Fibroblast growth factors as regulators of central nervous system development and function

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Dono, Rosanna. Fibroblast growth factors as regulators of central nervous system development and function. Am J Physiol Regul Integr Comp Physiol 284: R867–R881, 2003; 10.1152/ajpregu.00533.2002.—Fibroblast growth factors (FGFs) are multifunctional signaling proteins that regulate developmental processes and adult physiology. Over the last few years, important progress has been made in understanding the function of FGFs in the embryonic and adult central nervous system. In this review, I will first discuss studies showing that FGF signaling is already required during formation of the neural plate. Next, I will describe how FGF signaling centers control growth and patterning of specific brain structures. Finally, I will focus on the function of FGF signaling in the adult brain and in regulating maintenance and repair of damaged neural tissues.

neural stem cells; anterior neural ridge; isthmic organizer; neocortex; neural development

FIBROBLAST GROWTH FACTORS (FGFs) constitute a large family of structurally related polypeptide growth factors found in organisms ranging from nematodes to humans (17, 53, 71, 148, 189, 200). To date, the mammalian FGF proteins are encoded by twenty-two distinct genes known as Fgf1 to Fgf18 and Fgf20 to Fgf23 in the mouse (25, 71, 133, 189, 236). FGF proteins are small peptides of 155 to 268 amino acid residues (25, 71, 148, 189, 235). The degree of sequence identity between different family members is 30–60% in a “central domain” of ~120 amino acids. This domain confers to FGFs a common tertiary structure and the ability to bind to heparin (42, 245). Most FGFs are constitutively secreted using the endoplasmic reticulum-Golgi secretory pathway (77, 89, 203, 235). A small subgroup of FGF proteins, such as FGF1, -2, -9, -16, and -20, lacks the NH2-terminal signal sequence but is still transported in the extracellular space (36, 103, 135, 137). A third subgroup of FGF proteins, FGF11 to FGF14, lacks the signal peptide and remains intracellular (182).

Secreted FGFs signal to target cells by binding and activating cell-surface tyrosine kinase FGF receptors (FGFRs; 34, 82, 105). FGFRs are transcribed from four different genes and consist of an extracellular domain, a single transmembrane domain, and a cytoplasmic tyrosine kinase domain (34, 82, 105). The extracellular domain contains three immunoglobulin-like domains, called loops I, II, and III (81, 105). Although loops II and III contact the bound ligand, the region that dictates the specificity of binding is the COOH-terminal portion of loop III (20, 150). Alternative mRNA splicing of the COOH-terminal portion of loop III creates several forms of FGFRs with unique ligand-binding properties (81, 150, 212). Once an FGF ligand is bound, the receptor dimerizes and phosphorylates intermolecular tyrosine residues, triggering initiation of FGFR signal transduction (97, 138, 159). FGFR signaling activates a number of signal transduction molecules, including those of the Ras and phospholipase C-γ pathways (92, 97, 139, 221). Interestingly, the intracellular FGF12 and FGF14 do not bind to FGFRs; nevertheless, they interact with the mitogen-activated protein (MAP) kinase scaffold protein Islet-Brain-2 in neurons (145, 182, 222). The regulation of FGFR-ligand interaction is complex. Receptor isoforms can form heterodimers and share redundant ligand binding specificity (150). Moreover, ligand binding is affected by the distribution of heparan sulfate proteoglycans (HSPGs) on the cell surface and in the extracellular matrix (109, 151, 190). Extracellular FGFs, indeed, bind tightly to HSPGs, which may restrict FGF diffusion and favor interaction with receptors on nearby cells (11, 142). Finally, HSPGs promote and stabilize assembly of the FGF ligand-receptor complex (181).

The function of FGFs and FGFRs during embryonic development and adult physiology has been addressed by gain- and loss-of-function experiments in several animal model organisms. These studies have shown that FGFs act as key regulators of developmental
events. For example, FGFs control growth and survival of the postimplantation mouse embryos (7, 43), cell migration during gastrulation (21, 22, 199), and establishment of the anterior-posterior (A/P) body axis (22, 134). At later developmental stages, FGFs function in those organs and tissues in which reciprocal interactions between epithelial and mesenchymal cells are important for morphogenesis and differentiation (31, 183, 198). The discovery that certain human skeletal disorders are caused by point mutations in FgfR1, FgfR2, and FgfR3 (143, 149, 176, 228), and the genetic analysis of FGFR and FGF ligand functions in the mouse (23, 64, 112), have revealed essential roles for FGF signaling in chondrogenesis and osteogenesis. Disruption of FGF signaling may also underlie other pathologies, such as hypotension (37, 244), diabetes (67), and the hypophosphatemic rickets disorder (226).

In this review, I will focus on the function of FGF family members in the central nervous system (CNS). Several Fgfs and FgfRs are expressed in the embryonic and adult CNS (Table 1 and Refs. 51, 69, 70, 189, 193, 233, 241). I will summarize some of the findings showing that FGFs act as key regulators of CNS development and function. I will also discuss studies that address FGF signaling in the adult brain and neural stem cells.

**FGFS DURING INDUCTION AND EARLY PATTERNING OF THE NEURAL PLATE**

Neural induction is the first and fundamental step in the formation of the vertebrate CNS. During this process, pluripotent dorsal ectodermal cells undergo a “cell fate switch” and become neural stem cells instead of epidermal cell types (230). It is generally accepted that neural induction occurs via inhibiting BMP signaling in prospective neural cells, since BMP signaling promotes epidermal cell fate (231). Fgf ligands and receptors are expressed by the prospective neural cells and by the adjacent inducing tissues [Fgf2, -3, -4, and -8 (175, 194, 195, 232); FgfR1, FgfR2, and Fgf3 (219, 232)]. The involvement of FGF signaling in this process has been the subject of intense investigation since the discovery that FGFs promote expression of neuronal markers in *Xenopus laevis* ectodermal cells from an early gastrula stage (87, 88, 101). It has been proposed that FGFs are required for neural induction, since inhibition of FGF signaling in *Xenopus* embryos interferes with development of neural tissue (73, 102). Moreover, FGF2- and FGF4-soaked beads can induce ectopic neural structures when applied to chick primitive streak stage embryos (5).

Dorsal ectodermal cells of an early *Xenopus* gastrula acquire neural fates in response to signaling by Spemann organizer cells (230). These signals antagonize BMP activity and thereby prevent specification of epidermal cell fates (100, 180). Known organizer signals include the two BMP antagonists noggin and chordin (163, 179, 247). Strikingly, inhibition of FGF signaling in ectodermal explants from an *Xenopus* early gastrula precludes the induction of neural tissue by the Spemann’s organizer (73, 102). Moreover, noggin- and chordin-mediated neural induction is abolished in the absence of FGF signaling (102, 179). Taken together, these studies indicate that FGFs cooperate with BMP antagonists to induce neural cell fates.

In apparent controversy, other studies in mouse and chicken embryos have shown that in these vertebrates BMP antagonists are not required for neural induction.

**Table 1. Fgfs and FgfRs in the developing CNS**

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Summary of expression in rat and mouse embryos. Phenotypes data derived from gene targeting experiments in mice. Fgf, fibroblast growth factor gene; FgfR, fibroblast growth factor receptor gene; ANR, anterior neural ridge; AM, anterior midline; Is, isthmus; Te, telencephalon; Di, diencephalon; Ms, mesencephalon; Mt, metencephalon; My, myelencephalon; Sc, spinal cord, ND, not determined.
the more caudal diencephalon (Fig. 1). The forebrain gives rise to the anterior telencephalon and spinal cord (246). At later developmental stages, the forebrain, the midbrain, the hindbrain, and the neural tube become regionalized, and neural progenitors contributing to posterior CNS development (127). At later developmental stages, attenuation of FGF signaling is instead required to promote neuronal differentiation in the developing spinal cord (33).

FGF8 AS MEDIATORS OF NEUROEPITHELIAL ORGANIZER FUNCTIONS

Before neural tube closure, the developing CNS is subdivided along its A/P axis, also known as the rostrocaudal axis, into the following four distinct domains: the forebrain, the midbrain, the hindbrain, and the spinal cord (246). At later developmental stages, the forebrain gives rise to the anterior telencephalon and the more caudal diencephalon (Fig. 1A and Ref. 246). The midbrain will develop as one mesencephalic vesicle (Fig. 1A), whereas the hindbrain is divided in rhombomeres, with the most anterior rhombomere 1 and rhombomere 2, known as the metencephalon (Fig. 1A). As a result of these initial patterning events, the neural tube becomes regionalized, and neural progenitors acquire positional identity. For example, spinal cord progenitor cells will from now on generate spinal cord neurons, whereas telencephalic progenitors will only generate telencephalic neurons. It has been shown that A/P patterning of the early neuroectoderm is controlled by local signaling centers. These signaling centers act within the neural plate to induce and maintain regional identity in the surrounding neuroepithelium. For example, the anterior neural ridge (ANR), which lies at the junction between the anterior ectoderm and the anterior neural plate, is necessary for growth and maintenance of the forebrain identity (188). The isotheminc organizer (Iso) lies at the midhindbrain junction and regulates proper development of the mesencephalic and metencephalic derivatives [e.g., optic tectum and cerebellum (124)]. FGF family members, such as Fgf8, Fgf17, and Fgf18, are expressed at the ANR and Iso (Fig. 2A and Refs. 25, 125, 235, 236). Genetic and embryological studies have shown that they play important roles in executing neuroepithelium organizer functions (188). For example, removal of the ANR in neural plate explants leads to downregulation of Bf1 expression, a transcription factor essential for growth and patterning of the telencephalic vesicles (188, 238). The fact that beads soaked with FGF8 can induce Bf1 expression suggests that FGF8 can substitute for ANR functions (188). In agreement with these studies, Fgf8 is expressed by the ANR cells from day 8.5 onward (25), and embryos carrying a hypomorphic Fgf8 allele have small telencephalic vesicles (134). Moreover, zebrafish embryos lacking a functional FGF8 protein ("acerebellar" mutants) show disruption of the commissural axon pathway and patterning defects in the basal telencephalon (185). The phenotypes observed in acerebellar mutants are less severe than those resulting from ANR ablation (185, 188) suggesting that FGF8 is not the only medi-
ator of ANR function and acts in combination with other ANR signals.

In contrast to the situation in the ANR, FGF8 is a key mediator of the IsO functions. It is expressed by the IsO cells when the IsO is active (embryonic days 8–12.5; Fig. 3A and Ref. 25). When FGF8 protein beads are applied to different neural locations, for example diencephalon and mesencephalon, FGF8 can induce ectopic expression of genes normally present at the mes-metencephalic junction and additional cerebellar structures (26, 106, 123). Moreover, application of FGF8 beads to the hindbrain rhombomere 1 shifts the anterior boundary of the rhombomere 1 more posteriorly, as evidenced by changes in Hox gene expression patterns (79). In agreement with these gain-of-function studies, FGF8 loss-of-function mutations in zebrafish and mice lead to IsO tissue loss (134, 171). In addition, FGF8 zebrafish mutants lack cerebellar structure and show patterning defects in the developing tectum and its retinotectal projections (164, 171). Thus FGF8 secreted by the IsO cells influences cell specification, leading to the induction and patterning of specific CNS structures. Consistent with a role for FGF8 in cell fate specification, FGF8 in combination with SHH and FGF4 can induce dopaminergic and serotonergic neurons in neural plate explants (242). These and other

Fig. 2. Fibroblast growth factor (FGF) 2 functions during development of the neuronal layers in the embryonic neocortex. Left: schematic representation of a coronal section from an embryonic day 14 mouse brain at the level of the lateral ventricles (LV). The rectangle indicates the approximate position of the enlargement shown on right. Right: enlargement of the ventricular zone (VZ) and developing cortical plate (CP). During development of the neuronal layers (NL) of the neocortex, progenitor cells in the VZ divide asymmetrically (M) to generate one proliferating cell that remains in the VZ (arrowhead) and one postmitotic immature neuron. Postmitotic immature neurons leave the ventricular zone and migrate toward the margin of the cerebral wall, where they form the neuronal layers. Proliferating progenitors generate postmitotic neurons at precise time points. Neurons that become postmitotic during early development will form the deep layers, whereas those leaving the cell cycle at later stages will migrate through the existing cell layers and form more superficial ones. In FGF2-deficient mice, a fraction of postmitotic neurons fail to reach their target neocortical layers. FGF2 is highly expressed by the cells of the ventricular zone, indicating that FGF2 is part of the signaling network that determines the laminar fate of postmitotic migrating neurons. GE, ganglionic eminence. Modified from Ref. 177.

Fig. 3. Spatial distribution of Fgf8 and FGF2 during patterning of the vertebrate CNS. A: distribution of Fgf8 transcripts detected by whole mount in situ hybridization on a mouse embryo at embryonic day 9.25 (E9.25). Note the expression of Fgf8 by the anterior medial cells of the telencephalon (arrowhead) and by the isthmic organizer (IsO) cells (arrow). B: distribution of FGF2 proteins detected by whole mount antibody staining on a chicken embryo at stage 17 (staging according to Hamburger and Hamilton (65)). FGF2 proteins are present throughout the embryonic brain, and protein levels are higher in the developing telencephalon and metencephalon (arrowheads). FGF2 proteins are also found in the developing spinal cord (arrow). Ba, branchial arch; FL, forelimb; HL, hindlimb; S, somite.
findings have led to the proposal that FGF8 produced by the ANR and the IsO, in combination with SHH and FGF4, creates a grid of positional information in the neural tube that specifies forebrain and midbrain dopaminergic neurons and hindbrain serotonergic neurons (242).

Fgf17 is also expressed by IsO cells after the onset of Fgf8 expression (170, 236). Loss-of-function studies in mice have shown that these FGFs cooperate in regulating cerebellar growth and shape by maintaining the precursor cell pool in an undifferentiated proliferating state (236). Interestingly, FGF2 proteins are also present in the metencephalon (Fig. 3B and Ref. 37), and a single peripheral injection of FGF2 stimulates granule cell production and enhances cerebellar growth in newborn rats (19). The IsO and its adjacent cell layers also express Fgf18 and Fgf15, shortly after the onset of Fgf8 transcription (49, 125). Taken together, these observations suggest that at least four different FGFs may act sequentially to determine the final size and shape of the cerebellum.

In addition to control cerebellar development, FGFs appear to have more general roles during patterning of the vertebrate hindbrain. For example, it has been proposed that FGFs participate in the establishment of rhombomere identity during regionalization of the hindbrain (Fig. 1 and Ref. 117). In zebrafish embryos, signaling by rhombomere 4 cells influences segmental identity and promotes neuronal differentiation of adjacent rhombomeres (129). The presumptive rhombomere 4 cells specifically express Fgf3 and Fgf8, and development of rhombomere 5 and 6 is impaired by blocking FGF3 and FGF8 functions (129, 218). These studies indicate that FGF3 and FGF8 at the rhombomere 4 mediate the action of this signaling center in promoting development of more caudal rhombomeres. It is important to note that expression of Fgf3 in rhombomere 4 is conserved among vertebrates (119, 120), whereas Fgf8 expression is not. This raises the question whether this FGF-mediated signaling center also functions in other vertebrates. The fact that Fgf3 is coexpressed with Fgf4 in chicken embryos (184) suggests that other Fgfs may substitute for Fgf8 in other species.

**FGF PROTEINS AS REGULATORS OF NEOCORTEX DEVELOPMENT**

During mammalian CNS development, the neocortex arises from the dorsal telencephalon (Fig. 1B). This structure will undergo rapid expansion by midembryogenesis so that it will become the predominant brain structure (140). As development proceeds, the neocortex is partitioned into anatomically distinct areas along the A/P and mediolateral axis. For example, the motor and sensory cortices develop in the anterior and the visual cortex develops more posterior (140). Neurons of the neocortex will also be organized into six distinct layers running from the lumen of the neural tube to the margin of the cerebral wall (130). These cell arrangements have functional consequences, since neurons will develop synaptic connections according to the position they occupy within the neocortical areas and layers (130). It has been shown that growth and patterning of the neocortex is strictly dependent on localized production of instructive signals acting on the progenitor cells, which lie near the lateral ventricle in a layer known as the ventricular zone (VZ; Fig. 1B and Ref. 130). Several studies have shown that FGFs are among the regulatory signaling molecules. The expression of Fgfs and Fgfrs in the developing mouse and rat neocortices is spatially and temporally regulated. In particular, the cells of the VZ express high levels of FgfR1, FgfR2, and FgfR3 during the expansion of the neocortical progenitor pool and throughout neurogenesis (37, 158, 160, 168). In agreement with receptor distribution, FGF ligands are also found in the VZ. In particular, FGF2 proteins are abundant at early developmental stages and nearly absent by the end of neurogenesis (37, 147, 167). Other FGFs, such as Fgf7 and Fgf18, are also transiently expressed in the developing neocortex [embryonic days 14.5–15.5 (75, 126)], and their transcripts are found in the VZ and developing cortical plate, respectively. In contrast to these, Fgfs, Fg8, and Fgf17 are predominantly expressed by the anterior medial cells of the neocortical primordium (Fig. 3A and Refs. 25 and 236), a signaling center for neocortex A/P patterning (168). This suggests that these FGFs might act as paracrine factors to regulate development of the anterior neocortex. The biological effects of FGFs on neocortical progenitor cells have been first studied on cultured neocortical cells. FGF2 turned out to be among the most potent mitogenic and survival factors for many CNS cell types, including embryonic neocortical VZ cells (18, 169, 213, 214). Other studies have shown that FGF2 acts either alone or in combination with neurotrophins to promote differentiation of neocortical precursor cells (48, 144, 157). Finally, multipotential embryonic day 10 mouse cortical cells will generate either neurons or astrocytes in response to different concentrations of FGF2 in culture media (166). Interestingly, other FGFs have additional or distinct functions on neural progenitors. For example, FGF8 can induce dopaminergic neurons in explants of rostral fore- and midbrain, whereas FGF4 and FGF2, but not FGF8, can ectopically induce serotonergic neurons in midbrain explants (242). Moreover, FGF4 and FGF8b (an FGF8 isoform) promote proliferation and survival of neuronal precursors, whereas only FGF8b promotes differentiation along the astrocyte lineage (63). The in vivo function of some of these FGFs has been investigated by combining embryonic manipulation with mouse molecular genetics. Genetic analysis of FGF2 functions in mice has shown that FGF2 regulates neuronal density and cytoarchitecture of the developing neocortex (37, 153, 210). Neocortices of FGF2-deficient mice contain fewer neurons at maturity (37, 153, 210) because of possible defects in proliferation of progenitors (167). In addition, a fraction of postmitotic neurons fail to reach their target layer in the developing neocortex of FGF2-deficient mice. These cells either remain in deeper layers...
or accumulate in the corpus callosum (37). These genetic studies show that lack of FGF2 affects cell positioning in the developing neocortex. Development of the neuronal layers of the neocortex begins once newly generated postmitotic neurons leave the VZ and migrate to the cortical plate. It has been shown that the migratory paths of postmitotic neurons are defined by instructive signals acting on the progenitors in the VZ as these cells undergo their last mitotic division (130). Signals also act in the cortical plate and direct laminar organization of migrating neurons. Neuronal cells are exposed to FGF2 before or during onset of neuronal migration (Fig. 2 and Ref. 37). Indeed, FGF2 is expressed at high levels by the cells of the VZ, whereas it is not expressed by migrating neurons or other cortical plate cells (37). Thus it is likely that FGF2 is part of the signaling network that acts on the progenitor cells and defines the cell fate and migratory path of postmitotic neurons (2).

The neuronal defects observed in FGF2-deficient cortices predominantly affect the frontal motor sensory areas (37, 153, 211). This observation raises the possibility that FGFs are part of the signaling network regulating differential growth and patterning of neocortical areas. As discussed above, Fgf8 is expressed by the anterior medial cells of the telencephalon (25). Fukuchi-Shimogori and Grove (44) have shown that anterior expansion of the FGF8 source shifts the boundaries of the cortical areas more posterior, whereas reducing the endogenous FGF8 signal shifts these boundaries more anterior. Moreover, an ectopic posterior source of FGF8 instructs surrounding cells to acquire anterior identity. It is important to note that no changes in cortical size were observed in these experiments. Thus Fgf8 appears to specify positional identity in the neocortical primordium without affecting cell proliferation (44). Regional specification of the neocortical neural stem cells is mediated by gradients of transcriptional regulators such as Emx2 and Pax6. In particular, Emx2 is expressed at high levels by neocortical progenitors of the posterior VZ and at low levels by those of the anterior VZ. In contrast, Pax6 is high in the anterior VZ and low in the posterior (12, 121). FGF8 beads applied to the dorsal telencephalon of developing chicken embryos inhibit Emx2 expression (27). It will be important to understand whether FGF8 regulates regionalization of the neocortex by acting on these transcription factors or on other unknown regulators.

**FGFS IN THE DEVELOPING SPINAL CORD**

Development of the spinal cord leads to the establishment of different neuronal cell types along its dorsoventral axis (14). For example, motoneurons will differentiate ventrally, commissural neurons will form dorsally, and neurons of the autonomic nervous system will develop within the intermediate spinal cord layer (14, 192). Spinal motoneurons innervate the muscles, and their survival is dependent on trophic factors that are produced by the targeted muscle cells and by the neuron itself (39). Several Fgf genes (e.g., Fgf1 and -2 and -4 and -5) are expressed in the developing skeletal muscles (38, 66, 76) and by spinal motoneurons (e.g., Fgf1 and Refs. 9, 40, 83). Motoneurons also express FGFRs (162, 220). In vitro, FGF2, FGF5, and FGF9 promote survival of cultured chick and rat spinal motoneurons (62, 76, 83) and FGF2 and FGF9 upregulate the choline acetyltransferase (ChAT) activity in a dose-dependent manner (62, 83). The biological effects of FGFs on motoneurons have also been studied on experimentally induced motoneuron damages and in animal models of motoneuron diseases. As in vitro, also in vivo local infusion of FGFs can rescue motoneuron death induced by nerve fiber lesions (axotomy) or spinal cord injury (28, 108, 206). However, levels of ChAT activity remain low even in the presence of FGF2 (61). FGF2 also mediates motoneuron survival in the wobbler mouse affected by a motoneuron disease (78). FGF2-deficient mice show neuronal deficiencies in the cervical spinal cord region that also affect motoneuron density (37). However, mice do not show apparent defects resulting from the lack of a fraction of these motoneurons (37, 153, 244). Homozygous null Fgf9 mice die shortly after birth, and motoneuron development has not been analyzed in these mice (24). Additional studies need to be performed on these mutant strains to understand better if and how these FGFs contribute to motoneuron development and function. It is likely that this process requires a combination of different trophic factors, among which are FGFs (14).

Physiological and pharmacological studies on FGF2-deficient mice have shown that FGF2 is essential for other spinal cord functions. In particular, lack of FGF2 causes an impaired baroreceptor reflex response to hypotensive stimuli in adult mice (37). As a result of the neuronal regulation defect, FGF2-deficient mice show a reduced resting arterial blood pressure (37, 244). During CNS development, Fgf2 is expressed by progenitor cells of neuronal circuits involved in the central regulation of blood pressure, for example, in the myelencephalon (Fig. 3B and Ref. 36) and the intermediodorsal neurons of the spinal cord (192). Reexpression of FGF2 in the developing nervous system of FGF2-deficient embryos leads to a rescue of the baroreceptor reflex and of the hypotensive phenotype (36). These genetic studies indicate that FGF2 signaling is essential for development of the neural circuitry regulating central regulation of blood pressure (36). The distribution of certain classes of neurons of the intermediate cell layer is affected in FGF2-deficient embryos (E. ten Hove and R. Dono, unpublished observations), suggesting that positional identity of spinal cord neurons may be altered.

**FGFS IN BRAIN PHYSIOLOGY AND PATHOLOGY**

Most of the Fgf family members and Fgf receptors remain expressed in different cell types of the adult brain (Fig. 4 and Refs. 50, 69, 70, 189, 193, 233, 241). A number of recent studies are beginning to address the role of FGFs in brain physiology and pathology.
Most studies have focused on the possibility that FGF2 acts as a neurotrophic factor on mature brain neurons. For example, several laboratories have shown that FGF2 can promote survival of neocortical, hippocampal, cerebellar, dopaminergic, spinal cord, and sensory neurons isolated from adult CNS (1, 13, 60, 95, 116, 128). The neuronal survival promoted by FGF2 is independent of its mitogenic activity for glial cells (217). However, studies on FGF2-deficient mice have failed to reveal an increased cell death in the brain of embryos and adults (37, 167). Interestingly, FGF2 levels increase after CNS damage (50, 59), ischemia (47, 243), or seizure (243). Changes in FGF2 levels are also observed in patients with neurodegenerative disorders, such as Alzheimer’s and Parkinson’s diseases (52, 207). These observations raise the possibility that FGF2 acts to protect the brain from pathological events, where it promotes survival and/or has additional effects on neural cells. The biological effects of FGF2 in response to brain damage are currently being tested using a variety of experimental systems. As discussed above, neuronal cell death can be induced in vivo by performing axotomy. Peterson et al. (161) have shown that axotomy-induced death of glutamatergic neurons is prevented by grafting fibroblasts expressing FGF2 before axotomy. Brain damage can also be induced by injecting kainic acid, which causes seizures and neuronal cell death (113). When FGF2 is infused in the rat brain before seizure, it can prevent cell loss in the hippocampal region (113). FGF2 seems to rescue the injured neurons and promote brain regeneration through multiple strategies. Infusion of antibodies against FGF2 in the lateral ventricles leads to a significant reduction in sprouting of cholinergic neurons within a denervated hippocampus (41). These results are consistent with neurite-promoting effects of FGF-2 on cultured cholinergic neurons (6, 154). Progenitor cells of the hippocampus proliferate and differentiate in response to cerebral ischemia or seizures (110, 111, 201). Yoshimura et al. (243) have demonstrated that this process is affected in FGF2-deficient mice. Neurogenesis is restored upon delivering exogenous FGF2 to the hippocampus before cerebral ischemia and/or seizures (243). The molecular mechanisms underlying the potential FGF2-mediated repair of damaged brain cells are still unknown. Lenhard et al. (107) have shown that the neuroprotective effects of FGF2 on glutamate-induced hippocampus lesions are in part mediated by glial cell-derived neurotrophic factors. Instead, the neuroprotective action of FGF2 in stroke-induced cell death is dependent on the induction of activin A (208).

The potential use of FGF2 for treatment of brain disorders is very attractive. Clinical trials of intravenous administration of FGF2 for treatment of acute stroke are in progress (8, 9). However, further studies are required to determine if FGF2 is an efficient therapeutic reagent for treatment of disorders affecting the adult brain. Direct insight into a more general role for FGFs in brain physiology and pathology will come from detailed analysis of mouse mutant strains carrying loss-of-function mutation of FGFs or FGFRs expressed in the adult brain. Indeed, genetic analysis of FGF14 functions has shown that FGF14-deficient mice develop ataxia and hyperkinetic movement disorders similar to those found in patients affected by Huntington’s disease, Parkinson’s disease, and dystonia (222). These motor abnormalities are associated with dysfunction of the basal ganglia system and result from defects in axonal trafficking and synopsis.
FGFS AND THE NEURAL STEM CELLS IN THE ADULT CNS

For many years, the adult brain was wrongly considered an entirely postmitotic structure. More recently, research has corroborated previous findings by Altman and coworkers (4, 45, 84, 86, 98, 205) that established that specific areas of the adult brain retain the capacity for neurogenesis. Neurogenesis occurs in at least two sites of the adult brain: the subgranular zone of the hippocampus (3, 85) and the telencephalic subventricular zone (SVZ; 94, 114, 118). The subgranular zone generates the granule cells of the hippocampus (85, 86, 191), whereas the SVZ is the source of new olfactory bulb neurons (93, 114, 115, 118). Generation of neurons has also been reported in the primate prefrontal cortex, temporal cortex, and parietal cortex (4, 54, 84), and it has been proposed that the SVZ might also be the source of these neurons (4, 54, 84). Neurogenesis in the adult brain relies on neural stem cells (29, 90, 172, 225). Neural stem cells are defined as undifferentiated cell types that undergo self-renewal and thereby retain their multilineage potential (132). Recently, neural stem cells have been isolated from many CNS regions, including the previously mentioned neurogenic zones and nonneurogenic regions, such as the spinal cord (30, 90, 141, 225). In vitro, proliferating neural stem cells form aggregates, so-called neurospheres, that maintain both the capability of self-renewal and the ability to differentiate into neurons, astrocytes, and oligodendrocytes (46, 55, 173, 209, 213, 225). It has been shown that the in vitro expansion and differentiation of neural stem cells can be regulated by adding extracellular factors to the culture medium (46, 55, 58, 80, 156, 209, 213, 225). FGF2 and EGF are the most potent mitogens and survival factors for cultured neural stem cells (46, 141, 172, 173, 209, 213, 225). For example, FGF2 and EGF have been used both alone and in combination to isolate and maintain stem cells of the adult SVZ and the spinal cord in culture (55, 56, 141, 173, 187, 225). FGF2 alone is sufficient to maintain neural stem cells from either the adult striatum (57) or hippocampus (46) of rodents. Taupin et al. (204) have recently shown that cystatin C, a cystein proteinase inhibitor, is able to potentiate the mitogenic activity of FGF2 and enables expansion of rat hippocampal neural stem cells from single cells. It will be interesting to examine if cystatin C also cooperates with FGF2 to promote proliferation of neural stem cells isolated from other CNS regions.

One major question in stem cell biology concerns the type of progeny that neural stem cells can generate. Differentiation of cultured neural stem cells can be induced by mitogen withdrawal or by addition of extracellular factors that will drive differentiation along the neuronal or glia cell lineage (58, 80, 202). However, it is not clear whether differentiating neural stem cells are able to generate functional neuronal subtype, such as GABAergic interneurons or cortical pyramidal neurons, in addition to differentiation of a generic neuronal phenotype. Recent studies using FGF2-responsive hippocampal neural stem cells show that these cells can differentiate into cells with phenotypes of GABAergic, dopaminergic, and cholinergic neurons when exposed to both retinoic acid and neurotrophins (202). In addition, forced expression of the orphan nuclear receptor Nurr1 (104) in these cells induces predominant differentiation of tyrosine hydroxylase (TH)-positive dopaminergic neurons (178, 215).

Another way of testing the developmental potential of neural stem cells in vivo is their reimplantation in the adult CNS (15, 16). Suhonen et al. (197) have demonstrated that cultures of adult rat hippocampal neural stem cells undergo neuronal differentiation only when implanted in neurogenic sites. When transplanted in the hippocampus, cells that migrate to the neuronal layer of the dentate gyrus give rise to hippocampal-like neurons. In contrast, hippocampal progenitors differentiate into TH-positive neurons, a marker for dopaminergic neurons, when implanted in the rostral migratory stream leading to the olfactory bulb (197). As discussed previously, neural stem-like cells can be cultured from the adult spinal cord in the presence of FGF2 (187). Shihabuddin et al. (186) showed that these cells, although isolated from a nonneurogenic region, exhibit a broad developmental potential upon exposure to different environmental stimuli. Spinal cord neural stem cells give rise to glial cells if transplanted back into the adult spinal cord. When transplanted in the hippocampus, cells that integrate in the neuronal layer of the dentate gyrus differentiate into hippocampal-like neurons of the granular cell layer (186). Alternatively, they acquire an astroglial and oligodendroglial phenotype when integrated in nonneurogenic regions of the hippocampus (186). These studies indicate that neural stem cells expanded in the presence of FGF2 retain pluripotency and integrate into the host tissue where they respond to local differentiation signals.

The possibility that FGF2 may also promote proliferation of neural stem cells in vivo is currently being investigated. For example, Wagner et al. (216) have shown that subcutaneous injection of FGF2 increases the number of proliferating cells in the SVZ and olfactory tract. Injection of FGF2 in the lateral ventricle of an adult rat brain expands the SVZ progenitor pool and increases the number of neurons in the olfactory bulb (99). Finally, combined injection of FGF2 and cystatin C to the adult dentate gyrus stimulates proliferation and neurogenesis in the adult rat hippocampus (204).

CONCLUSIONS AND FUTURE DIRECTIONS

Research over the past years has advanced our understanding of the role of FGFS signaling in the embryonic and adult CNS. These studies have shed light on the role of FGFS during neural induction, patterning of specific CNS regions, and in the establishment of functional neuronal circuits. However, several major issues remain unanswered. The gene targeting approach in mice and analysis of zebrafish mutants have clarified the functions of some of the Fgfs expressed by neural
I am grateful to Liliana Minichiello and Klaus Unsicker for discussion on Fig. 4. I also thank Jacqueline Deschamps, Rolf Zeller, and members of the laboratory for critical comments that have improved this manuscript. I apologize to many researchers in this fast-moving field whose work I have not cited because of space limitations.

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