborn and adult (Weise *et al.,* 1993; Wanaka *et al.,* 1990, 1991). Since the mapping of FGF and its receptor variants at the mRNA and protein level is far from being complete, it seems to be premature to discuss clearly defined actions. These fragmentary results, however, point to important functions of the molecule during development.

Until recently, a protein exerting mitogenic activity upon binding to receptors with tyrosine kinase activity was not thought to mediate also neurotrophic activity. Therefore, reports documenting trophic actions of FGF for neurons *in vitro* were discussed controversially. *In vitro* activities of the molecules were not thought to reflect the *in vivo* situation. The finding, however, that tyrosine receptor kinases (*trk*) are the high affinity binding sites for the neurotrophins and that these molecules can also mediate mitogenic activity have relativized this difference (Cattaneo and McKay, 1990; Cordon-Cardo *et al.,* 1991).

The wide expression of FGF and its receptors in the nervous system was also thought to be not consistent with the idea of a specific function. Similar to the neurotrophin receptor (Ip *et al.,* 1993; Valenzuela *et al.,* 1993), it is now becoming apparent that the spatiotemporal distribution of different high-affinity FGFR variants might code for specific actions (Johnson and Williams, 1993). The fact that the low-affinity receptors for FGF, the heparan sulfate proteoglycans which are necessary for high-

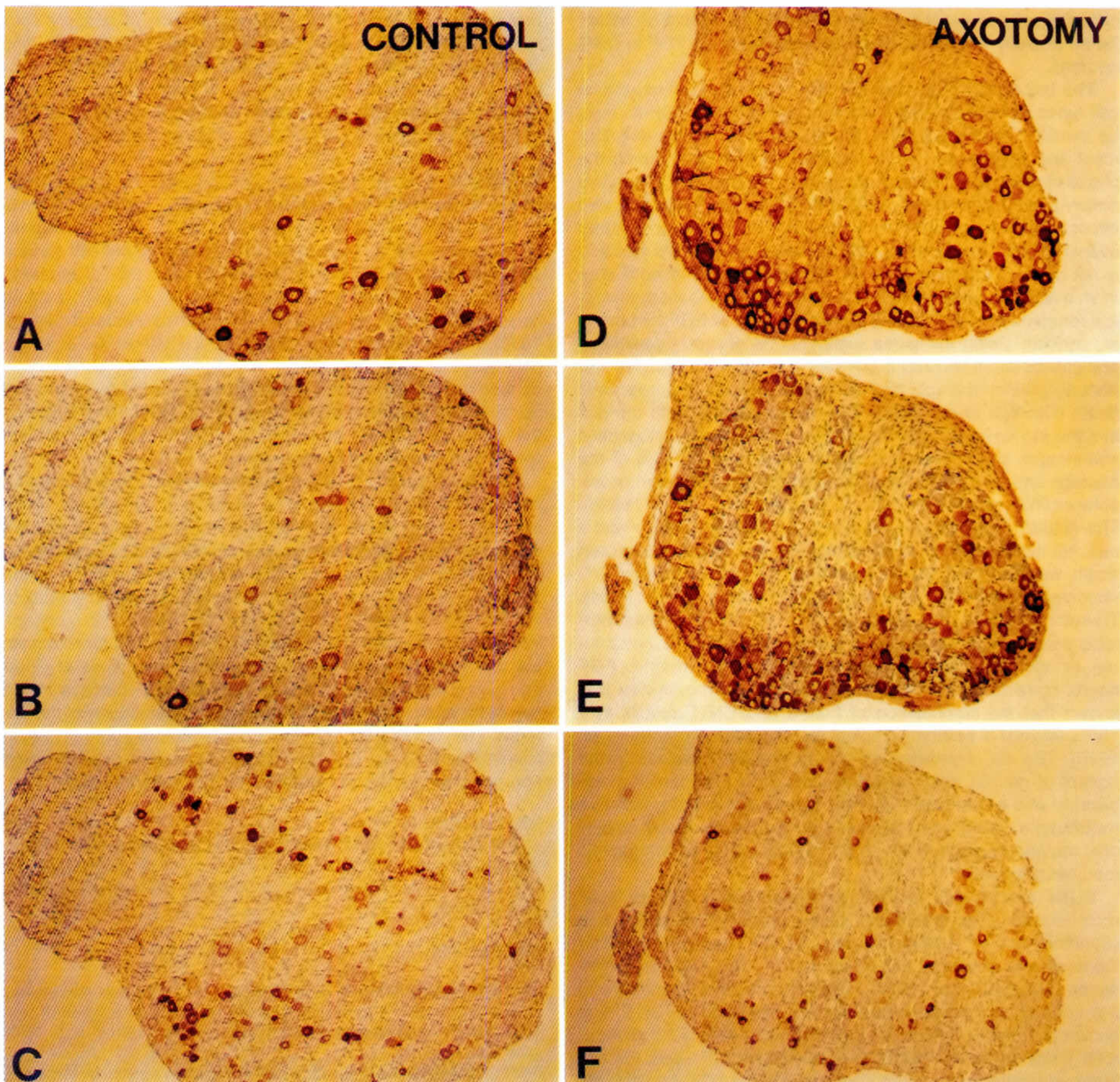

Fig. 3. L5 DRG 7 days after axotomy of the sciatic nerve at postnatal day 2. (A,B,C) *Contralateral control side;* **(D,E,F)** *ipsilateral lesioned side; consecutive sections are stained with anti-FGF-2* **(A,D)**, *anti-FGFR* **(B,E)**, *and anti-CGRP* **(C,F)**. *FGF-2 and FGFR are largely co-localized. Axotomy results in an increase of the number of FGF-2 and FGFR immunoreactive neurons* **(D,E)** *whereas the number of CGRP immunoreactive neurons decreases* **(F)**. *Magnification, 80x.*

affinity binding of FGF, are developmentally regulated adds a further level for generating cellular specificity (Nurcombe *et al.*, 1993).

Besides a possible involvement of FGF in maintenance or transmitter metabolism in the central nervous system, an alternative role of FGF-2 could be related to neuronal activity (Terlau and Seifert, 1990). In the sensory system FGF appears not to be axonally transported. The increase of FGF-2 and FGFR after crush lesion of the sciatic nerve seems to be a local reaction which is probably part of the degenerating process, e.g. correlated with the macrophage response, or, as we pointed out earlier, it might be crucial for fiber regeneration. The changing expression pattern of FGF-2 and FGFR in DRG neurons after axotomy is similar for neurotransmitters which are localized in

neuronal subpopulations as well. Experiments should be established to study a possible neuromodulatory role of FGF in DRG neurons.

The fact that FGF-2 promotes the *in vitro* survival of several cell types including motoneurons, suggested that FGF-2 might be involved in the regulation of neuron death during development and after lesion. *In vivo* application of FGF-2 during the phase of ontogenetic neuron death, however, did not confirm this notion (Oppenheim *et al.,* 1992a). Whether the lacking *in vivo* effect reflects a requirement for putative co-factors or whether events at the receptor level (e.g. down-regulation) are involved is not clear. Stimulation of ChAT-activity of the spinal cord after *in vivo* administration of FGF-2, however, excludes methodological reasons and underscores the significance of FGF (Grothe *et al.,* 1991a). Using the same experimental design, Oppenheim and collaborators showed that a variety of different peptides and partially purified extracts rescue motoneurons from ontogenetic death (Qin-Wei *et al.,* 1994). This, taken together with the fact that the *in vivo* application of CNTF or BDNF during ontogenetic motoneuron death does not rescue more than 20-30% of the neurons argues for a multifactorial control of motoneuron development (Wewetzer *et al.,* 1990; Oppenheim *et al.,* 1991, 1992b). This is further confirmed by studies using transgenic mice with a null mutation for BDNF or *trkB* where no significant motoneuron death was found in BDNF knockouts but approximately 30% motoneuron loss was revealed in *trkB* knockouts (Snider, 1994). Therefore, experiments applying a combination of purified proteins should be the best way to analyze regulation of motoneuron development and to answer the question whether FGF needs co-factors for *in vivo* activity. From *in vitro* studies it is known that the combination of FGF-2 and CNTF results in a 100% survival of embryonic spinal motoneurons (Arakawa *et al.,* 1990). The list of proteins which are probably involved in the control of neuron death also includes other FGF family members. *In vivo* experiments will have yet to demonstrate whether, for example FGF-5, which supports the *in vitro* survival of embryonic motoneurons (Hughes *et al.,* 1993a), prevents ontogenetic motoneuron death.

BDNF and NT-4/5, respectively, rescue about 50% of facial motoneurons after peripheral nerve lesion in newborn rats (Sendtner *et al.,* 1992; Koliatsos *et al.,* 1994). No further increase was observed when both factors were used in combination (Koliatsos *et al.,* 1994) suggesting that BDNF and NT-4/5 are likely to act on the same neuron population. FGF-2 was shown to have no effect on newborn lesioned facial motoneurons (Hughes *et al.,* 1993b) but did rescue 13% and 23% of lesioned hypoglossal motoneurons at P7 and P18, respectively (Grothe and Unsicker, 1992). Whether this reflects different requirements of different neuronal populations or whether this is a development-dependent effect is not yet clear. The rescue effect of purified CNTF on newborn facial motoneurons after lesion, as shown by Sendtner *et al.* (1990) could not be reproduced by a recent study (Clatterbuck *et al.,* 1994). In the adult hypoglossal system the lesion-induced loss of ChAT-activity could be prevented by BDNF but not by CNTF or FGF-2 (Chiu *et al.,* 1994; Grothe and Unsicker, 1992).

For sensory neurons a similar redundancy with regard to the number of active molecules is reported. In contrast to motoneurons it seems that neurotrophins could be sufficient to support development, maintenance, and regeneration of sensory neurons. Sensitivity of sensory neurons to different neurotrophins changes during development and it is known that different neurotrophins affect different sensory subpopulations (Davies, 1992). A possible non-neurotrophic physiological role for FGF in postnatal and adult DRGs has been discussed above.

A final point that has to be addressed is that FGF-1 and FGF-2 display no hydrophobic signal peptide which are normally necessary for secretion via the classical secretory pathway. There is evidence from model systems indicating that FGF-2 is transported, through currently undefined mechanisms, to the cell surface (e.g. Florkiewicz *et al.,* 1991). Thus, FGF-1 and FGF-2 appear to resemble proteins that are released although they lack a signal sequence, like for example the interleukins (Muesch *et al.,* 1990). Further experiments have to show whether FGF-5, which possesses a signal sequence, is actually acting as a target-derived neurotrophic factor like NGF, as proposed by Hughes *et al.* (1993a). Putative activities of FGF-5, however, do not exclude that other FGF family members, like FGF-1 or FGF-2 mediate their activity by different mechanisms.

The discovery of the neurotrophins has led to a more general neurotrophic factor concept. The situation is reminiscent of the immune system where one cell type is influenced by several molecules during development and maintenance. As postulated by Korsching (1993), a set of different parameters might define a multidimensional "neurotrophic state space", where a particular cell type possesses particular coordinates. This concept, however, requires new experimental designs, that, for example, are convenient for analyzing multifactorial interactions of different molecules. Further studies examining FGF function in the nervous system must address the specific distribution of the different FGF family members and their receptors in order to gain insight into possible non-neurotrophic functions and to design appropriate experimental approaches to test these ideas.

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