Molecular Neurobiology

ISSN0893-7648/96/13(3): 227–255/$12.25

The Role of the Insulin-Like Growth Factors in the Central Nervous System

A. Joseph D'Ercole, Ping Ye, Ali Suha Calikoglu, and Gabriel Gutierrez-Ospina*

Department of Pediatrics CB# 7220, The University of North Carolina, Chapel Hill, NC 27599-7220

Abstract

Increasing evidence strongly supports a role for insulin-like growth factor-I (IGF-I) in central nervous system (CNS) development. IGF-I, IGF-II, the type I IGF receptor (the cell surface tyrosine kinase receptor that mediates IGF signals), and some IGF binding proteins (IGFBPs; secreted proteins that modulate IGF actions) are expressed in many regions of the CNS beginning *in utero*. The expression pattern of IGF system proteins during brain growth suggests highly regulated and developmentally timed IGF actions on specific neural cell populations. IGF-I expression is predominantly in neurons and, in many brain regions, peaks in a fashion temporally coincident with periods in development when neuron progenitor proliferation and/or neuritic outgrowth occurs. In contrast, IGF-II expression is confined mainly to cells of mesenchymal and neural crest origin. While expression of type I IGF receptors appears ubiquitous, that of IGFBPs is characterized by regional and developmental specificity, and often occurs coordinately with peaks of IGF expression.

In vitro IGF-I has been shown to stimulate the proliferation of neuron progenitors and/or the survival of neurons and oligodendrocytes, and in some cultured neurons, to stimulate function. Transgenic (Tg) mice that overexpress IGF-I in the brain exhibit postnatal brain overgrowth without anatomic abnormality (20–85% increases in weight, depending on the magnitude of expression). In contrast, Tg mice that exhibit ectopic brain expression of IGFBP-1, an inhibitor of IGF action when present in molar excess, manifest postnatal brain growth retardation, and mice with ablated IGF-I gene expression, accomplished by homologous recombination, have brains that are 60% of normal size as adults. Taken together, these in vivo studies indicate that IGF-I can influence the development of most, if not all, brain regions, and suggest that the cerebral cortex and cerebellum are especially sensitive to IGF-I actions. IGF-I's growth-promoting in vivo actions result from its capacity to increase neuron number, at least in certain populations, and from its potent stimulation of myelination. These IGF-I actions, taken together with its neuroprotective effects following CNS and peripheral nerve injury, suggest that it may be of therapeutic benefit in a wide variety of disorders affecting the nervous system.

Index Entries: Insulin-like growth factors; insulin-like growth factor receptors; insulin-like growth factor binding proteins; brain; transgenic mice.

*Author to whom all correspondence and reprint requests should be addressed.

Abbreviations: BBB, blood–brain barrier; CNS, central nervous system; E, embryonic day; EGF, epidermal growth factor; EAE, experimental autoimmune encephalomyelitis; FGF, fibroblast growth factor; GH, growth hormone; ISHH, *in situ* hybridization histochemistry; IRS, insulin receptor substrate; IGF-I and IGF-II, insulin-like growth factor-I and -II; IGFBPs, insulin-like growth factor binding proteins; MBP, myelin basic protein; MT-1, metallothionein-1; P, postnatal day; PLP, proteolipid protein; Tg, transgenic.

Introduction

Increasing evidence strongly supports a role for the insulin-like growth factors (IGF-I and IGF-II) in central nervous system (CNS) development (Ishil, 1993; Raizada and LeRoith, 1993; DePablo and De la Rosa, 1995). The IGFs, as well as their cell surface receptors (type I and II IGF receptors) and IGF binding proteins (IGFBPs), are expressed early in the development of the CNS. IGFs (especially IGF-I) have been shown to stimulate the proliferation and/or survival of neural cells in culture, and, in some cases, differentiation and/or specialized neural function. Studies of transgenic (Tg) mice that overexpress IGF-I (Mathews et al., 1988; Behringer et al., 1990) and studies of mice with disruptions of the IGF-I gene or type I IGF receptor gene also point to an essential role for IGFs in brain development (Baker et al., 1993; Liu et al., 1993). This review provides an overview of the IGFs, their receptors, and binding proteins, summarizing recent research into their role in brain development and function. For a review of IGF actions in the peripheral nervous system, *see* Ishii et al. (1994).

Overview of the IGFs, IGF Receptors and IGFBPs

IGF-I and IGF-II

IGF-I and IGF-II are anabolic peptides of 70 and 67 amino acids (Fig. 1), respectively, that share homology with each other and proinsulin (Daughaday and Rotwein, 1989). Each is the product of single, large (~95 and ~35 kb, respectively), complex genes. Both are expressed in most tissues from early in embryonic development, with IGF-II being more abundantly expressed *in utero* in most tissues. During postnatal life, IGF-I is more abundantly expressed, is regulated in many tissues by growth hormone (GH), and mediates most of GH's growth promoting actions (Behringer et al., 1990).

Multiple studies of cultured cells have demonstrated that IGFs can stimulate cell proliferation and, in some cases, differentiation. For these reasons the IGFs have been considered important in development. The deduced importance of the IGFs in growth and development has been confirmed by studies in Tg mice in which each of the IGF genes has been disrupted by homologous recombination. Disruption of the IGF-I gene results in growth-retarded mice (an ~40% reduction in body weight at birth) that usually die at the time of birth (Baker et al., 1993; Liu et al., 1993), whereas disruption of the IGF-II gene yields similar growth-retarded mice who otherwise appear normal (DeChiara et al., 1990). Disruption of the type I IGF receptor gene, the cell surface receptor that mediates most, if not all, of the actions of both IGFs, yields mice that are about 40–45% of normal weight at the time of birth and invariably die (Liu et al., 1993).

IGF Receptors and Signal Transduction

The actions of the IGFs are mediated by interaction with cell surface receptors (Czech, 1989). The type I IGF receptor is the major transducer of IGF signals. It is a heterotetramer composed of paired, disulfide-linked α-and β-subunits (Fig. 2). The α-subunits are extracellular and bind IGFs, whereas the β-subunits span the cell membrane and possess tyrosine kinase activity. The type I IGF receptor is the product of a single gene that shares structural homology with the insulin receptor. The type I

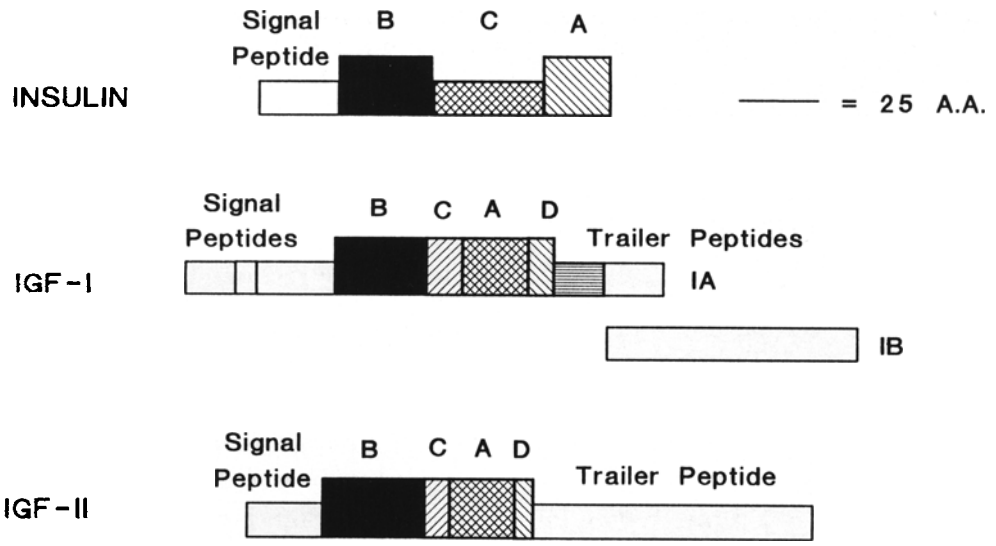

Fig. 1. Schematic of the structure of insulin, IGF-I, and IGF-II precursors. The large boxes depict the domains of the mature peptides; the small boxes depict domains (signal and trailer peptides) included in the precursors. Note that homologous domains are depicted with the same shading and are labeled.

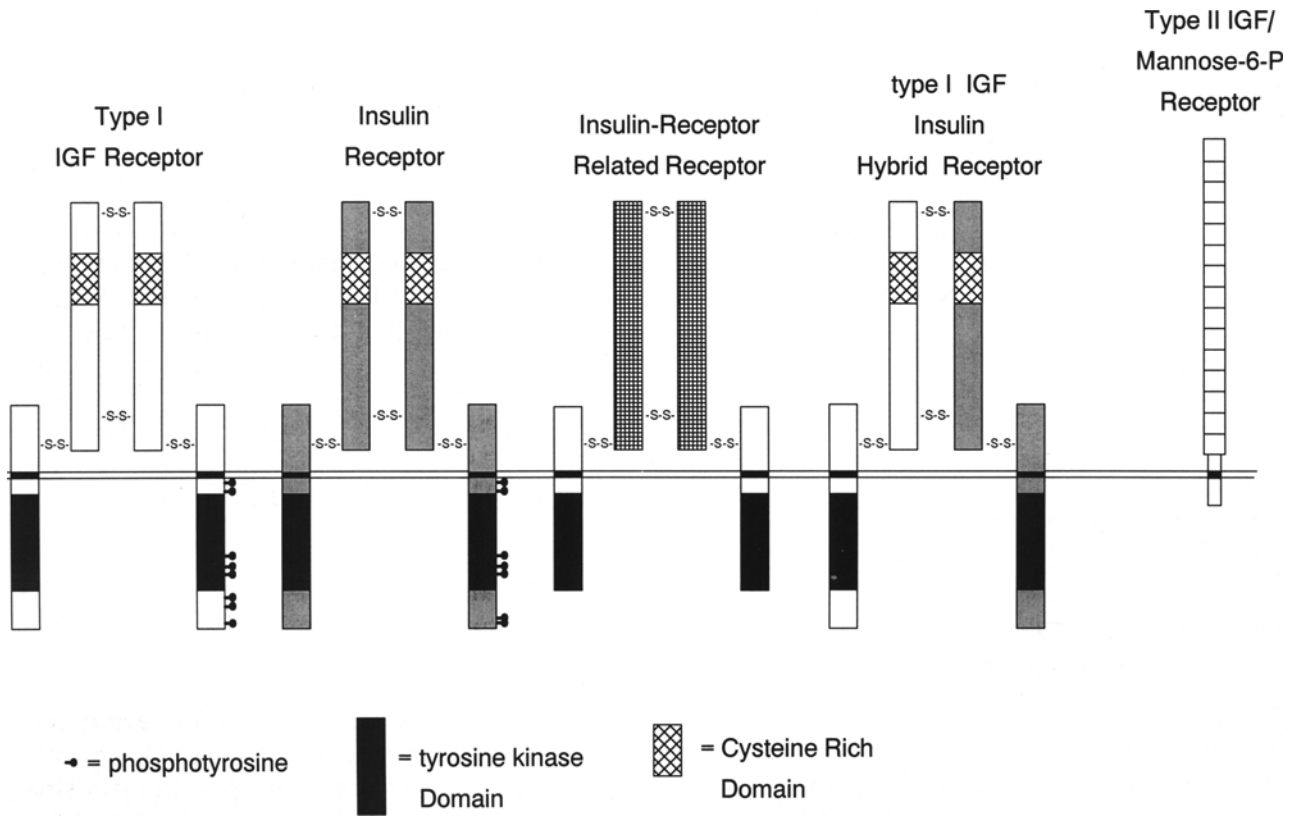

Fig. 2. Schematic of the structure of the type I IGF receptor, compared to that of the other related receptors and the type II IGF/mannose-6-phosphate receptor.

IGF receptor and the insulin receptor can form hybrid receptors by utilizing the α- and β-subunits of each to form heterodimers, but the functional significance of these hybrids is not known.

The type II IGF receptor (Fig. 2) is identical to the cation-independent, mannose-6-phosphate receptor, and acts to translocate proteins containing mannose-6-phosphate moieties and IGF-II to lysosomes for degradation. There is little convincing evidence that IGF interaction with this receptor signals IGF growth-promoting activity.

Binding of IGFs to the α-subunit of the type I IGF receptor induces a conformational change in the receptor that results in autophosphorylation of the β-subunit, and sets into motion signaling cascades that involve phosphorylation of a series of intracellular messengers (LeRoith et al., 1995). After autophosphorylation, the β-subunit's tyrosine kinase phosphorylates insulin receptor substrates (IRS-1 and/or IRS-2). When phosphorylated, the IRSs bind to the complex of Grb2, an src homology (SH) domain protein, and Sos. This complex of three proteins then translocates to the plasma membrane, where it activates Ras, a small membrane G protein. Activation of Ras in turn leads to the phosphorylation of Raf and the activation of the MAP kinase cascade. The latter pathway is common to the actions of many growth factors, and ultimately leads to transcription of early response genes. These genes generally encode transcription factors that then act to stimulate the transcription of late response genes. The products of these delayed response genes are the effectors of the end-result of growth-factor stimulation, such as progression through the cell cycle and differentiation. IRSs, however, also can bind to phosphoinositol-3 kinase, and in so doing initiate another signaling cascade involving phosphorylation of inositol moieties. The signaling pathways set in motion by the activation of the type I IGF receptor, however, are not completely elaborated. Some evidence suggests that type I IGF receptor signaling also may occur independent of IRS, probably by direct binding of SH domain proteins, such as Shc and Crk, to phosphorylated tyrosine motifs at the carboxy terminal end of the β-subunit.

IGFBPs

The IGFs exist in serum and tissues predominantly bound to high affinity extracellular proteins (probably >97%), termed IGFBPs (Baxter, 1991; Clemmons, 1991; Shimasaki and Ling, 1992). Six IGFBPs, named IGFBP-1 through IGFBP-6, have been identified. They comprise a family of proteins that share a common cysteine motif in their amino and carboxy terminal thirds, which accounts for their capacity to bind IGFs (Fig. 3). IGFBPs account for the relatively high levels of IGFs that circulate in the blood. They also modulate IGF actions, being capable of both inhibiting and augmenting IGFs' interactions with cell-surface receptors. The specific modulating activities of IGFBPs differ among IGFBPs and upon their posttranslational modification. Like the IGFs, IGFBPs are expressed in many, if not all, tissues. The expression of each IGFBP is cell specific and often developmentally regulated. The coordinate expression of IGFBPs and IGFs in many tissues suggests that the IGFBPs are important in the regulation of IGF action within the local milieu.

Sites and Ontogeny of Expression of IGF System Proteins in the CNS

The IGFs, IGF receptors, and IGFBPs are expressed in the CNS during development. The ontogeny and sites of expression of these proteins suggest a highly coordinated, complex interaction of these proteins in CNS development. IGF-I expressing neurons usually express type I IGF receptors, suggesting autocrine actions for IGF-I. However, cells nearby IGF-I expressing neurons typically also express type I IGF receptors, indicating paracrine actions. In addition, IGFBPs are often coordinately expressed near neurons expressing IGF-I, suggesting the possibility that they may be responsible for translocating IGF-I

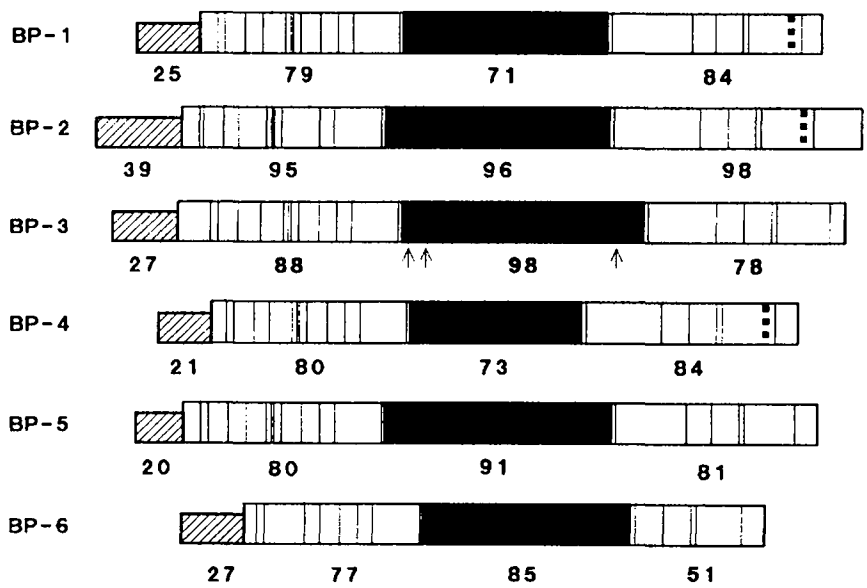

Fig. 3. Schematic of the precursor structure of the human IGFBPs. The small boxes depict the signal peptides; the large boxes depict the mature proteins with their three domains. The number of amino acids in each domain is shown below each diagram. The lines in the amino and carboxy terminal thirds of each IGFBP depict the positions of cysteine residues. The thick broken lines in the carboxy terminus of IGFBP-1, -2, and -3 represent potential integrin recognition sites. The arrows below IGFBP-3 point to glycosylation sites.

and/or modulating its interaction with cell-surface receptors.

IGFs not synthesized in the CNS also could have a role in CNS development and function. Recent evidence has demonstrated in vivo transport of IGFs across the blood–brain barrier (BBB) (Reinhardt and Bondy, 1994). When labeled IGF-I and IGF-II were infused into the common carotid arteries of adult rats, they could be detected in the choroid plexus, median eminence, brain arterioles, and parenchyma. Bovine (Frank et al., 1986), rat (Rosenfeld et al., 1987), and human (Duffy et al., 1988) brain capillary endothelial cells, the cells that form the BBB, each express type I IGF receptors. Because these receptors are capable of internalizing IGFs, they could provide a mechanism by which IGFs are transported to the CNS from the circulation. Such transport, however, appears to be limited, because there is no significant correlation between serum and cerebrospinal fluid (CSF) IGF concentrations in normal people and patients with pituitary disorders (Backstrom et al., 1984). In balance, therefore, it appears that IGFs in the circulation are not a major source of IGFs for the CNS.

IGF Expression

As determined by *in situ* hybridization histochemistry (ISHH), IGF-I expression in the developing rodent brain begins by at least embryonic day (E) 14 and occurs predominately in neurons (Bartlett et al., 1991; Bondy, 1991; Bondy and Lee, 1993; Lee et al., 1993). Analysis of IGF-I mRNA indicates that peak IGF-I expression occurs during the second week of postnatal life. While IGF-I is expressed in many types of neurons, it generally exhibits its highest expression at times when neurons that express IGF-I are undergoing proliferation (Table 1). For example, IGF-I is highly expressed in the mitral cell layer of olfactory bulb during late gestation and the first week of postnatal life, and thereafter expression decreases. In the hippocampus, IGF-I expression is high in several proliferative areas in the first 2 wk of

Table 1
Sites and Peak Developmental Times of IGF-I mRNA Expression in Rodent CNS[a]

	Sites	Peak time
Cerebellar system	Purkinje cells	P4–P28
	Inferior olive	E20–P21
	Deep cerebellar nuclei	E20–P21
	Red nucleus	P4–P28
	Ventrolateral nucleus	P4–P21
Somatosensory system	Spinal trigeminal nucleus	E20–D21
	Ventrobasilar nucleus	P4–P21
	Gelatinosa nucleus	E20–P14
	Intraluminar nucleus	E18–adult
	Anterior pretectal nucleus	E16–P28
	Dorsal column nucleus	P0–adult
Olfactory system	Mitrals cells	E16–adult
	Tufted cells	E18–adult
	Piriform cortex	P7–adult
Visual system	Retinal ganglion cells	E20–P10
	Superior colliculus	P0–adult
	Lateral geniculate nucleus	P7–P28
	Lateral posterior nucleus	P7–P28
	Anterior pretectal nucleus	E16–P28
Auditory–vestibular system	Medial vestibular nucleus	E20–P21
	Superior vestibular nucleus	E20–P21
	Dorsal cochlear nucleus	E20–P14
	Ventral cochlear nucleus	P0–P14
	Superior olive	E20–adult
	Lateral lemniscal nucleus	E20–P28
	Medial geniculate nucleus	P4–P21
	Inferior colliculus	P0–P28
	Interstitial nucleus	E20–P14
Hippocampus	Strata oriens, radiatum, and lacunosum-moleculare	E20–P28
	Dentate gyrus: stratum polymorph	E20–P21
Neocortex	Interneurons	P0–P40
	Subventricular zone	P0–P14

[a]The data in this table are comprehensive; the table lists some of the prominent sites of IGF-I mRNA expression, as reported in other references (Bartlett et al., 1991, 1992; Bondy, 1991; and Lee et al., 1993). The format of the table was adapted from Table 1, in Bondy (1991).

postnatal life, but peaks later in the dentate gyrus, where neuronal proliferation is more prolonged. Purkinje cells are the major cells of IGF-I expression in cerebellum. IGF-I expression in Purkinje cells commences at about the time of birth, declines by postnatal day (P) 20, and continues throughout adulthood (Aguado et al., 1992; Torres-Aleman et al., 1994). IGF-I also may be expressed by glia progenitors, because it is strongly expressed in the subventricular zone in early postnatal development (Bartlett et al., 1992). In contrast, IGF-II expression in the brain and head occurs predominately in mesenchymal and neural crest derivatives during embryonic life (Stylianopoulou et al., 1988). In adult rodent and human brain the expression of IGF-II appears to be confined to the choroid plexus, meninges, and blood vessels (Hynes et al., 1988; Harel and Tannenbaum, 1992a; Cavallaro et al., 1993; Lee

et al., 1993; Logan et al., 1994). IGF-II in these sites likely accounts for the abundance of IGF-II in CSF.

IGF-I immunoreactivity is more widespread in brain than would be expected from the localization of its mRNA. Immunocytochemistry studies reveal IGF-I associated with neurons from E15 to adulthood (Garcia-Segura et al., 1991), including neurons in olfactory bulb, cerebral cortex, hippocampus, striatum, diencephalon, and brain stem. Postnatally, IGF-I can be localized to capillary walls, ependymal cells, choroid plexus, glial cells, and nerve fiber paths. These results are consistent with quantitative studies of the regional distribution of IGF-I in adult rat brain, where significant IGF-I concentrations are found in all brain regions, as evaluated by radioimmunoassay (Yamaguchi et al., 1990). In the latter study, extracted IGF-I concentrations corresponded to the amount of IGF-I found in perfusates of the same brain regions after *in situ*, in vivo perfusion. Immunoreactive IGF-II also is widespread in the CNS and often found at locations remote from its sites of synthesis—for example, in myelinated nerve tracts where it colocalizes with IGFBP-2 (Logan et al., 1994; Sullivan and Feldman, 1994). As reviewed below, IGFBPs are often expressed by cells near the cells of IGF expression, and it is likely that binding of IGFs to IGFBPs results in an accumulation of IGF at sites distinct from its cells of synthesis.

IGF Receptor Expression

Both type I and II IGF receptors have been biochemically identified and characterized in whole adult mouse, rat, and human brain (Gammeltoft et al., 1988; Ocrant et al., 1988; Santos et al., 1994). Using quantitative autoradiography of labeled IGF-I binding, sites of IGF-I specific binding have been found to be widely distributed in adult rat brain (Bohannon et al., 1988), with virtually all brain regions possessing binding sites. Some of these sites, though specific for IGFs, could represent binding to IGFBPs. ISHH, however, has demonstrated that type I IGF receptor mRNA is exuberantly expressed throughout the brain of E14 fetal rats, with the ventral floor plate of the hindbrain exhibiting especially high expression (Bondy et al., 1990). Quantitative solution hybridization studies of type I IGF receptor mRNA indicate that peak expression occurs between E15 and E20 of rat gestation (Baron-Van Euercooren et al., 1991). Taken together, these data indicate that type I IGF receptors are ubiquitous in the brain and that expression occurs early in development. Studies of human autopsy specimens confirm that type I IGF receptors are expressed throughout the adult brain and that they are somewhat more abundant in early postnatal life (De Keyser et al., 1994).

In the brain, the size characteristics of the type I IGF receptor differ from those in non-neural tissues in that the α-subunit migrates at an apparent size ~10 kDa smaller and the β-subunit at a size 2–3 kDa smaller (McElduff et al., 1988). These differences are likely to be owing to differences in glycosylation, but other differences are possible. Studies of cultured cells indicate that neurons, and not glia, express this smaller receptor.

Expression of type II IGF receptor is more limited in the adult rat brain than that of the type I IGF receptor. Both ISHH and immunocytochemistry reveal the expression in neurons of the forebrain and hippocampus, as well as in the choroid plexus and meninges (Couce et al., 1992).

IGFBP Expression

IGFBP-2, IGFBP-4, and IGFBP-5 are the major IGFBPs expressed in the brain. IGFBP-2 expression, as judged by ISHH, begins early in rat embryogenesis (at least by E7) (Wood et al., 1992). At E10–11, IGFBP-2 is expressed in the rostral brain of the primary neural tube (Wood et al., 1992); in the E14 rat fetus it is well expressed in the choroid plexus epithelium, the ventral floor plate, and the infundibulum (Wood et al., 1990). Postnatally, IGFBP-2 mRNA expression is largely restricted to astroglia (Lee et al., 1993).

Throughout development, sites of IGFBP-2 and IGF expression are often temporally and

spatially related (Wood et al., 1990, 1992; Lee et al., 1992b, 1993), suggesting that IGFBP-2's interaction with IGFs may influence IGF physiology either by increasing its concentration near cells where it acts or by modulating its effects (either inhibiting or augmenting IGF actions). For example, throughout brain development, IGF-II expressing cells often are immediately adjacent to those expressing IGFBP-2 (Wood et al., 1990, 1992), with the peak expression of each mRNA occurring simultaneously (Lee et al., 1993). In other brain regions, IGFBP-2 is expressed during later development in a fashion coordinated with that of IGF-I (Lee et al., 1992b). Such brain regions include the cerebellum, retina, and developing sensory and cerebellar networks. In the developing hippocampus and neocortex, however, astrocyte IGFBP-2 expression is not anatomically linked to that of IGF-I expression (Lee et al., 1993).

By ISHH, IGFBP-4 expression becomes more widespread in advancing development (Brar and Chernausek, 1993; Stenuers et al., 1994). In E14 rat fetuses, its expression is limited to the epithelium of the choroid plexus and meninges; by E20 its expression is extended to basal ganglia neurons; in the early postnatal period, hippocampus expresses IGFBP-4; and, in the adult rat, IGFBP-4 also is expressed in neurons of the layers II and IV of the cerebral cortex, olfactory bulb, and amygdala.

IGFBP-5 mRNA shows a distinct pattern of expression by ISHH (Bondy and Lee, 1993; Brar and Chernausek, 1993), and, like IGFBP-2, exhibits a temporal pattern of expression that appears to be coordinated with IGF-I expression. For example, IGFBP-5 and IGF-I are coexpressed in neurons of sensory relay systems such as olfactory bulb, geniculate bodies, and vestibular nuclei (Bondy and Lee, 1993). In the cerebellum, IGFBP-5 is expressed in the developing external germinal zone of the cerebellar cortex, whereas IGF-I is expressed in anatomically adjacent Purkinje cells. In the cerebrum, IGFBP-5 is expressed in the lateral ventricular germinal zone at birth and later in the subventricular zone. It also is expressed in multiple thalamic nuclei, hippocampus, and perivascular cells.

While there have been few studies of the expression of IGFBP-3 in the brain, its mRNA has been demonstrated in the brain following ischemic/hypoxic injury (Gluckman et al., 1992). IGFBP-1 does not appear to be expressed in brain. However, it may be important to peripheral nerve function, because it (possibly together with other IGFBPs) has been immunochemically localized to synapses at the neuromuscular junction (Ma et al., 1994a, 1994b).

Regulation of IGF Expression in the CNS

GH and nutrition are the major factors that regulate IGF-I expression in the liver, as well as in a number of other organs (Daughaday and Rotwein, 1989; Clemmons and Underwood, 1991). In some tissue, however, IGF-I expression appears to be regulated by trophic factors that are specific for that tissue. For example, in uterus, estrogens (and not GH) stimulate IGF-I expression (Murphy and Friesen, 1988), whereas in ovary follicle stimulating hormone is a major regulator of IGF-I (Adashi et al., 1991). Little is known, however, about the factors and mechanisms that regulate IGF-I expression in the brain. There is evidence, however, that GH (Hynes et al., 1987), nutrition (Lowe et al., 1989; Philipps et al., 1989), and injury *(see* IGF Actions in the CNS*)* influence the in vivo brain expression of IGF-I. IGF-I mRNA abundance is reduced in the brain of hypophysectomized rats, and intracerebral infusion of GH restores IGF-I mRNA to 80% of normal (Hynes et al., 1987), indicating that GH has a role in modulating brain IGF-I. Similarly, hypothalamic IGF-I and IGF-II mRNA are decreased by ~60% following hypophysectomy, and continuous peripheral administration of GH restores hypothalamic IGF-I (but not IGF-II) to normal (Wood et al., 1991). Administration of thyroxine, corticosterone, and testosterone had no such effect. The finding that GH receptors are expressed in many regions of the brain at the time near that of peak IGF-I expression is consistent with GH regulation of brain IGF-I (Lobie et al., 1993).

Fasting for 48 h reduces whole brain IGF-I mRNA by about 35% in the adult rat, with no concurrent change in the abundance of type I IGF receptors (Lowe et al., 1989), whereas a greater reduction of IGF-I mRNA (~ 65%) was found in rat hypothalami following a 72-h fast, again with no change in type I IGF receptors (Olchousky et al., 1993). Protein restriction during gestation and early postnatal life modestly lowers cerebellar IGF-I content (Shambaugh et al., 1995). In contrast, P10 rats with altered nutrition were not found to exhibit changes in brain IGF-I concentrations, but did have lower levels of IGF-II (Philipps et al., 1989). Consistent with a role for nutrition in the regulation of IGF-I is the finding that glucose stimulates IGF-I expression in C6 glial cells (Straus and Burke, 1995).

Studies of cultured neural cells provide evidence that some growth factors may be capable of regulating IGF expression. For example, epidermal growth factor (EGF) appears to stimulate IGF-I expression in primary cultures of rat astroglia (Chernausek, 1993), and basic fibroblast growth factor (bFGF) stimulates the release of IGF-I from primary cultures of neuroepithelial cells from the neural crest (Drago et al., 1991) and hypothalamic neurons (Pons and Torres-Aleman, 1992). In both cases, IGF-I appears to mediate the growth-promoting actions of these growth factors.

Glucocorticoids appear to depress the expression of both IGFs, although few studies have addressed this issue. In primary cultures of neurons and glia cells derived from neonatal rats, glucocorticoids depress IGF-I mRNA (Adamo et al., 1988). They also have been shown to depress IGF-II mRNA in neonatal rat brain after in vivo administration (Levinovitz and Norstedt, 1989). Finally, a variety of injuries are capable of inducing IGF-I expression in astrocytes in vivo. For example, astrocytes express IGF-I in response to hypoxic/ischemic (Lee et al., 1992a), stereotactic (Garcia-Estrada et al., 1992), electrolytic (Yamaguchi et al., 1991), and cryogenic (Yao et al., 1995) injuries, as well as in response to cuprizone-induced demyelination (Komoly et al., 1992) and to experimental autoimmune encephalomyelitis (EAE; Liu et al., 1994, 1995). Following hypoxic/ischemic injury, IGF-II is also expressed by brain macrophages (Lee et al., 1992a), and microglia and/or astrocytes (Beilharz et al., 1995). These findings, taken together with the apparent neuroprotective actions of IGF-I *(see below)*, suggest that astrocytes are important in ameliorating brain injury.

IGF Actions in the CNS

IGFs have been documented experimentally to exert a wide variety of growth-promoting and functional effects on neural cells. In an attempt to provide a cogent, integrated review, our summary of IGF actions is divided as follows:

1. Studies on specific neural cell types. Except for the review of IGF actions on "oligodendrocytes and myelination," this section is restricted to in vitro studies.
2. In vivo studies of mice with experimental alterations in IGF, IGF receptor, and IGFBP expression.
3. Studies of IGF actions in specific brain regions. The section reviews relevant in vitro and in vivo studies. Reviews of IGF's role in neural injury and in CNS tumorigenesis follow in separate sections.

IGF Actions on Specific Neural Cell Types

In Vitro IGF Actions on Neurons and Neuronal Progenitors

Increasing evidence supports important actions for IGFs in neuronal development and function (Table 2). Påhlman et al. (1991) have proposed the SH-SY5Y neuroblastoma cell line as a paradigm for IGF-I's role in neural development. They have shown that IGF-I stimulates cellular proliferation of undifferentiated SH-SY5Y cells and augments their differentiation when induced by phorbol esters. Observations in primary cultures of neuronal cells are consistent with the hypothesis based on the SH-SY5Y paradigm. Cultured E10 murine neuroepithelial cells appear to be dependent on IGF-I for proliferation and survival (Drago

Table 2
Some IGF Actions on Cultured Neurons

Action	Cultured cell	Refs.
Proliferation	Undifferentiated SH-SY5Y cells	Påhlman et al., 1991
	Murine E10 neuroepithelium	Drago et al., 1991
	Murine E15–16 neuron precurors	Lenoir and Honegger, 1983
	Rat E15 sympathetic neuroblasts	DiCicco-Bloom and Black, 1989
	Rat olfactory bulb explants	Werther et al., 1993
Enhanced survival	Rat E15 Purkinje cells	Torres-Alemon et al., 1992
	Rat E16–17 cortical neurons	Aizeman and DeVellis, 1987
	Rat E15 Purkinje cells	Torres-Aleman et al., 1992
	Rat hypothalamic neurons	Torres-Aleman et al., 1990a, 1990b; Pons et al., 1991; Sortino and Canonico, 1996
	Rat P8 cerebellar granule cells	D'Mello et al., 1993
	Spinal cord motoneurons	Ang et al., 1992; Neff et al., 1993
Cell body hypertrophy	Rat E14 serotonin and dopamine neurons	Liu and Lauder, 1992
	Rat E16–17 cortical neurons	Aizeman and DeVellis, 1987
Neuritic outgrowth	Rat E16–17 cortical neurons	Aizeman and DeVellis, 1987
	Rat hypothalamic neurons	Torres-Aleman et al., 1990b
	Chick sympathetic neurons	Zackenfels et al., 1995
	Rat sensory dorsal root neurons	Zackenfels et al., 1995
Differentiation	Neural crest to catecholaminergic neuron	Nataf and Monier, 1992
Stimulated activity	Glutamine acid decarboxylase	Aizeman and DeVellis, 1987
	Choline acetyltransferase	Konishi et al., 1994
	Ca^{2+} influx	Kleppisch et al., 1992

et al., 1991). These cells synthesize IGF-I and, while they proliferate in response to bFGF, this response is completely inhibited by coincubation with antibodies to IGF-I, indicating that IGF-I mediates, at least in part, bFGF action on these CNS progenitor cells. Both IGF-I and -II stimulate the proliferation of aggregate cultures of mouse E15-16 neuron and glial precursors (Lenoir and Honegger, 1983). IGF-I also has been shown to stimulate mitosis in E15 rat cervical sympathetic neuroblasts (DiCicco-Bloom and Black, 1989). IGFs may influence neuron number, however, by mechanisms not related to the stimulation of proliferation. Studies of rat cortical neurons (Aizeman and DeVellis, 1987), embryonic chick (Neff et al., 1993) and mouse (Ang et al., 1992, 1993) spinal cord motor neurons indicate that IGFs enhance survival, but do not stimulate proliferation (Neff et al., 1993). IGF-I's effects on mouse motor neuron survival, however, appear to require an astrocyte-derived factor (Ang et al., 1992, 1993). In immortalized hypothalamic neurons, IGF-I appears to enhance survival by protecting against the effects of oxidants (Sortino and Canonico, 1996).

Evidence also suggests that IGFs stimulate the differentiation of certain neuronal populations. For example, IGF-I has been shown to increase the number of catecholaminergic cells in embryonic quail neural crest cells in culture (Nataf and Monier, 1992). IGFs also may stimulate hypertrophic growth of some neurons. IGF-I stimulates cell hypertrophy, neuritic outgrowth, and glutamic acid decarboxylase expression in cultured cortical neurons (Aizeman and DeVellis, 1987). IGF-II stimulates an increase in the cell body size of cultured rat E14 serotonin and dopamine neurons and increases neuritic outgrowth in dopamine neurons (Liu

and Lauder, 1992). Both IGFs stimulate mitosis and neuritic outgrowth in cultured sympathetic neurons from embryonic chick lumbosacral ganglia (Zackenfels et al., 1995). IGF-I, and to a lesser extent IGF-II, stimulates neuritic outgrowth of cultured postmitotic sensory neurons obtained from the dorsal root ganglia of adult rats (Fernyhough et al., 1993).

In other differentiated cultured neurons, IGF-I appears to promote differentiated functions. IGF-I stimulates choline acetyltransferase activity in primary cultures of rat E16 basal forebrain and pontine cultures, as well as dopamine uptake in mesencephalic primary cultures (Knusel and Hefti, 1991). Similar stimulation of choline acetyltransferase activity has been reported for cultured E15 mouse septal neurons (Konishi et al., 1994). Finally, IGF-I may facilitate Ca^{2+} influx in neurons (Kleppisch et al., 1992).

The mechanisms of IGF actions in neurons have not been precisely elucidated; however, IGF-I has been shown to stimulate the autophosphorylation of the type I IGF receptor, as well as a 70-kDa cytosolic substrate, in primary cultures of postmitotic embryonic chick forebrain neurons (Kenner and Heidenreich, 1991). This leads to the activation of Ras, which is proposed to lead in turn to IGF-I's effects on neuron survival (Robinson et al., 1994). IGF-I activation of its receptor also results in the transcription of c-*fos* by a protein kinase C pathway (Heidenreich et al., 1993), and to the activation of protein phosphatase-2A (Begum et al., 1993). In SH-SY5Y cells, the differentiating actions of IGF-I appear to be related to its capacity to decrease c-*myc* expression and to stimulation of the expression of a number of genes associated with differentiation, such as GAP-43 (Sumantran and Feldman, 1993), as well as to increase tubulin and neurofilament mRNA (Fernyhough et al., 1989; Wang et al., 1992), which are essential for neuritic outgrowth.

In Vitro IGF Actions on Astrocytes

Studies of astrocytes in primary culture demonstrate that these cells express IGF-I (Han et al., 1992; Chernausek, 1993) and several IGFBPs, including IGFBP-2 and IGFBP-3 (Bradshaw and Han, 1993). Late gestation rat astrocytes have been shown to express type I and II IGF receptors and to synthesize DNA in response to IGFs (IGF-I > IGF-II) (Ballotti et al., 1987). P1 rat astrocytes in culture synthesize IGF-I, and the rate of astrocyte DNA synthesis can be reduced by antibodies against IGF-I (Han et al., 1992), suggesting an autocrine role for IGF-I in astrocyte growth. In the latter cells, IGF-I also stimulates Glut-1 expression (and in turn glucose uptake), but this action is lacking in P21 astrocytes when the abundance of type I IGF receptors decreases (Masters et al., 1991). In perinatal rat astrocytes, IGF-I also stimulates glycogen accumulation (Dringen and Hamprecht, 1992). Astrocytes also appear to be capable of degrading IGFs (Auletta et al., 1992).

IGF Actions on Oligodendrocytes and in Myelination

Strong evidence supports a role for IGF-I in the autocrine proliferation of oligodendrocyte progenitor cells, as well as in oligodendrocyte differentiation. IGF-I mRNA can be readily detected in oligodendrocyte precursors purified from young rats, and appears to be less abundant in mature oligodendrocytes (Shinar and McMorris, 1995). Both progenitor cells and oligodendrocytes, isolated from P1 rat cerebrum, express functional type I IGF receptors (McMorris et al., 1986; Masters et al., 1991). In primary culture, IGF-I stimulates DNA synthesis, stimulates an increased number of oligodendrocyte progenitors (O-2A antigen positive cells), and induces commitment of these progenitors to oligodendrocyte differentiation (McMorris and Dabois-Dalcq, 1988). Using aggregate brain culture, IGF-I has been shown to dramatically increase oligodendrocyte number, as well as a parallel increase in myelin content (Mozell and McMorris, 1991). A role for IGF-I in regulating oligodendrocyte function is supported by the finding that IGF-I can stimulate increased myelin basic protein (MBP) synthesis in cultured oligodendrocytes, an effect augmented by the bFGF and transferrin (both factors have effects independent of IGF-I)

(Saneto et al., 1988). Also consistent with IGF-I's effects on oligodendrocyte progenitor proliferation is the observation that IGF-I stimulates the proliferation of cultured perinatal rat Schwann cells, provided that these cells are exposed to cAMP, which appears to act by increasing type I IGF receptor expression (Schumacher et al., 1993).

There also is strong evidence that IGF-I stimulates the proliferation of oligodendrocyte progenitors in vivo *(see also below)*. IGF-I knockout mice have a reduction in the total number of oligodendrocytes (Beck et al., 1995). While in one study of IGF-I-overexpressing Tg mice there was no apparent increase in oligodendrocyte number, as judged by the percentage of oligodendrocytes per total cell number (Carson et al., 1993), in another study a small increase was observed using ISHH for proteolipid protein (PLP) mRNA (Ye et al., 1995b). The fact that transgene expression in these mice does not begin until after birth may account for the modest increase in oligodendrocytes, or alternatively, native IGF-I's effects on oligodendrocyte progenitor development may be near maximal.

Despite the modest increase in oligodendrocyte number, IGF-I Tg mice exhibit a dramatic increase in PLP and MBP mRNA, coincident in time with the normal peaks in expression of these genes (Ye et al., 1995b), consequently, total brain myelin content is increased 2.3-fold (Carson et al., 1993) (Fig. 4 shows increased myelin in IGF-I Tg mouse brain). These results indicate that myelin associated protein gene expression is dramatically stimulated by IGF-I. The increase in myelin synthesis results in an increase in the number of myelinated axons and an increase in the thickness of myelin sheaths (Ye et al., 1995b). IGF-I also appears to stimulate the initiation of myelination, because in IGF-I Tg mice, axons with smaller diameters are myelinated (Ye et al., 1995a). The influence of IGF-I on myelination is further apparent by the dramatic decrease in white matter in IGF-I knockout mice (Beck et al., 1995).

Much evidence supports a role for IGF-I in remyelination following injury. When demyelination is induced in mouse spinal cord explants, IGF-I stimulated remyelination (Roth et al., 1995). As reviewed above, astrocytes express IGF-I following a variety of injuries in vivo. This astrocyte IGF-I expression also occurs in the animal model of multiple sclerosis, EAE

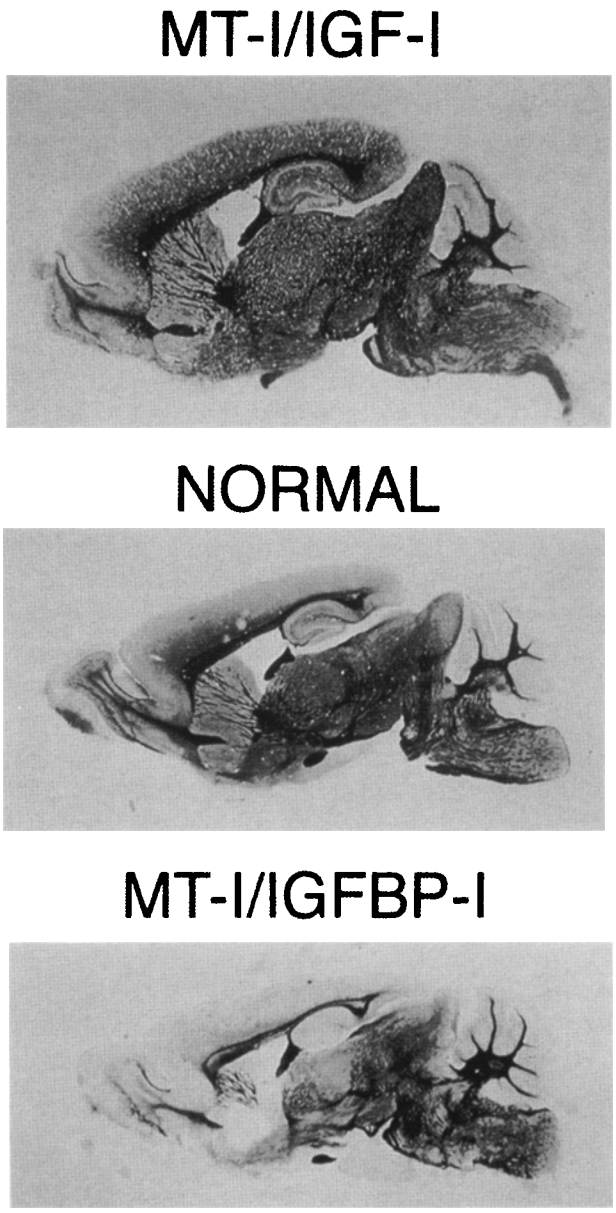

Fig. 4. Sagittal sections of brains from IGF-I overexpressing (MT-I/IGF-I) Tg, IGFBP-1 (MT-I/IGFBP-I) Tg, and normal mice. Sections are stained for myelin. The increased myelin content of IGF-I Tg brains and decreased myelin in IGFBP-1 Tg brains compared to normal mice is apparent.

(Liu et al., 1994). Furthermore, when IGF-I is administered systemically, the clinical consequences of EAE are ameliorated and oligodendrocytes exhibit increased myelin associated protein gene expression (Yao et al., 1995). The mechanisms by which IGF-I protects against EAE and promotes remyelination in EAE rats, however, are not clear. IGF-I may promote proliferation and/or survival of oligodendrocytes and their precursors, and/or reduce the permeability of the BBB (Liu et al., 1995). The latter would limit accessibility of T lymphocytes and soluble destructive immune factors to the brain. Other possibilities also exist. For example, IGF-I may influence the peripheral immune reaction; this in turn could reduce CNS inflammation, demyelination, and BBB permeability.

In Vivo IGF Actions: Studies of Mice with Altered IGF Expression

IGF-I Overexpressing Transgenic Mice

Tg mice that overexpress IGF-I in the brain under the control of the MT-I promoter exhibit dramatically increased brain sizes (Fig. 4) with weight increases of 25–85% greater than littermate controls, depending on the line of Tg mice and the degree of transgene expression in each line (Mathews et al., 1988; Behringer et al., 1990; Ye et al., 1995b; and unpublished data). Despite the increased size, these brains exhibit no anatomic abnormalities. In studies of the original line of these mice, IGF-I was found to be expressed in multiple organs. These Tg mice exhibited somatic overgrowth (~30%) and had elevated circulating IGF-I concentrations. Their pituitaries had a 60% reduction in GH mRNA and their serum contained no detectable GH (Mathews et al., 1988), indicating that they were partially GH deficient. These data indicate that IGF-I exerts negative feedback on the pituitary resulting in a suppression of GH synthesis and secretion. The source of the IGF-I exerting the negative feedback, however, may not be the serum; this is because, in other lines of IGF-I Tg mice without elevated serum IGF-I but with significant brain IGF-I transgene expression, there is evidence of GH suppression (unpublished data). It seems that IGF-I expressed in the brain, possibly in the hypothalamus, is capable of modulating GH expression. Given the widespread IGF-I expression in the brain, this may represent an important mechanism of GH regulation.

When these IGF-I Tg mice are cross-bred with a line of Tg mice made somatroph deficient by expression of a fusion gene linking the GH promoter and the gene for diphtheria toxin (Behringer et al., 1990), mice carrying both transgenes (i.e., mice that express near-normal IGF-I despite an absence of GH secretion) also exhibited increased brain growth. Additionally, GH-overexpressing Tg mice do not exhibit an increase in brain size; thus, there is little question that the increase in brain growth is a result of IGF-I overexpression. On the other hand, GH may have some influence on brain growth, because GH-deficient mice have significantly smaller brains then do normal mice, and it is possible that this GH action is mediated by IGF-I.

The brain growth of Tg mice with IGF-I overexpression is owing to increases both in cell number, as evidenced by increased DNA content (Behringer et al., 1990), and in myelination (Carson et al., 1993; Ye et al., 1995b). In MT-I driven IGF-I Tg mice there is enlargement of all brain regions with the cerebral cortex being the most enlarged, followed by the hippocampus, diencephalon, brain stem, and cerebellum (Ye et al., 1995b). While the relative size increases of these brain regions may reflect in part their sensitivity to the actions of IGF-I, the degree of regional transgene expression appears to be a major factor, because the magnitude of transgene expression in each region corresponds well to the degree of overgrowth (Ye et al., 1995b). Furthermore, in IGF-I Tg mice created with a different promoter in which transgene expression is greatest in the cerebellum (Ye et al., 1996), the cerebellum is the most enlarged region (twofold increases in weight; *see below*). Olfactory bulb is not significantly enlarged in IGF-I Tg mice. This structure, however, expresses endogenous IGF-I at high abun-

dance throughout life *(see below)*; therefore, the effects of IGF-I may be maximal under normal homeostatic conditions. These data indicate that IGF-I has the capacity to stimulate the growth of most, if not all, brain areas. Our studies of the cytoarchitecture of the S1 somatosensory cortex in IGF-I Tg mice indicate that IGF-I stimulates an increase in neuron cell body size and neuritic outgrowth (Gutierrez-Ospina et al., 1996). Specifically, we evaluated the posterior medial barrel subfield in IGF-I Tg mice. We found that, while neuron number was only increased modestly (~25%), the volume of this structure was increased as much as twofold in some lines of Tg mice. Neuron cell body size was increased ~33%, but most of the increased size was owing to increases in neuropil (again as much as twofold), indicating that the volume devoted to axons, dendrites, and their connections was markedly increased.

Transgenic Mice with Ablated IGF-I Expression and Reduced IGF-I Function

Whereas most mice with ablated IGF-I die in the immediate postnatal period, some survive (5–50%), depending on the background strain into which they are bred (Liu et al., 1993; Powell-Braxton et al., 1993). Those that survive have very small brains (~60% of normal) that are morphologically normal (Beck et al., 1995). These brains are characterized by a paucity of white matter owing to markedly decreased myelination and an apparent decrease in the number of axons. These findings are consistent with IGF-I promoting oligodendrocyte number and neuritic outgrowth, both of which are findings in IGF-I overexpressing Tg mice. Depletions in specific neuron populations also were found (the volume of the granular cell layer of the dentate gyrus was small, and striatal parvalbumin-containing neurons were reduced in number). It is difficult, however, to know whether IGF-I is an absolute requirement for the differentiation of these neurons. The absence of IGF-I expression may create a milieu that hinders their development, thus having an indirect role in their development. On the other hand, IGF-I could have roles that are not apparent in these knockout mice, if other factors can subserve IGF-I's function when it is absent.

Tg mice with ectopic brain expression of IGFBP-1, an inhibitor of IGF actions, also have small brains and exhibit many of the features seen in IGF-I knockout mice (Fig. 4) (Dai et al., 1994; D'Ercole et al., 1994). The transgene carried by these mice is driven by MT-1 and is not expressed until after birth; consequently, the brain growth retardation is first apparent in the second week of postnatal life, coincident with the peak time of IGF-I expression in brain (Rotwein et al., 1988). Other IGFBP-1 Tg mice in which the transgene is driven by a promoter that is expressed *in utero* have even smaller brains (Rajkumar et al., 1995). The characteristics of the brain in these mice, however, have yet to be studied. Consistent with blunted IGF-I actions, these Tg mice have a reduction in total brain DNA and protein content, and reduced myelination (Ye et al., 1995b). Neuron density in these mice, as in IGF-I knockout mice (Liu et al., 1993), is increased, indicating a decrease in neuropil and thus a probable decrease in neuritic outgrowth (Gutierrez-Ospina et al., 1996).

Knockout Mice with Altered IGF-Related Gene Expression

Mice carrying homozygously disrupted type I IGF receptor genes invariably die at birth (Liu et al., 1993). They have small brains with histologic features similar to IGF-I knockout mice, but detailed studies of brain histology are lacking. Knockout mice that do not express IGF-II have normal sized brains without apparent abnormality, and the same is the case for mice lacking the type II IGF receptor. Neither IGF-II nor the type II IGF receptor seems to be essential for brain growth and development. Mice lacking IRS-1, one substrate for type I IGF receptor phosphorylation (i.e., a potential mediator of IGF-I signaling), have somewhat small brains, demonstrate degeneration of some pyramidal neurons in the cerebral cortex, and exhibit aggregation of cerebellar Purkinje cells (Folli et al., 1995). These findings might mean that insulin is important to the survival of these cells or that IGF actions are mediated by IRS-1 in these cells.

IGF Actions in Specific Brain Regions

In some brain regions, a significant body of evidence points to important roles for the IGFs in development and/or function. Data on these specific regions is reviewed below. IGFs, however, may be equally or more important to the growth and development of other areas where studies have not been pursued. The cerebral cortex represents such a region. For example, parietal cortex explants exhibit remarkable growth when grafted intraocularly and treated with truncated IGF-I, a form not bound by IGFBPs (Giacobini et al., 1990). As mentioned, the cerebral cortex is the most overgrown region in the brains of IGF-I overexpressing mice (Ye et al., 1995b). The cortical mantle of these Tg mice is thicker than in normals (*see* Fig. 5). In addition, the pyramidal cell bodies appear increased in size, neuron density is reduced, and, concomitantly, neuropil is increased. In contrast, IGFBP-1 Tg mice with decreased IGF availability have thinner cortical mantles, apparently smaller neurons that are densely packed, and increased neuropil. These findings show that IGF-I stimulates cortical growth, and they suggest that elaboration of axons and dendrites may be an essential part of this growth.

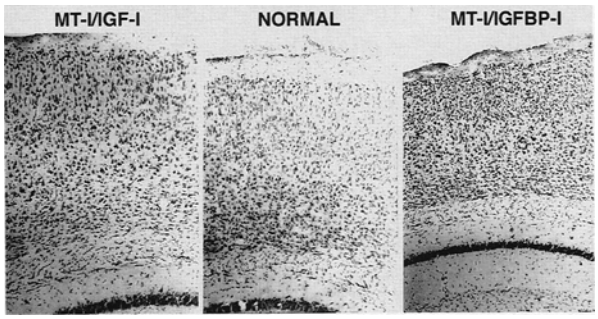

Fig. 5. Sagittal sections of nissle-stained cerebral cortices in IGF-I overexpressing (MT-I/IGF-I) Tg, IGFBP-1 (MT-I/IGFBP-I) Tg, and normal. Compared to normal, the cortex of IGF-I overexpressing Tg mice is thicker, has a decreased neuronal density, and more neuropil. IGFBP-1 Tg mice have a thinner cortex with more densely packed neurons.

IGF Actions in the Cerebellum

A growing number of studies point to roles for IGF-I in the growth and function of the cerebellum. IGF-I appears to be expressed exclusively by Purkinje cells during postnatal development (Bondy and Lee, 1993). IGF-I mRNA extracted from whole rat cerebellum peaks in the first week of postnatal life, and then decreases gradually, but continues to be expressed in the adult cerebellum (Torres-Aleman et al., 1994). Consistent with this observation is the finding that immunoreactive IGF-I is readily observed in migrating neuroglial cells in the inner portion of the external granular layer and in Purkinje cells, with peak immunostaining observed during the first 2 wk of postnatal life (Andersson et al., 1988). IGF-I has been localized to the endoplasmic reticulum and to microvesicular bodies of mature Purkinje cells (Aguado et al., 1992). IGF-I extracted from rat cerebellum increases in early postnatal life, peaking at about P4 (Torres-Aleman et al., 1994). The highest cerebellar IGF-I, however, is observed in adults (Torres-Aleman et al., 1994). Because IGF-I mRNA expression in adult cerebellum is relatively low, the high IGF-I content is likely the result of orthograde Purkinje cell transport from the inferior olive (Nieto-Bona et al., 1993).

The type I IGF receptor also is well expressed in cerebellum during development (Torres-Aleman et al., 1994). Type I IGF receptor abundance, as judged both by expression of mRNA and quantification of binding, is high during late gestation, decreases gradually in the first 2 wk of life, and thereafter remains stable (Torres-Aleman et al., 1994). While type I IGF receptors are likely expressed by all cerebellar cells, they are especially abundant in the molecular and external granular layers of the cerebellum (Werther et al., 1990). At least two IGFBPs, IGFBP-2 and IGFBP-5, are expressed in the cerebellum, and their peak expression appears to be in the first 2 wk of postnatal life (Bondy and Lee, 1993; Torres-Aleman et al., 1994). ISHH reveals that IGFBP-5 is expressed in the external granular layer (Bondy and Lee, 1993), where it may accumulate IGF-I and

modulate its actions. IGFBP-2 is expressed by astroglia predominately those in the Purkinje cell layer.

Studies of cultured cerebellar cells derived from P15 rats (Torres-Aleman et al., 1992) have demonstrated that IGF-I dramatically promotes the survival of Purkinje cells (a sevenfold increase in Purkinje cell number, compared to control cultures, without significant changes in thymidine incorporation). Because Purkinje cells synthesize IGF-I, it appears that IGF-I acts in an autocrine fashion to enhance their survival. In a similar study of cultured embryonic cerebellar cells, IGF-I was found to promote neuritic outgrowth as well as cell survival (Torres-Aleman et al., 1994). Both effects were blocked by an antibody to the type I IGF receptor. In cultured cerebellar granular neurons, IGF-I inhibits the apoptosis induced by exposure to low K^+ (D'Mello et al., 1993) and also sensitizes these neurons to excitatory, toxic effects of glutamate (Calissano et al., 1993), presumably by inducing NMDA receptors (Calissano et al., 1993).

Studies of Tg mice that overexpress IGF-I in the cerebellum indicate that IGF-I exerts similar growth-promoting actions in vivo (Ye et al., 1996). These Tg mice carry a fusion gene linking the mouse IGF-II 5' flanking region to a human IGF-I cDNA, and express the transgene from late in gestation. The expression of the transgene increases postnatally, reaching a peak at 2–3 wk that is maintained throughout life. Cerebelli in these Tg mice are twice the normal size by 50 d of age and exhibit no histologic abnormalities. The increased size is owing, at least in part, to increased cell number, because DNA content is similarly increased. Increases in the number of granule cells account for much of the increase in cell number, as they are 92% greater in number than those in their littermates. Increased granule progenitor cell proliferation, as judged by BrdU incorporation, occurs during the second week of life, and there is no apparent prolongation in the time of granule progenitor proliferation, because the external granular layer disappears at the same time in Tg mice as in their normal littermates. The increased proliferation, however, is modest; thus the large increase in the number of granule cells may be partly owing to IGF-I promotion of granule cell survival. The number of Purkinje cells also is modestly increased (~20%). Given that the transgene is only well-expressed postnatally, well after the time of Purkinje progenitor proliferation, this effect likely also is secondary to enhanced survival. The increased size of the molecular layer indicates other growth-promoting effects of IGF-I in the cerebellum.

Other evidence of IGF-I's role in cerebellar development comes from studies of mutant mice. In Lurcher mutant mice, there appears to be decreased IGF-I binding in the molecular layer of the cerebellum and defective tyrosine kinase phosphorylation in response to IGF-I (Vig et al., 1994), suggesting that the type I IGF receptor is abnormally expressed in the cerebellum of these mice. A similar defect may exist in patients with olivopontocerebellar atrophy. In the Weaver mutant mouse, IGFBP-5 expression is increased in the external germinal layer of the cerebellum (Lee et al., 1995). Because IGFBPs modulate IGF activity, it was postulated that IGFBP-5, synthesized by granule progenitors, inhibits IGF-I's interaction with these cells, leading to increased apoptosis and consequently a markedly reduced number of granule cells (and in turn a reduced number of Purkinje cells). While this is an attractive hypothesis, direct actions of IGFBP-5 that are independent of IGF-I also are possible, as are other mechanisms of disease in these mutant mice. Finally, when fetal cerebellar explants are grafted into mice with hereditary cerebellooolivary atrophy, the engrafted cells express IGF-I, and the ataxia and some of the other behavioral abnormalities in the mice are improved (Zhang et al., 1996).

In addition to IGF-I's effects on cerebellar growth, IGF-I clearly has neuromodulatory actions. Administration of IGF-I to the cerebellar cortex through a microdialysis probe inhibits glutamate-stimulated, but not KCl-stimulated, γ-aminobutyric acid (GABA) release (Castro-

Alamancos and Torres-Aleman, 1993). Electrical stimulation of the inferior olive stimulates IGF-I release and inhibits GABA release. These effects of IGF-I appear to be mediated by protein kinase C and nitric oxide (Castro-Alamancos et al., 1996). When inhibitors of each, stauroporine and a nitric oxide synthetase inhibitor, are administered in vivo by microdialysis, IGF-I's long-term depression of glutamate-induced GABA release (but not its short-term effect) is negated, whereas when activators of each are administered with glutamate, the short depressive effect of IGF-I is mimicked. When both inhibitors are given together, however, both short- and long-term IGF-I effects are ablated.

Motor learning also may be mediated by IGF-I to some extent (Castro-Alamancos and Torres-Aleman, 1994). When antisense oligonucleotides, known to be capable of blocking IGF-I synthesis, are instilled in the inferior olive (a source of cerebellar IGF-I) conditioned eye-blink learning is abolished. This manipulation, however, has no effect on the retention of this response once it is learned. While the mechanisms of these IGF-I actions have not been extensively explored, a number of other IGF-I actions on cerebellar cells may in part explain these effects. For example, IGF-I is known to stimulate calbindin-28D in Purkinje cells (Nieto-Bona et al., 1995) and to induce functional AMPA/kainate receptors in granule cells (Zona et al., 1995).

IGF Actions in the Hypothalamus

The hypothalamus in the rat expresses IGF-I, type I IGF receptors, and several IGFBPs (Pons et al., 1991). Type I IGF receptors are most abundant during late fetal life, as are several IGFBPs. IGF-I, type I IGF receptors, and IGFBPs are expressed throughout life, making it likely that IGF-I exerts actions important both to the developing and mature hypothalamus, and that IGFBPs modulate these actions. Studies of cultured embryonic rat hypothalamic cells indicate that IGF-I stimulates neuron survival and differentiation and astroglial proliferation (Torres-Aleman et al., 1990a, 1990b; Pons et al., 1991). In both mixed and neuron-rich hypothalamic cultures, IGF-I markedly increases the number of neurite-bearing cells (two- to fivefold, depending on the time in culture), and it increases DNA synthesis in astroglia (Torres-Aleman et al., 1990b). IGF-I's promotion of neuronal survival is additive to that of bFGF (Torres-Aleman et al., 1990a). Because bFGF increases neuronal release of IGF-I into culture media, the number of type I IGF receptors, and the abundance of media IGFBPs, it seems possible that the actions of bFGF on hypothalamic neurons are mediated, at least in part, by IGF-I (Pons and Torres-Aleman, 1992). IGF-I also could be responsible for some estrogen effects on the hypothalamus, because estradiol increases the expression of type I IGF receptors and some IGFBPs in embryonic rat hypothalamic cultures (Pons and Torres-Aleman, 1993). IGF-I also appears to be protective for hypothalamic neurons, because it ameliorates calcium-mediated neuronal damage caused by hypoglycemia (Cheng and Mattson, 1992) and oxidative stress (Sortino and Canonico, 1996).

In the mature hypothalamus, IGF-I plays an important role in the feedback regulation of GH expression and release. IGF-I negatively regulates the GH-releasing factor (Bourguignon et al., 1993; Fernández-Yázquez et al., 1993; Urchiyama et al., 1994) and increases somatostatin synthesis and release (Aguila et al., 1993; Ratajczak et al., 1994); it also appears to directly influence pituitary somatotroph synthesis and/or release of GH (Harel and Tannenbaum, 1992b). In vitro studies of cultured rat hypothalami suggest that IGF-I's major effects are at the level of the hypothalamus (Becker et al., 1995). IGF-II, but apparently not IGF-I, suppresses neuropeptide Y release from the paraventricular nucleus (Sahu et al., 1995), suggesting that IGF-II, like insulin, has effects on food intake.

IGF Actions in the Olfactory Bulb

IGF-I, together with bFGF, permits the survival of P1 rat olfactory bulb in primary explant culture (Werther et al., 1993). Pro-

longed culture of these explants with IGF-I increases the number of neurofilament-staining cells, presumably neurons, to nearly that of olfactory bulbs allowed to develop for the same time in vivo. IGF-I, however, has more marked effects on glial cell number, such that the number of glial cells is about threefold more than in olfactory bulbs remaining in vivo for the same time (Russo and Werther, 1994). A truncated form of IGF-I, des(1-3) IGF-I, which binds to IGFBPs poorly, had more marked effects, a finding consistent with the finding that olfactory bulb expresses three IGFBPs: IGFBP-2, -4, and -5 (Russo et al., 1994). While addition of bFGF to these explants did not enhance IGF-I's effects on cell number, it appeared to improve the cellular organization in the mitral layer.

The Role of IGFs in CNS and Peripheral Nerve Injury

It is becoming increasingly evident that IGF-I, and possibly IGF-II, has neuroprotective actions and a role in repair following CNS insult, as well as in nerve regeneration. IGF-I is expressed in astrocytes following a variety of in vivo CNS injuries *(see above)*. In each of these situations, mature astrocytes, in which IGF-I mRNA is not detectable under basal conditions (Garcia-Estrada et al., 1992), are induced to express IGF-I. Hypoxic–ischemic injury, induced in 3-wk-old rats by unilateral carotid artery ligation followed by exposure to 8% O_2, has been the most well-studied model of IGF's role in injury. In the first 24 h following hypoxic–ischemic injury, mRNAs for IGF-I (Lee et al., 1996), IGFBP-2 and -5 (Gluckman et al., 1992; Beilharz et al., 1993; Lee et al., 1996), IGFBP-4 (Beilharz et al., 1993), and type I IGF receptor (Lee et al., 1996) decline in the thalamus, hippocampus, and cortex on the lesioned side (Lee et al., 1996). After 3 d, however, the IGF-I and IGFBP-5 mRNAs are induced in reactive astrocytes on the side of the lesion (Gluckman et al., 1992; Beilharz et al., 1993; Lee et al., 1996), while the apparent abundance of IGFBP-2 and type I IGF receptor mRNA remain suppressed (Lee et al., 1996). At later times following injury, IGF-II (5–7 d post injury; Beilharz et al., 1995) and IGFBP-2 mRNA (7–10 d post injury; Klempt et al., 1993) also increased. IGFBP-3 mRNA, which may not be expressed in brain under basal conditions, appears to be induced 3–5 d after injury (Gluckman et al., 1992).

The pattern of IGF-I and IGFBP expression following hypoxic–ischemic injury suggests a role for IGF-I in repair or rescue that is modulated by IGFBPs. Evidence for this comes from studies showing that administration of IGF-I intraventricularly 2 h after hypoxic–ischemic insult is neuroprotective in that neuron loss is reduced in the dentate gyrus and lateral cortex (Gluckman et al., 1992). In a similar experiment, hypoxic–ischemic injury was induced in late-gestation fetal sheep and IGF-I was instilled in the lateral ventricle 2 h later (Johnston et al., 1996). IGF-I (1 μg) reduced neuron loss in a number of brain regions, including the cortex, hippocampus, and striatum 5 h later. This treatment also delayed the onset of seizures and reduced their incidence. Tenfold lower doses of IGF-I were less effective, and, interestingly, tenfold higher doses were ineffective. Experiments in mature rats with hypoxic–ischemic lesions show that IGF-I administered in the ventricle appears to traverse rapidly into the injured areas along white-matter tracts and through perivascular spaces (Guan et al., 1996), and, thus, IGF-I appears to be widely available to injured brain tissue.

Much evidence demonstrates an involvement of the IGFs in peripheral nerve regeneration (Ishii et al., 1994). IGF-I immunoreactivity can be localized to human and rat sciatic neurons (Hansson et al., 1989; Kerkhoff et al., 1994). IGF-I has been identified in sciatic nerve axons within 2 h of a crush injury (Hansson et al., 1987). Both IGF-I and IGF-II mRNAs increase following sciatic nerve crush injury and diminish following regeneration, but remain elevated if regeneration is prevented by nerve transection (Glazner et al., 1994). Schwann cells appear to

be the major, if not the sole, site of IGF-I mRNA expression (Pu et al., 1995). The increase in IGF-I mRNA is associated temporally with the proliferation of Schwann cells and the regeneration of axons. In contrast, the increase in IGF-II mRNA expression following crush injury occurs more distally near the innervated muscle, and the time-course of its expression is later, suggesting that IGF-I and IGF-II serve different functions during regeneration. Schwann cells also appear to be a major site of IGF-II expression. The muscle denervated by sciatic nerve injury, however, also expresses IGF mRNAs (IGF-II > IGF-I) (Glazner et al., 1994). Direct evidence for the involvement of the IGF in sciatic nerve repair comes from the findings that both local (Near et al., 1992; Svenningsen et al., 1994) and systemic (Contreras et al., 1995) administration of IGFs hasten regeneration. IGF-I also appears to enhance the regeneration of cultured adult rat sensory neurons (Fernyhough, 1993), but not that of sympathoadrenal projection neurons, where it appears to antagonize survival promoting effects of bFGF (Blottner and Baumgarten, 1992). In addition, both local and systemic IGF-I administration can ameliorate the deleterious effects of diabetes on sensory nerve regeneration (Ishii and Lupien, 1995).

Muscle-derived IGFs are likely important both to muscle innervation and to peripheral nerve regeneration. Muscle expression of IGF-I mRNA and protein increases either after botulinum toxin-induced paralysis or after denervation (Caroni and Schneider, 1994). There also is evidence that IGF-I may be involved in signaling between muscle and spinal motoneurons, because IGF-I mRNA decreases in neonatal rat muscle at the time of synapse elimination (Caroni and Becker, 1992). Further evidence of IGF-I signaling comes from the finding that immunoreactive IGF-I increases in peripheral nerve, following exposure to vibration (Hansson et al., 1988). Finally, direct intramuscular injection of IGFs stimulates peripheral nerve sprouting (Caroni and Grandes, 1990); this action is blocked by IGFBP-4 and IGFBP-5 (Caroni et al., 1994).

The Role of IGFs in CNS Tumorigenesis

Some evidence exists to suggest that aberrations in IGF expression or function are involved in brain tumorigenesis. The rat glioma cell line C6 has been shown to express IGF-I and type I and II IGF receptors (Kiess et al., 1989), and there is evidence that IGF-I acts in an autocrine fashion to stimulate its growth (Lowe et al., 1992). These cells form tumors when injected subcutaneously into rats; however, when IGF-I expression is ablated by transfection with an antisense transgene, C6 cells become incapable of forming tumors in vivo (Trojan et al., 1992). The simplest interpretation of this result is that the high IGF-I expression by C6 cells stimulates their in vivo growth. Other experiments suggest, however, that other mechanisms may be involved (Trojan et al., 1993). When the non-IGF-I synthesizing C6 cells are injected into glioblastomas in rats, CD8+ lymphocytes invade the tumor and cause it to regress. Thus, IGF-I's influence on the immune system may be as or more important to tumor formation than is its capacity to stimulate mitosis. Evidence that IGF-I may be involved in human brain tumors comes from several findings: that human gliomas often express abundant type I IGF receptors (Merrill and Edwards, 1990); that IGF-I, and sometimes IGF-II, can be detected in brain tumor cysts (Glick et al., 1991); and that IGFBP-2 is often elevated in the cerebrospinal fluid of children with solid brain tumors (Muller et al., 1994). High IGF-I expression also has been found in human tumors of neuroectodermal origin (Yee et al., 1990), and IGF-II may stimulate the growth of some human neuroblastomas (El-Badry et al., 1991).

Clinical Implications

Clinical trials of IGF-I in a variety of endocrinopathies (such as GH resistance, GH gene defects, and some forms of diabetes mellitus) are ongoing; they have shown IGF-I to be a use-

ful therapeutic agent (Bondy et al., 1994). The multiple actions of the IGFs (especially IGF-I) on neural cells suggest that the therapeutic use of IGFs also could be beneficial in a number of human neurologic diseases. Therapeutic trials of hIGF-I in Amyotrophic Lateral Sclerosis (ALS) are ongoing, and preliminary results indicate that the systemic administration of IGF-I is beneficial (Festoff et al., 1995). Because ALS involves motoneurons in both the central and peripheral nervous system, it is important to know the site(s) where IGF-I exerts its beneficial effects in ALS. Beneficial IGF-I effects could come from its actions on the musculature (as has been shown in the Wobbler mouse [Hantai et al., 1995], a mutant mouse with lower motoneuron disease) or from its neuroprotective effects on peripheral nerves. Because IGF-I crosses the BBB in rats to some extent (Reinhardt and Bondy, 1994), protective actions of IGF-I on CNS motoneurons are possible. Direct delivery of IGF-I to the CNS, however, seems more likely to provide optimal therapy for the CNS component of ALS and for neurologic disorders primarily involving the CNS.

IGF-I's capacity to promote myelination makes it attractive in the treatment of demyelinating diseases and, together with other neurotrophins, in the treatment of a wide variety of degenerative neurologic disorders. Perhaps the greatest potential therapeutic use of the IGF-I, however, is in the promotion of repair following injury *(see above)*.

Some Directions for Future Research

Although IGF-I has been shown to exert multiple actions on neurons, such as promoting the proliferation and/or survival of multiple neuronal populations and stimulating neuritic outgrowth, little is known about the consequences of these actions. For example, does IGF-I stimulation of neuron growth in vivo result in more axons and dendrites?... in more synapses? If the answers to these questions are yes, as appears to be the case, the questions become: "What is the result of the increase in neuronal connections? Is nerve conduction velocity faster? Is information transmission faster? Is more information transmitted, and is it more integrated and/or more discriminating? Is behavior affected?" Few studies of IGF-I's influence on behavior have been reported. However, there is some indication that IGF-I has behavioral effects. Intracerebral IGF-I administration to rat pups during the first week of life increases the frequency of ultrasonic calls (Santucci et al., 1994). As mentioned, there is strong evidence that IGF-I enhances motor learning (Castro-Alamancos and Torres-Aleman, 1994). Overexpression of IGF-I in Tg mice results in a marked decrease in ethanol-induced sleep; on the other hand, IGFBP-1 Tg mice with decreased IGF bioavailability exhibit an increase in ethanol-induced sleep (Pucilowski et al., 1996).

The mechanisms of IGF actions in the CNS are far from understood. Although it is clear that type I IGF receptors are involved in transmitting IGF signals, these receptors appear to be modified in the CNS, and neither the nature of the modifications nor their significance is clear. In addition, it seems possible that hybrid receptors composed of the type I IGF receptor and either the insulin receptor, the insulin-receptor related receptor, or both could participate in IGF signal transduction. Furthermore, the steps involved in the intracellular signaling cascade of the IGFs have not been defined, nor has the extent of gene expression stimulated by IGF signaling been determined. Other important questions regarding IGF actions in the CNS include: whether and how IGFs interact with other neurotrophins and the precise role of IGFBPs in modulating IGF actions.

Acknowledgments

The research from the authors' laboratory reported herein was supported by NIH grant HD08299 (to A. J. D.). P. Y. and A. S. C. were supported by Training Grant T32 DK07129, and G. G.-O. by a fellowship from the National Council of Science and Technology, Mexico.

References

Adamo M., Werner H., Farnsworth W., Roberts C. T., Jr., Raizada M., and LeRoith D. (1988) Dexamethasone reduces steady state insulin-like growth factor I messenger ribonucleic acid levels in rat neuronal and glial cells in primary culture. *Endocrinology* **123,** 2565–2570.

Adashi E. Y., Resnick C. E., Hurwitz A., Ricciarelli E., Hernandez E. R., Roberts C. T., LeRoith D., and Rosenfeld R. (1991) Insulin-like growth factors: the ovarian connection. *Hum. Reprod.* **6,** 1213–1219.

Aguado F., Sánchez-Franco F., Cacidedo L., Fernández T., Rodrigo J., and Martinez-Murillo R. (1992) Subcellular localization of insulin-like growth factor I (IGF-I) in Purkinje cells of the adult rat: an immunocytochemical study. *Neurosci. Lett.* **135,** 171–174.

Aguila M. C., Boggaram V., and McCann S. M. (1993) Insulin-like growth factor I modulates hypothalamic somatostatin through a growth hormone releasing factor increased somatostatin release and messenger ribonucleic acid levels. *Brain Res.* **625,** 213–218.

Aizeman Y. and De Vellis J. (1987) Brain neurons develop in a serum and glia free environment: effects of transferrin, insulin, insulin-like growth factor-I and thyroid hormones on survival, growth and differentiation. *Brain Res.* **406,** 32–42.

Andersson I. K., Edwall D., Norstedt G., Rozell B., Skottner A., and Hansson H.-A. (1988) Differing expression of insulin-like growth factor I in the developing and in the adult rat cerebellum. *Acta Physiol. Scand.* **132,** 167–173.

Ang L. C., Bhaumick B., and Juurlink B. H. J. (1993) Neurite promoting activity of insulin, insulin-like growth factor I and nerve growth factor on spinal motoneurons is astrocyte dependent. *Dev. Brain Res.* **74,** 83–88.

Ang L. C., Bhaumick B., Munoz D. G., Sass J., and Juurlink B. H. J. (1992) Effects of astrocytes, insulin and insulin-like growth factor I on the survival of motoneurons in vitro. *J. Neurol. Sci.* **109,** 168–172.

Auletta M., Nielsen F. C., and Gammeltoft S. (1992) Receptor-mediated endocytosis and degradation of insulin-like growth factor I and II in neonatal rat astrocytes. *J. Neurosci. Res.* **31,** 14–20.

Backstrom M., Hall K., and Sara V. (1984) Somatomedin levels in cerebrospinal fluid from adults with pituitary disorders. *Acta Endocrinol.* **101,** 171–178.

Baker J., Liu J., Robertson E. J., and Efstratiadis A. (1993) Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75,** 73–82.

Ballotti R., Nielsen F. C., Pringle N., Kowalski A., Richardson W. D., Van Obberghen E., and Gammeltoft S. (1987) Insulin-like growth factor I in culture rat astrocytes: expression of the gene, and receptor tyrosine kinase. *EMBO J.* **6,** 2620–2626.

Baron-Van Evercooren A., Olichon-Berthe C., Kowalski A., Visciano G., and Van Obberghen E. (1991) Expression of IGF-I and insulin receptor genes in the rat central nervous system: a developmental, regional, and cellular analysis. *J. Neurosci. Res.* **28,** 244–253.

Bartlett W. P., Li X.-S., and Williams M. (1992) Expression of IGF-I mRNA in the murine subventricular zone during postnatal development. *Mol. Brain Res.* **12,** 285–291.

Bartlett W. P., Li X.-S., Williams M., and Benkovic S. (1991) Localization of insulin-like growth factor-1 mRNA in murine central nervous system during postnatal development. *Dev. Biol.* **147,** 239–250.

Baxter R. C. (1991) Biochemical characterization of insulin-like growth factor binding proteins. *Acta Endocrinol. (Copenh.)* **124(Suppl. 2),** 33–40.

Beck K. D., Powell-Braxton L., Widmer H.-R., Valverde J., and Hefti F. (1995) Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal and striatal parvalbumin-containing neurons. *Neuron* **14,** 717–730.

Becker K., Stegenga S., and Conway S. (1995) Role of insulin-like growth factor I in regulating growth hormone release and feedback in the male rat. *Neuroendocrinol.* **61,** 573–583.

Begum N., Robinson L. J., Drazin B., and Heidenreich K. A. (1993) Protein phosphatase-1 and -2A activates in cultured chick neurons: differential regulation by insulin and insulin-like growth factor-I. *Endocrinology* **133,** 2085–2090.

Behringer R. R., Lewin T. M., Quaife C. J., Palmiter R. D., Brinster R. L., and D'Ercole A. J. (1990) Expression of insulin-like growth factor I stimulates normal somatic growth in growth hormone-deficient transgenic mice. *Endocrinology* **127,** 1033–1040.

Beilharz E. J., Bassett N. S., Sirimanne E. S., Williams C. E., and Gluckman P. D. (1995) Insulin-like growth factor II is induced during wound repair following hypoxic-ischemic injury in the developing rat brain. *Mol. Brain Res.* **29,** 81–91.

Beilharz E. J., Klempt N. D., Klempt M., Sirimanne E., Dragunow M., and Gluckman P. D. (1993) Differential expression of insulin-like growth factor binding proteins (IGFBP) 4 and 5 mRNA in the rat brain after transient hypoxic-ischemic injury. *Mol. Brain Res.* **18,** 209–215.

Blottner D. and Baumgarten H. G. (1992) Insulin-like growth factor-I counteracts bFGF-induced survival of nitric oxide synthase (NOS)-positive spinal cord neurons after target-lesion in vivo. *J. Neurosci. Res.* **32,** 471–480.

Bohannon N. J., Corp E. S., Wilcox B. J., Figlewictz D. P., Dorsa D. M., and Baskin D. G. (1988) Localization of binding sites for insulin-like growth factor-I (IGF-I) in the rat brain by quantitative autoradiography. *Brain Res.* **444,** 205–213.

Bondy C. A. (1991) Transient IGF-I gene expression during the maturation of functionally related central projection neurons. *J. Neurosci.* **11,** 3442–3455.

Bondy C. A., Underwood L. E., Clemmons D. R., Guler H.-P., Bach M. A., and Skarulis M. (1994) Clinical uses of insulin-like growth factor I. *Ann. Intern. Med.* **120,** 593–601.

Bondy C. A., Werner H., Roberts C. T. Jr., and LeRoith D. (1990) Cellular pattern of insulin-like growth factor-I (IGF-I) and type I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. *Mol. Endocrinol.* **4,** 1386–1398.

Bondy C. and Lee W.-H. (1993) Correlation between insulin-like growth factor (IGF)-binding protein 5 and IGF-I gene expression during brain development. *J. Neurosci.* **13,** 5092–5104.

Bourguignon J.-P., Gérard A., Alvarez Gonzalez M.-L., and Franchimont P. (1993) Acute suppression of gonadotropin-releasing hormone secretion by insulin-like growth factor I and subproducts: an age-dependent endocrine effect. *Neuroendocrinology* **58,** 525–530.

Bradshaw S. L. and Han V. K. M. (1993) Hormonal regulation of astroglial insulin-like growth factor (IGF)-binding protein gene expression by IGFs and insulin. *Endocrinology* **133,** 1767–1777.

Brar A. K. and Chernausek S. D. (1993) Localization of insulin-like growth factor binding protein-4 expression in the developing and adult rat brain: analysis by in situ hybridization. *J. Neurosci. Res.* **35,** 103–114.

Calissano P., Ciotti M. T., Battistini L., Zona C., Angelini A., Merlo D., and Mercanti D. (1993) Recombinant human insulin-like growth factor I exerts a trophic action and confers glutamate sensitivity on glutamate-resistant cerebellar granule cells. *Proc. Natl. Acad. Sci. USA* **90,** 8752–8756.

Caroni P. and Becker M. (1992) The down-regulation of growth-associated proteins in motoneurons at the onset of synapse elimination is controlled by muscle activity and IGF1. *J. Neurosci.* **12,** 3849–3861.

Caroni P. and Grandes P. (1990) Nerve spouting in innervated adult skeletal muscle induced by exposure to elevated insulin-like growth factors. *J. Cell Biol.* **110,** 1307–1317.

Caroni P. and Schneider C. (1994) Signaling by insulin-like growth factors in paralyzed muscle: rapid induction of IGF-I expression in muscle fibers and prevention of interstitial cell proliferation by IGF-BP5. *J. Neurosci.* **14,** 3378–3388.

Caroni P., Schneider C., Kiefer M. C., and Zapf J. (1994) Role of muscle insulin-like growth factors in nerve sprouting: suppression of terminal sprouting in paralyzed muscle by IGF-binding protein 4. *J. Cell Biol.* **125,** 893–902.

Carson M. J., Behringer R. R., Brinster R. L., and McMorris F. A. (1993) Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. *Neuron* **10,** 729–740.

Castro-Alamancos M. A. and Torres-Aleman I. (1993) Long-term depression of glutamate-induced gamma-aminobutyric acid release in cerebellum by insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **90,** 7386–7390.

Castro-Alamancos M. A. and Torres-Aleman I. (1994) Learning of the conditioned eye-blink response is impaired by an antisense insulin-like growth factor I oligonucleotide. *Proc. Natl. Acad. Sci. USA* **91,** 10,203–10,207.

Castro-Alamancos M. A., Arevalo M. A., and Torres-Aleman I. (1996) Involvement of protein kinase C and nitric oxide in the modulation by insulin-like growth factor-I of glutamate-induced GABA release in the cerebellum. *Neuroscience* **70,** 843–847.

Cavallaro T., Martone R. L., Stylianopoulou F., and Herbert J. (1993) Differential expression of the insulin-like growth factor II and transthyretin genes in the developing rat choroid plexus. *J. Neuropathol. Exp. Neurol.* **52,** 153–162.

Cheng B. and Mattson M. P. (1992) IGF-I and IGF-II protect cultured hippocampal and septal neurons against calcium-mediated hypoglycemic damage. *J. Neurosci.* **12,** 1558–1566.

Chernausek S. D. (1993) Insulin-like growth factor-I (IGF-I) production by astroglial cells: regulation and importance for epidermal growth factor-induced cell replication. *J. Neurosci. Res.* **34,** 189–197.

Clemmons D. R. (1991) Insulin-like growth factor binding protein control secretion and mechanisms of action. *Adv. Exp. Med. Biol.* **293,** 113–123.

Clemmons D. R. and Underwood L. E. (1991) Nutritional regulation of IGF-I and IGF binding proteins. *Ann. Rev. Nutr.* **11,** 393–412.

Contreras P. C., Steffler C., Yu E. Y., Callison K., Stong D., and Vaught J. L. (1995) Systemic administration of rhIGF-I enhanced regeneration after sciatic nerve crush in mice. *J. Pharmacol. Exp. Ther.* **274,** 1443–1449.

Couce M. E., Weatherington A. J., and McGinty J. F. (1992) Expression of insulin-like growth factor-II (IGF-II) and IGF-II/mannose-6-phosphate receptor in the rat hippocampus: an *in situ* hybridization and immunocytochemical study. *Endocrinology* **131,** 1636–1642.

Czech M. P. (1989) Signal transmission by the insulin-like growth factors. *Cell* **59,** 235–238.

D'Ercole A. J., Dai Z., Xing Y., Boney C., Wilkie M. B., Lauder J. M., Han V. K., and Clemmons D. R. (1994) Brain growth retardation due to the expression of human Insulin-like growth factor binding protein-1 (IGFBP-1) in transgenic mice: an in vivo model for analysis of IGF function in the brain. *Dev. Brain Res.* **82,** 213–222.

D'Mello S. R., Galli C., Ciotti T., and Calissano P. (1993) Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. *Proc. Natl. Acad. Sci. USA* **90,** 10,989–10,993.

Dai Z., Xing Y., Boney C., Clemmons D. R., and D'Ercole A. J. (1994) Human insulin-like growth factor binding protein-1 (IGFBP-1) transgenic mice: characterization and insights into the regulation of IGFBP-1 expression. *Endocrinology* **135,** 1316–1327.

Daughaday W. H. and Rotwein P. (1989) Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr. Rev.* **10,** 68–91.

De Keyser J., Wilczak N., and Goossens A. (1994) Insulin-like growth factor-I receptor densities in human frontal cortex and white matter during aging, in Alzheimer's disease, and in Huntington's disease. *Neurosci. Lett.* **172,** 93–96.

De Pablo F. and De la Rosa E. J. (1995) The developing CNS: a scenario for the actions of pro-insulin, insulin, and insulin-like growth factors. *TINS* **18,** 143–150.

DeChiara T. M., Efstratiadis A., and Robertson E. J. (1990) A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* **345,** 78–80.

DiCicco-Bloom E. and Black I. B. (1989) Depolarization and insulin-like growth factor-I (IGF-I) differentially regulate the mitotic cycle in cultured rat sympathetic neuroblasts. *Brain Res.* **491,** 403–406.

Drago J., Murphy M., Carroll S. M., Harvey R. P., and Bartlett P. F. (1991) Fibroblast growth factor-mediated proliferation of central nervous system precursors depends on endogenous production of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **88,** 2199–2203.

Dringen R. and Hamprecht B. (1992) Glucose, insulin, and insulin-like growth factor I regulate the glycogen content of astroglia-rich primary cultures. *J. Neurochem.* **58,** 511–517.

Duffy K. R., Pardridge W. M., and Rosenfeld R. G. (1988) Human blood-brain barrier insulin-like growth factor receptor. *Metabolism* **37,** 136–141.

El-Badry O. M., Helman L. J., Chatten J., Steinberg S. M., Evans A. E., and Israel M. A. (1991) Insulin-like growth factor II-mediated proliferation of human neuroblastoma. *J. Clin. Invest.* **87,** 648–657.

Fernández-Vázquez G., Cacicedo L., De los Frailes M. T., Lorenzo M. J., Tolón R., and Sánchez Franco F. (1993) Growth hormone-releasing factor regulation by somatostatin, growth hormone and insulin-like growth factor I in fetal rat hypothalamic-brain stem cell cocultures. *Neuroendocrinol.* **58,** 655–665.

Fernyhough P., Mill J. F., Roberts J. L., and Ishii D. N. (1989) Stabilization of tubulin mRNAs by insulin and insulin-like growth factor I during neurite formation. *Mol. Brain Res.* **6,** 109–120.

Fernyhough P., Willars G. B., Lindsay R. M., and Tomlinson D. R. (1993) Insulin and insulin-like growth factor I enhance regeneration in cultured adult rat sensory neurons. *Brain Res.* **607,** 117–124.

Festoff B. W., Yang S. X., Vaught J., Bryan C., and Ma J. (1995) The insulin-like growth factor signaling system and ALS neurotrophic factor treatment strategies. *J. Neurol. Sci.* **129(Suppl.),** 114–121.

Folli F., Bruning J. C., Patti M. E., Chen C., Ghidella S., Merighi A., and Kahn C. R. (1995) Roles of insulin-IGF-I receptor signalling network in the central nervous system. Programs & Abstracts of Annual Meeting of the Endocrine Society **515(Abstract).**

Frank H. J. L., Pardridge W. M., Morris W. L., Rosenfeld R. G., and Choi T. B. (1986) Binding and internalization of insulin and insulin-like growth factors by isolated brain microvessels. *Diabetes* **35,** 654–661.

Gammeltoft S., Ballotti R., Nielsen F. C., Kowalski A., and Van Obberghen E. (1988) Receptors for insulin-like growth factors in the central nervous system: structure and function. *Horm. Metab. Res.* **20,** 436–442.

Garcia-Estrada J., Garcia-Segura L. M., and Torres-Aleman I. (1992) Expression of insulin-like growth factor I by astrocytes in response to injury. *Brain Res.* **592,** 343–347.

Garcia-Segura L. M., Perez J., Pons S., Rejas M. T., and Torres-Aleman I. (1991) Localization of insulin-like growth factor I (IGF-I)-like immunoreactivity in the developing and adult rat brain. *Brain Res.* **560,** 167–174.

Giacobini M. M. J., Olson L., Hoffer B. J., and Sara V. R. (1990) Truncated IGF-1 exerts trophic effects on fetal brain tissue grafts. *Exp. Neurol.* **108,** 33–37.

Glazner G. W., Morrison A. E., and Ishii D. N. (1994) Elevated insulin-like growth factor (IGF) gene expression in sciatic nerves during IGF-supported nerve regeneration. *Mol. Brain Res.* **25,** 265–272.

Glick R. P., Unterman T. G., and Hollis R. (1991) Radioimmunoassay of insulin-like growth factors in cyst fluid of central nervous system tumors. *J. Neurosurg.* **74,** 972–978.

Gluckman P., Klempt N., Guan J., Mallard C., Sirmanne E., Dragunow M., Klempt M., Singh K., Williams C., and Nikolics K. (1992) A role for IGF-I in the rescue of CNS neurons following hypoxic-ischemic injury. *Biochem. Biophys. Res. Commun.* **182,** 593–599.

Guan J., Skinner S. J. M., Beilharz E. J., Hua K. M., Hodgkinson S., Gluckman P. D., and Williams C. E. (1996) Movement of IGF-I into the brain parenchyma after hypoxic-ischaemic injury. *Neuroreport* **7,** 632–636.

Gutierrez-Ospina G., Calikoglu A. S., Ye P., and D'Ercole A. J. (1996) *In vivo* effects of insulin-like growth factor-I (IGF-I) on the development of sensory pathways: analysis of the primary somatic sensory cortex (S1) of transgenic mice. *Endocrinology* (in press).

Han V. K. M., Smith A., Myint W., Nygard K., and Bradshaw S. (1992) Mitogenic activity of epidermal growth factor on newborn rat astroglia: interaction with insulin-like growth factors. *Endocrinology* **131,** 1134–1142.

Hansson H.-A., Dahlin L. B., Lowenadler B., Lundberg G., Paleus S., and Skottner A. (1988) Transient increase in insulin-like growth factor I immunoreactivity in rat peripheral nerves exposed to vibrations. *Acta Physiol. Scand.* **132,** 35–41.

Hansson H.-A., Rozell B., and Skottner A. (1987) Rapid axoplasmic transport of insulin-like growth factor I in the sciatic nerve of adult rats. *Cell Tissue Res.* **247,** 241–247.

Hantai D., Akaaboune M., Lagord C., Murawsky M., Houenou L. J., Festoff B. W., Vaught J. L., Riegr F., and Blondet B. (1995) Beneficial effects of insulin-like growth factor-I on wobbler mouse motoneuron disease. *J. Neurol.* **129,** 122–126.

Harel Z. and Tannenbaum G. S. (1992a) Centrally administered insulin-like growth factor II fails to alter pulsatile growth hormone secretion or food intake. *Neuroendocrinology* **56,** 161–168.

Harel Z. and Tannenbaum G. S. (1992b) Synergistic interaction between insulin-like growth factors-I and -II in central regulation of pulsatile growth hormone secretion. *Endocrinology* **131,** 758–764.

Heidenreich K. A., Zeppelin T., and Robinson L. J. (1993) Insulin and insulin-like growth factor I induce c-*fos* expression in postmitotic neurons by a protein kinase C-dependent pathway. *J. Biol. Chem.* **268,** 14,663–14,670.

Hynes M. A., Brooks P. J., Van Wyk J. J., and Lund P. K. (1988) Insulin-like growth factor ii messenger ribonucleic acids are synthesized in the choroid plexus of the rat brain. *Mol. Endocrinol.* **2,** 47–54.

Hynes M. A., Van Wyk J. J., Brooks P. J., D'Ercole A. J., Jansen M., and Lund P. K. (1987) Growth hormone dependence of somatomedin-C/insulin-like growth factor-I and insulin-like growth factor-II messenger ribonucleic acids. *Mol. Endocrinol.* **1,** 233–242.

Ishii D. N. (1993) Neurobiology of insulin and insulin-like growth factors, in *Neurotrophic Factors* (Loughlin S. E. and Fallon J. H., eds.), Academic, New York, pp. 415–442.

Ishii D. N. and Lupien S. B. (1995) Insulin-like growth factors protect against diabetic neuropathy: effects on sensory nerve regeneration in rats. *J. Neurosci. Res.* **40,** 138–144.

Ishii D. N., Glazner G. W., and Pu S.-F. (1994) Role of insulin-like growth factors in peripheral nerve regeneration. *Pharmacol. Ther.* **62,** 125–144.

Johnston B. M., Mallard E. C., Williams C. E., and Gluckman P. D. (1996) Insulin-like growth factor-1 is a potent neuronal rescue agent after hypoxic-ischemic injury in fetal lambs. *J. Clin. Invest.* **97,** 300–308.

Kenner K. A. and Heidenreich K. A. (1991) Insulin and insulin-like growth factors stimulate in vivo receptor autophosphorylation and tyrosine phosphorylation of a 70K substrate in cultured fetal chick neurons. *Endocrinology* **129,** 301–311.

Kerkhoff H., Hassan S. M., Troost D., Van Etten R. W., Veldman H., and Jennekens F. G. I. (1994) Insulin-like and fibroblast growth factors in spinal cords, nerve roots and skeletal muscle of human controls and patients with amyotrophic lateral sclerosis. *Acta Neuropathol. (Berl.)* **87,** 411–421.

Kiess W., Lee L., Graham D. E., Greenstein L., Tseng L. Y.-H., Rechler M. M., and Nissley S. P. (1989) Rat C6 glial cells synthesize insulin-like growth factor I (IGF-I) and express IGF-I receptors and IGF-II/mannose 6-phosphate receptors. *Endocrinology* **124,** 1727–1736.

Klempt M., Klempt N. D., and Gluckman P. D. (1993) Hypoxia and hypoxia/ischemia affect the expression of insulin-like growth factor binding protein 2 in the developing rat brain. *Mol. Brain Res.* **17,** 347–350.

Kleppisch T., Klinz F.-J., and Hescheler J. (1992) Insulin-like growth factor I modulates voltage-dependent Ca^{2+}-channels in neuronal cells. *Brain Res.* **591,** 283–288.

Knusel B. and Hefti F. (1991) Trophic actions of IGF-I, IGF-II and insulin on cholinergic and dopaminergic brain neurons. *Adv. Exp. Med. Biol.* **293,** 351–360.

Komoly S., Hudson L. D., Webster H. D., and Bondy C. A. (1992) Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. *Proc. Natl. Acad. Sci. USA* **89,** 1894–1898.

Konishi Y., Takahashi K., Chui D.-H., Rosenfeld R. G., Himeno M., and Tabira T. (1994) Insulin-like growth factor II promotes in vitro cholinergic development of mouse septal neurons: comparison with the effects of insulin-like growth factor I. *Brain Res.* **649,** 53–61.

Lee W. H., Wang G. M., Seaman L. B., and Vannucci S. J. (1996) Coordinate IGF-I and IGFBP5 gene expression in perinatal rat brain after hypoxia-ischemia. *J. Cereb. Blood Flow Metab.* **16,** 227–236.

Lee W.-H., Clemens J. A., and Bondy C. A. (1992a) Insulin-like growth factors in response to cerebral ischemia. *Mol. Cell. Neurosci.* **3,** 36–43.

Lee W.-H., Javedan S., and Bondy C. A. (1992b) Coordinate expression of insulin-like growth factor system components by neurons and neuroglia during retinal and cerebellar development. *J. Neurosci.* **12,** 4737–4744.

Lee W.-H., Michels K. M., and Bondy C. A. (1993) Localization of insulin-like growth factor binding protein-2 messenger RNA during postnatal brain development: correlation with insulin-like growth factors I and II. *Neuroscience* **53,** 251–265.

Lee W.-H., Wang G.-M., Lo T., Triarhou L. C., and Ghetti B. (1995) Altered IGFBP5 gene expression in the cerebellar external germinal layer of weaver mutant mice. *Mol. Brain Res.* **30,** 259–268.

Lenoir D. and Honegger P. (1983) Insulin-like growth factor I (IGF I) stimulates DNA synthesis in fetal rat brain cell cultures. *Exp. Br. Res.* **7,** 205–213.

LeRoith D., Werner H., Beitner-Johnson D., and Roberts C. T., Jr. (1995) Molecular and cellular aspects of the insulin-like growth factor I receptor. *Endocr. Rev.* **16,** 143–163.

Levinovitz A. and Norstedt G. (1989) Developmental and steroid hormonal regulation of insulin-like growth factor II expression. *Mol. Endocrinol.* **3,** 797–804.

Liu J. P. and Lauder J. M. (1992) S-100β and insulin-like growth factor-II differentially regulate growth of developing serotonin and dopamine neurons in vitro. *J. Neurosci. Res.* **33,** 248–256.

Liu J., Baker J., Perkins A. S., Robertson E. J., and Efstratiadis A. (1993) Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor. *Cell* **75,** 59–72.

Liu X., Yao D., and Webster H. D. (1995) Insulin-like growth factor I treatment reduces clinical deficits and lesion severity in acute demyelinating experimental autoimmune encephalomyelitis. *Multiple Sclerosis* **1,** 2–9.

Liu X., Yao D.-L., Bondy C. A., Brenner M., Hudson L. D., Zhou J., and Webster H. D. (1994) Astrocytes express insulin-like growth factor-I and its binding protein, IGFBP-2, during demyelination induced by experimental autoimmune encephalomyelitis. *Mol. Cell. Neurosci.* **5,** 418–430.

Lobie P. E., García-Aragón J., Lincoln D. T., Barnard R., Wilcox J. N., and Waters M. J. (1993) Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. *Dev. Brain Res.* **74,** 225–233.

Logan A., Gonzalez A.-M., Hill D. J., Berry M., Gregson N. A., and Baird A. (1994) Coordinated pattern of expression and localization of insulin like growth factor-II (IGF-II) and IGF-binding protein-2 in the adult rat brain. *Endocrinology* **135,** 2255–2264.

Lowe W. L., Jr., Adamo M., Werner H., Roberts C. T., Jr., and LeRoith D. (1989) Regulation by fasting of rat insulin-like growth factor I and its receptor. Effects on gene expression and binding. *J. Clin. Invest.* **84,** 619–626.

Lowe W. L., Jr., Meyer T., Karpen C. W., and Lorentzen L. R. (1992) Regulation of insulin-like growth factor I production in rat C6 glioma cells: possible role as an autocrine/paracrine growth factor. *Endocrinology* **130,** 2683–2691.

Ma J., Yang S. X., Ho G. J., and Festoff B. W. (1994a) Insulin-like growth factor binding protein-1 is pre-synaptic at mouse neuromuscular synapses and is transported in nerve. *Neurochem. Res.* **19,** 1363–1368.

Ma J., Yang S. X., Ho G. J., and Festoff B. W. (1994b) Insulin-like growth factor binding protein-1 at mouse neuromuscular synapses. *Synapse* **17,** 225–229.

Masters B. A., Werner H., Roberts C. T. Jr., LeRoith D., and Raizada M. K. (1991) Developmental regulation of insulin-like growth factor-I-stimulated glucose transporter in rat brain astrocytes. *Endocrinology* **128,** 2548–2557.

Masters B. A., Werner H., Roberts C. T., Jr., LeRoith D., and Raizada M. K. (1991) Insulin-like growth factor I (IGF-I) receptors and IGF-I action in oligodendrocytes from rat brains. *Regul. Pept.* **33,** 117–131.

Mathews L. S., Hammer R. E., Behringer R. R., D'Ercole A. J., Bell G. I., Brinster R. L., and Palmiter R. D. (1988) Growth enhancement of transgenic mice expressing human insulin-like growth factor I. *Endocrinology* **123,** 2827–2833.

McElduff A., Poronnik P., Baxter R. C., and Williams P. (1988) A comparison of the insulin and insulin-like growth factor I receptors from rat brain and liver. *Endocrinology* **122,** 1933–1939.

McMorris F. A. and Dubois-Dalcq M. (1988) Insulin-like growth factor I promotes cell proliferation and oligodendroglial commitment in rat glial progenitor cells developing in vitro. *J. Neurosci. Res.* **21,** 199–209.

McMorris F. A., Smith T. M., DeSalvo S., and Furlanetto R. W. (1986) Insulin-like growth factor I/somatomedin C: a potent inducer of oligodendrocyte development. *Proc. Natl. Acad. Sci. USA* **83,** 822–826.

Merrill M. and Edwards N. A. (1990) Insulin-like growth factor-receptors in human glial tumors. *J. Clin. Endocrinol. Metab.* **71,** 199–209.

Mozell R. L. and McMorris F. A. (1991) Insulin-like growth factor I stimulates oligodendrocyte development and myelination in rat brain aggregate cultures. *J. Neurosci. Res.* **30,** 382–390.

Muller H. L., Oh Y., Lehrnbecher T., Blum W. F., and Rosenfeld R. G. (1994) Insulin-like growth factor-binding protein-2 concentrations in cerebral-spinal fluid and serum of children with malignant solid tumors or acute leukemia. *J. Clin. Endocrinol. Metab.* **79,** 428–434.

Murphy L. J. and Friesen H. G. (1988) Differential effects of estrogens and growth hormone on uterine and hepatic insulin-like growth factor I gene expression in the ovariectomized hypophysectomized rat. *Endocrinology* **122,** 325–332.

Nataf V. and Monier S. (1992) Effect of insulin and insulin-like growth factor I on the expression of the catecholaminergic phenotype by neural crest cells. *Dev. Brain Res.* **69,** 59–66.

Near S. L., Whalen L. R., Miller J. A., and Ishii D. N. (1992) Insulin-like growth factor II stimulates motor nerve regeneration. *Proc. Natl. Acad. Sci. USA* **89,** 11,716–11,720.

Neff N., Prevette D., Houenou L. J., Lewis M. E., Glicksman M. A., Yin Q.-W., and Oppenheim R. W. (1993) Insulin-like growth factors: putative muscle-derived trophic agents that promote motoneuron survival. *J. Neurobiol.* **24,** 1578–1588.

Nieto-Bona M. P., Busiguina S., and Torres-Aleman I. (1995) Insulin-like growth factor I is an afferent trophic signal that modulates calbindin-28kD in adult Purkinje cells. *J. Neurosci. Res.* **42,** 371–376.

Nieto-Bona M. P., Garcia-Segura L. M., and Torres-Aleman I. (1993) Orthograde transport and release of insulin-like growth factor I from the inferior olive to the cerebellum. *J. Neurosci. Res.* **36,** 520–527.

Ocrant I., Valentino K. L., Eng L. F., Hintz R. L., Wilson D. M., and Rosenfeld R. G. (1988) Structural and immunohistochemical characterization of insulin-like growth factor I and II receptors in the murine central nervous system. *Endocrinology* **123,** 1023–1034.

Olchovsky D., Song J., Gelato M. C., Sherwood J., Spatola E., Bruno J. F., and Berelowitz M. (1993) Pituitary and hypothalamic insulin-like growth factor-I (IGF-I) and IGF-I receptor expression in food-deprived rats. *Mol. Cell. Endocrinol.* **93,** 193–198.

Påhlman S., Meyerson G., Lindgren E., Schalling M., and Johansson I. (1991) Insulin-like growth factor I shifts from promoting cell division to potentiating maturation during neuronal differentiation. *Proc. Natl. Acad. Sci. USA* **88,** 9994–9998.

Philipps A., Drakenberg K., Persson B., Sjögren B., Eklöf A. C., Hall K., and Sara V. (1989) The effects of altered nutritional status upon insulin-like growth factors and their binding proteins in neonatal rats. *Pediatr. Res.* **26,** 128–134.

Pons S. and Torres-Aleman I. (1992) Basic fibroblast growth factor modulates insulin-like growth factor-I, its receptor, and its binding proteins in hypothalamic cell cultures. *Endocrinology* **131,** 2271–2278.

Pons S. and Torres-Aleman I. (1993) Estradiol modulates insulin-like growth factor I receptors and binding proteins in neurons from the hypothalamus. *J. Neuroendocrinol.* **5,** 267–271.

Pons S., Rejas M. T., and Torres-Aleman I. (1991) Ontogeny of insulin-like growth factor I, its receptor, and its binding proteins in the rat hypothalamus. *Dev. Brain Res.* **62,** 169–175.

Powell-Braxton L., Hollingshead P., Warburton C., Dowd M., Pitts-Meek S., Dalton D., Gillett N., and Stewart T. A. (1993) IGF-I is required for normal embryonic growth in mice. *Genes Dev.* **7,** 2609–2617.

Pu S. F., Zhuang H. X., and Ishii D. N. (1995) Differential spatio-temporal expression of the insulin-like growth factor genes in regenerating sciatic nerve. *Mol. Brain Res.* **34,** 18–28.

Pucilowski O., Ayensu W. K., and D'Ercole A. J. (1996) Insulin-like growth factor I expression alters acute sensitivity and tolerance to ethanol in transgenic mice. *Eur. J. Pharmacol.* **305,** 57–62.

Raizada M. K. and LeRoith D. (1993) *The Role of Insulin-Like Growth Factors in the Nervous System.* The New York Academy of Sciences, New York.

Rajkumar K., Barron D., Lewitt M. S., and Murphy L. J. (1995) Growth retardation and hyperglycemia in insulin-like growth factor binding protein-1 transgenic mice. *Endocrinology* **136,** 4029–4034.

Ratajczak M. Z., Kuczynski W. I., Onodera K., Moore J., Ratajczak J., Kregenow D. A., DeRiel K., and Gewirtz A. M. (1994) A reappraisal of the role of insulin-like growth factor I in the regulation of human hematopoiesis. *J. Clin. Invest.* **94,** 320–327.

Reinhardt R. R. and Bondy C. A. (1994) Insulin-like growth factors cross the blood-brain barrier. *Endocrinology* **135,** 1753–1761.

Robinson L. J., Leitner W., Draznin B., and Heidenreich K. A. (1994) Evidence that p21ras mediates the neurotrophic effects of insulin and insulin-like growth factor I in chick forebrain neurons. *Endocrinology* **135,** 2568–2573.

Rosenfeld R. G., Pham H., Keller B. T., Borchardt R. T., and Pardridge W. M. (1987) Demonstration and structural comparison of receptors for insulin-like growth factor-I and -II (IGF-I and -II) in brain and blood-brain barrier. *Biochem. Biophys. Res. Commun.* **149,** 159–166.

Roth G. A., Spada V., Hamill K., and Bornstein M. B. (1995) Insulin-like growth factor I increases myelination and inhibits demyelination in cultured organotypic nerve tissue. *Dev. Brain Res.* **88,** 102–108.

Rotwein P., Burgess S. K., Milbrandt J. D., and Krause J. E. (1988) Differential expression of insulin-like growth factor genes in rat central nervous system. *Proc. Natl. Acad. Sci. USA* **85,** 265–269.

Russo V. C. and Werther G. A. (1994) Des (1–3) IGF-I potently enhances differentiated cell growth in olfactory bulb organ culture. *Growth Factors* **11,** 301–311.

Russo V. C., Edmondson S. R., Mercuri F. A., Buchanan C. R., and Werther G. A. (1994) Identification, localization, and regulation of insulin-like growth factor binding proteins and their messenger ribonucleic acids in the newborn rat olfactory bulb. *Endocrinology* **135,** 1437–1446.

Sahu A., Dupe M. G., Phelps C. P., Sninsky C. A., Kalra P. S., and Kalra S. P. (1995) Insulin and insulin-like growth factor II suppress neuropeptide Y release from the nerve terminals in the paraventricular nucleus: a putative hypothalamic site for energy homeostasis. *Endocrinology* **136,** 5718–5724.

Saneto R. P., Low K. G., Melner M. H., and De Vellis J. (1988) Insulin/insulin-like growth factor I and other epigenetic modulators of myelin basic protein expression in isolated oligodendrocyte progenitor cells. *J. Neurosci. Res.* **21,** 210–219.

Santos A., Yusta B., Fernández-Moreno M. D., and Blázquez E. (1994) Expression of insulin-like growth factor-I (IGF-I) receptor gene in rat brain and liver during development and in regenerating adult rat liver. *Mol. Cell. Endocrinol.* **101,** 85–93.

Santucci D., Cagiano R., and Calamandrei G. (1994) IGF-I and IGF-I^{24-41} but not IGF-I^{57-70} affect somatic and neurobehavioral development of newborn male mice. *Brain Res. Bull.* **35,** 367–371.

Schumacher M., Jung-Testas I., Robel P., and Baulieu E.-E. (1993) Insulin-like growth factor I: a mitogen for rat Schwann cells in the presence of elevated levels of cyclic AMP. *Glia* **8,** 232–240.

Shambaugh G. I., Natarajan N., Davenport M. L., Ochler D., and Unterman T. (1995) Nutritional insult and recovery in the neonatal rat cerebellum: insulin-like growth factors (IGFs) and their binding proteins (IGFBPs). *Neurochem. Res.* **20,** 475–489.

Shimasaki S. and Ling N. (1992) Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5 and -6). *Prog. Growth Factor Res.* **3,** 243–266.

Shinar Y. and McMorris F. A. (1995) Developing oligodendroglia express mRNA for insulin-like growth factor-I, a regulator of oligodendrocyte development. *J. Neurosci. Res.* **42,** 516–527.

Sortino M. A. and Canonico P. L. (1996) Neuroprotective effect of insulin-like growth factor I in immortalized hypothalamic cells. *Endocrinology* **137,** 1418–1422.

Stenvers K. L., Zimmermann E. M., Gallagher M., and Lund P. K. (1994) Expression of insulin-like growth factor binding protein-4 and -5 mRNAs in adult rat forebrain. *J. Comp. Neurol.* **339,** 91–105.

Straus D. S. and Burke E. J. (1995) Glucose stimulates IGF-I gene expression in C6 glioma cells. *Endocrinology* **136,** 365–368.

Stylianopoulou F., Efstratiadis A., Herbert J., and Pintar J. (1988) Pattern of the insulin-like growth factor II gene expression during rat embryogenesis. *Development* **103,** 497–506.

Sullivan K. A. and Feldman E. L. (1994) Immunohistochemical localization of insulin-like growth factor-II (IGF-II) and IGF-binding protein-2 during development in the rat brain. *Endocrinology* **135,** 540–547.

Sumantran V. N. and Feldman E. L. (1993) Insulin-like growth factor I regulates c-*myc* and GAP-43 messenger ribonucleic acid expression in SH-SY5Y human neuroblastoma cells. *Endocrinology* **132,** 2017–2023.

Svenningsen Å. F., Kanje M., and Sjöberg J. (1994) Insulin-like growth factor II (IGF-II) stimulates regeneration and proliferation in the rat sciatic nerve. *Neurosci. Res. Commun.* **15,** 111–118.

Torres-Aleman I., Naftolin F., and Robbins R. J. (1990a) Trophic effects of basic fibroblast growth factor on fetal rat hypothalamic cells: interactions with insulin-like growth factor I. *Dev. Brain Res.* **52,** 253–257.

Torres-Aleman I., Naftolin F., and Robbins R. J. (1990b) Trophic effects of insulin-like growth factor-I on fetal rat hypothalamic cells in culture. *Neuroscience* **35,** 601–608.

Torres-Aleman I., Pons S., and Arévalo M. A. (1994) The insulin-like growth factor I system in the rat cerebellum: developmental regulation and role in neuronal survival and differentiation. *J. Neurosci. Res.* **39,** 117–126.

Torres-Aleman I., Pons S., and Santos-Benito F. F. (1992) Survival of Purkinje cells in cerebellar cultures is increased by insulin-like growth factor I. *Eur. J. Neurosci.* **4,** 864–869.

Trojan J., Blossey B. K., Johnson T. R., Rudin S. D., Tykocinski M., and Ilan J. (1992) Loss of tumorigenicity of rat glioblastoma directed by episome-based antisense cDNA transcription of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **89,** 4874–4878.

Trojan J., Johnson T. R., Rudin S. D., Ilan J., and Tykocinski M. L. (1993) Treatment and prevention of rat glioblastoma by immunogenic C6 cells expressing antisense insulin-like growth factor I RNA. *Science* **259,** 94–97.

Uchiyama T., Kaji H., Abe H., and Chihara K. (1994) Negative regulation of hypothalamic growth hormone-releasing factor messenger ribonucleic acid by growth hormone and insulin-like growth factor I. *Neuroendocrinol.* **59,** 441–450.

Vig P. J. S., Desaiah D., Joshi P., Subramony S. H., Fratkin J. D., and Currier R. D. (1994) Decreased insulin-like growth factor I-mediated protein tyrosine phosphorylation in human olivopontocerebellar atrophy and lurcher mutant mouse. *J. Neurol. Sci.* **124,** 38–44.

Wang C., Li Y., Wible B., Angelides K. J., and Ishii D. N. (1992) Effects of insulin and insulin-like growth factors on neurofilament mRNA and tubulin mRNA content in human neuroblastoma SH-SY5Y cells. *Mol. Brain Res.* **13,** 289–300.

Werther G. A., Abate M., Hogg A., Cheesman H., Oldfield B., Hards D., Hudson P., Power B., Freed K., and Herington A. C. (1990) Localization of insulin-like growth factor-I mRNA in rat brain by in situ hybridization—relationship to IGF-I receptors. *Mol. Endocrinol.* **4,** 773–778.

Werther G. A., Cheesman H., and Russo V. (1993) Olfactory bulb organ culture is supported by combined insulin-like growth factor-I and basic fibroblast growth factor. *Brain Res.* **617,** 339–342.

Wood T. L., Berelowitz M., Gelato M. C., Roberts C. T., Jr., LeRoith D., Millard W. J., and McKelvy J. F. (1991) Hormonal regulation of rat hypothalamic neuropeptide mRNAs: effect of hypophysectomy and hormone replacement on growth-hormone-releasing factor, somatostatin and the insulin-like growth factors. *Neuroendocrinol.* **53,** 298–305.

Wood T. L., Brown A. L., Rechler M. M., and Pintar J. E. (1990) The expression pattern of an insulin-like growth factor (IGF)-binding protein gene is distinct from IGF-II in the midgestational rat embryo. *Mol. Endocrinol.* **4,** 1257–1263.

Wood T. L., Streck R. D., and Pintar J. E. (1992) Expression of the IGFBP-2 gene in post-implantation rat embryos. *Development* **114,** 59–66.

Yamaguchi F., Itano T., Miyamoto O., Janjua N. A., Ohmoto T., Hosokawa K., and Hatase O. (1991) Increase of extracellular insulin-like growth factor I (IGF-I) concentration following electrolytical lesion in rat hippocampus. *Neurosci. Lett.* **128,** 273–276.

Yamaguchi F., Itano T., Mizobuchi M., Miyamoto O., Janjua N. A., Matsui H., Tokuda M., Ohmoto T., Hosokawa K., and Hatase O. (1990) Insulin-like growth factor I (IGF-I) distribution in the tissue and extracellular compartment in different regions of rat brain. *Brain Res.* **533,** 344–347.

Yao D.-L., Liu X., Hudson L. D., and Webster H. D. (1995) Insulin-like growth factor I treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis. *Proc. Natl. Acad. Sci. USA* **92,** 6190–6194.

Yao D.-L., West N. R., Bondy C. A., Brenner M., Hudson L. D., Zhou J., Collins G. H., and Webster H. D. (1995) Cryogenic spinal cord injury induces astrocytic gene expression of insulin-like growth factor I and insulin-like growth factor binding protein 2 during myelin regeneration. *J. Neurosci. Res.* **40,** 647–659.

Ye P., Carson J., and D'Ercole A. J. (1995a) Insulin-like growth factor-I influences the initiation of myelination: studies of the anterior commissure of transgenic mice. *Neurosci. Lett.* **201,** 235–238.

Ye P., Carson J., and D'Ercole A. J. (1995b) *In vivo* actions of insulin-like growth factor-I (IGF-I) on brain myelination: studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J. Neurosci.* **15,** 7344–7356.

Ye P., Xing Y. Z., Dai Z. H., and D'Ercole A. J. (1996) In vivo actions of insulin-like growth factor-I (IGF-I) on cerebellum development in transgenic mice: evidence that IGF-I increases proliferation of granule cell progenitors. *Dev. Brain Res.* **95,** 44–54.

Yee D., Favoni R. E., Lebovic G. S., Lombana F., Powell D. R., Reynolds C. P., and Rosen N. (1990) Insulin-like growth factor I expression by tumors of neuroectodermal origin with the t(11;22) chromosomal translocation. A potential autocrine growth factor. *J. Clin. Invest.* **86,** 1806–1814.

Zackenfels K., Oppenheim R. W., and Rohrer H. (1995) Evidence for an important role of IGF-I and IGF-II for the early development of chick sympathetic neurons. *Neuron* **14,** 731–741.

Zhang W., Lee W. H., and Triarhou L. C. (1996) Grafted cerebellar cells in a mouse model of hereditary ataxia express IGF-I system genes and partially restore behavioral function. *Nature Med.* **2,** 65–71.

Zona C., Ciotti M. T., and Calissano P. (1995) Human recombinant IGF-I induces the functional expression of AMPA/kainate receptors in cerebellar granule cells. *Neurosci. Lett.* **186,** 75–78.