7 IGF-1 in Brain Growth and Repair Processes

C. Bondy · C. Cheng · J. Zhong · W.-H. Lee

Abstract: This chapter addresses the role of insulin-like growth factor 1 (IGF-1) and the IGF-1 receptor (IGF1R) in brain development, injury response, and aging. We concentrate mainly on recent information from murine model systems, with consideration of interesting and relevant data from invertebrates and humans. IGF-1 and its cognate receptor are both highly expressed in the developing brain, supporting both autocrine and paracrine activity for this anabolic peptide. IGF-1 deletion or inhibition during brain development attenuates brain growth, with reductions in both cell number and cell size. Cell numbers are notably reduced in the olfactory system, the dentate gyrus of the hippocampus, and the striatum. Brain volume is globally decreased due to a loss of neuropil, with significant reductions in neuronal soma volume, dendritic length and complexity, and synapse number. Myelination is reduced in proportion to the decreases in neuron number and nerve processes in the IGF-1-null brain. Conversely, transgenic IGF-1 overexpression results in increased brain size with increases in cell number, cell size, and dendrite growth with proportionate increases in myelination. Metabolic activity as measured by glucose utilization is significantly decreased in the IGF-1-null brain and increased in the transgenic IGF-1-overexpressing brain. IGF-1 deletion in humans is associated with mental retardation and sensorineural deafness. IGF-1 deletion is also associated with deafness in mice, but no other obvious neurological or behavioral phenotypes have been identified.

IGF-1 prevents neuronal death in response to a variety of insults in vitro, but cell death appears to be a minor effect in the IGF-1-null brain. IGF-1's physiological effects in brain depend on when and where the peptide is expressed. For example, IGF-1 is expressed in an olfactory neuron germinal zone early in development, enhancing proliferation of these neurons, which are correspondingly reduced in number in the IGF-1-null mouse. IGF-1 is expressed in long-axon projection neurons at a later, postmitotic stage, promoting somatic and dendritic growth for these neurons, which are normal in number but small with hypotrophic dendritic arbors in the IGF-1-null brain. Increased circulating or brain IGF-1 is associated with increased hippocampal neurogenesis in adult rodents, and treatment with exogenous IGF-1 may protect against neurodegeneration in response to brain injury. IGF-1's anabolic effects in brain are executed via the IRS2-PI3K-Akt signaling system. The multifunctional enzyme glycogen synthase kinase 3 (GSK3) is a major target of this pathway. Inhibitory phosphorylation of GSK3 by IGF-1 enhances glucose utilization and protein synthesis, promoting somatic growth and dendritogenesis in IGF-1-expressing projection neurons. Brain IGF-1 also inhibits the phosphorylation of tau, a microtubule-associated protein, via the PI3K-Akt-GSK3 pathway. This neurofibrillary tangle (NFT) protein is hyperphosphorylated in both IGF-1- and IRS2-null brains. IGF-1's role in brain aging is unclear at present. Data obtained from worms to primates suggest that suppression of the IGF system slows the aging process, but it is not yet known if brain aging is altered in IGF-1-null or -deficient mice.

List of Abbreviations: AD, Alzheimer's disease; Akt, serine/threonine protein kinase; BAD, bcl-associated death promoter; BBB, blood–brain barrier; BRDU, bromodeoxyuridine; CNPase, 2′,3′-cyclic nucleotide, 3′-phosphodiesterase; EGF, epidermal growth factor; eIF2B, eukaryotic initiation factor 2B; FOXO, forkhead transcription factors; GH, growth hormone; GLUT, glucose transporter; GSK3, glycogen synthase kinase 3; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; IGF1R, IGF-1 receptor; IRS, insulin receptor substrate; MAG, myelin-associated protein; MAPK, mitogen-activated protein kinase; MBP, myelin basic protein; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; NFT, neurofibrillary tangle; NO, nitric oxide; PCR, polymerase chain reaction; PDK1, 2, 3-phosphoinositide-dependent protein kinase 1, 2; PI3K, phosphoinositide-3 kinase; PIP3, phosphatidylinositol-3,4,5-trisphosphate; PLP, myelin proteolipid protein; PTEN, phosphatase and tensin homolog; S6K, ribosomal protein S6 kinase

1 Introduction

Members of the insulin-like growth factor (IGF) family, including insulin, IGF-1, and IGF-2, promote carbohydrate, lipid, and protein metabolism in support of cell growth and survival. Insulin has a specialized role in peripheral glucose homeostasis, and a neuroendocrine role at the hypothalamic level, promoting the

integration of nutrient acquisition, storage, and expenditure. IGF-1 promotes postnatal somatic growth while IGF-2 promotes similar, proportionate growth in utero. Insulin deficiency leads to the metabolic derangements of diabetes mellitus, while IGF deficiency is associated with proportionate dwarfism. The fundamental importance of this insulin/IGF system is reflected by the fact that insulin and IGF peptides and receptor homologs are found in evolutionarily distant and diverse organisms such as *Caenorhabditis elegans* and *Drosophila melanogaster*. Recent genetic studies have shown that the insulin:IGF-1 receptor/PI3K/Akt signaling pathway is largely conserved down to the metazoan level and plays an essential role in regulating life span as well as body, organ, and cell size (Finch and Ruvkun, 2001).

An interesting feature observed in Drosophila is that insulin-like peptides are expressed in neural cells in the brain. Ablation of these "neurons" causes developmental delay and growth retardation (Rulifson et al., 2002). In fact, single-gene mutations targeting the insulin/IGF receptor or downstream signaling components result in significant extension of the life span in yeast, nematodes, fruit fly, and rodents (reviewed in Richardson et al., 2004; Katic and Kahn, 2005). In many cases, these animals are healthier than normal, like animals on food-restricted diets, although fecundity may be impaired. Thus it seems that the insulin/IGF system promotes anabolic effects that increase growth rate and fertility, but also accelerates the aging process through impaired responses to oxidative and other types of stress. Suppressed insulin/IGF signaling impairs somatic growth, but minimizes damage to and increases repair of cell macromolecules. The question is how these anabolic growth-promoting and age-accelerating peptides function in the brain. IGF-1 is clearly important in brain development and function, as individuals homozygous for mutations/deletions in *IGF1* are profoundly mentally retarded (Woods et al., 1997; Bonapace et al., 2003; Walenkamp et al., 2005).

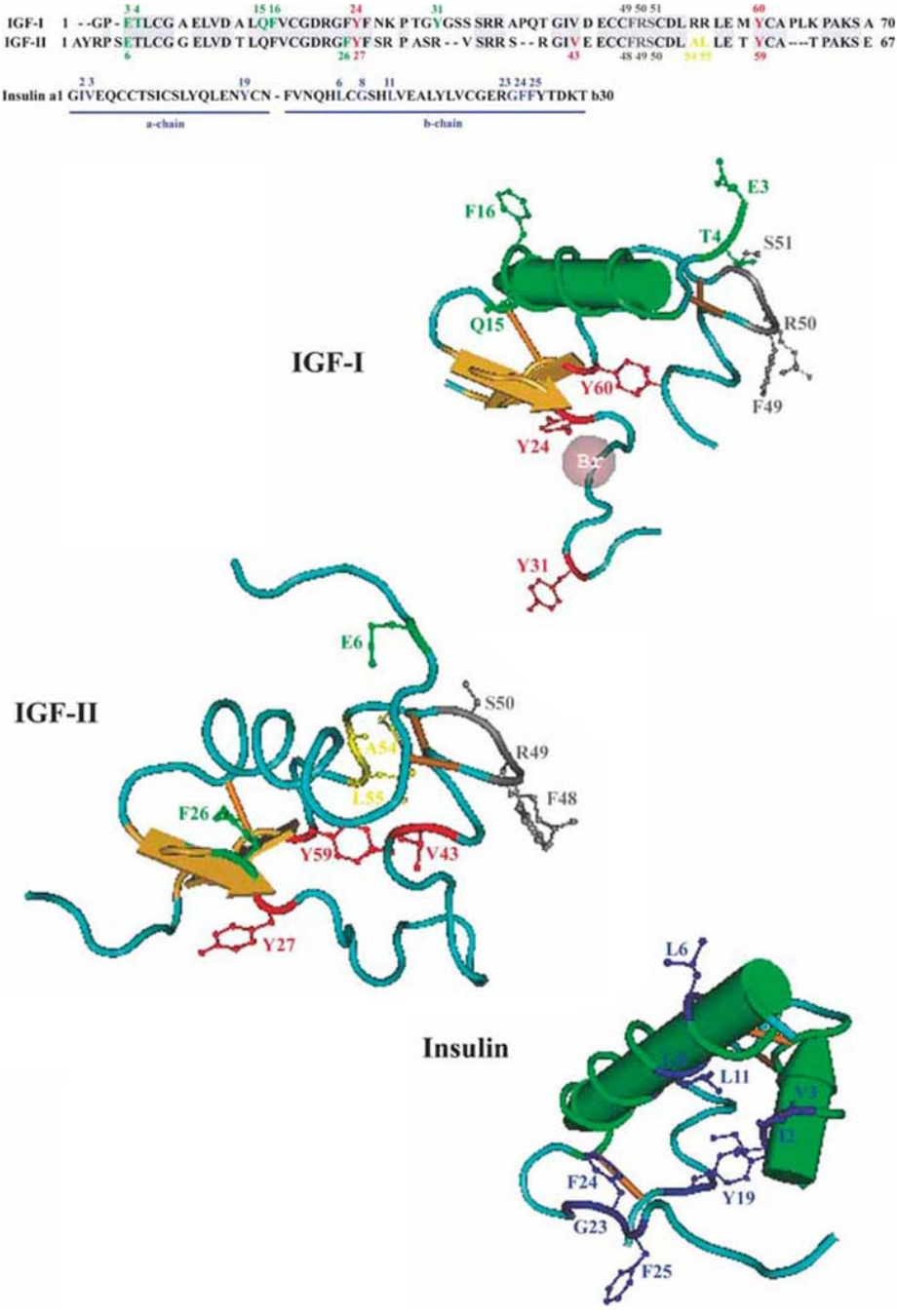
2 IGF/Insulin Peptides and Binding Proteins

IGF-1, IGF-2, and insulin (❯ *Figure 7-1*) belong to an ancient family of peptides sharing a common evolutionary origin (LeRoith et al., 1986; LeRoith et al., 1993; Reinecke and Collet, 1998; Navarro et al., 1999). An ancestral gene encoding an insulin-like peptide gave rise to multiple genes encoding more specialized peptides about the time gastroenteric and central nervous systems (CNS) differentiated (Reinecke and Collet, 1998). From that time insulin became progressively more specialized in terms of secondary processing (proteolytic excision of the "C" peptide and joining of the A and B peptides by disulfide bonds), packaging in acidic secretory granules, and association with the gastrointestinal tract. Insulin expression is largely restricted to pancreatic beta cells, where its synthesis and secretion are tightly coupled to ingested substrates (Tager et al., 1981). IGF-1 and IGF-2, in contrast, did not acquire such extensive posttranslational processing, and have continued to be widely expressed in many cell types demonstrating constitutive secretion (Clemmons, 1989; Sussenbach, 1989). IGF-1 and IGF-2 are single-chain polypeptides of 70 amino acids with three intramolecular disulfide bridges. The IGFs share about 50% homology with insulin in amino acid sequences in addition to very similar tertiary structures and functional binding sites (❯ *Figure 7-1*).

In mammals, insulin production is centrally localized in the beta cells of the pancreas, from which insulin is released in bolus fashion in response to nutrient stimuli. Insulin serves in classic endocrine hormone fashion to regulate glucose, lipid, and protein metabolism in many peripheral tissues, but excluding the brain. IGF-1 is produced in great abundance by the liver where its synthesis is regulated by pituitary growth hormone (GH) (Laron, 2001). IGF-1 is also synthesized locally in many tissues (Daughaday and Rotwein, 1989; Le Roith et al., 2001), including the brain (Rotwein et al., 1988; Bartlett et al., 1991; Bondy, 1991), where GH does not regulate its synthesis (Wang et al., 1999; Lupu et al., 2001; Sun et al., 2005). Circulating insulin levels peak after meals but are very low most other times, while circulating IGF-1 levels are severalfold higher than insulin, and stable around the clock. Also, in contrast to insulin, IGFs in the circulation and interstitial fluids are bound to high-affinity IGF-binding proteins (IGFBPs) that prolong IGF half-life by impeding proteolysis and renal clearance (Clemmons, 1998; Duan, 2002). Insulin levels are relatively stable throughout the life span in normal individuals, while IGF-1 levels peak during childhood and decline steadily as people age (Laron, 2001).

Figure 7-1

Comparison of amino acid sequence and predicted tertiary structure of the IGF-1, IGF-2, and insulin peptides. This figure was reproduced from article "Insulin-like growth factor ligands, receptors, and binding proteins in cancer" by Foulstone et al. 2005. J Pathol 205: 148. Copyright of the Pathological Society of Great Britain and Ireland. Reproduced with permission from John Wiley and Sons



IGFs serve as endocrine and paracrine/autocrine regulators of somatic growth both in utero (IGF-2) and during postnatal growth (IGF-1) (Sara and Carlsson-Skwirut, 1986; Daughaday and Rotwein, 1989; Baker et al., 1993). IGF-2 is important for somatic growth during embryonic development, but its role, if any, during postnatal life is unknown. Targeted gene deletion showed that IGF-2 expression is imprinted, that is, preferentially expressed from the paternal allele in most tissues, with deletion of the paternal allele producing a 30–40% reduction in somatic size (DeChiara et al., 1990), but even homozygous IGF-2 deletion produces no discernible effect on the CNS or peripheral nervous system (PNS) (C.A. Bondy and R.R. Reinhardt, unpublished data). Indeed, IGF-2 overexpression in brain appears to have no effect on brain size or structure or mouse behavior (Reijnders et al., 2004). IGF-2's lack of any apparent effect in brain development may be explained by the brain's high-level expression of the IGF-2-mannose-6-phosphate receptor, which sequesters IGF-2 into lysosomes (Hawkes and Kar, 2004).

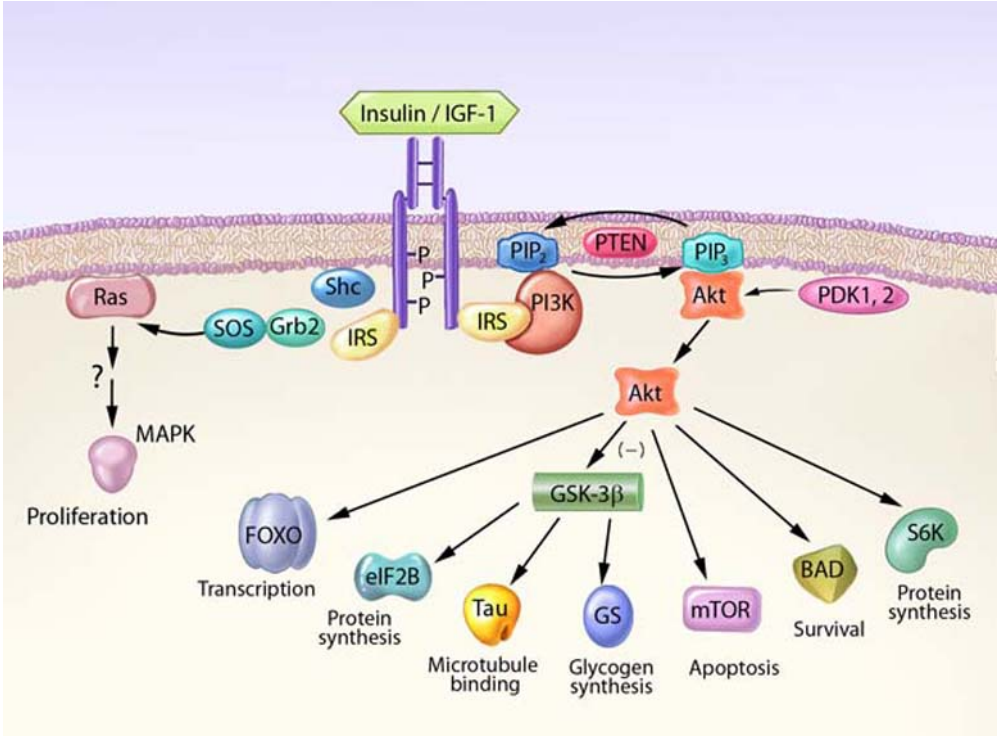
IGF-1 and IGF-2 bind with high affinity to a number of IGFBPs, which protect the IGFs from proteolysis and modulate their interaction with their receptor (Clemmons, 1998). IGFBPs are expressed in the brain in addition to being expressed in diverse peripheral tissues (Bondy and Lee, 1993a; Brar and Chernausek, 1993; Lee and Bondy, 1993; Lee et al., 1993; Logan et al., 1994; Sullivan and Feldman, 1994; Ye and D'Ercole, 1998). IGFBP2 and 5 are most abundant in the brain, and are expressed in spatiotemporal coordination with IGF-1 (Lee et al., 1992b; Lee et al., 1993). Early in development IGFBP5 messenger RNA (mRNA) is concentrated in germinal zones and is colocalized with IGF-1 in developing sensory and cerebellar relay neurons (Bondy and Lee, 1993a), and IGF-1 appears to induce IGFBP5 expression (Ye and D'Ercole, 1998). IGFBP2 mRNA is concentrated in astroglia adjacent to IGF-1-expressing neurons (Lee et al., 1993) and colocalizes with IGF-2 in the meninges and choroid plexi (Logan et al., 1994). IGFBP2 is also highly abundant in capillary endothelium, median eminence, and other circumventricular sites (Lee et al., 1993), suggesting a potential role in carrier-mediated transcytosis of circulating IGFs into the brain. Thus, each IGFBP may play a specific role in modulating IGF-1's bioactivity in brain development. These theoretical modulatory roles appear nonessential, however, since targeted deletion of IGFBPs, singly or in combination, produces no apparent neurological phenotype (J. Pintar, personal communication).

3 IGF-1/Insulin Receptors and Signaling Pathways

Like the cognate peptides, the insulin and IGF-1 receptors (IGF1Rs) demonstrate close structural homology and sequence identity (reviewed in Clemmons, 1989; LeRoith, 1996), having evolved from a common ancestor, in parallel with the ligands' evolution (LeRoith et al., 1993; Reinecke and Collet, 1998; Navarro et al., 1999). The type 1 IGF receptor, or the IGF1R, actually binds and transduces both IGF-1 and IGF-2 with high affinity. There is a so-called IGF-2-mannose-6-phosphate receptor unrelated to the insulin/IGF receptor family (Kiess et al., 1988) that binds and clears IGF-2 by sequestration into lysosomes (Wylie et al., 2003). Insulin and IGFs bind their cognate receptors with highest affinity, but cross-reactivity occurs at higher hormone concentrations (Clemmons, 1989). The insulin and IGF1Rs are membrane-bound tyrosine kinases that are covalent dimers in the absence of ligand. One molecule of insulin or IGF binds to the extracellular alpha-chains, triggering transautophosphorylation of the intracellular beta-chains (Luo et al., 1999). The tyrosine kinase domains of the insulin and IGF1Rs are highly conserved, with ~85% amino acid sequence identity (Hubbard, 1999) and very similar tertiary structures (Favelyukis et al., 2001). Not unexpectedly, the two receptors engage the same signaling pathways (❯ *Figure 7-2*). Receptor activation triggers phosphorylation of IRS proteins, which serve as binding sites for proteins containing src homology 2 domains, including the p85 regulatory subunit of phosphoinositide-3 kinase (PI3K). Activation of PI3K leads to the generation of phosphatidylinositol-3,4,5-trisphosphate (PIP3), which triggers phosphoinositide-dependent kinases to activate the ser/thr kinase Akt (also known as protein kinase B), upon recruitment to the plasma membrane (Summers and Birnbaum, 1997). The lipid phosphatase, PTEN, negatively impacts this pathway by dephosphorylating PIP3. Activated Akt, through subsequent phosphorylation of several downstream targets, is primarily responsible for the ability of this family of growth factors to stimulate glucose uptake and protein synthesis culminating in cell growth (reviewed in Saltiel and Kahn, 2001). The ras/MAPK pathway has also been associated with insulin/IGF receptor activation (❯ *Figure 7-2*) in studies on cultured

Figure 7-2

Schematic diagram of signaling pathways involved in IGF-1's activity in brain. Ligand binding to insulin/IGF-1 receptors (IGF1Rs) triggers receptor autophsophorylation and association with IRS-docking proteins. Activation of phosphoinositide-3 kinase (PI3K) generates phospholipids that activate Akt. Akt may then interact with multiple downstream substrates, including GSK-3β, FOXO, eIF2B, mTOR, bcl-associated death promoter (BAD), and S6K. For example, Akt serine phosphorylates GSK3-β, causing its inhibition. Since GSK-3β normally inhibits glycogen synthase and eIF2B, inactivation of GSK-3β promotes both glycogen and protein synthesis. The microtubule-associated protein tau is also a target for GSK-3β and is hyperphosphorylated in the IGF-1-null brain, providing further evidence that IGF-1 normally inhibits brain GSK-3β activity. The MAPK pathway has been implicated in insulin/IGF action by in vitro studies and some in vivo observations on peripheral tissues, but its relevance to IGF action in brain is unknown



cells. The in vivo significance of this association remains unclear, since most of the known physiological effects of insulin/IGF-1 involve the PI3K-Akt pathway (Katic and Kahn, 2005). This review focuses on the latter pathway, which has been specifically implicated in IGF signaling in the brain.

3.1 IGF-1 Signaling in Brain

Activation of PI3K and Akt kinase is central to insulin/IGF-1-induced anabolic effects. For example, Akt activation results in translocation of glucose transporters (GLUTs), from intracellular pools to the plasma membrane, promoting glucose entry into cells (Kohn et al., 1996; Summers and Birnbaum, 1997). In the periphery, glucose transport is promoted by insulin at the insulin receptor, activating the IRS/PI3K/Akt system, but in the brain, IGF-1 is responsible for local glucose transport and utilization by the same pathway (Cheng et al., 2000). IGF-1-induced Akt phosphorylation appears linked to translocation of neuronal

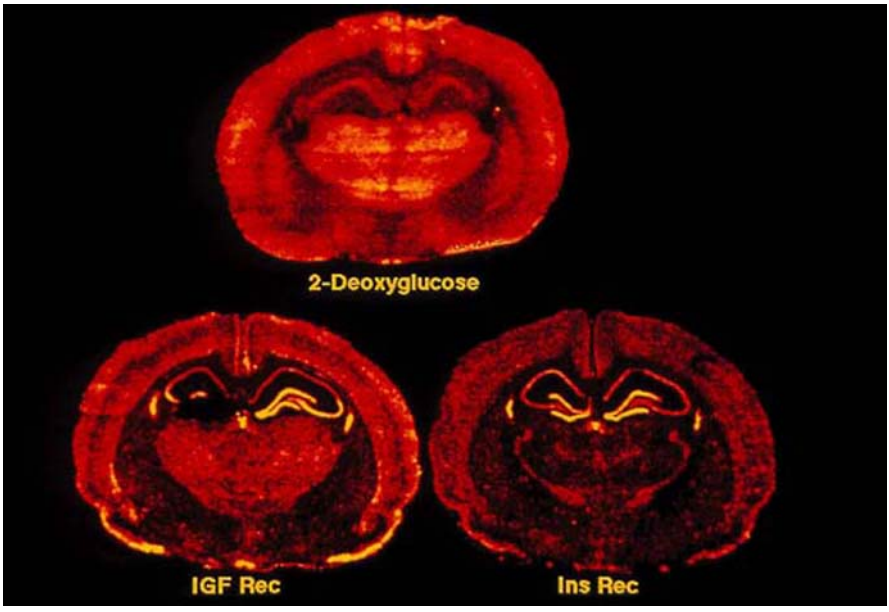
GLUT4 from intracellular pools to membranes of nerve processes in the normal developing brain. Another target of insulin/IGF signaling via Akt kinase is glycogen synthase kinase 3 (GSK3). Insulin and IGF-1 both stimulate the inhibitory serine phosphorylation of this multifunctional enzyme in neurons (Hong and Lee, 1997). Insulin/IGF-induced inhibitory phosphorylation of GSK3 (Summers et al., 1999) relieves GSK3's inhibition of glycogen synthase and of the eukaryotic translation initiation factor 2B (eIF2B), thus promoting glycogen and protein synthesis. The convergence of IGF1R, phospho-Akt, membranous GLUT4, phospho-GSK3, and abundant glycogen stores specifically in large IGF-1-expressing neurons (Cheng et al., 2000) suggests that IGF-1 acts in a cell-autonomous or in an autocrine manner, via the PI3K-Akt-GSK3 pathway, to promote nutrient acquisition, protein, and lipid synthesis supporting the growth of maturing projection neurons.

3.2 IGF-1 and Brain Glucose Utilization

Reflecting IGF-1's role in brain glucose utilization, IGF-1 and IGF1R expression closely parallel regional glucose utilization (see *Figure 7-3*, Cheng et al., 2000). High-level IGF-1 expression is seen in concert with

Figure 7-3

Autoradiography comparing gene expression patterns for the IGF-1 receptor (IGF1R), the insulin receptor, and 2-deoxyglucose uptake in the early postnatal rat brain



intense glucose uptake in maturing cerebellar, somatosensory, auditory–vestibular, olfactory, and visual system neurons. Glucose utilization is reduced by 30–60% in the IGF-1-null brain, with the greatest decrease in structures where IGF-1 expression is normally highest (Cheng et al., 2000). The defect in glucose utilization is demonstrable at the nerve terminal level in synaptosomes prepared from IGF-1-null brains, and is completely reversed by IGF-1, showing that the defect in glucose utilization is not due to reduced neural activity or reduced brain blood flow, neither of which affects the synaptosome preparation. Furthermore, the finding of reduced glucose uptake in isolated nerve terminals shows that IGF-1 normally

promotes glucose uptake by nerve terminals independent of glial effects, since glial cells are not present in the synaptosome preparation.

Conversely, brain glucose utilization is globally increased in IGF-1-overexpressing adult mice (Gutierrez-Ospina et al., 1997). It is not certain which cell types are responsible for the ectopic IGF-1 expression in these mice, but apparently IGF-1 is in excess through most of the brain for much of development. Thus, the generalized increase in glucose use likely reflects local field potentials originating from more highly ramified dendritic arbors with greater synaptic density in IGF-1-overexpressing brains, along with direct IGF-1-enhanced glucose transport and utilization. The fact that pentobarbital anesthesia suppressed glucose uptake in both transgenic and wild-type (WT) mice in that study was thought to suggest that IGF-1 does not promote brain glucose utilization (Gutierrez-Ospina et al., 1997). However, pentobarbital interferes with GLUT function per se (Haspel et al., 1999) and so suppresses any stimulus of glucose transport.

4 IGF-1 Versus Insulin in the Brain

A theory that neurodegeneration in the aging human brain may be linked to loss of insulin trophic effects has recently been put forward (Hoyer, 2004). Despite the fact that there is very little insulin within the brain, both the insulin and IGF1Rs are widely expressed in the developing and mature brain (*Figure 7-3*) (Hill et al., 1986; Bohannon et al., 1988; Bondy et al., 1992a, b). IGF-1 and insulin receptors are coexpressed in many brain regions, such as the granule cell layers of the olfactory bulb, dentate gyrus, and cerebellar cortex (Bondy et al., 1992a). The insulin receptor is most highly expressed in anterior thalamic and hypothalamic nuclei, including the periventricular, reticular, and anterior thalamic nuclear complex and the paraventricular and supraoptic nuclei (*Figure 7-3*) (Bondy et al., 1992a, b), consistent with insulin's neuroendocrine role in connecting peripheral metabolic signals to central control of appetite and metabolic activity (reviewed in Porte et al., 2005). The significance of insulin receptor expression throughout the brain is unclear, given that little insulin is found in brain outside the hypothalamus. One explanation is that the ancestral insulin/IGF receptor was heavily expressed in the nervous system, and that regulation of "offspring" receptor gene expression continued this pattern, despite the evolutionary specialization of the ligand insulin as regulator of peripheral metabolism.

Circulating insulin and IGF-1 may influence hypothalamic and other periventricular regions by interacting with receptors localized in the median eminence and circumventricular structures outside the blood–brain barrier (BBB). Both insulin and IGF1Rs are expressed on brain capillaries, but IGF-1 crosses the BBB with greater efficiency than insulin (Reinhardt and Bondy, 1994). A number of factors may explain IGF-1's relative facility in crossing the BBB. The coexpression of insulin and IGF1Rs in brain capillary endothelium may result in formation of hybrid receptors, which bind IGF-1 with substantially greater affinity than insulin (Soos et al., 1993). In addition, IGFBP2 is abundant in capillary endothelium, median eminence, and other circumventricular sites (Lee et al., 1993), suggesting possible carrier-mediated IGF transport across the BBB. While IGF-1 is abundant, very little insulin is detected within the murine brain (Coker et al., 1990), although small foci of insulin mRNA have been detected in the anterior hypothalamus (Young, 1986). A very recent study reported detection of insulin, IGF-1, and IGF-2 mRNA in postmortem human brain tissue using quantitative polymerase chain reaction (PCR) (Steen et al., 2005), but this novel report awaits confirmation.

Given IGF-1's abundant expression within the brain, and its apparent facility in crossing the BBB, both in contrast to insulin, it seems unlikely that insulin is required as an additional trophic factor for brain. The brain requires trophic support for developmental needs and responses to new learning or injury, but insulin secretion is tightly coupled to the timing and composition of meals. It seems unlikely that a gastrointestinal peptide, for which synthesis and secretion are tightly coupled to the contents of the duodenum, should be critically involved in brain development, function, or protection from degeneration. If this notion were true, then lean vegetarian individuals with very low levels of insulin secretion would be mentally deficient and at risk for premature neurodegeneration, while consumers of refined carbohydrates, which is associated with a high level of insulin secretion, would be intellectually superior and spared from senility. Further

evidence that insulin is not involved in brain development or function is the finding that brain-specific insulin receptor knockouts have normal brains and brain function, although neuroendocrine regulation of appetite is disturbed (Bruning et al., 2000).

5 Regulation of Brain IGF System

While it has been known for many years that IGF-1 produced in the liver is closely regulated by GH, there is limited understanding of factors that regulate brain IGF-1 gene expression. Certainly, expression of brain IGF-1 and IGF1R are developmentally controlled (Bondy et al., 1990; Bondy, 1991). Expression of IGF-1 is also elevated dramatically after hypoxia, ischemia, and other brain injury (see Section 8 for details). Thus, high-level expression of IGF-1 appears in situations where extraordinary energy needs for brain cell growth or repair processes are engaged. However, the molecular mechanisms underlying IGF-1's developmental stage and cell-specific expression in brain are still unclear. Recent evidence shows that modest caloric restriction significantly reduces brain IGF-1 and IGF1R mRNA levels in rats on a carbohydrate-dominant diet. A diet with the same calorie content composed primarily of lipid, however, "increased" brain IGF1R expression (Cheng et al., 2003a). Additional studies in rats (Chowen et al., 2002) and *Drosophila* (Ikeya et al., 2002) support the view that nutrient supply has important and complex effects on brain IGF system gene expression. Further study is required to elucidate the specific mechanisms regulating brain IGF system expression and the functional consequences of these changes.

6 IGF-1 and Normal Brain Growth

IGF-1 deletion or inhibition during brain development attenuates brain growth, with reductions in both cell number and cell size (Beck et al., 1995; Cheng et al., 2000). This effect is more profound in the nullizygous state, but even partial IGF-1 deficiency, as in IGF-1(+/−) mice, results in significantly diminished brain growth (Cheng et al., 2000). Cell numbers are notably reduced in the olfactory system, the dentate gyrus of the hippocampus, and the striatum. Brain volume is globally decreased due to a loss of neuropil, with significant reductions in neuronal soma volume, dendritic length and complexity, and synapse number. A fundamental requirement for cell, organ, and organism growth is nutrient acquisition and utilization. This most basic of functions is a prerequisite for cell division as well as somatic and process growth. IGF-1's fundamental role in the brain, as in peripheral tissues, is to promote nutrient acquisition and thus enhance cell proliferation, growth of the cell soma and processes, and more differentiated functions at later stages of development. Depending on where and when IGF-1 and its receptor are expressed during brain development, it may predominantly impact cell proliferation, or postmitotic growth processes.

IGF-1's anabolic functions on brain growth involve IRS2, PI3K, and Akt, as demonstrated by the growth phenotypes in genetic models with altered expression of each of these signaling molecules. Overexpression of PI3K/Akt or deletion of PTEN leads to increased brain size (Backman et al., 2001; Kwon et al., 2001). Activation of this pathway early in brain development is associated with augmented proliferation of neural stem cells (Groszer et al., 2001), while activation later, when more cells are in a more differentiated, postmitotic state, results in increased soma size (Kwon et al., 2003). Deletion of Akt3 results in a major brain growth deficit, due to both decreased cell numbers and decreased cell size, suggesting that this specific Akt isoform mediates IGF-1 effects on brain growth (Easton et al., 2005). Deletion of IRS2 results in significant reduction in brain growth, largely due to reduced cell proliferation (Schubert et al., 2003), thus implicating IGF-1 as the driver of this pathway in brain development.

6.1 Neurogenesis

The IGF1R is expressed at high levels in the developing nervous system, with highest expression concentrated in the germinal and subventricular zones that give rise to new neurons (Bondy, 1991; Bondy et al., 1992b). IGF-1 is coexpressed with the IGF1R in the subventricular zone of the anterior lateral ventricles that

give rise to olfactory system neurons (Bartlett et al., 1991) where it most likely acts as an autocrine factor to stimulate, alone or together with other neurotrophic factors, the proliferation of neural stem and precursor cells. For example, epidermal growth factors (EGFs) or fibroblast growth factor-2 (FGF-2) is known to stimulate neural stem cells to renew, expand, and differentiate into neural precursors, but they are effective only in the presence of IGF-1 (Arsenijevic et al., 2001). Given what is known about IGF-1's anabolic signaling in neural cells, it seems likely that IGF-1 supports proliferation triggered by the other growth factors by providing essential anabolic support through nutrient acquisition and protein synthesis.

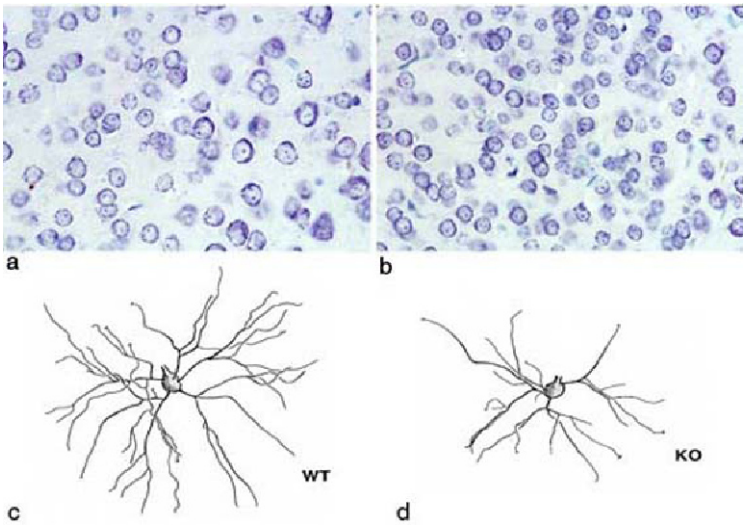
IGF-1 normally has a very selective, cell-specific, and developmentally timed pattern of expression in normal brain maturation. Elucidation of the phenotype of these IGF-1-expressing and neighboring cells in the IGF-1-null mouse provides insight into IGF-1's role in normal brain development. The study of transgenic mice that overexpress IGF-1 ectopically under transgene control reveals what may happen when IGF-1 is expressed at abnormally high levels in various different cell types at various developmental stages. Depending on the cellular pattern and developmental timing of IGF-1 transgene expression, neuron numbers are increased in the cerebral cortex (Gutierrez-Ospina et al., 1996), cerebellar cortex (Ye et al., 1996), hippocampus (O'Kusky et al., 2000), and brainstem (Dentremont et al., 1999). This increase in neuron numbers is due to, at least partially, increased neurogenesis (Ye et al., 1996; O'Kusky et al., 2000). These observations reveal what happens as a result of abnormal IGF-1 expression in brain, but do not reveal the nature of IGF-1's role in normal brain development. In fact, the high-level IGF-1 expression under transgene control may suppress normal IGF-1 production, and alter IGF1R and IGFBP expression as well, thus distorting normal developmental patterns.

6.2 Neuronal Somatic Growth and Dendritogenesis

The 30–40% reduction in brain size in adult IGF-1-null mice is due to a reduction in cell size and neuropil, or neuronal processes. Cell density is significantly increased throughout the IGF-1-null brain (❯ *Figure 7-4*)

❑ Figure 7-4

Cortical neurons are smaller with hypotrophic dendritic trees in the IGF-1-null brain. Panels a (wild type, WT) and b (IGF-1-null) are micrographs of Nissl-stained cortical sections. The soma size is distinctly smaller and the cell density increased in IGF-1-null brains. Camera lucida drawings of Golgi-stained cortical pyramids (layers II–III) reveal dramatically reduced dendritic profiles of the IGF-null neurons (c and d). Adapted from Cheng et al. 2003. J Neurosci Res 73: 3

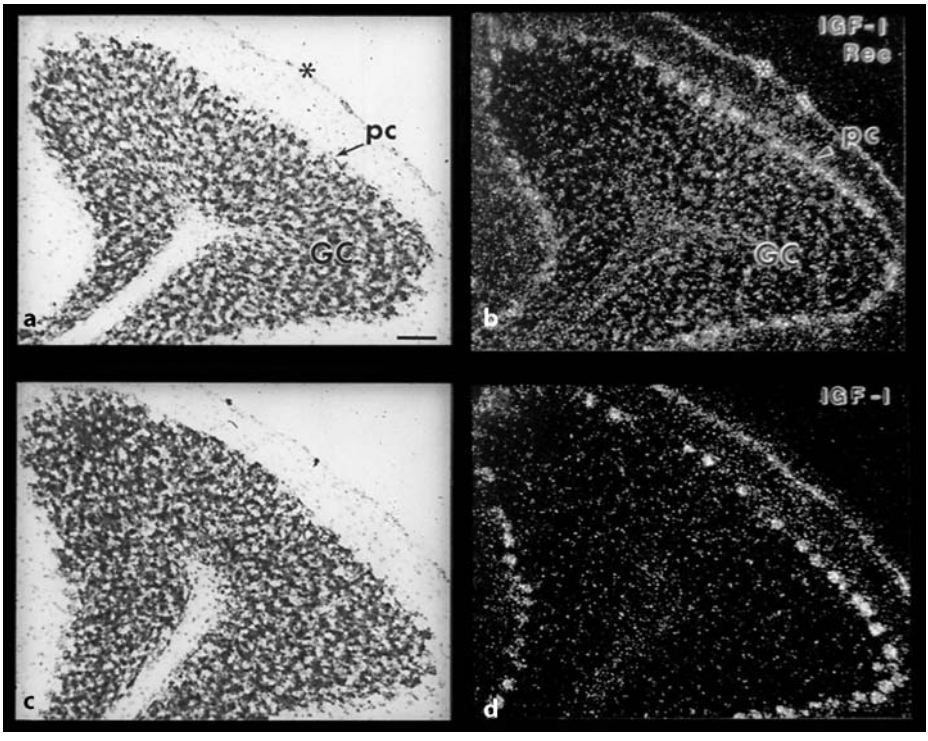


(Beck et al., 1995; Cheng et al., 1998; Cheng et al., 2003b). This observation is explained by reduced process growth, since the space between neurons is normally occupied by extensively branched neuronal processes. Soma size of projection neurons in the IGF-1-null brain is reduced by ~25%, and dendritic length, branching, and synapses are reduced by a similar amount (*Figure 7-4*). IGF-1 mRNA is most abundant in growing projection neurons in sensory and cerebellar relay systems (Bondy, 1991). Interestingly, very high-level IGF-1 expression is concentrated in all the auditory system nuclei: the medial geniculate, inferior colliculus, inferior olives, and cochlear nuclei (Bondy, 1991). These auditory system way stations are known to exhibit extremely high levels of glucose utilization. Humans with IGF-1 deficiency suffer sensory-neural hearing loss, in addition to mental retardation (Woods et al., 1997; Bonapace et al., 2003; Walenkamp et al., 2005). A recent study using auditory brainstem response testing in IGF-1-null mice has shown that the hearing loss is composed of both peripheral and central defects, consistent with IGF-1's extensive expression throughout the auditory neural system (Cediel et al., 2006). The IGF-1-expressing neurons attain exceptionally large soma size and develop extraordinarily extensive and complex dendritic formations. For example, the Purkinje cell expresses the highest level of IGF-1 mRNA of any cell type in the brain and at maturity has the largest soma and most elaborate dendritic arbor of any brain cell (*Figure 7-5*).

Thus it appears that impaired neuronal somatic growth and process formation accounts in large part for the reduction in IGF-1-null brain size. In the transgenic IGF-1-overexpressing brain, neuropil and synapses are significantly increased (O'Kusky et al., 2000). IGF-1 treatment significantly increased dendritic growth in cortical slices, which supports these in vivo findings (Niblock et al., 2000). In addition, IGF-1 has been shown to stimulate neuritic outgrowth in rat embryonic day 16–17 cortical neurons, rat hypothalamic

Figure 7-5

Coexpression of IGF1 receptor (IGF1R) (a and b) and IGF-1 (c and d) mRNAs by Purkinje cells of the cerebellar cortex in the early postnatal mouse brain. Bright and dark-field photomicrographs of in situ hybridization are shown in pairs (a and b, c and d). In the dark field, white sliver grains are hybridized mRNA signals. Arrowheads point to Purkinje cells (*PC*). *GC* granule cells



neurons (Torres-Aleman et al., 1990), chicken sympathetic neurons (Zackenfels et al., 1995), and mouse Purkinje cells (Fukudome et al., 2003).

6.3 Neuronal Survival

The dentate gyrus is selectively reduced in size and cell number in the adult IGF-1 knockout mouse (Beck et al., 1995; Cheng et al., 2001). IGF-1, however, is not expressed in the germinal zone supplying progenitors for the dentate gyrus, though it is expressed by unidentified cells scattered throughout the hippocampal formation. Unexpectedly, bromodeoxyuridine (BRDU) incorporation was actually increased in the IGF-1-null subventricular zone (Cheng et al., 2001). Apoptotic cells were also increased throughout the dentate gyrus in the IGF-1-null brain, however, indicating that IGF-1 normally promotes neuronal survival in this structure. There is abundant evidence from in vitro models supporting IGF-1's role in promoting neuronal survival.

IGF-1 promotes the in vitro survival of many different types of cultured neurons derived from many regions of the nervous system, including cortical neurons (Aizenman and de Vellis, 1987; Harper et al., 1996) and hippocampal neurons (Zheng and Quirion, 2004) in situations of serum or glucose deprivation. IGF-1 also protects hippocampal neurons from toxic effects of corticosterone (Nitta et al., 2004), nitric oxide, hypoxia (Tamatani et al., 1998; Yamaguchi et al., 2001), and amyloid (Dore et al., 1997). In primary cerebellar neuronal cultures, IGF-1 increased the survival of Purkinje cells (Torres-Aleman et al., 1992) and granule neurons in situations of serum, potassium (D'Mello et al., 1993), or glucose deprivation (Harper et al., 1996). IGF-1 also protects granule neurons against toxicity induced by dopamine (Offen et al., 2001) and polyQ-huntingtin (Humbert et al., 2002), which is involved in the pathogenesis of Huntington's disease. Moreover, IGF-1 partially prevents apoptosis of granule neurons isolated from Weaver mutant mice (Zhong et al., 2002), a mouse model of hereditary cerebellar ataxia. Finally, IGF-1 also enhances the survival of spinal cord motoneurons (Ang et al., 1992), parasympathetic neurons (Crouch and Hendry, 1991), hypothalamic neurons (Torres-Aleman et al., 1990), and striatal neurons (Nakao et al., 1996) in culture.

The neuronal survival signaling of IGF-1 has been investigated in primary cerebellar granule neuron cultures (D'Mello et al., 1993). Upon IGF-1's binding to its cognate receptor, both MAPK and PI3K are normally activated (❯ *Figure 7-2*). Under serum and potassium deprivation, most cerebellar granule neurons die unless IGF-1 is added in culture media. IGF-1's survival effect on cerebellar granule neurons is mediated by the PI3 kinase/Akt signaling pathway, since specific PI3 kinase inhibitors block IGF-1's survival-promoting activity, while the MAP kinase specific inhibitor PD98059 had no effect (D'Mello et al., 1997; Miller et al., 1997). The involvement of PI3K/Akt in this pathway has been confirmed by expressing WT or dominant-negative forms of Akt (Dudek et al., 1997). In fact, activation of PI3 kinase/Akt cascade is a common mechanism that mediates IGF-1's actions not only on cerebellar granule neurons, but also on other neuronal culture models (Matsuzaki et al., 1999; Yamaguchi et al., 2001; Zhong et al., 2002; Rangone et al., 2005). Downstream from Akt, different substrates are required to mediate IGF-1's survival effects depending on the type of neurons and the kind of adverse stimuli, e.g., potentiation of L calcium channels (Blair et al., 1999), NF-κB activation (Koulich et al., 2001), and Bim induction (Linseman et al., 2002). In hippocampal neurons, Akt mediates IGF-1's survival action against hypoxia or NO by inhibiting p53 transcriptional activity (Yamaguchi et al., 2001), but mediates IGF-1's rescue action from dehydroepiandrosterone-induced apoptosis by inactivation of GSK3 (Lin et al., 2004). Overall, IGF-1 promotes the in vitro survival of many types of differentiated neurons through the PI3/Akt pathway and multiple downstream signaling molecules that are specific for particular neuronal types.

Given all these data from in vitro studies, one might expect to see massive, widespread apoptosis in the IGF-1-null mouse brain. However, cell numbers are normal in the cerebellar and cerebral cortices and other brain regions, except for the dentate gyrus, olfactory bulb, and striatum, as documented in two independent targeted deletions in different outbred mouse lines (Beck et al., 1995; Cheng et al., 1998). While there were increased apoptotic figures in the dentate gyrus, there were few apoptotic cells in other regions (Cheng et al., 2001). Moreover, the IRS2 deletion model does not demonstrate increased brain cell death, despite a 30–40% reduction in brain size (Schubert et al., 2003). Thus, it seems likely that IGF-1 is essential for cell

survival under the stress of in vitro conditions and in response to brain insult, more than in normal brain development.

6.4 Myelination

IGF-1 promotes the survival and production of myelin by cultured oligodenrdocytes (Mozell and McMorris, 1991; McMorris and McKinnon, 1996). These in vitro observations led to the view that IGF-1 has a role in oligodendrocyte generation or differentiation and myelin synthesis. However, IGF-1 and IGF1R expression are lowest in white matter (Bondy and Lee, 1993b) and there is no apparent sign of central or peripheral myelinopathy in IGF-1-null mice (Cheng et al., 1998; Gao et al., 1999). Myelin concentration, normalized to brain weight or protein, is equal in IGF-1-null and WT littermate mice. Likewise, concentrations of myelin-specific proteins (MBP, PLP, MAG, and CNPase) are equal in IGF-1 null and WT littermate mice. Oligodendrocyte numbers and myelin are reduced in the IGF-1-null olfactory system, which is profoundly reduced in size and depleted of neurons, with efferent tracts correspondingly diminished, associated with decreased myelin in anterior white matter tracts that include a large olfactory component (Beck et al., 1995).

In brain structures where neurons are preserved, however, such as the cerebellum, myelination appears normal. This observation suggests that if the system projection neurons survive despite the lack of IGF-1, as in the cerebellum, oligodendrocytes prosper and appropriate myelination occurs. The PNS of IGF-1-null mice demonstrates reduced axonal diameter and proportionately reduced myelin sheath thickness, with no evidence of peripheral myelinopathy (Gao et al., 1999). The IGF-1-null mice show no neurological signs of myelinopathy, with normal motor function, coordination, and gait (Cheng et al., 1998), all functions normally impaired by myelin defects. Finally, the mentally retarded individual with IGF-1 gene deletions shows no evidence of dysmyelination or myelinopathy (Woods et al., 1996, 1997).

Observations of increased myelin content in the brains of transgenic mice overexpressing IGF-1 have been invoked to support a primary role for IGF-1 in myelination (Carson et al., 1993). The study reported that both brain size and myelin content, but not DNA content and oligodendrocyte numbers, are increased in the transgenic mice, suggesting that the increased brain mass is primarily due to increased cell size and/or process growth. Further investigation showed that myelin sheath thickness was increased in proportion to increased axonal diameter in this transgenic model (Ye et al., 1995). These findings in IGF-1-null and overexpressing brains are consistent with the current view (Barres and Raff, 1999) that myelination is induced by neuronal fiber growth and/or activity. IGF-1 overexpression stimulates excessive growth in size and number of neuronal processes and possibly also the survival of additional neurons, which, in turn, stimulates additional oligodendrocyte biosynthetic activity and myelination. The findings that myelination in IGF-1-null and IGF-1-overexpressing mice is essentially matched to neuroaxonal mass is best explained by the simple hypothesis that IGF-1 stimulates neuronal process growth, which in turn stimulates myelin formation.

The fact that IGF-1 does not seem to have an essential role in developmental myelination does not mean that it is not important in repair processes after nervous system injury. IGF-1 expression is induced in reactive astrocytes responding to demyelinating insults and IGF1R expression is enhanced in injured oligodendrocytes (Komoly et al., 1992). When cuprizone induced demyelination in the CNS of mice whose IGF1R was selectively mutated, oligodendrocyte progenitors did not accumulate, proliferate, or survive, indicating that signaling through IGF1R plays a critical role in remyelination (Mason et al., 2003). Administration of exogenous IGF-1 improves remyelination after injury, which supports the significance of these expression patterns (Yao et al., 1995). IGF-1's prominent effects on oligodendrocytes in vitro may actually reflect the fact that cell culture is essentially an injury model system.

7 IGF-1 and Neurogenesis in the Mature Brain

Adulthood neurogenesis occurs continuously within the subventricular zone of the hippocampal dentate gyrus and is important in learning and memory (Nilsson et al., 1999; Shors et al., 2001). This neurogenesis

can be enhanced by exercise (Neeper et al., 1995; Trejo et al., 2001), an enriched environment (Nilsson et al., 1999; Shors et al., 2001), and by IGF-1 (Aberg et al., 2000). Subcutaneous infusion of IGF-1 significantly increased the proliferation of neural progenitors in the hippocampal dentate gyrus in adult hypophysectomized rats (Aberg et al., 2000) or in rats after cerebral ischemia insults (Dempsey et al., 2003). Interestingly, exercise-induced neurogenesis in the adult hippocampus appears to be mediated by uptake of IGF-1 into the brain. This is because exercise-induced increases in the number of new neurons in the hippocampus were blocked by the administration of an antibody that prevents passage of systemic IGF-1 into the brain (Trejo et al., 2001). On the other hand, neurogenesis was significantly increased in the dentate of dwarf mice that have virtually no circulating IGF-1, although IGF-1 production is normal in the brain (Sun et al., 2005).

Interestingly, cerebral ischemia appears to increase the proliferation of progenitor cells in the cortex and subventricular zone of adult rats (Zhang et al., 2001). Since ischemia also activates astrocytic IGF-1 expression at a late stage, it may be that this ischemia-induced neurogenesis is partially mediated by IGF-1 released from astrocytes. This hypothesis is supported by the increase in neuron numbers in astrocyte-specific IGF-1 transgenic mice upon induction (Ye et al., 2004).

8 IGF-1 and Brain Injury

There has been a great deal of interest in the idea of treating brain injury with trophic agents including IGF-1. This was prompted in part by the finding that many components of the IGF system are induced in response to diverse types of brain injury. In contrast to the predominantly neuronal pattern of IGF-1 expression during normal brain development, this injury-invoked IGF-1 expression is generally observed in astrocytes (Komoly et al., 1992; Lee et al., 1992a; Gehrmann et al., 1994; Li et al., 1998). Interestingly, local IGF-1 expression at brain injury sites is also strongly correlated with local [^{14}C]-2-deoxyglucose uptake (Cheng et al., 2000). Potential roles for IGF-1 in response to brain injury have been studied in animal models, such as hypoxia and/or ischemia (Tagami et al., 1997a, b; Guan et al., 2003) and various models of traumatic brain injury (Saatman et al., 1997; Walter et al., 1997; Li et al., 1998; Kazanis et al., 2004). Regardless of primary insult, IGF-1 expression decreases in the early phases of the injury (Lee et al., 1992a, 1996; Clawson et al., 1999). This immediate suppression of neuronal IGF-1 gene expression is best characterized in an animal model of hypoxic–ischemic encephalopathy, where neuronal IGF-1 expression decreased within the hypoxic–ischemic hemisphere as early as 1 h (the earliest time studied) following the insult. IGF-1 mRNA levels are inversely correlated with the length of the hypoxia and the number of apoptotic cells (Clawson et al., 1999). IGF-1 mRNA levels continued to decrease with a nadir at 24 h of recovery (Lee et al., 1996), when the number of apoptotic cells was also at the maximum (Clawson et al., 1999). This correlation would indicate that the early decrease in neuronal IGF-1 expression likely contributes to hypoxia–ischemia-induced neuronal death. At a delayed phase of the recovery, endogenous IGF-1 genes become activated in astrocytes as they react to the injury (Lee et al., 1992a, 1996; Clawson et al., 1999). These observations provide a rationale for restoring IGF-1 during the early phase of hypoxia–ischemia.

Encouraging results have been obtained in adult rats (Guan et al., 1993) and fetal sheep (Johnston et al., 1996; Guan et al., 2000a). In these animal models, supplying IGF-1 to the injured brain intraventricularly within 2 h of hypoxia–ischemia promoted neuronal survival (Guan et al., 2000b). In both these models, IGF-1 treatment reduced infarct size and, more impressively, improved somatosensory function as evaluated by bilateral tactile test (Guan et al., 2001). In another injury model, IGF-1's effect on long-term recovery can be attributed to specific effects on oligodendrocytes. During myelinogenesis, younger rats are more sensitive to hypoxic–ischemic insult, manifested as ipsilateral necrosis originating in and spreading from myelinogenic foci (Rice et al., 1981). This hypoxia–ischemia-induced injury to immature oligodendrocytes may be alleviated by IGF-1. In fact, infusion of IGF-1 (3 μg over 1 h) into the cerebroventricles of fetal sheep at 90 min after recovery from hypoxia–ischemia prevented the delayed oligodendrocyte loss and associated demyelination (Guan et al., 2001).

9 IGF-1, Brain Aging, and Neurodegeneration

Normal aging is often accompanied by cognitive decline associated with reduced glucose utilization, altered synaptic plasticity, decreased hippocampal neurogenesis, impaired brain angiogenesis, and in more severe cases, accumulation of amyloid plaques and neurofibrillary tau-containing tangles as well as neuronal cell death (Hof and Mobb, 2001). Both circulating and endogenous brain IGF-1 are reduced with aging (Breese et al., 1991). IGF-1 mRNA is significantly decreased in hippocampal neurons (Lai et al., 2000) and IGF-1 and IGF1R levels are decreased in the cerebral cortex of aged rats (Sonntag et al., 1999). In addition, IGF-1 and IGFBPs are altered in many types of neural disease, including Alzheimer's disease (AD), amyotrophic lateral sclerosis, and inherited neurodegenerative conditions (Torres-Aleman et al., 1996, 1998; Busiguina et al., 2000). Serum IGF-1 is positively correlated with cognitive performance in older men (Aleman et al., 1999). It is unknown, however, if reduced circulating or brain IGF-1 is a cause or effect of brain disease.

Although IGF-1's effects on synaptogenesis and dendritic growth are most profound during brain development, recent findings indicate that IGF-1 and other neurotrophic factors may continually modulate neuronal circuits where the reshaping of the synapse contacts continues throughout life (Caroni, 1993; Schuman, 1999). For example, IGF-1 infusion increases synaptic density and number in the hippocampus in aged rats (Shi et al., 2005). Moreover, IGF-1 infusion improves memory and some age-related behavioral deficits in aged rats (Markowska et al., 1998). In summary, IGF-1 treatment seems to enhance neurogenesis and synaptogenesis and possible cognitive function in rodents, suggesting a role of IGF-1 in ameliorating age-related cognitive impairment. This view contrasts with the finding that IGF-1-deficient and IGF-1-receptor-deficient mice have longer life spans (Katic and Kahn, 2005) and maintain physiological functions, including cognitive function, at youthful levels into old age (Kinney et al., 2001).

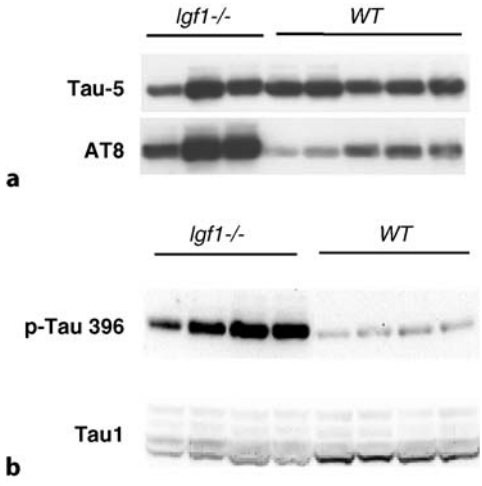
9.1 IGF-1 and Alzheimer's disease

Alzheimer's disease (AD) has two pathological hallmarks, the accumulation of neurofibrillary tangles (NFTs) and deposition of β-amyloid plaques (Dani, 1997). Recent evidence suggests that IGF-1 deficiency may contribute to the development of both these pathological features. First, IGF-1 regulates the phosphorylation of tau (Hong and Lee, 1997; Bondy and Cheng, 2004; Cheng et al., 2005), a microtubule-associated protein involved in microtubule assembly and stabilization (Barghorn et al., 2000). Hyperphosphorylated tau disrupts normal microtubule-dependent processes (Lee et al., 2001) and is resistant to degradation and prone to aggregation, culminating in the formation of NFT (Spillantini and Goedert, 1998; Lee et al., 2001). Hyperphosphorylated tau is associated with cognitive dysfunction in normal aged and disease brains. Tau is hyperphosphorylated in the IGF-1-null mouse brain (*Figure 7-6*) (Bondy and Cheng, 2004; Cheng et al., 2005). In addition, inhibition of IGF-1 signaling in IRS2 knockout mice increased tau phosphorylation and led to NFT accumulation (Schubert et al., 2003). Tau hyperphosphorylation in IGF-1-null mice appears due to overactivity of GSK3. In addition to regulating glycogen synthesis, GSK3 is also involved in tau phosphorylation in brain (Ishiguro et al., 1993; Jope and Johnson, 2004); thus reduced inhibition of GSK3 in IGF-1-deficient brains (Cheng et al., 2000) is associated with tau hyperphosphorylation (Bondy and Cheng, 2004; Cheng et al., 2005). In AD, NFTs consistently contain tau phosphorylated on GSK3 target residues, and GSK3 is physically associated with pretangle and tangle-bearing neurons in human brains (Pei et al., 1999).

IGF-1 protects neurons from β-amyloid toxicity (Dore et al., 1997) and appears to promote clearance of brain β amyloid (Gasparini et al., 2001; Carro et al., 2002). Increased serum IGF-1 levels are associated with reduced brain β-amyloid burden (Carro et al., 2002). Insulin, while its structure and function are closely related to IGF-1, may have distinct mechanisms in modulating brain amyloid levels (for a review see Carro and Torres-Aleman, 2004). It can directly stimulate the release of β amyloid from neurons and also increase extraneuronal accumulation of β amyloid by competing with β amyloid for insulin-degrading enzyme (Gasparini et al., 2001; Watson and Craft, 2003). Therefore, insulin seems to increase brain β-amyloid

Figure 7-6

Hyperphosphorylated tau in the IGF-1-null brain. Total and phospho-tau were examined in IGF-1-null (*Igf1*(−/−) and wild-type (WT) brains by immunoblots. (a) Total tau was detected by anti-Tau 5, which recognizes tau protein irrespective of its phosphorylation status. This blot was striped and reprobed with antibody AT-8 that recognizes PHT-tau with phosphorylated ser202 residue. (b) Phospho-tau was detected by antibody p-Tau 396, which specifically recognizes tau phosphorylation on serine 396, a site prominently phosphorylated in PHF-tau. The same blot was then stripped and reprobed with tau-1 antibody, which detects dephosphorylated tau. These blots show that total tau protein is preserved in *Igf1*(−/−) brain, while tau phosphorylation is dramatically increased in specific epitope in aged *Igf1*(−/−) brains as compared with wild types (WTs). Adapted from Cheng et al. 2005. Endocrinology



release. Taken together, dysfunction of insulin/IGF-1 signaling contributes to the major pathological events occurring in the brains of patients with AD.

10 Summary

The brain requires enormous supplies of fuel and substrate to support neuroglial growth and process formation during early postnatal development. Murine and human brains consume over half the energy available to the organism as a whole during this critical period that is characterized more by synapse formation than by synaptic activity. Purkinje cells grow into giant cells with surface areas exceeding all other cells in the body. How this remarkable anabolic feat is achieved when all brain cells are exposed to the same extracellular nutrient supply is unclear. Evidence from in vivo studies of murine brain development suggests that IGF-1's role in normal brain development is to promote these extraordinary growth processes via PI3K/Akt/GSK3β pathways that are similar to insulin signaling pathways in peripheral tissues. IGF-1 promotes hypertrophy of muscle cells using these same molecular signals, including GSK3 (Rommel et al., 2001). These observations in the mouse are supported by data from *Drososphila*, in which inactivation of paralogs of the insulin/IGF receptor, IRS, PI3K, and Akt all result in globally reduced cell size, which results in proportionate dwarfism, while overexpression of any of these molecules results in increased cell size and gigantism (Potter and Xu, 2001). As a further comment on IGF-1 action in general, all of these studies in mice and in *Drosophila* suggest that IGF-1 effects are neutral with respect to cellular differentiation.

This is not to suggest that IGF-1's only role in brain is to promote neuronal growth. Reduced cell numbers in specific brain regions of the IGF-1-null mouse support an IGF-1 role in developmental neurogenesis, through either increased proliferation or increased survival of nascent neurons. However,

further investigation is required to elucidate whether IGF-1 mainly promotes faster rates of mitosis or increases the number of cells entering the mitotic cycle, what signaling pathways are involved, and whether IGF-1 acts in an autocrine, paracrine, or even endocrine fashion to promote neurogenesis. In addition, further work is necessary to identify cell populations where IGF-1 promotes survival, and to discover the cellular interactions and signaling molecules active in this function. A major area for future investigation is the role of IGF-1 in brain aging. As we have described, there are opposing views as to potential positive versus negative effects of insulin/IGF action on aging. Evidence from diverse organisms shows that these growth peptides are important for somatic growth and reproduction, but at a cost of accelerated aging. Suppression of insulin/IGF signaling results in smaller size and reduced fecundity, but prolongation of life span. The natural reduction in IGF-1 levels with aging could be protective, allowing cells to devote more resources to repair processes, thus preventing cell death from oxidative damage, and retarding abnormal growths. However, since IGF-1 signaling clearly represses tau phosphorylation, a major feature of degenerative aging in brain, the story could be different in this unique organ. This should be a very productive area for future research.

References

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. 2000. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 20: 2896-2903.

Aizenman Y, de Vellis J. 1987. Brain neurons develop in a serum and glial free environment: effects of transferrin, insulin, insulin-like growth factor-I and thyroid hormone on neuronal survival, growth, and differentiation. Brain Res 406: 32-42.

Aleman A, Verhaar HJ, De Haan EH, De Vries WR, Samson MM, et al. 1999. Insulin-like growth factor-I and cognitive function in healthy older men. J Clin Endocrinol Metab 84: 471-475.

Ang LC, Bhaumick B, Munoz DG, Sass J, Juurlink BH. 1992. Effects of astrocytes, insulin, and insulin-like growth factor I on the survival of motoneurons in vitro. J Neurol Sci 109: 168-172.

Arsenijevic Y, Weiss S, Schneider B, Aebischer P. 2001. Insulin-like growth factor-I is necessary for neural stem cell proliferation and demonstrates distinct actions of epidermal growth factor and fibroblast growth factor-2. J Neurosci 21: 7194-7202.

Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, et al. 2001. Deletion of Pten in mouse brain causes seizures, ataxia, and defects in soma size resembling Lhermitte-Duclos disease. Nat Genet 29: 396-403.

Baker J, Liu JP, Robertson EJ, Efstratiadis A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. Cell 75: 73-82.

Barghorn S, Zheng-Fischhofer Q, Ackmann M, Biernat J, von Bergen M, et al. 2000. Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias. Biochemistry 39: 11714-11721.

Barres BA, Raff MC. 1999. Axonal control of oligodendrocyte development. J Cell Biol 147: 1123-1128.

Bartlett WP, Li XS, Williams M, Benkovic S. 1991. Localization of insulin-like growth factor-1 mRNA in murine central nervous system during postnatal development. Dev Biol 147: 239-250.

Beck KD, Powell-Braxton L, Widmer HR, Valverde J, Hefti F. 1995. Igf1 gene disruption results in reduced brain size, CNS hypomyelination, and loss of hippocampal granule and striatal parvalbumin-containing neurons. Neuron 14: 717-730.

Blair LA, Bence-Hanulec KK, Mehta S, Franke T, Kaplan D, et al. 1999. Akt-dependent potentiation of L channels by insulin-like growth factor-1 is required for neuronal survival. J Neurosci 19: 1940-1951.

Bohannon NJ, Corp ES, Wilcox BJ, Figlewicz DP, Dorsa DM, et al. 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.

Bonapace G, Concolino D, Formicola S, Strisciuglio P. 2003. A novel mutation in a patient with insulin-like growth factor 1 (IGF1) deficiency. J Med Genet 40: 913-917.

Bondy C, Bach M, Lee W. 1992a. Mapping of brain insulin and insulin-like growth factor receptor gene expression by in situ hybridization. Neuroprotocols 1: 240-249.

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

Bondy C, Werner H, Roberts CT Jr, LeRoith D. 1992b. Cellular pattern of type-I insulin-like growth factor receptor gene expression during maturation of the rat brain: comparison with insulin-like growth factors I and II. Neuroscience 46: 909-923.

Bondy CA. 1991. Transient IGF-I gene expression during the maturation of functionally related central projection neurons. J Neurosci 11: 3442-3455.

Bondy CA, Cheng CM. 2004. Signaling by insulin-like growth factor 1 in brain. Eur J Pharmacol 490: 25-31.

Bondy CA, Lee WH. 1993b. Patterns of insulin-like growth factor and IGF receptor gene expression in the brain: functional implications. Ann N Y Acad Sci 692: 33-43.

Bondy CA, Werner H, Roberts CT, Leroith D. 1990. Cellular pattern of insulin-like growth factor-I and type-I IGF receptor gene expression in early organogenesis: comparison with IGF-II gene expression. Mol Endocrinol 4: 1386-1398.

Brar AK, Chernausek SD. 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.

Breese CR, Ingram RL, Sonntag WE. 1991. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. J Gerontol 46: B180-B187.

Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, et al. 2000. Role of brain insulin receptor in control of body weight and reproduction. Science 289: 2122-2125.

Busiguina S, Fernandez AM, Barrios V, Clark R, Tolbert DL, et al. 2000. Neurodegeneration is associated to changes in serum insulin-like growth factors. Neurobiol Dis 7: 657-665.

Caroni P. 1993. Activity-sensitive signaling by muscle-derived insulin-like growth factors in the developing and regenerating neuromuscular system. Ann N Y Acad Sci 692: 209-222.

Carro E, Torres-Aleman I. 2004. The role of insulin and insulin-like growth factor I in the molecular and cellular mechanisms underlying the pathology of Alzheimer's disease. Eur J Pharmacol 490: 127-133.

Carro E, Trejo JL, Gomez-Isla T, Le Roith D, Torres-Aleman I. 2002. Serum insulin-like growth factor I regulates brain amyloid-beta levels. Nat Med 8: 1390-1397.

Carson MJ, Behringer RR, Brinster RL, McMorris FA. 1993. Insulin-like growth factor I increases brain growth and central nervous system myelination in transgenic mice. Neuron 10: 729-740.

Cediel R, Riquelme R, Contreras J, Diez A, Varela-Neito I. 2006. Sensorineural hearing loss in insulin-like growth factor 1-null mice: a new model of human deafness. Eur J Neurosci 23: 587-590.

Cheng CM, Cohen M, Tseng V, Bondy CA. 2001. Endogenous IGF1 enhances cell survival in the postnatal dentate gyrus. J Neurosci Res 64: 341-347.

Cheng CM, Joncas G, Reinhardt RR, Farrer R, Quarles R, et al. 1998. Biochemical and morphometric analyses show that myelination in the insulin-like growth factor 1 null brain is proportionate to its neuronal composition. J Neurosci 18: 5673–5681.

Cheng CM, Kelley B, Wang J, Strauss D, Eagles DA, et al. 2003a. A ketogenic diet increases brain insulin-like growth factor receptor and glucose transporter gene expression. Endocrinology 144: 2676-2682.

Cheng CM, Mervis RF, Niu SL, Salem N Jr, Witters LA, et al. 2003b. Insulin-like growth factor 1 is essential for normal dendritic growth. J Neurosci Res 73: 1-9.

Cheng CM, Reinhardt RR, Lee WH, Joncas GS, Patel C, et al. 2000. Insulin-like growth factor 1 regulates developing brain glucose metabolism. Proc Natl Acad Sci USA 97: 10236-10241.

Cheng CM, Tseng V, Wang J, Wang D, Matyakhina L, Bondy, CA. 2005. Tau is hyperphosphorylated in the insulin-like growth factor-1 null brain. Endocrinology 146: 5086-5091.

Chowen JA, Goya L, Ramos S, Busiguina S, Garcia-Segura LM, et al. 2002. Effects of early undernutrition on the brain insulin-like growth factor-I system. J Neuroendocrinol 14: 163-169.

Clawson TF, Vannucci SJ, Wang GM, Seaman LB, Yang XL, et al. 1999. Hypoxia–ischemia-induced apoptotic cell death correlates with IGF-I mRNA decrease in neonatal rat brain. Biol Signals Recept 8: 281-293.

Clemmons DR. 1989. Structural and functional analysis of insulin-like growth factors. Br Med Bull 45: 465-480.

Clemmons DR. 1998. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol 140: 19-24.

Coker GT, 3rd, Studelska D, Harmon S, Burke W, O'Malley KL. 1990. Analysis of tyrosine hydroxylase and insulin transcripts in human neuroendocrine tissues. Brain Res Mol Brain Res 8: 93-98.

Crouch MF, Hendry IA. 1991. Co-activation of insulin-like growth factor-I receptors and protein kinase C results in parasympathetic neuronal survival. J Neurosci Res 28: 115-120.

Dani S. 1997. Histological markers of neuronal aging and their meaning. Dani S, Hori A, Walter HJ, editors. Amsterdam: Elsevier; Oxford, p. 263.

Daughaday WH, 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.

DeChiara TM, Efstratiadis A, Robertson EJ. 1990. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 345: 78-80.

Dempsey RJ, Sailor KA, Bowen KK, Tureyen K, Vemuganti R. 2003. Stroke-induced progenitor cell proliferation in adult

spontaneously hypertensive rat brain: effect of exogenous IGF-1 and GDNF. J Neurochem 87: 586-597.

Dentremont KD, Ye P, D'Ercole AJ, O'Kusky JR. 1999. Increased insulin-like growth factor-I (IGF-I) expression during early postnatal development differentially increases neuron number and growth in medullary nuclei of the mouse. Brain Res Dev Brain Res 114: 135-141.

D'Mello SR, Borodezt K, Soltoff SP. 1997. Insulin-like growth factor and potassium depolarization maintain neuronal survival by distinct pathways: possible involvement of PI 3-kinase in IGF-1 signaling. J Neurosci 17: 1548-1560.

D'Mello SR, Galli C, Ciotti T, 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: 10989-10993.

Dore S, Kar S, Quirion R. 1997. Insulin-like growth factor I protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity. Proc Natl Acad Sci USA 94: 4772-4777.

Duan C. 2002. Specifying the cellular responses to IGF signals: roles of IGF-binding proteins. J Endocrinol 175: 41-54.

Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, et al. 1997. Regulation of neuronal survival by the serine–threonine protein kinase Akt. Science 275: 661-665.

Easton RM, Cho H, Roovers K, Shineman DW, Mizrahi M, et al. 2005. Role for Akt3/protein kinase Bγ in attainment of normal brain size. Mol Cell Biol 25: 1869-1878.

Favelyukis S, Till JH, Hubbard SR, Miller WT. 2001. Structure and autoregulation of the insulin-like growth factor 1 receptor kinase. Nat Struct Biol 8: 1058-1063.

Finch CE, Ruvkun G. 2001. The genetics of aging. Annu Rev Genomics Hum Genet 2: 435-462.

Foulstone E, Prince S, Zaccheo O, Burns JL, Harper J, et al. 2005. Insulin-like growth factor ligands, receptors, and binding proteins in cancer. J Pathol 205: 145-153.

Fukudome Y, Tabata T, Miyoshi T, Haruki S, Araishi K, et al. 2003. Insulin-like growth factor-I as a promoting factor for cerebellar Purkinje cell development. Eur J Neurosci 17: 2006-2016.

Gao WQ, Shinsky N, Ingle G, Beck K, Elias KA, et al. 1999. IGF-I deficient mice show reduced peripheral nerve conduction velocities and decreased axonal diameters and respond to exogenous IGF-I treatment. J Neurobiol 39: 142-152.

Gasparini L, Gouras GK, Wang R, Gross RS, Beal MF, et al. 2001. Stimulation of beta-amyloid precursor protein trafficking by insulin reduces intraneuronal beta-amyloid and requires mitogen-activated protein kinase signaling. J Neurosci 21: 2561-2570.

Gehrmann J, Yao DL, Bonetti B, Bondy CA, Brenner M, et al. 1994. Expression of insulin-like growth factor-I and related peptides during motoneuron regeneration. Exp Neurol 128: 202-210.

Groszer M, Erickson R, Scripture-Adams DD, Lesche R, Trumpp A, et al. 2001. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. Science 294: 2186-2189.

Guan J, Bennet L, Gluckman PD, Gunn AJ. 2003. Insulin-like growth factor-1 and post-ischemic brain injury. Prog Neurobiol 70: 443-462.

Guan J, Bennet TL, George S, Waldvogel HJ, Faull RL, et al. 2000a. Selective neuroprotective effects with insulin-like growth factor-1 in phenotypic striatal neurons following ischemic brain injury in fetal sheep. Neuroscience 95: 831-839.

Guan J, Gunn AJ, Sirimanne ES, Tuffin J, Gunning MI, et al. 2000b. The window of opportunity for neuronal rescue with insulin-like growth factor-1 after hypoxia–ischemia in rats is critically modulated by cerebral temperature during recovery. J Cereb Blood Flow Metab 20: 513-519.

Guan J, Miller OT, Waugh KM, McCarthy DC, Gluckman PD. 2001. Insulin-like growth factor-1 improves somatosensory function and reduces the extent of cortical infarction and ongoing neuronal loss after hypoxia–ischemia in rats. Neuroscience 105: 299-306.

Guan J, Williams C, Gunning M, Mallard C, Gluckman P. 1993. The effects of IGF-1 treatment after hypoxic–ischemic brain injury in adult rats. J Cereb Blood Flow Metab 13: 609-616.

Gutierrez-Ospina G, Calikoglu AS, Ye P, D'Ercole AJ. 1996. In vivo effects of insulin-like growth factor-I on the development of sensory pathways: analysis of the primary somatic sensory cortex (S1) of transgenic mice. Endocrinology 137: 5484-5492.

Gutierrez-Ospina G, Saum L, Calikoglu AS, Diaz-Cintra S, Barrios FA, et al. 1997. Increased neural activity in transgenic mice with brain IGF-I overexpression: a [3H]2DG study. Neuroreport 8: 2907-2911.

Harper SJ, Macaulay AJ, Hill RG, Priestley T. 1996. The effects of insulin-like growth factor analogues on survival of cultured cerebral cortex and cerebellar granule neurones. Brain Res 709: 303-310.

Haspel HC, Stephenson KN, Davies-Hill T, El-Barbary A, Lobo JF, et al. 1999. Effects of barbiturates on facilitative glucose transporters are pharmacologically specific and isoform selective. J Membr Biol 169: 45-53.

Hawkes C, Kar S. 2004. The insulin-like growth factor-II/mannose-6-phosphate receptor: structure, distribution, and function in the central nervous system. Brain Res Rev 44: 117-140.

Hill JM, Lesniak MA, Pert CB, Roth J. 1986. Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. Neuroscience 17: 1127-1138.

Hof P, Mobb C. 2001. Functional neurobiology of aging. San Diego: Academic Press.

Hong M, Lee VM. 1997. Insulin and insulin-like growth factor-1 regulate tau phosphorylation in cultured human neurons. J Biol Chem 272: 19547-19553.

Hoyer S. 2004. Glucose metabolism and insulin receptor signal transduction in Alzheimer's disease. Eur J Pharmacol 490: 115-125.

Hubbard SR. 1999. Structural analysis of receptor tyrosine kinases. Prog Biophys Mol Biol 71: 343-358.

Humbert S, Bryson EA, Cordelieres FP, Connors NC, Datta SR, et al. 2002. The IGF-1/Akt pathway is neuroprotective in Huntington's disease and involves Huntingtin phosphorylation by Akt. Dev Cell 2: 831-837.

Ikeya T, Galic M, Belawat P, Nairz K, Hafen E. 2002. Nutrient-dependent expression of insulin-like peptides from neuroendocrine cells in the CNS contributes to growth regulation in Drosophila. Curr Biol 12: 1293-1300.

Ishiguro K, Shiratsuchi A, Sato S, Omori A, Arioka M, et al. 1993. Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. FEBS Lett 325: 167-172.

Johnston BM, Mallard EC, Williams CE, Gluckman PD. 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.

Jope RS, Johnson GV. 2004. The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29: 95-102.

Katic M, Kahn CR. 2005. The role of insulin and IGF-1 signaling in longevity. Cell Mol Life Sci 62: 320-343.

Kazanis I, Giannakopoulou M, Philippidis H, Stylianopoulou F. 2004. Alterations in IGF-I, BDNF, and NT-3 levels following experimental brain trauma and the effect of IGF-I administration. Exp Neurol 186: 221-234.

Kiess W, Blickenstaff G, Sklar M, Thomas C, Nissley S, et al. 1988. Biochemical evidence that the type II insulin-like growth factor receptor is identical to the cation-independent mannose 6-phosphate receptor. J Biol Chem 263: 9339-9344.

Kinney BA, Meliska CJ, Steger RW, Bartke A. 2001. Evidence that Ames dwarf mice age differently from their normal siblings in behavioral and learning and memory parameters. Horm Behav 39: 277-284.

Kohn AD, Summers SA, Birnbaum MJ, Roth RA. 1996. Expression of a constitutively active Akt Ser/Thr kinase in 3T3-L1 adipocytes stimulates glucose uptake and glucose transporter 4 translocation. J Biol Chem 271: 31372-31378.

Komoly S, Hudson LD, Webster HD, Bondy CA. 1992. Insulin-like growth factor I gene expression is induced in astrocytes during experimental demyelination. Proc Natl Acad Sci USA 89: 1894-1898.

Koulich E, Nguyen T, Johnson K, Giardina C, D'Mello S. 2001. NF-κB is involved in the survival of cerebellar granule neurons: association of Iκβ phosphorylation with cell survival. J Neurochem 76: 1188-1198.

Kwon C-H, Zhu X, Zhang J, Baker SJ. 2003. mTor is required for hypertrophy of Pten-deficient neuronal soma in vivo. Proc Natl Acad Sci USA 100: 12923-12928.

Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, et al. 2001. Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. Nat Genet 29: 404-411.

Lai M, Hibberd CJ, Gluckman PD, Seckl JR. 2000. Reduced expression of insulin-like growth factor 1 messenger RNA in the hippocampus of aged rats. Neurosci Lett 288: 66-70.

Laron Z. 2001. Insulin-like growth factor 1 (IGF-1): a growth hormone. Mol Pathol 54: 311-316.

Lee VM, Goedert M, Trojanowski JQ. 2001. Neurodegenerative tauopathies. Annu Rev Neurosci 24: 1121-1159.

Lee W, Clemens J, Bondy C. 1992a. Insulin-like growth factors in the response to cerebral ischemia. Mol Cell Neurosci 3: 36-43.

Lee WH, Bondy C. 1993. Insulin-like growth factors and cerebral ischemia. Ann N Y Acad Sci 679: 418-422.

Lee WH, Javedan S, Bondy CA. 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 WH, Michels KM, Bondy CA. 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 WH, Wang GM, Seaman LB, Vannucci SJ. 1996. Coordinate IGF-I and IGFBP5 gene expression in perinatal rat brain after hypoxia–ischemia. J Cereb Blood Flow Metab 16: 227-236.

Le Roith D. 1996. Insulin-like growth factor receptors and binding proteins. Baillieres Clin Endocrinol Metab 10: 49-73.

Le Roith D, Bondy C, Yakar S, Liu JL, Butler A. 2001. The somatomedin hypothesis: 2001. Endocr Rev 22: 53-74.

Le Roith D, Delahunty G, Wilson GL, Roberts CT Jr, Shemer J, et al. 1986. Evolutionary aspects of the endocrine and nervous systems. Recent Prog Horm Res 42: 549-587.

Le Roith D, Kavsan VM, Koval AP, Roberts CT Jr. 1993. Phylogeny of the insulin-like growth factors (IGFs) and receptors: a molecular approach. Mol Reprod Dev 35: 332-6; discussion 337–338.

Li XS, Williams M, Bartlett WP. 1998. Induction of IGF-1 mRNA expression following traumatic injury to the postnatal brain. Brain Res Mol Brain Res 57: 92-96.

Lin SY, Cui H, Yusta B, Belsham DD. 2004. IGF-I signaling prevents dehydroepiandrosterone (DHEA)-induced

apoptosis in hypothalamic neurons. Mol Cell Endocrinol 214: 127-135.

Linseman DA, Phelps RA, Bouchard RJ, Le SS, Laessig TA, et al. 2002. Insulin-like growth factor-I blocks Bcl-2 interacting mediator of cell death (Bim) induction and intrinsic death signaling in cerebellar granule neurons. J Neurosci 22: 9287-9297.

Logan A, Gonzalez AM, Hill DJ, Berry M, Gregson NA, et al. 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.

Luo RZ-T, Beniac DR, Fernandes A, Yip CC, Ottensmeyer FP. 1999. Quaternary structure of the insulin–insulin receptor complex. Science 285: 1077-1080.

Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. 2001. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev Biol 229: 141-162.

Markowska AL, Mooney M, Sonntag WE. 1998. Insulin-like growth factor-1 ameliorates age-related behavioral deficits. Neuroscience 87: 559-569.

Mason JL, Xuan S, Dragatsis I, Efstratiadis A, Goldman JE. 2003. Insulin-like growth factor (IGF) signaling through type 1 IGF receptor plays an important role in remyelination. J Neurosci 23: 7710-7718.

Matsuzaki H, Tamatani M, Mitsuda N, Namikawa K, Kiyama H, et al. 1999. Activation of Akt kinase inhibits apoptosis and changes in Bcl-2 and Bax expression induced by nitric oxide in primary hippocampal neurons. J Neurochem 73: 2037-2046.

McMorris FA, McKinnon RD. 1996. Regulation of oligodendrocyte development and CNS myelination by growth factors: prospects for therapy of demyelinating disease. Brain Pathol 6: 313-329.

Miller TM, Tansey MG, Johnson EM Jr, Creedon DJ. 1997. Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. J Biol Chem 272: 9847-9853.

Mozell RL, McMorris FA. 1991. Insulin-like growth factor I stimulates oligodendrocyte development and myelination in rat brain aggregate cultures. J Neurosci Res 30: 382-390.

Nakao N, Odin P, Lindvall O, Brundin P. 1996. Differential trophic effects of basic fibroblast growth factor, insulin-like growth factor-1, and neurotrophin-3 on striatal neurons in culture. Exp Neurol 138: 144-157.

Navarro I, Leibush B, Moon TW, Plisetskaya EM, Banos N, et al. 1999. Insulin, insulin-like growth factor-I (IGF-I) and glucagon: the evolution of their receptors. Comp Biochem Physiol B Biochem Mol Biol 122: 137-153.

Neeper SA, Gomez-Pinilla F, Choi J, Cotman C. 1995. Exercise and brain neurotrophins. Nature 373: 109.

Niblock MM, Brunso-Bechtold JK, Riddle DR. 2000. Insulin-like growth factor I stimulates dendritic growth in primary somatosensory cortex. J Neurosci 20: 4165-4176.

Nilsson M, Perfilieva E, Johansson U, Orwar O, Eriksson PS. 1999. Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39: 569-578.

Nitta A, Zheng WH, Quirion R. 2004. Insulin-like growth factor 1 prevents neuronal cell death induced by corticosterone through activation of the PI3k/Akt pathway. J Neurosci Res 76: 98-103.

Offen D, Shtaif B, Hadad D, Weizman A, Melamed E, et al. 2001. Protective effect of insulin-like-growth-factor-1 against dopamine-induced neurotoxicity in human and rodent neuronal cultures: possible implications for Parkinson's disease. Neurosci Lett 316: 129-132.

O'Kusky JR, Ye P, D'Ercole AJ. 2000. Insulin-like growth factor-I promotes neurogenesis and synaptogenesis in the hippocampal dentate gyrus during postnatal development. J Neurosci 20: 8435-8442.

Pei J-J, Braak E, Braak H, Grundke-Iqbal I, Iqbal K, et al. 1999. Distribution of active glycogen synthase kinase 3β (GSK-3β) in brains staged for Alzheimer disease neurofibrillary changes. J Neuropathol Exp Neurol 58: 1010-1019.

Porte D, Jr., Baskin DG, Schwartz MW. 2005. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. Diabetes 54: 1264-1276.

Potter CJ, Xu T. 2001. Mechanisms of size control. Curr Opin Genet Dev 11: 279-286.

Rangone H, Pardo R, Colin E, Girault JA, Saudou F, Humbert S. 2005. Phosphorylation of arfaptin 2 at Ser260 by Akt Inhibits PolyQ-huntingtin-induced toxicity by rescuing proteasome impairment. J Biol Chem 280: 22021-22028.

Reijnders C, Koster J, van Buul-Offers S. 2004. Overexpression of human IGF-II mRNA in the brain of transgenic mice modulates IGFBP-2 gene expression in the medulla oblongata. J Endocrinol 182: 445-455.

Reinecke M, Collet C. 1998. The phylogeny of the insulin-like growth factors. Int Rev Cytol 183: 1-94.

Reinhardt RR, Bondy CA. 1994. Insulin-like growth factors cross the blood–brain barrier. Endocrinology 135: 1753-1761.

Rice JE 3rd, Vannucci RC, Brierley JB. 1981. The influence of immaturity on hypoxic–ischemic brain damage in the rat. Ann Neurol 9: 131-141.

Richardson A, Liu F, Adamo ML, Remmen HV, Nelson JF. 2004. The role of insulin and insulin-like growth factor-I in

mammalian ageing. Best Prac Res Clin Endo Metab 18: 393-406.

Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, et al. 2001. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI(3)K/Akt/mTOR and PI(3)K/Akt/GSK3 pathways. Nat Cell Biol 3: 1009-1013.

Rotwein P, Burgess SK, Milbrandt JD, Krause JE. 1988. Differential expression of insulin-like growth factor genes in rat central nervous system. Proc Nat Acad Sci USA 85: 265-269.

Rulifson EJ, Kim SK, Nusse R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. Science 296: 1118-1120.

Saatman KE, Contreras PC, Smith DH, Raghupathi R, McDermott KL, et al. 1997. Insulin-like growth factor-1 (IGF-1) improves both neurological motor and cognitive outcome following experimental brain injury. Exp Neurol 147: 418-427.

Saltiel AR, Kahn CR. 2001. Insulin signaling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.

Sara VR, Carlsson-Skwirut C. 1986. The biosynthesis of somatomedins and their role in the fetus. Acta Endocrinol Suppl (Copenhagen) 279: 82-85.

Schubert M, Brazil DP, Burks DJ, Kushner JA, Ye J, et al. 2003. Insulin receptor substrate-2 deficiency impairs brain growth and promotes tau phosphorylation. J Neurosci 23: 7084-7092.

Schuman EM. 1999. Neurotrophin regulation of synaptic transmission. Curr Opin Neurobiol 9: 105-109.

Shi L, Linville MC, Tucker EW, Sonntag WE, Brunso-Bechtold JK. 2005. Differential effects of aging and insulin-like growth factor-1 on synapses in CA1 of rat hippocampus. Cereb Cortex 15: 571-577.

Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, et al. 2001. Neurogenesis in the adult is involved in the formation of trace memories. Nature 410: 372-376.

Sonntag WE, Lynch CD, Bennett SA, Khan AS, Thornton PL, et al. 1999. Alterations in insulin-like growth factor-1 gene and protein expression and type 1 insulin-like growth factor receptors in the brains of ageing rats. Neuroscience 88: 269-279.

Soos MA, Field CE, Siddle K. 1993. Purified hybrid insulin/insulin-like growth factor-I receptors bind insulin-like growth factor-I, but not insulin, with high affinity. Biochem J 290: 419-426.

Spillantini MG, Goedert M. 1998. Tau protein pathology in neurodegenerative diseases. Trends Neurosci 21: 428-433.

Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, et al. 2005. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimer's disease—is this type 3 diabetes? J Alzheimers Dis 7: 63-80.

Sullivan KA, Feldman EL. 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.

Summers SA, Birnbaum MJ. 1997. A role for the serine/threonine kinase, Akt, in insulin-stimulated glucose uptake. Biochem Soc Trans 25: 981-988.

Summers SA, Kao AW, Kohn AD, Backus GS, Roth RA, et al. 1999. The role of glycogen synthase kinase 3β in insulin-stimulated glucose metabolism. J Biol Chem 274: 17934-17940.

Sun LY, Evans MS, Hsieh J, Panici J, Bartke A. 2005. Increased neurogenesis in dentate gyrus of long-lived Ames dwarf mice. Endocrinology 146: 1138-1144.

Sussenbach JS. 1989. The gene structure of the insulin-like growth factor family. Prog Growth Factor Res 1: 33-48.

Tagami M, Ikeda K, Nara Y, Fujino H, Kubota A, et al. 1997a. Insulin-like growth factor-1 attenuates apoptosis in hippocampal neurons caused by cerebral ischemia and reperfusion in stroke-prone spontaneously hypertensive rats. Lab Invest 76: 613-617.

Tagami M, Yamagata K, Nara Y, Fujino H, Kubota A, et al. 1997b. Insulin-like growth factors prevent apoptosis in cortical neurons isolated from stroke-prone spontaneously hypertensive rats. Lab Invest 76: 603-612.

Tager HS, Steiner DF, Patzelt C. 1981. Biosynthesis of insulin and glucagon. Methods Cell Biol 23: 73-88.

Tamatani M, Ogawa S, Nunez G, Tohyama M. 1998. Growth factors prevent changes in Bcl-2 and Bax expression and neuronal apoptosis induced by nitric oxide. Cell Death Differ 5: 911-919.

Torres-Aleman I, Barrios V, Berciano J. 1998. The peripheral insulin-like growth factor system in amyotrophic lateral sclerosis and in multiple sclerosis. Neurology 50: 772-776.

Torres-Aleman I, Barrios V, Lledo A, Berciano J. 1996. The insulin-like growth factor I system in cerebellar degeneration. Ann Neurol 39: 335-342.

Torres-Aleman I, Naftolin F, Robbins RJ. 1990. Trophic effects of insulin-like growth factor-I on fetal rat hypothalamic cells in culture. Neuroscience 35: 601-608.

Torres-Aleman I, Pons S, Santos-Benito FF. 1992. Survival of Purkinje cells in cerebellar cultures is increased by insulin-like growth factor I. Eur J Neurosci 4: 864-869.

Trejo JL, Carro E, Torres-Aleman I. 2001. Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. J Neurosci 21: 1628-1634.

Walenkamp MJ, Karperien M, Pereira AM, Hilhorst-Hofstee Y, van Doorn J, et al. 2005. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab 90: 2855-2864.

Walter HJ, Berry M, Hill DJ, Logan A. 1997. Spatial and temporal changes in the insulin-like growth factor (IGF) axis indicate autocrine/paracrine actions of IGF-I within wounds of the rat brain. Endocrinology 138: 3024-3034.

Wang J, Zhou J, Powell-Braxton L, Bondy C. 1999. Effects of Igf1 gene deletion on postnatal growth patterns. Endocrinology 140: 3391-3394.

Watson GS, Craft S. 2003. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. CNS Drugs 17: 27-45.

Woods KA, Camacho-Hubner C, Barter D, Clark AJ, Savage MO. 1997. Insulin-like growth factor I gene deletion causing intrauterine growth retardation and severe short stature. Acta Paediatr Suppl 423: 39-45.

Woods KA, Camacho-Hubner C, Savage MO, Clark AJ. 1996. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Engl J Med 335: 1363-1367.

Wylie AA, Pulford DJ, McVie-Wylie AJ, Waterland RA, Evans HK, et al. 2003. Tissue-specific inactivation of murine M6P/IGF2R. Am J Pathol 162: 321-328.

Yamaguchi A, Tamatani M, Matsuzaki H, Namikawa K, Kiyama H, et al. 2001. Akt activation protects hippocampal neurons from apoptosis by inhibiting transcriptional activity of p53. J Biol Chem 276: 5256-5264.

Yao DL, West NR, Bondy CA, Brenner M, Hudson LD, et al. 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, D'Ercole AJ. 1995. 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, D'Ercole J. 1998. Insulin-like growth factor I (IGF-I) regulates IGF binding protein-5 gene expression in the brain. Endocrinology 139: 65-71.

Ye P, Popken GJ, Kemper A, McCarthy K, Popko B, et al. 2004. Astrocyte-specific overexpression of insulin-like growth factor-I promotes brain overgrowth and glial fibrillary acidic protein expression. J Neurosci Res 78: 472-484.

Ye P, Xing Y, Dai Z, D'Ercole AJ. 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. Brain Res Dev Brain Res 95: 44-54.

Young WS. 1986. Periventricular hypothalamic cells in rat brain contain insulin mRNA. Neuropeptides 8: 93-97.

Zackenfels K, Oppenheim RW, 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 RL, Zhang ZG, Zhang L, Chopp M. 2001. Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 105: 33-41.

Zheng WH, Quirion R. 2004. Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. J Neurochem 89: 844-852.

Zhong J, Deng J, Ghetti B, Lee WH. 2002. Inhibition of insulin-like growth factor I activity contributes to the premature apoptosis of cerebellar granule neuron in weaver mutant mice: in vitro analysis. J Neurosci Res 70: 36-45.