Molecular Neurobiology

ISSN0893-7648/96/13(2): 155–183/$12.25

The Early Intracellular Signaling Pathway for the Insulin/Insulin-Like Growth Factor Receptor Family in the Mammalian Central Nervous System

Franco Folli,[1,2] Silvana Ghidella,[3] Luca Bonfanti,[3] C. Ronald Kahn,[1] and Adalberto Merighi*[,3]

[1]Research Division, Joslin Diabetes Center, Department of Medicine, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA 02115; [2]Department of Medicine, H. San Raffaele, I-20132 Milano, Italy; and [3]Dipartimento di Morfofisiologia Veterinaria, Università degli Studi di Torino, Via Nizza 52, I-10126 Torino, Italy

Abstract

Several studies support the idea that the polypeptides belonging to the family of insulin and insulin-like growth factors (IGFs) play an important role in brain development and continue to be produced in discrete areas of the adult brain. In numerous neuronal populations within the olfactory bulb, the cerebral and cerebellar cortex, the hippocampus, some diencephalic and brainstem nuclei, the spinal cord and the retina, specific insulin and IGF receptors, as well as crucial components of the intracellular receptor signaling pathway have been demonstrated. Thus, mature neurons are endowed with the cellular machinery to respond to insulin and IGF stimulation. Studies in vitro and in vivo, using normal and transgenic animals, have led to the hypothesis that, in the adult brain, IGF-I not only acts as a trophic factor, but also as a neuromodulator of some higher brain functions, such as long-term potentiation and depression. Furthermore, a trophic effect on certain neuronal populations becomes clearly evident in the ischemic brain or neurodegenerative disorders. Thus, the analysis of the early intracellular signaling pathway for the insulin/IGF receptor family in the brain is providing us with new intriguing findings on the way the mammalian brain is sculpted and operates.

Index Entries: Insulin; IGF-I; IGF-II; IGF receptor; IRS-1; IRS-2; PI-3 kinase; neurons; glial cells; central nervous system.

Introduction

Over the past decade, a considerable amount of data have been added to our knowledge concerning the intracellular signaling pathways for members of the insulin/insulin-like growth factor (IGF) family. Evidence has been obtained that the gene products and specific receptors

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

for these molecules are widely distributed in the developing and adult central nervous system (CNS) of several vertebrates. Since previous articles have summarized the biochemistry of the intracellular signaling cascade of these hormones and their role in the CNS development (Werner et al., 1993; White and Kahn, 1994; De Pablo and De la Rosa, 1995; LeRoith et al., 1995; Pawson, 1995), this article will focus on the localization of insulin, IGFs, and related molecules (insulin-like growth factor binding proteins [IGFBPs]), their specific receptors and intracellular signaling substrates in the adult mammalian CNS, as well as on in vitro and genetic models for the study of the putative functions of these growth factors in the brain. A brief summary of the state of the art on the biochemistry and pharmacology of the intracellular signal transduction pathway for insulin and IGFs and the major findings regarding the localization and functional role of these polypeptides in the developing CNS will help to put this in perspective.

The Intracellular Signal Transduction Pathway of the Insulin/IGF Receptor Family

The biological effects of insulin and IGFs are triggered by high-affinity binding to specific receptors on the cell plasma membrane (Fig. 1). The intracellular cascade of events initiated by receptor binding is well characterized for the insulin and IGF-I receptors, but is still far from clear for the IGF-II receptor. The existence of hybrid receptors formed by an insulin receptor α-β dimer and an IGF-I receptor α-β dimer has been demonstrated in vitro and in vivo, but their functional role is still not understood (Treadway et al., 1991; Soos et al., 1993). An insulin receptor-related receptor (IRR) has also been recently cloned in mammals, but its ligand is unknown, and thus does not appear to play a role in insulin or IGF action (Reinhardt et al., 1994).

The complexity in the process of intracellular signal transduction accounts for a very fine modulation of the biological activity of both insulin and IGF-I. In addition, it is important to remember here that the activity of the IGFs, but not of insulin, is also modulated by at least six soluble binding proteins, commonly referred to as the IGFBPs (Jones and Clemmons, 1995).

The Insulin/IGF-I Receptor and Substrates

The insulin and IGF-I receptors (Fig. 2) are heterotetrameric proteins composed of two α-extracellular subunits and two β-transmembrane subunits (Kasuga et al., 1982a; Massague et al., 1982). In the absence of ligand, the unoccupied α-subunits of the receptors inhibit the tyrosine kinase activity of the β-subunit (White and Kahn, 1994). When insulin and IGF-I bind to the restricted ligand binding domains of the α-subunits of their respective receptors, the tyrosine kinase activity of the β-subunit is activated (Kasuga et al., 1982b,c; Petruzzelli et al., 1982). The identification of the insulin receptor as a tyrosine kinase and its subsequent cloning have stimulated a variety of studies aiming at the identification of the structural determinants of its multifaceted intracellular activities (Ebina et al., 1985; Ullrich et al., 1985). Several functional domains have been defined in the β-subunit of the insulin receptor, including an ATP-binding domain and seven potential autophosphorylation sites in the intracellular juxtamembrane region, the regulatory region, and the COOH terminus (Tornqvist and Avruch, 1988; Tornqvist et al., 1988; White et al., 1988a; Feener et al., 1993). Autophosphorylation of three tyrosine residues (1157, 1158, 1162) in the YXXXY motif of the regulatory region stimulates kinase activity 10- to 20-fold (White et al., 1988a). Mutation of one, two, or three tyrosine residues in this region progressively reduces insulin-stimulated kinase activity with a parallel loss of biological activity (Wilden et al., 1992a,b). The results of these mutagenesis studies have been further clarified by resolution of the crystal structure of the tyrosine kinase domain of the insulin receptor in which tyrosine 1162 in the regulatory subunit has been shown to play a critical role in the regula-

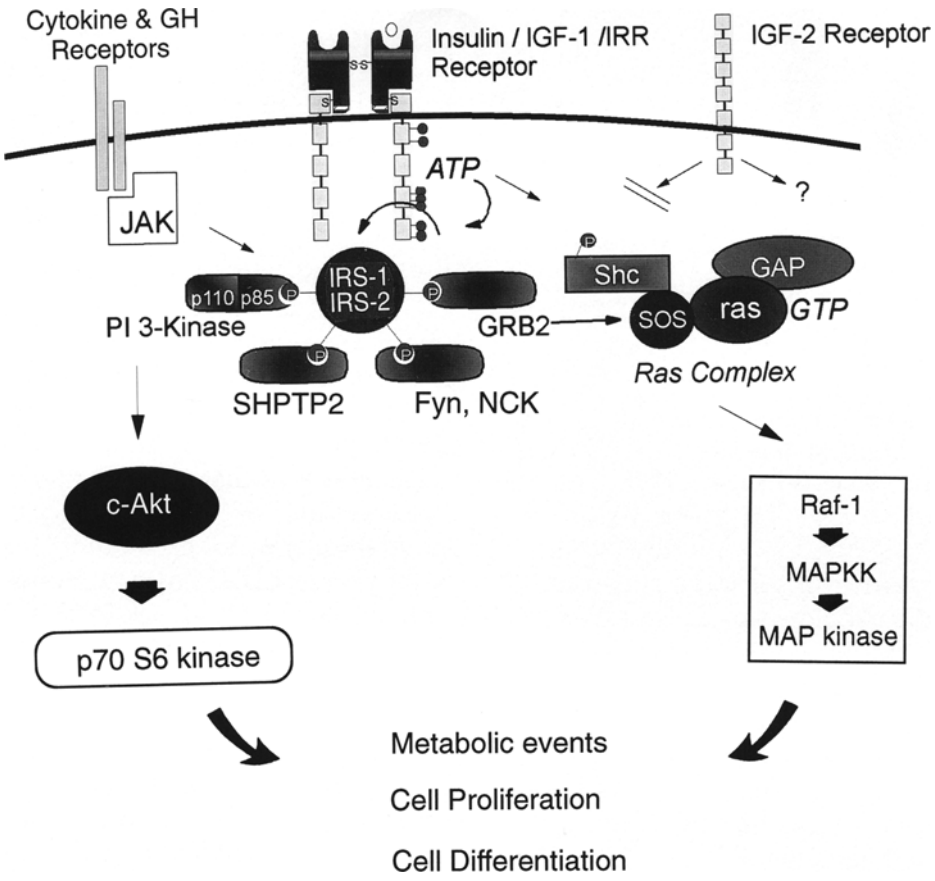

Fig. 1. Current model of intracellular signaling for the insulin/IGF-I and cytokine receptors. Insulin/IGF-I receptor autophosphorylation or cytokine and growth hormone receptor-induced phosphorylation of associated kinases (i.e., JAK) elicit tyrosyl-phosphorylation of several intracellular proteins (IRS-1, IRS-2, and Shc). Tyrosin-phosphorylated motifs in the IRSs bind, via SH2 domains, a variety of proteins with enzymatic or adapter functions, such as PI 3-kinase, SHPTP2, *fyn*, GRB2, and NCK. Tyrosine-phosphorylated Shc activates the *ras*/Raf-1/MAPKK/MAP-kinase cascade. PI-3 kinase stimulation leads to activation of the serine kinase c-Akt, which turns on the p70 S6 kinase. The activation of these multiple early intracellular substrates leads to a series of different metabolic events, including cell proliferation and differentiation.

tion of the tyrosine kinase activity of the receptor. These studies revealed a novel autoinhibitory mechanism whereby, in the unphosphorylated state, tyrosine 1162 is engaged in the active site, and both substrate and ATP-binding sites are inaccessible. When insulin binds to the α-chain, a change in the quaternary structure of the receptor places the phosphorylation sites of one β-chain within the active site of the other β-chain. Intramolecular trans-autophosphorylation can then occur when tyrosine 1162 is disengaged and Mg-ATP is bound. When phosphorylation occurs in the activation loop tyrosine, the loop is shifted toward a noninhibiting conformation, and tyrosine 1162 disengages from the active site. The result is a marked increase in kinase activity (up to 200-fold for the triphosphorylated state in vitro). These studies have also revealed the determinants of substrate preference for tyrosine, rather than serine or threonine as well as peptide substrate specific-

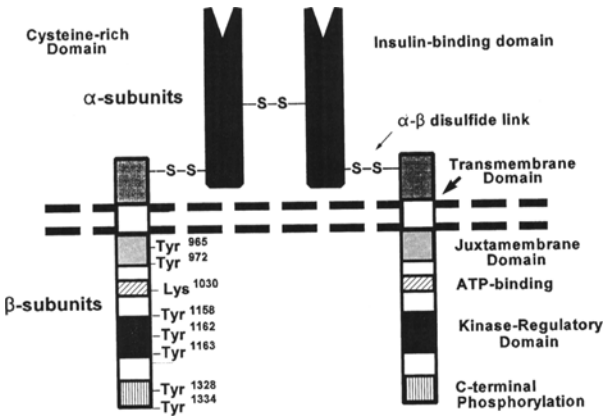

Fig. 2. A schematic model of the insulin receptor with the various structural and functional domains (redrawn from Cheatham and Kahn, 1995).

ity whereby the insulin receptor substrate-1 (IRS-1) protein is predicted to be an excellent substrate of the insulin receptor kinase at specific tyrosine sites, previously identified by in vitro phosphorylation studies (Sun et al., 1993; Hubbard et al., 1994). Although the IGF-I receptor has not been studied by crystallography, in vivo mutagenesis studies (Ullrich et al., 1986; Lammers et al., 1989; Pillay et al., 1991; Schumacher et al., 1991; Treadway et al., 1991) suggest it has similar structural determinants.

A major advance in the understanding of the intracellular signaling pathways employed by the insulin and IGF-I receptors was the identification, purification, and cloning of two major high-mol-wt substrates initially termed pp185 and subsequently renamed (IRS-1 and IRS-2) (White et al., 1985; Rothenberg et al., 1991; Sun et al., 1991, 1995). The importance of these substrates was illustrated by the fact that mutation of the tyrosine residue 960 in the insulin receptor, which does not affect receptor autophosphorylation, abolishes tyrosine phosphorylation of IRS-1 and IRS-2 and leads to marked inhibition of the intracellular metabolic and growth-promoting effects of insulin (White et al., 1988b). Both IRS-1 and IRS-2 are cytosolic proteins containing about 20 potential tyrosyl-phosphorylation and over 40 potential serine phosphorylation sites. The two IRSs show remarkable structural similarities, i.e., a highly conserved amino-terminus containing a plekstrin-homology domain, a phosphotyrosine-binding domain, as well as homology at many tyrosine phosphorylation sites. IRS-1 and IRS-2 are the intracellular substrates for insulin, IGF-I, growth hormone, interleukin (IL)-4-, IL-9-, and IL-13-linked receptors, and other cytokines (Wang et al., 1992, 1993a,b; Myers et al., 1993; Argetsinger et al., 1995; Welham et al., 1995). A variety of biological effects are regulated by IRS-1, including modulation of gene expression, mitogenesis, and glucose transport (Myers et al., 1995). Although IRS-2 was originally recognized as a specific intracellular substrate for the IL-4 receptor (Wang et al., 1993a), it is likely to mediate at least partially overlapping functions with IRS-1, as suggested by studies on the IRS-1 (−/−) mouse (Araki et al., 1994; Tamemoto et al., 1994; Patti et al., 1995). Tyrosyl-phosphorylation at specific sites on the IRS proteins enables their engagement with a variety of SH2-containing proteins. These include protein with known enzymatic function, like the p85/p110α phosphatidylinositol 3-kinase (PI 3-kinase) complex, *Fyn* tyrosine kinase, and SHPTP2 or with adaptor proteins, such as grb2, nck, and 14.3.3 (Backer et al., 1992; Folli et al., 1992; Kuhne et al., 1993; Sun et al., 1993). As discussed in the following paragraphs, a variety of in vivo and in vitro studies have established a crucial role of tyrosyl-phosphorylation of these intracellular substrates for a fine tuning of the enzymatic activities of their targets (Sun et al., 1991; Backer et al., 1992; Folli et al., 1992; Myers et al., 1992; Chuang et al., 1994).

The Shc proteins are additional intracellular substrates of the insulin and IGF-I receptors. Interestingly, they interact at the NPXY (tyrosine 960) consensus sequence of the juxtamembrane region of the insulin receptor, which is also essential for efficient tyrosyl phosphorylation of the IRS-1 protein via a PTB-binding domain with some homology to that of IRS-1 (Wolf et al., 1995).

PI 3-kinases (Fig. 3) are a rapidly growing family of dual-specificity kinases (protein/

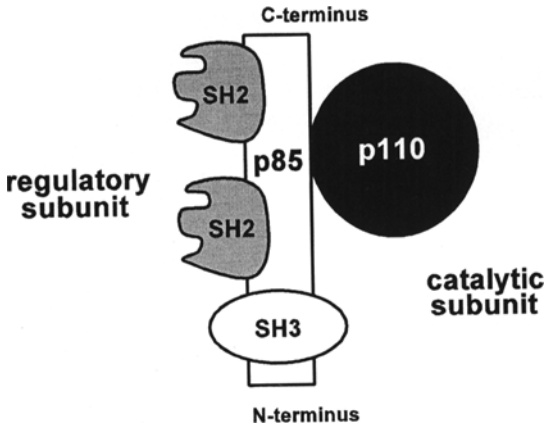

Fig. 3. A schematic model of the PI 3-kinase complex. The enzyme is composed of a regulatory subunit (p85), with two SH2 domains and one SH3 domain, and a catalytic subunit (p110), which is responsible for the kinase activity of the molecule (redrawn from Varticovski et al., 1994).

lipid kinases) with different cellular function (Hunter, 1995). Tyrosine kinase-coupled receptors and substrates like the IRSs bind via YXXM/YMXM to SH2 domains of the p85 regulatory subunit, and further enhance the enzymatic function of the p110α catalytic subunit of PI 3-kinases to give rise to PI 3-P, PI 3,4-P_2, and PI 3,4,5-P_3 (Escobedo et al., 1991; Otsu et al., 1991; Skolnik et al., 1991; Sun et al., 1991; Backer et al., 1992; Hiles et al., 1992; Fantl et al., 1993). The p85 regulatory subunit of PI 3-kinase is also endowed with one SH3 domain, which is involved in the interaction with proteins containing a proline-rich domain, especially dynamin (Gout et al., 1993). The latter is a recently discovered protein that plays an important role in the vesicular recycling-endocytotic mechanisms at the synaptic terminal (Takei et al., 1995).

Recently, a novel regulatory subunit of PI 3-kinase ($p55^{PIK}$) has been identified in the adult mouse brain and testis (Pons et al., 1995). $p55^{PIK}$ is composed of a unique 30-residue NH_2 terminal followed by a proline-rich motif and two SH2 domains with about 70% sequence identity to those in p85; $p55^{PIK}$ forms a stable complex with p110 and associates with IRS-1 during insulin stimulation. Activation of PI 3-kinase has been shown to be necessary for insulin and IGF-I stimulation of p70 S6 kinase ($p70^{S6k}$) and GLUT4-mediated glucose transport (Cheatham et al., 1994; Hung et al., 1994; Myers et al., 1994).

A possible intermediate in this PI 3-kinase pathway is protein kinase B (c-Akt). C-Akt mRNA is present in a variety of organs, including the brain. This serine/threonine kinase is the cellular homolog of the transforming v-Akt and is activated to stimuli, such as IGF-I, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and basic fibroblast growth factor (bFGF). Activation of protein kinase B is inhibited by the PI 3-kinase inhibitor wortmannin and by coexpression of a dominant-negative mutant of PI 3-kinase. A constructed Gag-PKB fusion protein, homologous to the v-Akt oncogene, displays significantly increased ligand-independent kinase activity, which is sufficient to activate the $p70^{S6k}$, a known downstream target of PI 3-kinase. Furthermore, Akt activity is induced following the addition of PI 3-phosphate to Akt protein immunoprecipitated from serum-starved cells in vitro (Franke et al., 1995).

Grb2 (Fig. 1) is an adapter protein composed of one SH2 domain and two flanking SH3 domains that specifically bind tyrosine-phosphorylated IRS-1 at tyrosine 1172 (Sun et al., 1993). One of the functions of grb2 is to link tyrosine kinase receptors to *ras* activation via the binding of grb2 to Sos, a guanine nucleotide exchange factor for *ras* (Lowenstein et al., 1992).

The IGF-II Receptor

In mammals only, IGF-II and to a lesser extent IGF-I bind to the IGF-II/mannose 6- phosphate receptor (Gammeltoft et al., 1985; Kornfeld, 1987; Nielsen et al., 1991), which seems to be a nonsignaling receptor and to function as a scavenger receptor for IGF-II and IGF-I. There is, in fact, a general consensus that the cellular effects of IGF-II are mediated by the IGF-I receptor via activation of its intrinsic tyrosine kinase, whereas the IGF-II receptor has an important role in lysosomal enzyme sorting

and endocytosis (Nielsen et al., 1991; Kornfeld, 1992; Doré et al., 1995a). However, in certain neuronal precursors, mannose-6-phosphate acts as a mitogen (Nielsen and Gammeltoft, 1990). The intracellular signal transduction pathway for this receptor is, at present, unknown (Kornfeld, 1992; Gammeltoft et al., 1991).

Developmental Studies

The exact role of insulin in brain development remains unclear. Several studies have demonstrated the existence of preproinsulin gene transcripts in the developing CNS, although at low levels (Bondy, 1991; Scavo et al., 1991; Deltour et al., 1993; Devaskar et al., 1994; Perez-Villamil et al., 1994). In most instances, the localization of the mRNA and the nature of the translation product(s) have remained elusive. However, it appears that transcripts are particularly abundant in the neural tube and the retina (Mermaridis et al., 1990). Insulin is also immunocytochemically detected in the retina of mice between E17 and 18. Transgenic animals in which expression of the reporter gene *Tag* is driven by the preproinsulin-II promoter demonstrate expression of *Tag*-positive cells in the basal plate of the neural lobe, the rostral mesencephalon, the rhombencephalon, and later in development, the floor plate of the myelencephalon (Alpert et al., 1988).

A much more significant wealth of data is available for the distribution of the IGFs and the IGFBPs during neurogenesis. As a general rule, it appears that IGF-I is associated with neurons, whereas IGF-II is present in nonneuronal cells, and the IGFBPs have a more widespread localization. IGF-I mRNA is detected during the early organogenesis of the rodent brain only as a weak signal in the olfactory bulb (Ayer-le-Lievre et al., 1991). Between E16 and E20, transcripts become evident in the olfactory bulb, thalamus, hippocampus, cerebellum, and retina (Bondy, 1991; DeChiara et al., 1991). IGF-II mRNA is present at early embryonic stages of gastrulation/neurulation (Stylianopoulou et al., 1988a) and is subsequently localized in the choroidal plexus, leptomeninges, and pituitary gland (Stylianopoulou et al., 1988b; Lee et al., 1990; DeChiara et al., 1991).

The distribution of the insulin and IGF receptors has been studied using ligand binding and autoradiography techniques (Havrankova et al., 1978; Werner et al., 1993; Baskin et al., 1994). In general, insulin and IGF-I receptors are largely confined to the gray matter; *in situ* hybridization experiments reveal IGF-I receptor-mRNAs in virtually all neuronal cell types, although at different levels (Bondy et al., 1992). There is a remarkable parallelism between the IGF-I and the IGF-I receptor gene expression in the olfactory bulb, thalamus, and cerebellum, whereas in the retina, there is high level of receptor mRNA, but a low content of IGF-I mRNA, limited to the ganglion cells. Considering the widespread overlapping in the distribution of the insulin and IGF-I receptors, it is reasonable to infer that the targets of insulin and IGF-I in early development are similar, and that any regional specificity of action appears only in the late neurogenesis (Kar et al., 1993). The presence of IGF-II receptor mRNA in neuronal precursors within the cortex, hypothalamus, and retina (Valentino et al., 1990; Nielsen et al., 1991) is puzzling, since the IGF-II gene product was not observed in neurons. However, it may be possible that IGF-I could be the ligand for the IGF-II receptor in these cells, as shown in other cell systems.

An interesting finding concerns the very limited cell distribution of the IRR compared to the insulin and IGF-I receptors. IRR mRNA is present only in those neuronal populations that also express the high-affinity nerve growth factor (NGF) receptor (trkA) mRNA. Thus, it is reasonable to infer the existence of a functional linkage between these two receptors in NGF-sensitive neurons (Reinhardt et al., 1994).

IGFBP2, IGFBP4, and IGFBP5 are also expressed during early neurogenesis (Green et al., 1994). Early in development, IGFBP2 mRNA is present in rapidly dividing cells of nonneuronal phenotype, whereas IGFBP5 is found in neuroblasts and postmitotic neurons. In the later embryogenesis, the two IGFBP

mRNAs are detected, synchronously with IGF-I, in the olfactory bulb, cerebellum and retina (De Pablo and De la Rosa, 1995). The effects of insulin and/or IGF gene knockout on CNS development are discussed later in this article.

Expression of the Insulin, IGF-I, IGF-II, and IGFBP Genes and Their Products in the Adult Brain (Table 1)

Insulin and IGF-I

Only a limited number of studies are available on the localization of the insulin gene transcripts in the adult brain. By *in situ* hybridization in the rabbit, insulin mRNA is localized to the anatomical regions involved with olfaction and higher association of the limbic system (Devaskar et al., 1994). The same authors also mapped the distribution of the insulin-II mRNA in the adult rat brain by RT-PCR with similar results (Devaskar et al., 1993).

On the other hand, numerous data can be found on the distribution of the IGF-I mRNA in the CNS. In general, the neuronal expression of the IGF-I gene is reduced after birth, whereas the expression of the IGF-I receptor gene increases in the same brain areas. Northern blot analysis has revealed that the IGF-I mRNA concentration is high in perinatal life and then declines dramatically, particularly in the cerebral cortex and hypothalamus, whereas in the brainstem and cerebellum, the IGF-I mRNA levels are unchanged from E16 up to P82 (Bach et al., 1991). The IGF-I gene is also expressed in the senescent rat brain (Park and Buetow, 1991). *In situ* studies reported the presence of IGF-I mRNA in numerous areas of the rat CNS, including the olfactory bulb, piriform cortex, lateral geniculate body, hippocampus, ventral tier, the cochlear, lemniscal and vestibular nuclei, cerebellum, spinal cord, and retina (Rothwein et al., 1988; Werner et al., 1989b; Bondy, 1991; Bondy et al., 1992; Bondy and Lee, 1993a). A study in the mouse has confirmed localization in the olfactory bulb, hippocampal complex, and cerebellum (Bartlett et al., 1991). As a general trend, IGF-I mRNA is mainly present within the principal projection neurons in specific sites of sensory and cerebellar systems, but can be also detected in the local circuit interneurons. For example, in the adult olfactory bulb, IGF-I mRNA is localized to the mitral and tufted neurons, whereas in the cerebral cortex and hippocampus, the IGF-I gene transcripts are detected in medium- to large-sized (pyramidal) neurons.

IGF-I protein has been detected in the brain using radioimmunoassay (Pons and Torres-Aleman, 1992) and immunocytochemistry (Noguchi et al., 1987; Hansson et al., 1988; Andersson et al., 1988; Garcia-Segura et al., 1991). After colchicine administration, IGF-I immunoreactivity is detected in neuronal cell bodies of the olfactory bulb, cerebral cortex, tenia tecta, hippocampus, islands of Calleja, septal nuclei, striatum, endopiriform nucleus, and amygdala. Most diencephalic nuclei, the substantia nigra, the mesencephalic colliculi, the cerebellar Purkinje cells, and several nuclei in the mesencephalon, pons, and medulla oblongata also contain immunoreactive IGF-I, whereas glial cells are negative (Garcia-Segura et al., 1991).

IGF-II

Analysis of expression of the IGF-II gene in the adult rat brain has demonstrated that the IGF-II mRNA is not detected in neurons or glial cells, but rather in the meninges, choroid plexus, and mesenchymal cells surrounding the blood vessels (Brown et al., 1986; Lund et al., 1986; Hynes et al., 1988; Rotwein et al., 1988; Stylianopoulou et al., 1988a; Bondy et al., 1992). IGF-II mRNA is also observed in the CNS of the senescent rat (Park and Buetow, 1991). In the human brain, the IGF-II mRNA was detected in the hypothalamus (Irminger et al., 1987). By immunocytochemistry, IGF-II protein can be localized to the choroid plexus and presumptive glial cells of the hippocampus and hypothalamus, as well as a small population of

Table 1
Brain Distribution of Members of the Insulin/IGF Family of Growth Factors, Their Receptors, and IGF Binding Proteins[a]

Areas of localization	Insulin	Insulin rec	IGF-I	IGF-I rec
Cerebral	**Olfactory cortex** OB **Cerebral cortex** LIM	**Olfactory cortex** OB **Cortex** **Basal forebrain** SFO **Hippocampus** **Thalamus** **Hypothalamus** ARH **Brainstem** **Cerebellum** CBX, DNC	**Olfactory cortex** OB, TT, PIR, EP **Cortex** **Basal ganglia** STR, AMY **Basal forebrain** isl, SEP **Hippocampus** **Brainstem** LG, SN, MRN, CN PRN, VNC, MDRN **Cerebellum** CBX **Spinal cord** **Retina**	**Olfactory cortex** OB, PIR **Cortex** **Basal ganglia** AMY, COA **Hippocampus** **Hypothalamus** PHV, SO, SCH **Brainstem** MG, ME, SOC **Cerebellum** CBX
Nonnervous structures		Choriod plexus		Choroid plexus
Cell type(s)	Neurons	Neurons	Neurons	Neurons

Areas of localization	IGF-II	IGF-II rec	IGBP2	IGBP5
	Olfactory cortex OB **Hippocampus** **Hypothalamus** **Brainstem** **Cerebellum**	**Olfactory cortex** OB **Hippocampus** **Brainstem** **Cerebellum**	**Olfactory cortex** OB **Cerebral cortex** **Hippocampus** **Cerebellum** CBX **Retina**	**Olfactory cortex** OB **Hippocampus** DG **Thalamus** **Brainstem** PG
Nonnervous structures	Meninges Choroid plexus Blood vessels	Meninges Choroid plexus Blood vessels		
Cell type(s)	Astrocytes, neurons (brainstem), mesenchymal cells	Astrocytes, neurons (brainstem, hippocampus), mesenchymal cells	Astrocytes	Neurons

[a]Localization of insulin, IGFs, their receptors, and binding proteins in the mammalian brain after *in situ* hybridization, immunocytochemistry, or receptor autoradiography studies *(see text)*. Major brain divisions are in bold; when applicable, specific areas/nuclei have also been indicated. Abbreviations: Insulin rec, insulin receptor; IGF-I, insulin-like growth factor-I; IGF-I rec, insulin-like growth factor-I receptor; IGF-II, insulin-like growth factor-II; IGF-II rec, insulin-like growth factor-II receptor; IGBP2, insulin-like growth factor binding protein 2; IGBP5, insulin-like growth factor binding protein 5; AMY, amygdala; ARH, arcuate nucleus; CBX, cerebellar cortex; CN, cochlear nuclei; COA, cortical nucleus amygdala; DG, dentate gyrus; DNC, deep cerebellar nuclei; EP, endopiriform nucleus; isl, islands of Calleja; LG, lateral geniculate complex; LIM, limbic region, telencephalon; ME, median eminence; MG, medial geniculate complex; MRN, mesencephalic reticular nucleus; MDRN, medullary reticular nucleus; OB, olfactory bulb; PG, pontine gray; PIR, piriform area; PRN, pontine reticular nucleus; PVH, paraventricular nucleus hypothalamus; SCH, suprachiasmatic nucleus; SEP, septal region; SFO, subfornical organ; SO, supraoptic nucleus; SOC, superior olivary complex; SN, substantia nigra; STR, striatum; TT, taenia tecta; VNC, vestibular nuclei.

brainstem neurons (Sullivan and Feldman, 1994). One recent study has demonstrated that IGF-II is produced predominantly in the leptomeninges, choroid plexus, and parenchymal microvasculature, but becomes localized remote from the site of synthesis, in the myelin sheaths of individual axons and axonal tracts throughout the brain (Logan et al., 1994).

IGFBPs

As mentioned above, the IGFBPs potently modulate the interaction of IGFs with the IGF-I receptor. Accordingly, there is a good degree of correlation with the distribution of the IGF-I and IGF-II mRNAs, except in the neocortex, in which IGFBP2 mRNA disappears soon after birth (Lee et al., 1993). The localization of the IGBP2 mRNA and protein has been found to match closely that of IGF-II protein (Logan et al., 1994). The mRNA is localized almost exclusively to astrocytes in the adult brain, especially in the olfactory bulb, the cerebral and cerebellar cortices, the hippocampus, and the retina (Lee et al., 1993). In agreement, astrocytes, but not neurons produce IGFBP2 in primary cultures from the rat brain (Olson et al., 1991; Ocrant, 1993). Although the IGFBP2 is localized to glial cells, IGFBP5 mRNA has a completely different neuroanatomical distribution, being mainly detected in neurons of sensory relay systems, including the olfactory bulb, the thalamus, the hippocampal dentate gyrus, and the pons (Bondy and Lee, 1993b).

Distribution of Insulin and IGF Receptors in the Adult Brain (Table 1)

Initial studies on the distribution of receptors for insulin and IGFs were carried out using membrane receptor binding or in vitro receptor autoradiography. Later, the development of the immunocytochemical and molecular approaches has allowed for the localization of receptor proteins and mRNAs. We will briefly summarize the main finding derived from the large number of studies on this matter.

Autoradiography Studies

The first reports on the presence of specific insulin receptors in the brain date back to the mid-1970s (Posner et al., 1974; Havrankova et al., 1978). Most subsequent studies have been qualitative (Young et al., 1980; Havrankova et al., 1981; Sara et al., 1982; Hill et al., 1986; Lesniak et al., 1988; Pomerance et al., 1988). However, more recently, a thorough study has been carried out using quantitative autoradiographic localization of [^{125}I]insulin receptor binding sites in the developing and adult rat brain (Kar et al., 1993). [^{125}I]insulin labeling reached its maximal level during the first postnatal week. The regions of the brain that show relatively high densities of binding sites in the adult brain are the olfactory bulb, cerebral cortex, hypothalamus, cerebellum, and choroid plexus. The thalamus, caudate-putamen, and some mesencephalic and brainstem nuclei show a low density of labeling sites in adult rats, in contrast with the high density observed in these areas during neurogenesis (Kar et al., 1993).

As in the case for the insulin receptor, [^{125}I]IGF-I receptor binding sites display marked differences in localization during development and adulthood. The adult level of receptor density is attained in the rat by the third postnatal week, and thereafter there are no significant qualitative and/or quantitative modifications (Kar et al., 1993). High-density IGF-I receptor binding sites in the adult rat brain are found in the olfactory bulb, cerebral cortex, hippocampus, median eminence, cerebellum, and choroid plexus (Bohannon et al., 1988; Lesniak et al., 1988; Pomerance et al., 1988; Araujo et al., 1989; Werther et al., 1989; Matsuo et al., 1991; Kar et al., 1993). As previously noted for insulin receptor binding sites, other brain areas, such as the caudate-putamen, and most hypothalamic, thalamic, mesencephalic, and brainstem nuclei display low levels of IGF-I receptor.

[^{125}I]IGF-II receptor binding sites are evident in several regions of the adult brain, including the olfactory bulb, hippocampus, cerebellum, certain brainstem nuclei, and choroid plexus (Kar et al., 1993; Devaskar et al., 1995). Again, other brain areas, i.e., the caudate-putamen, most hypothalamic and thalamic nuclei, and some mesencephalic nuclei, show a low density of IGF-II binding in adulthood, although very high densities are observed in these regions during development.

Taken together, autoradiographic studies on insulin and IGF's binding sites in the adult brain indicate the existence of substantially overlapping distribution prolifes, with slight temporal differences related to the postnatal age at which the adult pattern is attained. However, it is worth noting that, in any given region, receptor binding sites are concentrated in different anatomical areas.

In Situ *Hybridization and Immunocytochemical Studies*

In studies using Northern blot analysis of whole rat brain homogenates, the patterns of expression of insulin and IGF-I receptor mRNAs have been found to be substantially overlapping during development and in perinatal ages (Baron-Van Evercooren et al., 1991). At the cellular level, insulin receptor mRNA is localized primarily in the olfactory bulb, hippocampus, arcuate nucleus, cerebellum, and choroid plexus (Marks et al., 1990). Similarly, the IGF-I receptor has been localized by *in situ* hybridization in different neuronal populations within numerous areas of the rat brain, including the olfactory bulb, the piriform, cerebral and cerebellar cortices, the suprachiasmatic nucleus, median eminence, supraoptic and paraventricular nuclei, hippocampus, and posteromedial cortical nucleus (Werner et al., 1989b; Bondy, 1991; Marks et al., 1991). Additional areas of localization of the IGF-I receptor mRNA include the medial geniculate and superior olivary nuclei (Bondy and Lee, 1993b). The insulin and IGF-I receptor proteins have been visualized immunocytochemically in neuronal cell bodies and processes that are scattered throughout several areas of the adult brain, such as the olfactory bulb, subfornical organ, cerebral and cerebellar cortices, hippocampus, thalamus, hypothalamus, brainstem, and deep cerebellar nuclei (Fig. 4C,D; 5A) (Noguchi et al., 1987; Andersson et al., 1988; Unger et al., 1989, 1991a,b; Yamaguchi et al., 1990; Bondy, 1991; Garcia-Segura et al., 1991; Folli et al., 1994). Northern blots have demonstrated the presence of substantial amounts of IGF-II receptor mRNA in total brain extracts of the postnatal rat (Ballesteros et al., 1990). The IGF-II receptor mRNA is not observed in neurons, but in the meninges, choroid plexus, and blood vessel mesenchymal cells (Brown et al., 1986; Hynes et al., 1988; Stylianopoulou et al., 1988b; Rothwein et al., 1988). Immunocytochemistry has demonstrated IGF-II receptor immunoreactivity in the olfactory bulb, cerebellum, and choroid plexus, in which it is mainly associated with astrocyte-like cells (Ocrant et al., 1988; Valentino et al., 1988). Recently, by use of a highly selective IGF-II receptor antibody, hippocampal neurons have also been demonstrated to contain immunoreactive receptor (Doré et al., 1996a).

Localization of the Components of the Early Signaling Pathway for the Insulin/IGF Family in the Adult Brain

Phosphorylation of the β-subunit of the insulin/IGF-I receptor initiates a cascade of intracellular events, which eventually end up in regulation of many cellular functions, including gene expression, growth, and proliferation (Fig. 1). Only recently has attention been drawn to the localization of some of the components of the early intracellular signaling pathway for the insulin/IGF-I family of receptors in the adult brain.

High levels of expression of IRS-1 mRNA in the brain have been observed in both humans and rats by Northern blot analysis (Sun et al.,

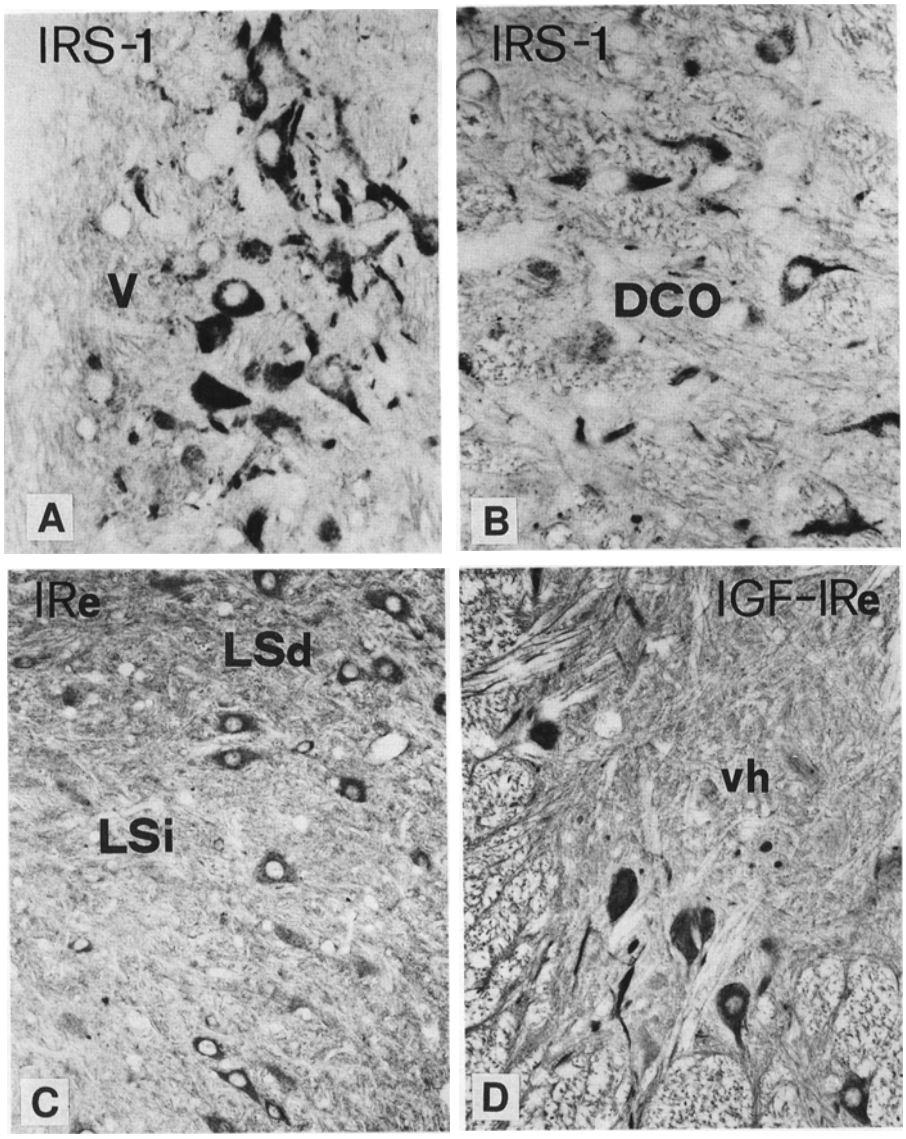

Fig. 4. Immunocytochemical distribution of some components of the early intracellular signaling pathway for the insulin/IGF receptor family in some regions of the CNS of the adult rat. **(A,B)** IRS-1 immunopositive neurons in the brainstem; **(C)** insulin receptor (IRe)-immunoreactive neurons in the septal region; **(D)** IGF-I receptor (IGF-IRe) immunolabeled motor neurons in the spinal cord ventral horn. Abbreviations: V, motor nucleus of the trigeminal nerve; DCO, dorsal choclear nucleus; LSd, lateral septal nucleus, dorsal part; Lsi, lateral septal nucleus, intermediate part; vh, ventral horn. Magnifications: (A–D) 200×.

1992; Araki et al., 1993). More recently, the availability of specific antibodies and cDNA probes has allowed for *in situ* analysis at the cell level. Substantial amounts of the IRS-1 protein have been demonstrated by immunoprecipitation and Western blotting in the adult CNS (Folli et al., 1994). In parallel, immunocytochemical studies have shown that the IRS-1 protein is largely confined to neurons (Figs. 4A,B, 5B), with the exception of the nonneuronal cells of the choroid plexus. Nevertheless, preliminary PCR analysis showed

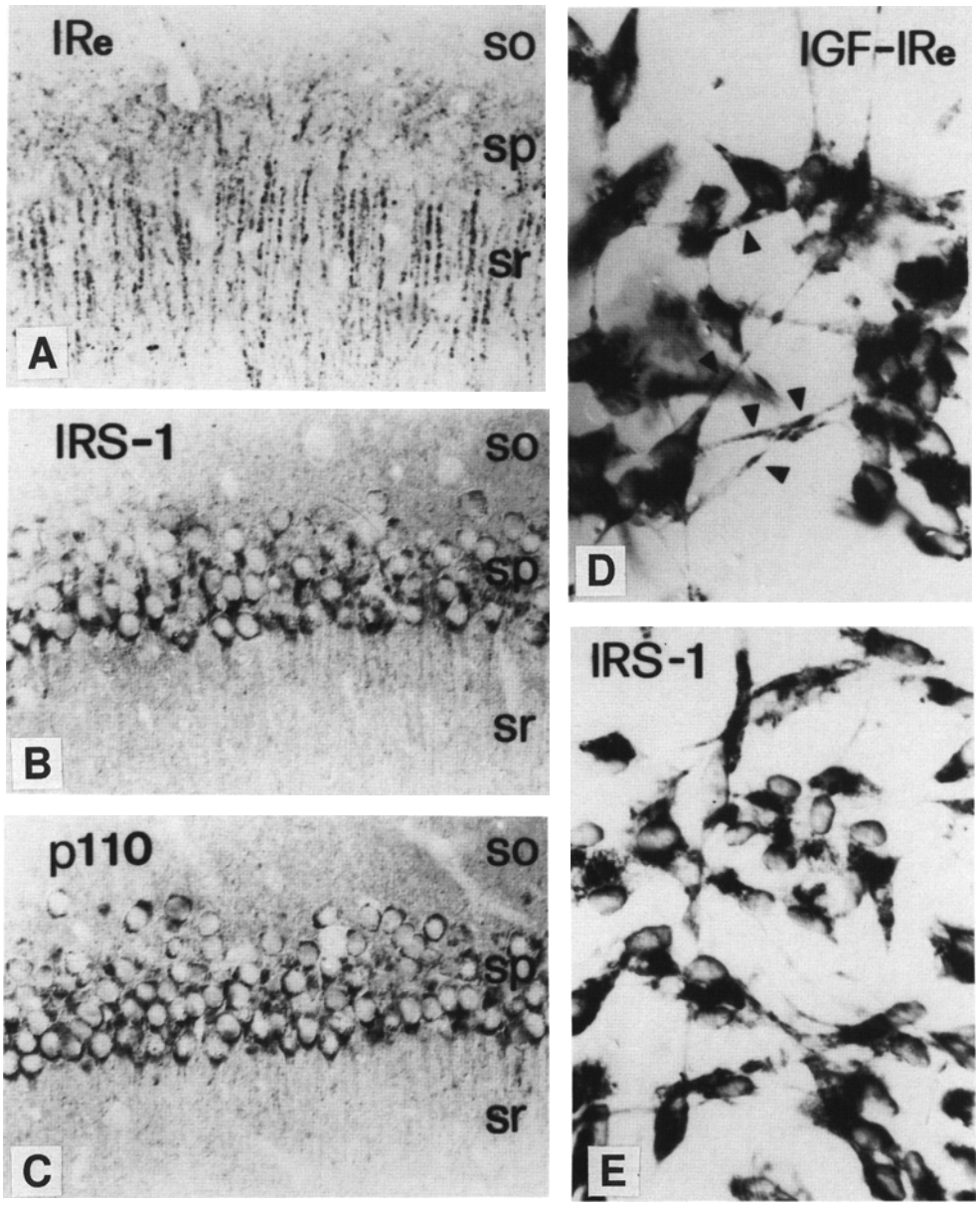

Fig. 5. Immunocytochemical distribution of some components of the early intracellular signaling pathway for the insulin/IGF receptor family in the hippocampal formation **(A–C)** and in the neuroblastoma cell line SY5Y **(D,E)**. In the Ammon's horn, insulin receptor (IRe; A) immunoreactivity is observed at the level of the dendritic trees of the pyramidal hippocampal neurons, which display strong positive staining of their cell bodies after incubation with anti-IRS-1 (IRS-1; B) and anti-PI 3-kinase catalytic subunit (p110; C) antibodies. SY5Y neuroblastoma cells in culture are highly immunoreactive for IGF-I receptor (IGF-IRe; D) and IRS-1 (IRS-1; E). Note that IGF-I receptor immunoreactivity is distributed within cell bodies and processes (D; arrowheads) of virtually all cultured cells, whereas IRS-1 immunoreactivity is mostly localized to the cell somata. Abbreviations: so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum. Magnifications: (A–C) 300×; (D–E) 800×.

the presence of detectable amounts of IRS-1 mRNA in cultured newborn rat oligodendrocytes (McMorris, personal communication).

IRS-1 immunoreactivity has been demonstrated in numerous areas of the rat brain, including the cerebral cortex, hippocampus, many hypothalamic and thalamic nuclei, basal ganglia, cerebellar cortex, brainstem nuclei, lamina X of the spinal cord, and retina (Baskin et al., 1993, 1994; Folli et al., 1994). The presence of IRS-1 immunoreactivity in the olfactory bulb and in the hypothalamic supraoptic and paraventricular nuclei is controversial. In one of their studies, which was focused on the olfactory bulb and hippocampus only, Baskin et al. localized the IRS-1 mRNA to these two areas of the brain (Baskin et al., 1994).

We have correlated the immunocytochemical distributions of the IRS-1 protein and insulin and IGF-I receptors, and have found substantial overlap in the cerebral cortex, the basal ganglia, the hippocampus, the Purkinje neurons of the cerebellum, and the spinal cord. Other neuronal populations, such as the magnocellular neurons of the supraoptic and paraventricular nuclei, and the neurons of the mesencephalic trigeminal nucleus, although being devoid of IRS-1, displayed IGF-I receptor immunoreactivity (Folli et al., 1994). In the brain areas in which such an overlapping distribution was detected, e.g., the hippocampus (Fig. 5A–C), neurons express most of the intracellular receptor signaling molecules and, therefore, are endowed with the cellular machinery to respond to insulin and IGF stimulation.

Since publication of the above studies, the gene for the IRS-2 protein has been cloned (Sun et al., 1995) and significant levels of IRS-2 protein and mRNA have been demonstrated in brain extracts (Folli et al., 1995; Sun et al., 1995). There are no data at present on the tissue distribution of the IRS-2 protein and mRNA within the CNS. However, it seems reasonable to hypothesize that IRS-2 may function in parallel with IRS-1 in certain neuronal populations, or act as an alternative receptor substrate in the neurons that do not express IRS-1, but show significant amounts of insulin and/or IGF-I receptor immunoreactivity.

Other components of the early intracellular signaling pathway of the insulin/IGF-I receptor family have also been localized in neurons of the mature brain. Using an antiserum directed against the p85 regulatory subunit of the PI 3-kinase, we found a completely overlapping distribution of p85 with IRS-1 (Folli et al., 1994). Similar observations have been made on selected brain areas using an antiserum directed against the p110 catalytic subunit of the protein (Fig. 5C) and, at least in hippocampus, there appears to be a high degree of colocalization of PI 3-kinase and IRS-1 in single neurons of the Ammon's horn (Fig. 5B,C). Nonreceptor tyrosine kinases of the Src family, such as *Src*, *Yes*, and *Fyn*, are strongly expressed in the brain and have been suggested to play important functions in the CNS (Grant et al., 1992; Yagi et al., 1995). *Fyn* can be immunocytochemically detected in developing axons throughout the rat brain, whereas in adult animals, immunoreactivity is limited to neuronal cell bodies and processes of the main olfactory bulb, vomero-nasal system, hypothalamus, brainstem, and to certain glial cell types (Bare et al., 1993). In transgenic animals in which the *lac-z* reporter gene expression is driven by the *Fyn* promoter, the *lac-z* positivity is observed in neurons of the hippocampus and olfactory bulb (Yagi et al., 1995). In keeping with the previously mentioned association of *Fyn* and IRS-1 (Fig. 1), the immunocytochemical distribution of the two proteins in the adult brain seems to be remarkably similar. On the other hand, it should be mentioned here that *in situ* hybridization has localized the *Fyn* mRNA both in neurons and oligodendrocytes (Umemori et al., 1994), whereas IRS-1 is low or absent in nonneuronal cells.

Grb2, an adapter protein that interacts with IRS-1 (Fig. 1), is present in synaptic fractions and binds to four major protein components: mSos, dynamin, p145, and synapsin I (McPherson et al., 1994a). Although there are no data on immunocytochemical distribution of Grb2 in the brain, the localization of p145 and

dynamin in the hippocampus (McPherson et al., 1994b) is in general agreement with the association of Grb2 and IRS-1.

In Vitro Studies on Neural Cells and Cell Lines

In vitro observations have helped to elucidate some of the functions of insulin and IGFs on nerve cell proliferation, differentiation, and maintenance. In general, these studies have shown that cultured neurons and neuroblastoma cell lines are capable of responding to insulin and IGFs in terms of cell growth and metabolic effects in a manner similar to that of nonneural cells (Puro and Agardh, 1984; Heidenreich et al., 1991; Raizada, 1991; Unger et al., 1991a; Ang et al., 1993; Wozniak et al., 1993). We will only briefly summarize here the more recent findings related to this issue.

IGF-I promotes survival and stimulates neurite outgrowth from cultured central and peripheral neurons, including mesencephalic dopaminergic nerve cells, the forebrain cholinergic neurons, and spinal cord motor neurons (Bozyczko-Coyne et al., 1993; Beck, 1994). In primary cultures from E15 rat brain, IGF-I receptors are present and the proliferation of neuronal precursor cells is stimulated by IGF-I and IGF-II via the activation of this receptor (Nielsen et al., 1991). In keeping with these findings, in primary cultures of E15 mouse septal neurons, IGF-I and, to a much greater extent, IGF-II have been shown to promote the differentiation, maintenance, and regeneration of cholinergic nerve cells (Konishi et al., 1994). More recent studies using serum-free cultures of lumbosacral sympathetic ganglia have indicated an important role for both IGFs in the control of neurogenesis and neurite outgrowth (Zachenfels et al., 1995). IGF-I has also been shown to promote differentiated cell growth in olfactory bulb organotypic cultures when added to the culture medium in association with bFGF (Russo and Werther, 1994). A cooperative effect of IGF-I and bFGF has also been demonstrated on the developmental expression/survival of NPY-expressing neurons from cultures of 17-d-old rat cerebral cortex (Barnea and Cho, 1993) and on the proliferation and survival of neuroepithelial cells (Drago et al., 1991).

IGF-I stimulates oligodendrocyte development and myelination in rat brain aggregated cultures (Mozell and McMorris, 1991). This has been confirmed in other experiments that show that IGF-I and IGF-II increase the number of oligodendrocytes in culture, and IGF-I promotes the proliferation and regeneration of oligodendrocytes and their precursors in parallel with increased myelin gene expression (McMorris et al., 1993).

The intracellular cascade of events mediated in vitro by stimulation of the insulin and IGF receptors is similar to that reported in vivo. In reaggregated cortical culture of both neurons and astrocytes, insulin and IGF-I enhance tyrosine phosphorylation of several proteins (Heidenreich et al., 1991; Girault et al., 1992). These effects are likely owing to increased RNA synthesis with an involvement of cAMP and protein kinase-C (Cortizo et al., 1991). Likewise, studies on primary cultures of neuronal and glial cells from newborn rats have demonstrated that stimulation by insulin and/or IGF-I induces phosphotyrosine phosphorylation of the receptor β-subunit and pp185 (IRS-1 or IRS-2) (Shemer et al., 1987a,b).

Other insights on the functional role of the IGFs have come from studies on neuronal-derived cell lines. Observations on the N18 mouse neuroblastoma cell line have shown that these cells respond to insulin and IGF-I with rapid phosphorylation of a 185-kDa protein that very likely corresponds to one of the IRS molecules (Adamo et al., 1993; Shemer et al., 1989). In keeping with these findings, we have observed that the neuroblastoma cell line SY5Y contains high levels of IGF-I receptor and IRS-1 protein immunoreactivity (Fig. 5D,E). In these cells, IRS-1 as well as phosphotyrosine-containing proteins are functionally coupled to PI 3-kinase after insulin and IGF-I, but not NGF challenge (Fig. 6). These findings further emphasize that receptors of the insulin/IGF family and neurotrophin receptors utilize dif-

metabolic studies relying on glucose-uptake techniques. Indeed, the neuroanatomical patterns of IGF receptor gene expression and binding sites closely correlate with the patterns of metabolic activity as revealed by autoradiographic localization of ^{14}C-2-deoxyglucose (2-DG) in many brain regions and particularly in sensory projection systems, such as the olfactory bulb, the medial geniculate, and the superior olivary nuclei (Sokoloff, 1981; Nehlig et al., 1988; Guthrie et al., 1990). Microinjection of IGF-1 into the cerebral cortex induces an immediate increase in 2-DG uptake, whereas bFGF and NGF have no effect (Bondy and Lee, 1993a). In vivo ^{14}C-leucine administration also reveals impressive similarities in the pattern of protein synthetic activity and IGF-I receptor gene expression (Werner et al., 1989a; Smith, 1991). Therefore, the IGF-I receptor seems to be deeply involved in the regulation of CNS metabolism.

Insulin and IGFs may also play a role in neuromodulatory and neuroendocrine processes, such as the regulation of feeding behavior, possibly by interaction with classic neurotransmitters (Unger et al., 1991a; Bondy and Lee, 1993a). IGF-I has also been reported to act as a neuromodulator of specific somatosensory systems (Bohannon et al., 1988).

Posttranslational processing of the IGF-I prohormone in the human brain results in two peptides that display distinct functions in the CNS (Sara et al., 1986, 1989). The first is a truncated form of IGF-I, which lacks the amino-terminal tripeptide GPE (gly-pro-glu), and likely represents the major gene product and biologically active form of IGF-I in the CNS, since no evidence for the presence of intact IGF-I has been obtained in the nervous tissue (Sara and Hall, 1990). Truncated IGF-I displays enhanced neurotrophic activity in vivo and in vitro (Sara et al., 1991), but systemic administration of truncated IGF-I, as opposed to intact IGF-I, fails to induce growth response in neonatal rats, possibly because of its short half-life in the circulation (Drakenberg et al., 1990). On the other hand, truncated IGF-I, and not intact IGF-I, displays potent growth-promoting activity after grafting of embryonic brain tissue in various areas of the CNS (Giacobini et al., 1990).

The second proteolytic product of the IGF-I prohormone is the tripeptide GPE (Sara and Carlsson-Swirut, 1990). GPE does not interact with the IGF receptors, and does not display growth promoting activity either in vivo or in vitro (Sara et al., 1989). Instead, GPE interacts with the NMDA glutamate receptor (Sara et al., 1989, 1991), potentiating the release of dopamine in rat striatal slices and the release of acetylcholine, in this latter case with a yet unclarified mechanism. Thus, GPE is believed to act as a neuromodulator.

Other studies have strenghtened the idea that IGF-I might act as a neuromodulator, rather than a trophic factor, in the adult brain. For example, it has been demonstrated that coadministration of IGF-I and glutamate through a microdyalisis probe stereotaxically implanted into the cerebellar cortex and the deep cerebellar nuclei greatly depresses the release of GABA, which normally follows a glutamate pulse (Castro-Alamancos and Torres-Aleman, 1993). Also, evidence has accumulated suggesting that IGF-I is produced by the inferior olive neurons, transported orthogradely, and released in the cerebellar cortex on electrical stimulation (Nieto-Bona et al., 1993). Even more recently, it has been demonstrated that learning of the conditioned eyeblink response is impaired after injection of an IGF-I antisense oligonucleotide in the inferior olive neurons (Castro-Alamancos and Torres-Aleman, 1994). These findings opened the intriguing possibility that IGF-I might be involved in the learning-related phenomenon of the cerebellar long-term depression (LTD) (Ito, 1989). In addition, since LTD is triggered by the activation of the NMDA receptor, it is possible that the GPE peptide, rather than intact IGF-I, is responsible for the neuromodulatory effect in the CNS. Several tyrosine kinase inhibitors block long-term potential (LTP) in the hippocampus, further emphasizing the possible role of IGF-I in synaptic transmission (O'Dell et al., 1991), as suggested for other

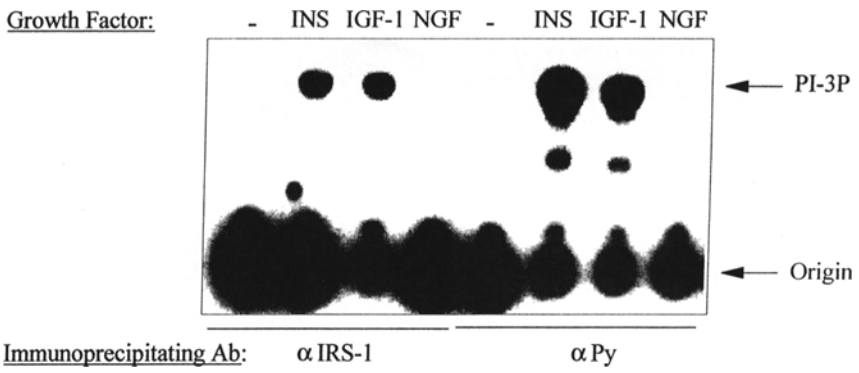

Fig. 6. SY5Y neuroblastoma cells cultured in a chemically defined medium were challenged with insulin (100 n*M*), IGF-I (10 n*M*), and NGF (10 n*M*) for 10 min. At the end of the incubation period, cells were lysed and cleared lysates were immunoprecipitated with anti-IRS-1 or antiphosphotyrosine antibodies and protein A-Sepharose. PI-3 kinase activity was assayed as previously described (Folli et al., 1992). Origin, origin of phospholipid migration; PI-3P, position of PI-3P standard.

ferent intracellular signal transduction pathways (Saltiel and Ohmichi, 1993).

Recent data suggest that both IGFs activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3 (Ma et al., 1994), a finding of particular interest in view of the hypothesized involvement of the estrogen receptor in the prenatal maturation of nerve cells. Finally, in another neuroblastoma cell line (IMR-5), the IRR has been demonstrated (Kovacina and Roth, 1995). Taken together, these observations indicate that transformed neuronal cells express all three types of receptors for the members of the insulin/IGF-I family and are endowed with at least some of the intracellular molecules that enable the cell to transduce the receptor-mediated signal.

In Vivo Studies on Insulin/IGF Function in the Adult Brain

The concept that neurons require functionally active cellular signaling machinery to respond to a growth factor during development appears to be an obvious consequence of the growth factor paradigm. However, the functional significance of numerous neurons within the adult CNS, which are endowed with some of the molecular structures to respond to the growth factors of the insulin/IGF family, is more difficult to understand.

The presence of insulin and IGFs, their receptors, and the receptor substrates in certain specific areas of the CNS, which physiologically display a certain degree of plasticity immediately after birth and in adulthood, such as the olfactory bulb, the dentate gyrus of the hippocampus, the supraoptic nucleus, and the cerebellar cortex (Gage et al., 1995), seems likely to be related to a "classic" neurotrophic effect. In commenting on the IGF-I mRNA localization in the olfactory bulb, hippocampus, and cerebellum, it was suggested that IGF-I might have a role in the stimulation of the neurogenesis in vivo (Bartlett et al., 1991), although the spatial correlation with the known sites of neurogenesis in the postnatal and adult brain does not completely match at the cell level.

However, what is the function of insulin and IGFs in the several other areas of the brain, which apparently do not display any significant degree of physiological remodeling after birth? In the following discussion, we will summarize the most significative data on the biological effects of these polypeptides in vivo, with special reference to IGF-I.

Some clues on the possible function(s) of IGF-I in the mature brain may be obtained from

growth factors, such as EGF (Terlau and Seifert, 1989) and bFGF (Terlau and Seifert, 1990).

Genetic Models

The development of transgenic animals by recombinant DNA technology has added further data to our knowledge of the biological effects of insulin and IGFs in the brain. Developmental analysis of growth kinetics in mouse embryos carrying null mutation of the IGF-I/IGF-II and IGF-I receptor genes, alone or in combination, indicates that between E11 and 12.5, the IGF-I receptor serves the in vivo mitogenic signaling of IGF-II, whereas from E13.5 onward, IGF-I receptor interacts with both IGF-I and IGF-II (Backer et al., 1993). IGF-II also appears to recognize an additional receptor (XR), which may or may not be the insulin receptor.

Mutations of the genes encoding IGF-I or IGF-II result in severe growth deficiency (60% of normal birthweight) in homozygous mice (DeChiara et al., 1990, 1991; Liu et al., 1993). Depending on the genetic background, some of the IGF-I(−/−) dwarfs die shortly after birth, whereas others survive and reach adulthood. In contrast, null mutants for the IGF-I receptor gene invariably die at birth and exhibit severe growth deficiency (45% normal size). In this case, the IGF-I receptor (−/−) embryos show increased cellular densities in the spinal cord mantle zone at E14.5–E18.5, and the ratio of mutant to wild-type cell densities is progressively reduced with developmental age. On this basis, it has been hypothesized that the increase in cell density in the mutant is not absolute, but rather depends on the reduction in the area of the surrounding neuropil (Liu et al., 1993). In addition, preliminary data suggest that, in the mutants, significantly fewer oligodendrocyte precursors develop in vitro to a mature stage (Liu et al., 1993). Taken together, these observations indicate a role for IGF-I in the differentiation of specific cell populations, especially oligodendrocytes, rather than a direct effect on cell proliferation.

The results arising from other experiments in transgenic animals overexpressing the IGF-I gene substantiate the above assumption (Carson et al., 1993; D'Ercole, 1993; McMorris et al., 1993; Ye et al., 1995). In these animals, brains were larger than those of control mice, owing to an increased cell size. Most, if not all areas of the brain appear to show hypermyelination. At the same time, the myelin content of the transgenic mouse brain is 130% greater than that of controls, primarily as a consequence of an increase in myelin production per oligodendrocyte. In a very recent paper, it has been suggested that the IGF-I-induced hypermyelination is the result of an increase in the number of myelinated axons, as well as in the thickness of myelin sheaths. The latter is owing to stimulation of expression of myelin basic protein and cerebral cortical proteolipid protein by up to 200% (Ye et al., 1995).

Brain growth retardation is also observed in IGFBP1 transgenic mice. These animals carry a fusion gene linking the metallothionin I promoter to a cDNA encoding human IGFBP1. The construct partially mimics the effect of IGF-I gene deletion, possibly by the sequestration of IGF-I from its target receptor (D'Ercole et al., 1994). The phenotype of these mice is less severe than that of the IGF-I receptor (−/−) animals, with only about a 15% reduction of brain weight. Localization of the transgene is consistent with the previously reported distribution of the IGFBPs. More recently, a reduction in brain myelination has been reported in these animals, mainly affecting the cerebral cortex and corpus callosum (Ye et al., 1995). Other relevant information regarding the role of the IGFBP1 has come from studies on transgenic animals overexpressing the protein. Adult heterozygous mice with this transgene displayed retarded brain growth as manifested in a 8–16% reduction of brain weight (D'Ercole et al., 1994).

The above studies have provided some crucial observations concerning the effects of IGFs on glial cells in vivo. However, an in-depth analysis focused on the neuronal cell populations within the CNS has not been carried out in these transgenic models. Considering the

wide body of evidence for an effect of insulin and IGFs on growth, differentiation, and survival of cultured neurons, these unique animals represent important tools for the dissection of the significance in vivo for data obtained in vitro.

In order to understand better the intracellular signaling pathway of the insulin/IGF-I receptor in vivo, IRS-1(−/−) mice have been generated by homologous recombination (Araki et al., 1994; Tamemoto et al., 1994). These mice display a marked reduction in body weight at birth, as well as growth retardation postnatally, remaining at about 50% of normal size throughout life. Furthermore, they are insulin- and IGF-I-resistant with regard to glucose metabolism. Several lines of evidence indicate that in these animals, IRS-2 acts, at least partially, as an alternative substrate to IRS-1 (Patti et al., 1995). We have recently started to analyze the CNS of the IRS-1(−/−) mouse. These brains show a reduction of about 10% in weight, but no macroscopic morphological anomalies. However, some histological changes, including neuronal shrinkage and, possibly, a modest reduction in the number of neurons in the cerebral and cerebellar cortices, are observed (Folli et al., unpublished data). Myelin content in IRS-1(−/−) animals is also reduced to about 75% of normal (McMorris, personal communication).

A number of gene ablation experiments have been carried out with the nonreceptor tyrosine kinases, such as *Src*, *Fyn*, *Yes*, and *Abl*. Thus far, only *Fyn* (−/−) mice have shown clear histological abnormalities in the hippocampus, as well as impaired LTP and spatial learning (Grant et al., 1992). Another strain of *Fyn*-deficient mice has been generated by replacing the SH2, SH3, and tyrosine kinase region of *Fyn* by the *lacZ* gene (Yagi et al., 1995). These animals show a marked alteration of the olfactory system and hippocampus leading to an anomalous suckling behavior. Csk is a recently discovered protein tyrosine kinase that inactivates members of the *Src* family, including *Fyn*, in vitro (Okada et al., 1991; Nada et al., 1993). Interestingly, in csk (−/−) mouse embryo, there is an arrest at the 10–20 somite stage and necrosis of neural tissues, with death at E9–E10 (Imamoto and Soriano, 1993; Nada et al., 1993). This result further emphasizes the possible significance of *Fyn* and other nonreceptor tyrosine kinases in brain neurogenesis.

Implications for Pathology

The wealth of data on the functional role of the IGFs in the developing and adult brain, in parallel with the ongoing human genome analysis studies, have led to some exploitation of the possible implications of these growth factors in certain pathologic conditions, particularly neuronal rescue after hypoxic-ischemic injury and hereditary neurodegenerative diseases.

Recent findings indicate that the IGF system has a protective effect on neuronal death, which normally follows an hypoxic-ischemic condition in the CNS (Gluckman et al., 1993). The effect is demonstrated in the mature brain and is probably owing to the interruption of the mechanisms involved in delayed cell death. The mechanism of action of IGF-I is still poorly understood, but it is tempting to speculate that this growth factor, like NGF, might prevent apoptosis by inhibiting RNA and protein synthesis and suppressing activation of endonucleases that are responsible for cell death (Batistatou and Greene, 1991; Altman, 1992).

Experimental autoimmune encephalomielytis is an animal model for multiple sclerosis in humans, and is characterized by demyelination and inflammatory changes in the CNS. In this model, IGF-I administration has been shown to produce a significant reduction in the number and areas of the demyelinating lesions. The relative mRNA levels for certain myelin-synthesizing enzymes and constitutive proteins is significantly higher in IGF-I-treated rats compared to controls, suggesting a stimulating effect on myelin synthesis (Yao et al., 1995).

In clinical trials, treatment with IGF-I has also been shown to slow the progression of amyotrophic lateral sclerosis (ALS) (Reinhardt et al., 1994). Interestingly, significant differences in the [^{125}I]IGF-I and [^{125}I]IGF-II binding

levels exist in the spinal cord of ALS patients, suggesting an upregulation of the two IGF receptors in this disease state (Doré et al., 1996b; Reinhardt et al., 1994).

Ataxia-telangectasia (A-T) is a multisystem autosomal-recessive disorder defined by cerebellar ataxia, which results in progressive neurodegeneration, teleangectases of the eyes, ears, and parts of the face, immune deficiencies, and a greatly elevated incidence of tumors, especially of the lymphoreticular system. Recently, a candidate gene for A-T was isolated, and although the reported cDNA sequence is incomplete, it clearly is homologous to PI 3-kinase gene family (Savitsky et al., 1995). Particularly interesting members of this family are the *rad3* gene of *Schizosaccharomyces pombe* and its homolog *ESR1 (MEC1)* of *Saccharomyces cereviasiae*. These genes are involved in the checkpoint response to DNA damage and in the dependence of mitosis on completion of DNA replication. In yeasts that are mutated in any one of a group of six "checkpoint rad" genes, which include *rad3*, there is no block in G_2 after radiation damage (Al-Khodairy and Carr, 1992); cells progress into mitosis with damaged DNA and eventually die. Since *rad3* null mutant cells are defective in a DNA-damage checkpoint, it is tempting to speculate that the A-T gene product and *rad3* have homologous functions in different biological systems. On the other hand, the high degree of similarity of the A-T gene product to the PI 3-kinase family members, which are known to be involved in a variety of signal transduction pathways, suggests the possibility that the A-T gene product is in someway involved in preventing programmed cell death (apoptosis) of specific neuronal subpopulations. Indeed, in certain neuronal systems, NGF prevents apoptotic cell death utilizing a PI 3-kinase-dependent pathway (Yao and Cooper, 1995).

Future Directions

The protein tyrosine kinases of the growth factor receptor family and the cytosolic nonreceptor family are commonly regarded as fundamental components of mitogenic pathways. Nevertheless, in the adult CNS, which is typically composed of postmitotic, nonproliferating cells, substantial amounts of tyrosine kinase activity are observed (Adamo et al., 1993). Numerous neuronal populations of the mature brain not only express specific receptors for insulin and IGFs, but are also endowed with some of the components of the early intracellular receptor signaling pathway. Studies on transgenic models have clearly demonstrated a crucial role in brain development for the growth factors of the family, some regulatory molecules (IGFBPs), and intracellular substrates (IRS-1). These studies have also pointed out the existence of an intimate crosstalk between neuron-to-neuron and neuron-to-glial cells, a crosstalk in which the IGFs likely act as leading characters in the scenario of the developing CNS. Clearly more work on these unique and precious models is needed to understand fully the function(s) of insulin and IGFs in the adult brain.

Nevertheless, electrophysiological experiments suggest that in the fully developed brain, the growth factors of the family might not have a trophic role under normal conditions, but rather modulate synaptic transmission in very selected, important brain areas, such as the hippocampus and cerebellum, where the IGFs might be involved in the fundamental phenomena of LTP and LTD, which are crucial to higher brain function. A more "classic" trophic effect is likely to explain the results of some experimental and clinical trials showing that IGF-I is of benefit in the treatment of certain neurodegenerative disorders. Also, as in the case of the A-T gene, mapping of the human genome will likely offer some more hints about the function(s) of protein and lipid kinases and their cellular substrates in the fully mature brain.

The advent of the recombinant DNA technology has dramatically widened our knowledge of the biological activity and mechanism of action of several growth factors and cytokines in the nervous system. Although studies have

mainly focused on effects on cell proliferation, differentiation, and maintenance during development, it is clear that additional, as yet unexpected, functions do occur and are fundamental in the mature brain.

A curious and stimulating parallelism can be drawn with the function of the biologically active neuropeptides, whose discovery in the 1970s represented a major breakthrough in neurobiologic research (Hökfelt et al., 1980). Neuropeptides were initially believed to act almost exclusively as modulators of the synaptic transmission, but later it became clear that they also had a role as trophic factors during development. The ongoing research related to growth factors of the insulin/IGF family is proceeding following a somewhat reverse direction, i.e., these molecules were initially implicated in brain development and now evidence is accumulating that in the adult brain, they might act as modulators of synaptic transmission. This would represent an effective way to assign to the same molecules different functions in the various temporal windows of the development, maturation, and maintenance of the CNS.

Acknowledgments

Part of the experimental work described here was made possible by grants of the Italian MURST (fondi 40 and 60%), CNR, and N.I.H. (DK 31036). Franco Folli was supported by H.S.R., Milano, Italy. We are greatly indebted to Stefano Gustincich and Jes Bruning for stimulating discussion, and to Laura Chiappino for photographic artwork.

References

Adamo M. L., Shemer J., Roberts C. T. Jr., and LeRoith D. (1993) Insulin and insulin-like growth factor-I induced phosphorylation in neurally derived cells. *Ann. NY Acad. Sci.* **692,** 113–125.

Al-Khodairy F. and Carr A. M. (1992) DNA repair mutants defining G2 checkpoint pathways in *Schizosaccharomyces pombe*. *EMBO J.* **11,** 1343–1350.

Alpert S., Hanahan D., and Teitelman G. (1988) Hybrid insulin genes reveal a developmental lineage for pancreatic endocrine cells and imply a relationship with neurons. *Cell* **53,** 295–308.

Altman J. (1992) Programmed cell death: the paths to suicide. *Trends Neurol. Sci.* **15,** 278–280.

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 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.

Araki E., Sun X., Haag B. L. III, Chuang L., Zhang Y., Yang-Feng T. L., Morris F. W., and Kahn C. R. (1993) Human skeletal muscle insulin receptor substrate-1. Characterization on the cDNA, gene, and chromosomal localization. *Diabetes* **42,** 1041–1054.

Araki E., Lipes M. A., Patti M. E., Bruning J. C., Haag B. I., Johnson R. S., and Kahn C. R. (1994) Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* **7,** 43–58.

Araujo D. M., Lapchak P. A., Collier B., Chabot J.-G., and Quirion R. (1989) Insulin-like growth factor-I (somatomedin-C) receptors in the rat brain: distribution and interaction with the hippocampal cholinergic system. *Brain Res.* **484,** 130–138.

Argetsinger L. S., Hsu G. W., Myers M. G. J., Billestrup N., White M. F., and Carter-Su C. (1995) Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. *J. Biol. Chem.* **270,** 14,685–14,692.

Ayer-le-Lievre A., Stählbom P. A., and Sara V. R. (1991) Expression of IGF-I and II mRNA in the brain and cranio-facial region of the rat fetus. *Development* **111,** 105–111.

Bach M. A., Shen-Orr Z., Lowe W. L. Jr., Roberts C. T. Jr., and LeRoith D. (1991) Insulin-like growth factor I mRNA levels are developmentally regulated in specific regions of the rat brain. *Mol. Brain Res.* **10,** 43–48.

Backer J. M., Myers M. G., Shoelson S. E., Chin D. J., Jian Sun X., Miralpeix M., Hu P., Margolis B., Skolnik Y., Schlessinger J., and White M. F. (1992) Phosphatidylinositol 3'-kinase is activated by association with IRS-1 during insulin stimulation. *EMBO J.* **11,** 3469–3479.

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

Ballesteros M., Scott C. D., and Baxter R. C. (1990) Developmental regulation of insulin-like growth factor-II/mannose 6-phosphate receptor mRNA in the rat. *Biochem. Biophys. Res. Commun.* **172,** 775–779.

Bare D. J., Lauder J. M., Wilkie M. B., and Maness P. F. (1993) p59*fyn* in rat brain is localized in developing axon tracts and subpopulations of adult neurons and glia. *Oncogene* **8,** 1429–1436.

Barnea A. and Cho G. (1993) Basic fibroblast growth factor selectively amplifies the functional state of neurons producing neuropeptide Y but not somatostatin in cultures of fetal brain cells: evidence for a cooperative interaction with insulin-like growth factor I. *Endocrinology* **133,** 1895–1898.

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., 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.

Baskin D. G., Sipols A. F., Schwartz M. W., and White M. F. (1993) Immunocytochemical detection of insulin receptor substrate-1 (IRS-1) in rat brain: colocalization with phosphotyrosine. *Regul. Pept.* **48,** 257–266.

Baskin D. G., Schwartz M. W., Sipols A. J., D'Alessio D. A., Goldstein B. J., and White M. F. (1994) Insulin receptor substrate-1 (IRS-1) expression in rat brain. *Endocrinology* **134,** 1952–1955.

Batistatou A. and Greene L. A. (1991) Auriinitricarboxylic acid rescues PC12 cells and sympathetic neurons from cell death caused by nerve growth factor deprivation: correlation with suppression of endonuclease activity. *J. Cell Biol.* **115,** 461–471.

Beck K. D. (1994) Functions of brain-derived neurotrophic factor, insulin-like growth factor-I and basic fibroblast growth factor in the development and maintenance of dopaminergic neurons. *Prog. Neurobiol.* **44,** 497–516.

Bohannon N. J., Corp E. S., Wilcox B. J., Figlewicz 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. and Lee W. (1993a) Patterns of insulin-like growth factor and IGF receptor gene expression in the brain. *Ann. NY Acad. Sci.* **692,** 33–43.

Bondy C. A. and Lee W. (1993b) 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. A., Werner H., Roberts C. T. Jr., and LeRoith D. (1992) 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.

Bozyczko-Coyne D., Glicksman M. A., Prantner J. E., McKenna B., Connors T., Friedman C., Dasgupta M., and Neff N. T. (1993) IGF-I supports the survival and/or differentiation of multiple types of central nervous system neurons. *Ann. NY Acad. Sci.* **692,** 311–313.

Brown A. L., Graham D. E., Nissley S. P., Hill D. J., Strain A. J., and Rechler M. M. (1986) Developmental regulation of insulin-like growth factor II mRNA in different rat tissues. *J. Bio. Chem.* **261,** 13,144–13,150.

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.

Cheatham B. and Kahn C. R. (1995) Insulin action and the insulin signaling network. *Endocrine Rev.* **16,** 117–142.

Cheatham B., Vlahos C., Cheatham L., Wang L., Blenis J., and Kahn C. R. (1994) Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis and glucose transporter translocation. *Mol. Cell. Biol.* **14,** 4902–4911.

Chuang L. M., Hausdorff S. F., Myers M. G. J., White M. F., Birnbaum M. J., and Kahn C. R. (1994)

Interactive roles of Ras, insulin receptor substrate-1, and proteins with Src homology-2 domains in insulin signaling in *Xenopus* oocytes. *J. Biol. Chem.* **269,** 27,645–27,649.

Cortizo A. M., van Arnaldo J., Burgess S. K., and Espinal J. (1991) Insulin and IGF-I stimulated RNA synthesis in primary cultures of neuronal cells: involvement of cyclic AMP and protein kinase-C. *Acta Pysiol. Pharmalcol. Ther. Latinoamericana* **41,** 295–307.

Deltour L., Leduque P., Blume N., Madsen O., Dubois P., Jami J., and Bucchini D. (1993) Differential expression of the two nonallelic proinsulin genes in the developing mouse embryo. *Proc. Natl. Acad. Sci. USA* **90,** 527–531.

D'Ercole A. J. (1993) Expression of insulin-like growth factor-I in transgenic mice. *Ann. NY Acad. Sci.* **692,** 149–160.

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

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.

DeChiara T. M., Robertson E. J., and Efstratiadis A. (1991) Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* **64,** 849–859.

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

Devaskar S. U., Singh B. S., Carnaghi L. R., Rajakumar P. A., and Giddings S. J. (1993) Insulin II gene expression in rat central nervous system. *Regul. Pep.* **48,** 55–63.

Devaskar S. U., Giddings S. J., Rajakumar P. A., Carnaghi L. R., Menon R. K., and Zahm D. S. (1994) Insulin gene expression and insulin synthesis in mammalian neuronal cells. *J. Biol. Chem.* **269,** 8445–8454.

Devaskar S. U., Holekamp N., Karycki L., and Devaskar U. P. (1995) Ontogenesis of the insulin receptors in the rabbit brain. *Horm. Res.* **24,** 319–327.

Doré S., Kar S., and Quirion R. (1995a) Evidence for the presence of two distinct insulin-like growth factor (IGF-I and IGF-II) receptors in cultured rat hippocampal neurons and different process of internalization. *Neuroscience,* in press.

Doré S., Krieger C., Kar S., and Quirion R. (1996b) Distribution and levels of insulin-like growth factors (IGF-I and IGF-II) and insulin receptor binding sites in spinal cords of amyotrophic lateral sclerosis (ALS) patients. *Mol. Brain Res.* **41,** 128–223.

Drago J., Murphy M., Carroll S. M., Harvey A. R., 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.

Drakenberg K., Östenson G.-C., and Sara V. R. (1990) Circulating forms and biological activity of intact and truncated insulin-like growth factor-1 (IGF-1) in adult and neonatal rat. *Acta Endocrinol.* **123,** 43–51.

Ebina Y., Ellis L., Jarnagin K., Edery M., Graf L., Clauser E., Ou J., Masiar F., Kan Y. W., Goldfine I. D., Roth R. A., and Rutter W. J. (1985) The human insulin receptor cDNA: the structural basis for hormone activated transmembrane signalling. *Cell* **40,** 747–758.

Escobedo J. A., Navankasattusas S., Kavanaugh W. M., Milfay D., Fried V. A., and Williams L. T. (1991) cDNA cloning of a novel 85 kD protein that has SH2 domains and regulates binding of PI3-kinase to the PDGF beta-receptor. *Cell* **65,** 75–82.

Fantl W. J., Johnson D. E., and Williams L. T. (1993) Signalling by receptor tyrosine kinases. *Ann. Rev. Biochem.* **62,** 453–481.

Feener E. P., Backer J. M., King G. L., Wilden P. A., Sun X., Kahn C. R., and White M. F. (1993) Insulin stimulates serine and tyrosine phosphorylation in the juxtamembrane region of the insulin receptor. *J. Biol. Chem.* **268,** 11,256–11,264.

Folli F., Saad M. J. A., Backer J. M., and Kahn C. R. (1992) Insulin stimulation of phosphatidylinositol 3-kinase activity and association with insulin receptor substrate 1 in liver and muscle of the intact rat. *J. Biol. Chem.* **267,** 22,171–22,177.

Folli F., Bonfanti L., Renard E., Kahn C. R., and Merighi A. (1994) Insulin receptor substrate-1 (IRS-1) distribution in the rat central nervous system. *J. Neurosci.* **14,** 6412–6422.

Folli F., Patti M. E., Chen C., Ghidella S., Merighi A., and Kahn C. R. (1995) Roles of the insulin-IGF-1 receptor signalling network in the central nervous system. *Proc. 77th Ann. Mtg. Endocrine Soc.,* p. 515 (abstract).

Franke T. F., Yang S., Chan T. O., Datta K., Kazlauskas A., Morrison D. K., Kaplan D. R., and Tsichlis P. N. (1995) The protein kinase encoded by the Akt protooncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* **81,** 727–736.

Gage F. H., Ray J., and Fisher L. J. (1995) Isolation, characterization, and use of stem cells from the CNS. *Ann. Rev. Neurosci.* **18,** 159–192.

Gammeltoft S., Haselbacher G. K., Humbel R. E., Fehlmann M., and Van Obberghen E. (1985) Two types of receptor for insulin-like growth factors in mammalian brain. *EMBO J.* **4,** 3407–3412.

Gammeltoft S., Christiansen J., Nielsen F. C., and Verland S. (1991) Insulin-like growth factor II: complexity of biosynthesis and receptor binding, in *Molecular Biology and Physiology of Insulin and Insulin-Like Growth Factors* (Raizada M. K. and LeRoith D., eds.), Plenum, New York, pp. 31-44.

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. B., Olson M. J., Hoffer B. J., and Sara V. R. (1990) Truncated IGF-1 exerts trophic effects on fetal brain tissue grafts. *Exp. Neurol.* **108,** 33–39.

Girault J. A., Chamak B., Bertuzzi G., Tixier H., Wang J. K., Pang D. T., and Greengard P. (1992) Protein phosphotyrosine in mouse brain: developmental changes and regulation by epidermal growth factor, type I insulin-like growth factor, and insulin. *J. Neurochem.* **58,** 518–528.

Gluckman P. D., Guan J., Beilharz E. J., Klempt N. D., Klempt M., Miller O., Sirimanne E., Dragunow M., and Williams C. E. (1993) The role of the insulin-like growth factor system in neuronal rescue. *Ann. NY Acad. Sci.* **262,** 138–148.

Gout I., Dhand R., Hiles I. D., Fry M. J., Panayotou G., Das P., Truong O., Totty N. F., Hsuan J., and Booker G. W. (1993) The GTPase dynamin binds to and is activated by a subset of SH3 domains. *Cell* **75,** 25–36.

Grant S. G., O'Dell T. J., Karl K. A., Stein P. L., Soriano P., and Kandel E. R. (1992) Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. *Science* **258,** 1903–1910.

Green B. N., Jones S. B., Streck R. D., Wood T. L., Rotwein P., and Pintar J. E. (1994) Distinct expression patterns of insulin-like growth factor binding proteins 2 and 5 during fetal and postnatal development. *Endocrinology* **134,** 954–962.

Guthrie K. M., Wilson D. A., and Leon M. (1990) Early unilateral deprivation modifies olfactory bulb function. *J. Neurosci.* **10,** 3402–3412.

Hansson H.-A., Nilsson A., Isgaard J., Billing H., Isaksson O., Skottner A., Andersson I. K., and Rozell B. (1988) Immunohistochemical localization of insulin-like growth factor I in the adult rat. *Histochemistry* **89,** 403–410.

Havrankova J., Roth J., and Brownstein M. (1978) Insulin receptors are widely distributed in the central nervous system of the rat. *Nature* **272,** 827–829.

Havrankova J., Brownstein M., and Roth J. (1981) Insulin and insulin receptors in rodent brain. *Diabetologia* **20,** 268–273.

Heidenreich K. A., Toledo S. P., and Kenner K. A. (1991) Regulation of protein phosphorylation by insulin and insulin like growth factors in cultured fetal neurons. *Adv. Exp. Med. Biol.* **293,** 379–384.

Hiles I. D., Otsu M., Volinna S., Fry M. J., Gout I., Dhand R., Panayotou G., Ruiz-Larrea F., Thompson A., Totty N. F., Hsuan J., Courtneidge S. A., Parker P. J., and Waterfield M. D. (1992) Phosphatidylinositol 3-kinase: structure and expression of the 110 kD catalytic subunit. *Cell* **70,** 419–429.

Hill J. M., Lesniak M. A., Pert C. B., and Roth J. (1986) Autoradiographic localization of insulin receptors in rat brain: prominence in olfactory and limbic areas. *Neuroscience* **17,** 1127–1138.

Hökfelt T., Johansson O., Ljungdahl Å., Lundberg J. M., and Schultzberg M. (1980) Peptidergic neurones. *Nature* **284,** 515–521.

Hubbard S. R., Wei L., Ellis L., and Hendrickson W. A. (1994) Crystal structure of the tyrosine kinase domain of the human insulin receptor. *Nature* **372,** 746–-754.

Hung J., Granner T. C., Emos K. P., Kazlauskas A., and Blenis J. (1994) PDGF and insulin-dependent pp70S6k activation mediated by phospatidylinositol 3-OH kinase. *Nature* **370,** 71–75.

Hunter T. (1995) When is a lipid kinase not a lipid kinase? When it is a protein kinase. *Cell* **83,** 1–4.

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.

Imamoto A. and Soriano P. (1993) Disruption of the csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. *Cell* **73,** 1117–1124.

Irminger J., Rosen K. M., Humbel R. E., and Villa-Komaroff L. (1987) Tissue-specific expression of insulin-like growth factor II mRNAs with distinct 5' untranslated regions. *Proc. Natl. Acad. Sci. USA* **84,** 6330–6333.

Ito M. (1989) Long-term depression. *Ann. Rev. Neurosci.* **12,** 85–102.

Jones J. I. and Clemmons D. R. (1995) Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* **16,** 3–34.

Kar S., Chabot J.-G., and Quirion R. (1993) Quantitative autoradiographic localization of [^{125}I] insulin-like growth factor I, [^{125}I] insulin-like growth factor II, and [^{125}I]insulin receptor binding sites in developing and adult rat brain. *J. Comp. Neurol.* **333,** 375–397.

Kasuga M., Hedo J. A., Yamada K. M., and Kahn C. R. (1982a) The structure of the insulin receptor and its subunits: evidence for multiple nonreduced forms and a 210K possible proreceptor. *J. Biol. Chem.* **257,** 10,392–10,399.

Kasuga M., Karlsson F. A., and Kahn C. R. (1982b) Insulin stimulates the phosphorylation of the 95,000-dalton subunit of its own receptor. *Science* **215,** 185–187.

Kasuga M., Zick Y., Blithe D. L., Crettaz M., and Kahn C. R. (1982c) Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system. *Nature* **298,** 667–669.

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.

Kornfeld S. (1987) Trafficking of lysosomal enzymes. *FASEB J.* **1,** 462–468.

Kornfeld S. (1992) Structure and function of the mannose 6-phospate/insulin-like growth factor II receptors. *Ann. Rev. Biochem.* **61,** 307–330.

Kovacina K. S. and Roth R. A. (1995) Characterization of the endogenous insulin receptor-related receptor in neuroblastomas. *J. Biol. Chem.* **270,** 1881–1887.

Kuhne M. R., Pawson T., Lienhard G. E., and Feng G. S. (1993) The insulin receptor substrate-1 associates with the SH2-containing phosphotyrosine phosphatase 5YP. *J. Biol. Chem.* **268,** 11,479–11,481.

Lammers R., Gray A., Schlessinger J., and Ullrich A. (1989) Differential signaling potential of insulin and IGF-I receptor cytoplasmic domains. *EMBO J.* **8,** 1369–1375.

Lee J. E., Pintar J., and Efstratiadis A. (1990) Pattern of the insulin-like growth factor II gene expression during early mouse embryogenesis. *Development* **110,** 151–159.

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

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.

Lesniak M. A., Hill J. M., Kiess W., Rojeski M., Pert C. B., and Roth J. (1988) Receptors for insulin-like growth factors I and II: autoradiographic localization in rat brain and comparison to receptors for insulin. *Endocrinology* **123,** 2089–2099.

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 (Igf1r). *Cell* **75,** 59–72.

Logan A., Gonzales 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.

Lowenstein E. J., Daly R. G., Batzer A. G., Li W., Margolis B., Lammers R., Ullrich A., Skolnik E. Y., Bar-Sagi D., and Schlessinger J. (1992) The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* **70,** 431–442.

Lund P. K., Moats-Staats B. M., Hynes M. A., Simmons J. G., Jansen M., D'Ercole A. J., and Van Wyk J. J. (1986) Somatomedin-C/insulin-like growth factor I and insulin-like growth factor II mRNAs in rat fetal and adult tissues. *J. Biol. Chem.* **261,** 14,539–14,544.

Ma Z. Q., Santagati S., Patrone C., Pollio G., Vegeto E., and Maggi A. (1994) Insulin-like growth factors activate estrogen receptor to control the growth and differentiation of the human neuroblastoma cell line SK-ER3. *Mol. Endocrinol.* **8,** 910–918.

Marks J. L., Porte D., Stahl W. L., and Baskin D. G. (1990) Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology* **127,** 3234–3236.

Marks J. L., Porte D., and Baskin D. G. (1991) Localization of type I insulin-like growth factor recep-

tor messenger RNA in the adult rat brain by in situ hybridization. *Mol. Endocrinology* **5,** 1158–1168.

Massague J., Pilch P. F., and Czech M. P. (1982) Electrophoretic resolution of three major insulin receptor structures with unique subunit stoichiometries. *Proc. Natl. Acad. Sci. USA* **77,** 7137–7141.

Matsuo K., Niwa M., Kurihara M., Shigematsu K., Yamashita S., Ozaki M., and Nagataki S. (1991) Receptor autoradiographic analysis of insulin-like growth factor-I (IGF-I) binding sites in rat forebrain and pituitary gland. *Cell. Mol. Neurobiol.* **9,** 357–367.

McMorris F. A., Mozell R. L., Carson M. J., Shinar Y., Meyer R. D., and Marchetti N. (1993) Regulation of oligodendrocyte development and central nervous system myelination by insulin-like growth factors. *Ann. NY Acad. Sci.* **262,** 321–334.

McPherson P. S., Czernik A. J., Chilcote T. J., Onofri F., Benfenati F., Greengard P., Schlessinger J., and DeCamilli P. (1994a) Interaction of Grb2 via its Src homology 3 domains with synaptic proteins including synapsin I. *Proc. Natl. Acad. Sci. USA* **91,** 6486–6490.

McPherson P. S., Takei K., Schmid S. L., and DeCamilli P. (1994b) p145, a major grb2-binding protein in brain, is co-localized with dynamin in nerve terminals where it undergoes activity-dependent dephosphorylation. *J. Biol. Chem.* **269,** 30,132–30,139.

Mermaridis D. G., Morse D. E., Pansky B., and Budd G. C. (1990) Insulin immunoreactivity in the fetal and neonatal rat retina. *Neurosci. Lett.* **118,** 116–119.

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.

Myers M. G., Jr., Granner T. C., Wang L. M., Sun X. J., Pierce J. H., Blenis J., and White M. F. (1994) Insulin receptor substrate-1 mediates phospatidylinositol 3' kinase and $p70^{S6K}$ signaling during insulin, insulin-like growth factor 1 and interleukin-4 stimulation. *J. Biol. Chem.* **269,** 28,783–28,789.

Myers M. G. J., Backer J. M., Sun X., Shoelson S. E., Hu P., Schlessinger J., Yoakim M., Schaffhausen B., and White M. F. (1992) IRS-1 activates phosphatidylinositol 3'-kinase by associating with src homology 2 domains of p85. *Proc. Natl. Acad. Sci. USA* **89,** 10,350–10,354.

Myers M. G. J., Sun X., Cheatham B., Jachna B. R., Glasheen E. M., Backer J. M., and White M. F. (1993) IRS-1 is a common element in insulin and insulin-like growth factor-I signaling to the phosphatidylinositol 3'-kinase. *Endocrinology* **132,** 1421–1430.

Myers M. G. J., Sun X., and White M. F. (1995) The IRS-1 signaling system. *TIBS* **19,** 289–294.

Nada S., Yagi T., Takeda H., Tokunaga T., Nakagawa H., Ikawa Y., Okada M., and Aizawa S. (1993) Constitutive activation of Src family kinases in mouse embryos that lack Csk. *Cell* **73,** 1125–1135.

Nehlig A., De Vasconcelos A. P., and Boyet S. (1988) Quantitative autoradiographic measurement of local cerebral glucose utilization in freely moving rats during post-natal development. *J. Neurosci.* **8,** 2321–2333.

Nielsen F. C. and Gammeltoft S. (1990) Mannose-6-phosphate stimulates proliferation of neuronal precursor cells. *FEBS Lett.* **262,** 142–144.

Nielsen F. C., Wang E., and Gammeltoft S. (1991) Receptor binding, endocytosis, and mitogenesis of insulin-like growth factors I and II in fetal rat brain neurons. *J. Neurochem.* **56,** 12–21.

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.

Noguchi T., Kurata L. M., and Sugisaki T. (1987) Presence of somatomedin-C immunoreactive substance in the central nervous system: immunohistochemical mapping studies. *Neuroendocrinology* **46,** 277–282.

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

Ocrant I. (1993) Insulin-like growth factor binding proteins in nervous-tissue-derived cells. *Ann. NY Acad. Sci.* **692,** 44–50.

O'Dell T. J., Kandel E. R., and Grant S. G. N. (1991) Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. *Letters to Nature* **353,** 558–560.

Okada M., Nada S., Yamanashi Y., Yamamoto T., and Nakagawa H. (1991) CSK: a protein-tyrosine kinase involved in regulation of src family kinases. *J. Biol. Chem.* **266,** 24,249–24,252.

Olson J. A. J., Shiverick K. T., Ogilvie S., Buhi W. C., and Raizada M. K. (1991) Developmental expression of rat insulin-like growth factor binding protein-2 by astrocytic glial cells in culture.

Endocrinology **129,** 1066–1074.
Otsu M., Hiles I., Gout I., Fry M. J., Ruis-Larrea F., Panayotou G., Thompson A., Dhand R., Hsuan J., Totty N., Smith A. D., Morgan S. J., Courtneidge S. A., Parker P. J., and Waterfield M. D. (1991) Characterization of two 85 kD proteins that associate with receptor tyrosine kinases, middle-T/pp60c-src complexes and PI3-kinase. *Cell* **65,** 91–104.
Park G. H. and Buetow D. E. (1991) Genes for insulin-like growth factors I and II are expressed in senescent rat tissues. *Gerontology* **37,** 310–316.
Patti M., Sun X., Bruening J. C., Araki E., Lipes M. A., White M. F., and Kahn C. R. (1995) 4PS/insulin receptor substrate (IRS)-2 is the alternative substrate of the insulin receptor in IRS-1-deficient mice. *J. Biol. Chem.* **270,** 24,670–24,673.
Pawson T. (1995) Protein modules and signalling networks. *Nature* **373,** 573–580.
Perez-Villamil B., De la Rosa E. J., Morales A. V., and De Pablo F. (1994) Developmentally regulated expression of the preproinsulin gene in the chicken embryo during gastrulation and neurulation. *Endocrinology* **135,** 2342–2350.
Petruzzelli L. M., Gangaly S., Smith C. R., Cobb M. H., Rubin C. S., and Rosen O. M. (1982) Insulin activates a tyrosine-specific protein kinase in extracts of 3T3-L1 adipocytes and human placenta. *Proc. Natl. Acad. Sci. USA* **79,** 6792–6796.
Pillay T. S., Whittaker J., Lammers R., Ullrich A., and Siddle K. (1991) Multisite serine phosphorylation of the insulin and IGF-I receptors in transfected cells. *FEBS Lett.* **288,** 206–211.
Pomerance M., Gavaret J., Jacquemin C., Matricon C., Toru-Delbauffe D., and Pierre M. (1988) Insulin and insulin-like growth factor I receptors during postnatal development in the rat brain. *Dev. Brain Res.* **62,** 169–175.
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., Asano T., Glasheen E. M., Miralpeix M., Weiland A., Zhang Y., Myers M. G. J., Sun X., and White M. F. (1995) The structure and function of p55pik reveals a new regulatory subunit for the phosphatidyinositol-3 kinase. *Mol. Cell. Biol.* **15,** 4453–4465.
Posner B. I., Kelly P. A., Shiu R. P. C., and Friesen H. G. (1974) Studies of insulin, growth hormone and prolactin binding: tissue distribution, species variations and characterization. *Endocrinology* **95,** 521–531.
Puro D. G. and Agardh E. (1984) Insulin-mediated regulation of neuronal maturation. *Science* **225,** 1170–1172.
Raizada M. K. (1991) Insulin-like growth factor I: a possible modulator of intercellular communication in the brain, in *Molecular Biology and Physiology of Insulin and Insulin-Like Growth Factors* (Raizada M. K. and LeRoith D, eds.), Plenum, New York, pp. 493–505.
Reinhardt R. R., Chin E., Zhang B., Roth R. A., and Bondy C. A. (1994) Selective coexpression of insulin receptor-related receptor (IRR) and TRK in NGF-sensitive neurons. *J. Neurosci.* **14,** 4674–4683.
Rothenberg P. L., Lane W. S., Karasik A., Backer J., White M., and Kahn C. R. (1991) Purification and partial sequence analysis of pp185, the major cellular substrate of the insulin receptor tyrosine kinase. *J. Biol. Chem.* **266,** 8302–8311.
Rothwein 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.
Saltiel A. R. and Ohmichi M. (1993) Pleiotropic signaling from receptor tyrosine kinases. *Curr. Opin. Neurobiol.* **3,** 352–359.
Sara V. R. and Carlsson-Swirut C. (1990) Insulin-like growth factors in the central nervous system: biosynthesis and biological role, in *Growth Factors: From Genes to Clinical Application* (Sara V. R., Hall K., and Löw H., eds.), Raven, New York, pp. 179–199.
Sara V. R. and Hall K. (1990) Insulin-like growth factors and their binding proteins. *Physiol. Rev.* **70,** 591–605.
Sara V. R., Hall K., Holtz H. V., Humbel R. E., Sjogren B., and Wetterberg L. (1982) Evidence for the presence of specific receptors for insulin-like growth factors 1 (IGF-1) and 2 (IGF-2) and insulin throughout the adult human brain. *Neurosci. Lett.* **34,** 39–44.
Sara V. R., Carlsson-Swirut C., Andersson C., Hall E., Sjogren B., Holmgren A., and Jornvall H. (1986) Characterization of somatomedins from human brain: identification of a variant form of IGF-I. *Proc. Natl. Acad. Sci. USA* **83,** 4904–4907.
Sara V. R., Carlsson-Swirut C., Bergman T., Jornvall H., Roberts P. J., Crawford M., Hakansson N., Civalero I., and Nordberg A. (1989) Identification

of gly-pro-glu (GPE) the aminoterminal tripeptide of IGF-I which is truncated in brain, as a novel neuroactive peptide. *Biochem. Biophys. Res. Commun.* **165,** 766–771.

Sara V. R., Sandberg-Nordqvist A., Carlsson-Swirut C., Bergman T., and Ayer-LeLievre C. (1991) Neuroactive products of IGF-I and IGF-2 gene expression in the CNS, in *Molecular Biology and Physiology of Insulin and Insulin-like Growth Factors* (Raizada M. K. and LeRoith D., eds.), Plenum, New York, pp. 439–448.

Savitsky K., Bar-Shira A., Gilad S., Rotman G., Vanagaite L., Tagle D. A., Smith S., Uziel T., Sfez S., Ashkenazi M., Pecker I., Frydman M., Harnik R., Patanjali S. R., Simmons A., Clines G. A., Sartiel A., Gatti R. A., Chessa L., Sanal O., Lavin M. F., Jaspers N. G. J., Taylor A. M. R., Arlett C. F., Miki T., Weissman S. M., Lovett M., Collins F. S., and Shiloh Y. (1995) A single ataxia telangectasia gene with a product similar to PI 3-kinase. *Science* **268,** 1749–1753.

Scavo L., Shuldiner A. R., Serrano J., Dashner R., Roth J., and De Pablo F. (1991) Genes encoding receptors for insulin and insulin-like growth factor I are expressed in *Xenopus* oocytes and embryos. *Proc. Natl. Acad. Sci. USA* **88,** 6214–6218.

Schumacher R., Mosthaf L., Schlessinger J., Brandenburg D., and Ullrich A. (1991) Insulin and insulin-like growth factor-1 binding specificity is determined by distinct regions of their cognate receptors. *J. Biol. Chem.* **266,** 19,288–19,295.

Shemer J., Adamo M., Wilson G. L., Heffez D., Zick Y., and LeRoith D. (1987a) Insulin and insulin-like growth factor-I stimulate a common endogenous phosphoprotein substrate (pp185) in intact neuroblastoma cells. *J. Biol. Chem.* **262,** 15,476–15,482.

Shemer J., Raizada M. K., Masters B. A., Ota A., and Lerdith D. (1987b) Insulin-like growth factor-I receptors in neuronal and glial cells. *J. Biol. Chem.* **262,** 7693–7699.

Shemer J., Adamo M., Raizada M. K., Heffez D., Zick Y., and Le Roith D. (1989) Insulin and IGF-I stimulate phosphorylation of their respective receptors in intact neuronal and glial cells in primary culture. *J. Mol. Neurosci.* **1,** 3–8.

Skolnik E. Y., Margolis B., Mohammadi M., Lowenstein E., Fischer R., Drepps A., Ullrich A., and Schlessinger J. (1991) Cloning of PI3 kinase-associated p85 utilizing a novel method for expression/cloning of target proteins for receptor tyrosine kinases. *Cell* **65,** 83–90.

Smith C. B. (1991) The measurement of regional rates of cerebral protein synthesis. *Neurochem. Res.* **16,** 1037–1045.

Sokoloff L. (1981) Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *J. Cerebral Blood Flow Metab.* **1,** 7–36.

Soos M. A., Field C. E., and 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.

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

Stylianopoulou F., Herbert J., Soares M. B., and Efstratiadis A. (1988b) Expression of the insulin-like growth factor II gene in the choroid plexus and the leptomeninges of the adult rat central nervous system. *Proc. Natl. Acad. Sci.* **85,** 141–144.

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.

Sun X., Rothenberg P., Kahn C. R., Backer J. M., Araki E., Wilden P. A., Cahill D. A., Goldstein B. J., and White M. F. (1991) The structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* **352,** 73–77.

Sun X., Miralpeix M., Myers M. G. J., Glasheen E. M., Backer J. M., Kahn C. R., and White M. F. (1992) The expression and function of IRS-1 in insulin signal transmission. *J. Biol. Chem.* **267,** 22,662–22,672.

Sun X., Crimmins D. L., Myers M. G. J., Miralpeix M., and White M. F. (1993) Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. *Mol. Cell. Biol.* **13,** 7418–7428.

Sun X. J., Wang L., Zhang Y., Yenush L., Myers M. G., Jr., Giasheen E., Lane W. S., Pierce J. H., and White M. F. (1995) Role of IRS-2 in insulin and cytokine signalling. *Nature* **377,** 173–177.

Takei K., McPherson P. S., Schmid S. L., and De Camilli P. (1995) Tubular membrane invaginations coated by dynsmin ring are induced by GTP-ase in nerve terminals. *Nature* **374,** 186–190.

Tamemoto H., Kadowaki T., Tobe K., Yagi T., Sakura H., Hayakawa T., Terauchi Y., Ueki K., Kaburagi Y., Satoh S., Sekihara H., Yoshioka S., Horikoshi H., Furuta Y., Ikawa Y., Kasuga M., Yazaki Y., and Aizawa S. (1994) Insulin resistance and growth

retardation in mice lacking insulin receptor substrate-1. *Nature* **372,** 182–186.

Terlau H. and Seifert W. (1989) Influence of epidermal growth factor on long-term potentiation in hippocampal slice. *Brain Res.* **484,** 352–356.

Terlau H. and Seifert W. (1990) Fibroblast growth factor enhances long-term potentiation in the hippocampal slice. *Eur. J. Neurosci.* **2,** 973–977.

Tornqvist H. E. and Avruch J. (1988) Relationship of site-specific tyrosine autophosphorylation to insulin activation of the insulin receptor (tyrosine) protein kinase activity. *J. Biol. Chem.* **263,** 4593–4601.

Tornqvist H. E., Gunsalus J. R., Nemenoff R. A., Frackelton A. R., Pierce M. W., and Avruch J. (1988) Identification of the insulin receptor tyrosine residues undergoing insulin-stimulated phosphorylation in intact rat hepatoma cells. *J. Biol. Chem.* **263,** 350–359.

Treadway J. L., Morrison B. D., Soos M. A., Siddle K., Olefsky J., Ullrich A., McClain D. A., and Pessin J. E. (1991) Transdominant inhibition of tyrosine kinase activity in mutant insulin/insulin-like growth factor I hybrid receptors. *Proc. Natl. Acad. Sci. USA* **88,** 214–218.

Ullrich A., Bell J. R., Chen E. Y., Herrera R., Petruzzelli L. M., Dull T. J., Gray A., Coussens L., Liao Y., Tsubokawa M., Mason A., Seeburg P. H., Grunfeld C., Rosen O. M., and Ramachandran J. (1985) Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* **313,** 756–761.

Ullrich A., Gray A., Tam A. W., Yang-Feng T. L., Tsubokawa M., Collins C., Henzel W., Le Bon T., Kathuria S., Chen E., Jacobs S., Franke U., Ramachandran J., and Fujita-Yamaguchi Y. (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. *EMBO J.* **5,** 2503–2512.

Umemori H., Sato S., Yagi T., Aizawa S., and Yamamoto T. (1994) Initial events of myelination involve Fyn tyrosine kinase signalling. *Nature* **367,** 572–576.

Unger J., McNeill T. H., Moxley R. T. III, White M., Moss A., and Livingston J. N. (1989) Distribution of insulin receptor-like immunoreactivity in the rat forebrain. *Neuroscience* **31,** 143–157.

Unger J. W., Livingston J. N., and Moss A. M. (1991a) Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. *Prog. Neurobiol.* **36,** 343–362.

Unger J. W., Moss A. M., and Livingston J. N. (1991b) Immunohistochemical localization of insulin receptors and phosphotyrosine in the brainstem of the adult rat. *Neuroscience* **42,** 853–861.

Valentino K. L., Pham H., Ocrant I., and Rosenfeld R. G. (1988) Distribution of insulin-like growth factor II receptor immunoreactivity in rat tissues. *Endocrinology* **122,** 2753–2763.

Valentino K. L., Ocrant I., and Rosenfeld R. G. (1990) Developmental expression of insulin-like growth factor II receptor immunoreactivity in the rat central nervous system. *Endocrinology* **126,** 914–920.

Varticovski L., Harrison-Findik, D., Keeler M. L., and Susa M. (1994) Role of PI 3-kinase in mitogenesis. *Biochem. Biophys. Acta* **1226,** 1–11.

Wang L. M., Keegan A. D., Paul W. E., Heidaran M. A., Gutkind J. S., and Pierce J. H. (1992) IL-4 activates a distinct signal transduction cascade from IL-3 in factor dependent myeloid cells. *EMBO J.* **11,** 4899–4908.

Wang L. M., Keegan A. D., Li W., Lienhard G. E., Pacini S., Gutkind J. S., Myers M. G. J., Sun X., White M. F., Aaronson S. A., Paul W. E., and Pierce J. H. (1993a) Common elements in IL4 and insulin signaling pathways in factor dependent hematopoietic cells. *Proc. Natl. Acad. Sci. USA* **90,** 4032–4036.

Wang L. M., Myers M. G. J., Sun X., Aaronson S. A., White M. F., and Pierce J. H. (1993b) IRS-1: essential for insulin and IL-4-stimulated mitogenesis in hematopoietic cells. *Science* **261,** 1591–1594.

Welham M. J., Learmont L., Bone H., and Schrader J. W. (1995) Interleukine-13 signal transduction in linphohemopoietic cells: similarities and differences in signal transduction with interleukin-4 and insulin. *J. Biol. Chem.* **270,** 12,286–12,296.

Werner H., Raizada M. K., Mudd L. M., Foyt H. F., Simpson I. A., Roberts C. T., Jr., and Le Roith D. (1989a) Regulation of rat brain/hepG2 glucose transporter gene expression by insulin and IGF-I in primary cultures of neuronal and glial cells. *Endocrinology* **125,** 314–320.

Werner H., Woloschak M., Adamo M., Shen-Orr Z., Roberts C. T., Jr., and LeRoith D. (1989b) Developmental regulation of the rat insulin-like growth factor I receptor gene. *Proc. Natl. Acad. Sci. USA* **86,** 7451–7455.

Werner H., Roberts C. T., Jr., Raizada M. K., Bondy C. A., Adamo M., and Le Roith D. (1993) Developmental regulation of the insulin-like growth factor receptors in the central nervous system, in

Growth Factors and Hormones (Zagon I. S. and McLaughlin P. J., eds.), Chapman & Hall, New York, 109–127.

Werther G. A., Hogg A., Oldfield B. J., McKinley M. J., Figdor R., and Mendelsohn F. A. O. (1989) Localization and characterization of IGF-I receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry: a distinct distribution from insulin receptors. *J. Neuroendocrinol.* **1,** 369–377.

White M. F. and Kahn C. R. (1994) The insulin signaling system. *J. Biol. Chem.* **269,** 1–5.

White M. F., Maron R., and Kahn C. R. (1985) Insulin rapidly stimulates tyrosine phosphorylation of a M_r 185,000 protein in intact cells. *Nature* **318,** 183–186.

White M. F., Shoelson S. E., Keutmann H., and Kahn C. R. (1988a) A cascade of tyrosine autophosphorylation in the β-subunit activates the insulin receptor. *J. Biol. Chem.* **263,** 2969–2980.

White M. F., Livingston J. N., Backer J. M., Lauris V., Dull T. J., Ullrich A., and Kahn C. R. (1988b) Mutation of the insulin receptor at tyrosine 960 inhibits signal transmission but does not affect its tyrosine kinase activity. *Cell* **54,** 641–649.

Wilden P. A., Kahn C. R., Siddle K., and White M. F. (1992a) Insulin receptor kinase domain autophosphorylation regulates receptor enzymatic function. *J. Biol. Chem.* **267,** 16,660–16,668.

Wilden P. A., Siddle K., Haring E., Backer J. M., White M. F., and Kahn C. R. (1992b) The role of insulin receptor kinase domain autophosphorylation in receptor-mediated activities. *J. Biol. Chem.* **267,** 13,719–13,727.

Wolf G., Trüb T., Ottinger E., Groninga L., Lynch A., White M. F., Miyazaki M., Lee J., and Shoelson S. E. (1995) PTB domains of IRS-1 and Shc have distinct but overlapping binding specificities. *J. Biol. Chem.* **370,** 27,407–27,410.

Wozniak M., Rydzewski B., Baker S. P., and Raizada M. K. (1993) The cellular and physiological actions of insulin in the central nervous system. *Neurochem. Int.* **22,** 1–10.

Yagi T., Aizawa S., Tokunaga T., Shigetani Y., Takeda N., and Ikawa Y. (1995) A role for fyn tyrosine kinase in the suckling behaviour of neonatal mice. *Nature* **366,** 742–745.

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 R. and Cooper G. M. (1995) Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science* **267,** 2003–2006.

Yao D., Liu X., Hudson L. D., and De F. Webster H. (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.

Ye P., Carson J., and D'Ercole A. J. (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.

Young W., Kuhar M., Roth J., and Brownstein M. (1980) Radiohistochemical localization of insulin receptors in the adult and developing rat brain. *Neuropeptides* **1,** 15–22.

Zachenfels K., Oppenheim R. W., and Roher 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.