Insulin and Insulin-Like Growth Factor Receptors in the Nervous System

Martin Adamo,[1] Mohan K. Raizada,[2] and Derek LeRoith,[1]

[1]*Section of Molecular and Cellular Physiology, Diabetes Branch, NIDDK, National Institutes of Health, Bldg 10, Rm 8S243, 9000 Rockville Pike, Bethesda, MD 20892; and*[2] *Department of Physiology, University of Florida, Gainesville, FL 32610*

Contents

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

Abstract

Insulin and the insulin-like growth factors (I and II) are homologous peptides essential to normal metabolism as well as growth. These peptide hormones are present in the brain, and, based on biosynthetic labeling studies as well as evidence for local gene expression, they are synthesized by nervous tissue as well as being taken up by the brain from the peripheral circulation. Furthermore, the presence of insulin and IGF receptors in the brain, on both neuronal and glial cells, also suggests a role for these peptides in the nervous system. Thus, these ligands affect brain electrical activity, either as neurotransmitters or as neuromodulators, altering the release and reuptake of other neurotransmitters.

The insulin and IGF-I and -II receptors found in the brain exhibit a lower molecular weight than corresponding receptors on peripheral tissues, primarily caused by alterations in glycosylation. Despite these alterations, both brain insulin and IGF-I receptors exhibit tyrosine kinase activity in cell-free systems, as do their peripheral counterparts. Brain insulin and IGF-I receptors are developmentally regulated, with the highest levels appearing in fetal or perinatal life. However, the altered glycosylation of brain receptors does not appear until late in fetal development. The receptors are widely distributed in the brain, but especially enriched in the circumventricular organs, choroid plexus, hypothalamus, cerebellum, and olfactory bulb. These studies on the insulin and IGF receptor in brain, add strong support to the suggestion that insulin and IGFs are important neuroactive substances, regulating growth, development, and metabolism in the brain.

Index Entries: Insulin; insulin-like growth factors; neuropeptides; receptors; tyrosine kinase; neurons and glial neural-derived cells.

Introduction

Insulin and the insulin-like growth factors (IGF-I/SmC and IGF-II/MSA) appear to be neuroactive peptides. They are readily detectable in various regions of the brain, as are their specific receptors, and, importantly, they act through these receptors to bring about bioeffects. Convincing evidence exists supporting the suggestion that the IGFs are synthesized in the brain. In the case of insulin, available evidence, primarily from use of cultured brain cells in vitro, suggests, but does not prove conclusively, that this peptide is also synthesized in the central nervous system. Thus, we will begin by summarizing evidence indicating that brain levels of the family of insulin-related growth factors reflect both local synthesis as well as uptake from peripheral circulation. The reader is referred to Table 1 for a brief summary of the chemistry, source, nonmenclature, and binding proteins for this family of insulin-related growth factors.

Insulin and IGFs in the Brain

Insulin in the Brain

Insulin is found in various regions of the brain, both by radioimmunoassay of extracts, and immunocytochemistry, with the highest concentrations present in the olfactory bulb and hypothalamus (Havrankova et al., 1978; Rosenzweig et al., 1980; Baskin et al., 1983a,b). Insulin is also present in extracts of peripheral nerves (Uvnas-Moberg et al., 1982, 1988). Insulin isolated from brain is immunologically similar to pancreatic insulin and has similar bioactivity (Havrankova et al., 1978). Several lines of evidence suggest that insulin is actually synthesized by the brain (LeRoith et al., 1983, 1988). First, the concentration of brain insulin has been reported to be higher than that of plasma (Havrankova et al., 1978; Rosenzweig et al., 1980; Agardh et al., 1986). Furthermore, in situations of experimentally induced or naturally occurring hyper- or hypoinsulinemia, neither

Table 1
Insulin-Related Family of Neuropeptides[a]

	Insulin	IGF-I/SmC	IGF-II/MSA
~7500	~7500; 10,000		
Other name	—	Somatomedin C	Multiplication Stimulating Activity
Domains in peptide	B,A[b]	B, C, A, D, E[c]	B, C, A, D, E[c]
Binding proteins	—	150 kDa; 25–40 kDa	150 kDa; 25–40 kDa[d]
Receptors	Transmembrane heterotetramer	Transmembrane heterotetramer	Transmembrane monomer
Major source	Pancreas	Liver	Liver, brain in adults
Function	Metabolic neuromodulatory	Mitogenesis, differentiation, neuromodulation, local tissue repair	Unknown
pI	5.8	8.0–8.5	7.0

[a]Data taken from Perdue et al., 1984; Binoux et al., 1982, 1986; Ebina et al., 1985; Ullrich et al., 1985, 1986; Jansen et al., 1983; Bell et al., 1984; Shiu and Paterson, 1988.

[b]Domains as revealed by sequence analysis of cDNA clones. Sequence domains code for A and B chains of mature insulin, C-peptide is cleaved off the proinsulin molecule (Perdue et al., 1984).

[c]Domain present in IGFs codes for C-peptide, which is not cleaved off the pro IGF-I molecule. D domain is an additional peptide sequence at carboxyterminus and E domain is predicted by cDNA sequence (Jansen et al., 1983; Bell et al., 1984).

[d]This binding protein has greater affinity for IGF-II than for IGF-I (Binoux et al., 1982, 1986).

brain concentrations of insulin (Havrankova et al., 1979; Agardh et al., 1986) nor insulin receptors (Havrankova et al., 1979; Pacold and Blackard, 1979; Simon et al., 1986; Shemer et al., 1988) are altered. These results indicate that the plasma insulin does not rapidly equilibrate with the brain insulin pool, and have been interpreted as evidence favoring local synthesis of insulin. Also along these lines, insulin-like material has been detected in chick embryos before formation of a discrete pancreas (De Pablo et al., 1982). Second, proinsulin and insulin immunoreactivity have been detected in neural-derived cells in primary culture maintained in serum free medium (Weyhenmeyer and Fellows, 1983; Raizada, 1973; Birch et al., 1984a,b; Budd et al., 1986). Third, primary neuronal cell cultures from 1-d-old rat brains incorporate ^{3}H-leucine into and release, a substance that is immunoprecipitated by anti-insulin antibody and that behaves identically with insulin on HPLC. Furthermore, this insulin-related material is released in response to chemical depolarization (Clarke et al., 1986). Primary cultures of a subset of rabbit brain neurons, but not glial cells, were shown to possess immunoreactive insulin, which was increased when neurosecretion was inhibited (Schechter et al., 1988). These latter studies thus strongly support neuronal synthesis and release of insulin. The best evidence for insulin synthesis by brain is the demonstration of insulin gene expression by nervous tissue. Insulin specific mRNA has been demonstrated in cultured pituitary cells and primary neuronal cell cultures from rat and rabbit brain (but not glial cell cultures) as well as in hypothalamic periventricular nuclei next to the ependymal lining of the third ventricle (Budd and Pansky, 1986; Young, 1986; Clarke et al., 1987; Schechter et al., 1988). Though these preliminary studies are very

suggestive of insulin gene expression in neural tissue, the possibility of cross hybridization of the IGFs with the insulin probe used in Northern and *in situ* hybridizations cannot be fully ruled out. The actual size of the mRNA transcript is still to be determined, as is the translatability of the mRNA.

The evidence cited above suggests at the least that neurally derived cultured cells synthesize insulin or an insulin related molecule. The issue of local synthesis is clearly more controversial in the intact brain in vivo, and circulating insulin may also contribute to the brain content. The circumventricular organs (CVO, which include the subfornical organ, the organum vasculosum, lamina terminalis, and the median eminence) lack a blood–brain barrier (BBB), and, thus, are directly accessed by the plasma (Phillips, 1987). Infused ^{125}I-insulin has been found to be concentrated in the CVO (Van Houten and Posner, 1979a; Van Houten et al., 1979, 1980). These regions are enriched in glial cells, which apparently restrict insulin's subsequent movement (Phillips, 1987). Insulin bound to neurons here, however, may activate neuronal pathways and, thus, initiate a signal that is relayed deeply into the brain (Baskin et al., 1987; Phillips, 1987). An alternative pathway for the transfer of plasma insulin to the brain is by its transfer across the BBB into the cerebrospinal fluid (Baskin et al., 1983). Such a transfer is felt to occur by utilizing specific insulin receptors in receptor mediated transcytosis. Thus, endothelial cells of the capillary network making up the BBB (e.g., at the choroid plexus) possess specific insulin receptors, which bind, internalize, and transport the ligand (Pardridge et al., 1985; Frank et al., 1986; Baskin et al., 1986). Uptake of plasma insulin by the CSF has been shown to occur (Baskin et al., 1983b), and, indeed, hyperinsulinemia in genetically obese rats or normal human volunteers can lead to elevated CSF insulin levels (Stein et al., 1983; Wallum et al., 1987). Collectively, these latter studies indicate that brain insulin is comprised of both local synthesis as well as a contribution from the peripheral circulation.

Insulin-Like Growth Factors in the Brain

Evidence for IGF-I synthesis in brain is similar to that for insulin. Thus, IGF-I immunoreactivity is detected in brain (Sara et al., 1982; D'Ercole et al., 1984; Noguchi et al., 1987; Andersson et al., 1988), and IGF-I and its binding protein are released from cultured pituitary explants (Binoux et al., 1981). Also, both fetal and adult human brain contain a variant form of IGF-I with a truncated *N*-terminus, presumably a result of posttranslational modification (Sara et al, 1986; Carlsson-Skwirut et al., 1986). Most importantly, IGF-I specific mRNA is readily detectable in various regions of brain tissue from fetuses and adults (Lund et al., 1986; Murphy et al., 1987; Lowe et al., 1987, 1988; Han et al., 1987, 1988; Rotwein et al., 1988). The olfactory bulb and cervical thoracic spinal cord from adult rat brain are especially rich in IGF-I mRNA (Rotwein et al., 1988). Neuronal and glial cell primary cultures both contain typical IGF-I mRNA (Ballotti et al., 1987; Rotwein et al., 1988; Adamo et al., 1988) although *in situ* hybridization studies will be required in order to determine which of these cell types in the intact brain is responsible for IGF-I synthesis. Like insulin, IGF-I may also reach the brain from the peripheral circulation. IGF-I receptors are present in high levels in the median eminence (Bohannon et al., 1986, 1988; Lesniak et al., 1988), suggesting IGF-I uptake into the CVO from the plasma, as well as in the choroid plexus (Bohannon et al., 1986, 1988; Lesniak et al., 1988), thus representing a site for receptor mediated transcytosis across the BBB. Furthermore, central microvessels possess IGF-I receptors (Rosenfeld et al., 1987; Duffy et al., 1988), which, in analogy to microcapillary insulin receptors, could transport IGF-I from blood to CSF.

IGF-II immunoreactivity is also found in the brain (in higher levels than IGF-I), with the highest concentrations in the anterior pituitary, dorsomedial hypothalamus, and supraoptic nucleus (Haselbacher et al., 1985). Higher molecular weight forms (i.e., >10 kDa) are also de-

tectable and may represent IGF-II precursor molecules (Haselbacher et al., 1985; Zumstein et al., 1985), although a recent report utilizing biosynthetic labeling techniques, indicated that although brain and pituitary synthesized multiple IGF-II peptides, they secreted only the higher M_r forms, especially an 8.7 kDa form (Shiu and Patterson, 1988). Brain may be a predominant source of IGF-II in the adult rat relative to other tissues based on analysis of specific mRNA levels (Brown et al., 1986; Soares et al., 1986). IGF-II mRNA is more uniformly distributed in various brain regions of adult rats than is IGF-I mRNA (Rotwein et al., 1988) although *in situ* hybridization reveals especially high levels of IGF-II mRNA in the choroid plexus (Hynes et al., 1988; Stylianopoulou et al., 1988). As mentioned earlier for insulin, the choroid plexus is a major site of brain-CSF interaction, and thus IGF-II may enter the CSF from this region. Furthermore, the human blood–brain barrier exhibits an IGF-I receptor that preferentially binds IGF-II vs IGF-I or insulin (Duffy et al., 1988). Thus, a high rate of receptor mediated transcytosis, as well as high rates of local synthesis could form the basis for the higher level of IGF-II, relative to insulin or IGF-I in the CSF (Haselbacher and Humbel, 1982). Both neuronal and glial cultures from brain express IGF-I mRNA, but only glial cultures express IGF-II mRNA (Rotwein et al., 1988). In addition, IGF-binding proteins of molecular size 25–40 kDa and that have a higher affinity for IGF-II than IGF-I are found in hypothalamus, cortex, and CSF (Binoux et al., 1982, 1986) and are also produced by astrocytic glial cells in culture (Han et al., 1988). The functional relevant binding protein specific for each tissue has yet to be determined.

Receptors for Insulin and the IGFs

Peptide hormones and neurotransmitters bind to specific receptors on the plasma membrane of the cell and thereby stimulate a number of cellular events leading to their specific biological effects. As shown in Fig. 1, the insulin and IGF-I receptors, which are very similar in structure, are integral membrane glycoproteins composed of two α subunits with apparent M_r 130,000–135,000, and two β subunits with apparent M_r 95,000 linked by disulphide bonds. The α and β subunits derive from a glycoprotein precursor of M_r 190,000 and the apparent M_r of the heterotetrameric ($\alpha_2\beta_2$) receptors is 350,000–400,000 (Massague and Czech, 1982; Kasuga et al., 1982a; McElduff et al., 1986; Fujita-Yamaguchi et al., 1986; Morgan et al., 1986).

The α subunit, which lies extracellularly and is significantly glycosylated, binds the ligand. The β subunit comprises a short extracellular domain, a transmembrane domain, and a long cytoplasmic domain that contains an ATP-binding site and intrinsic protein tyrosine kinase activity, making these receptors part of the tyrosine kinase family of peptide growth factor receptors and oncogene products (Ebina et al., 1985; Ullrich et al., 1985, 1986). Binding of the ligand to the α subunit stimulates phosphorylation of the β subunit on serine and tyrosine residues (Kasuga et al., 1982b; Roth et al., 1983; Zick et al., 1983; Rosen et al., 1983; Petruzzelli et al., 1984). This autophosphorylation of the β subunit then leads to phosphorylation of cellular substrates that may lead to the final signal transfer cascade for insulin and IGF-I (Rees-Jones and Taylor, 1985; White et al., 1985; Accili et al., 1986; Chou et al., 1987; Ebina et al., 1987; Morgan and Roth, 1987; Machicao et al., 1987; Kadowaki et al., 1987; Bernier et al., 1987; Shemer et al., 1987a; Haring et al., 1987; Madoff et al., 1988). The structural similarities between insulin and IGF-I and their respective receptors form the basis for the overlapping array of biological responses to these peptides (Perdue et al., 1984; Zapf et al., 1984).

In contrast to the insulin and IGF-I receptor, the IGF-II receptor is a single polypeptide of Mr 250,000 without intrinsic kinase activity (Massague and Czech, 1982; Morgan et al., 1987). It contains only one transmembrane region, a large extracellular domain, and a relatively small cytoplasmic domain. It bears no homol-

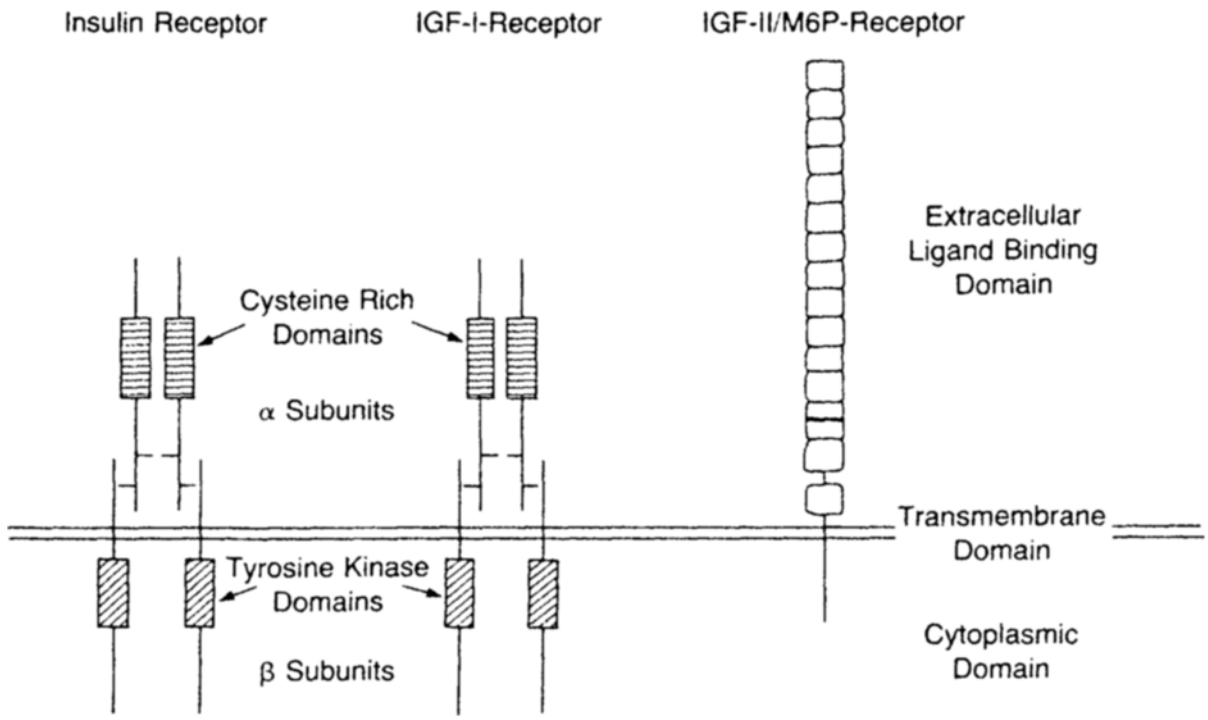

Fig. 1. Schematic representation of the insulin and IGF-I and -II receptors. The structures of the receptors for insulin and IGF-I and -II have been determined using affinity crosslinking, autophosphorylation, surface labeling, biosynthetic labeling, and molecular cloning techniques. As indicated, the subunit structures for the IGF-I and insulin receptors are similar. Each has two cysteine-rich extracellular α-subunits (M_r of 135 kDa, shown by hatched boxes), which bind the ligands and are themselves joined by disulfide bonds. Each α-subunit is joined to a β-subunit (M_r = 95 kDa, shown by diagonal hatched boxes), by disulfide bonds, in the extracellular domain. The β-subunits traverse the plasma membrane and extend intracellularly, giving rise to the transmembrane and cytoplasmic domains, respectively. A portion of the cytoplasmic domain of the insulin and IGF-I receptor β-subunits possess ATP binding sites and catalytic sites, giving rise to tyrosine kinase activity. Ligand binding to the α-subunit activates the β-subunit tyrosine kinase, causing autophosphorylation as well as tyrosine-specific phosphorylation of substrates. As described in the text, the brain insulin and IGF-I receptors differ from the peripheral type (e.g., liver, adipocyte) primarily in having less glycosylation of α-subunits, causing them to be ~10 kDa smaller. At the right side of the figure is shown a schematic of the IGF-II receptor, recently reported to be identical to the cation-independent mannose-6-phosphate receptor. The IGF-II receptor consists of a single protein subunit oriented almost exclusively extracellularly where it is comprised of 15 cysteine-rich "repeat" regions (indicated by the boxes). In region 13, the dark band represents a homology with the type-II fibronectin receptor. The IGF-II receptor has no intrinsic tyrosine kinase activity (Adapted from Morgan et al., 1987).

ogy with either insulin or IGF-I receptors, but, interestingly, is identical to the mannose-6-phosphate receptor, which is involved in intracellular targeting of lysosomal proteins (Morgan et al., 1987; Roth et al., 1987; Tang et al., 1988; Roth, 1988; Waheed et al., 1988). This recent finding has added further confusion to the as-yet-undefined role for IGF-II.

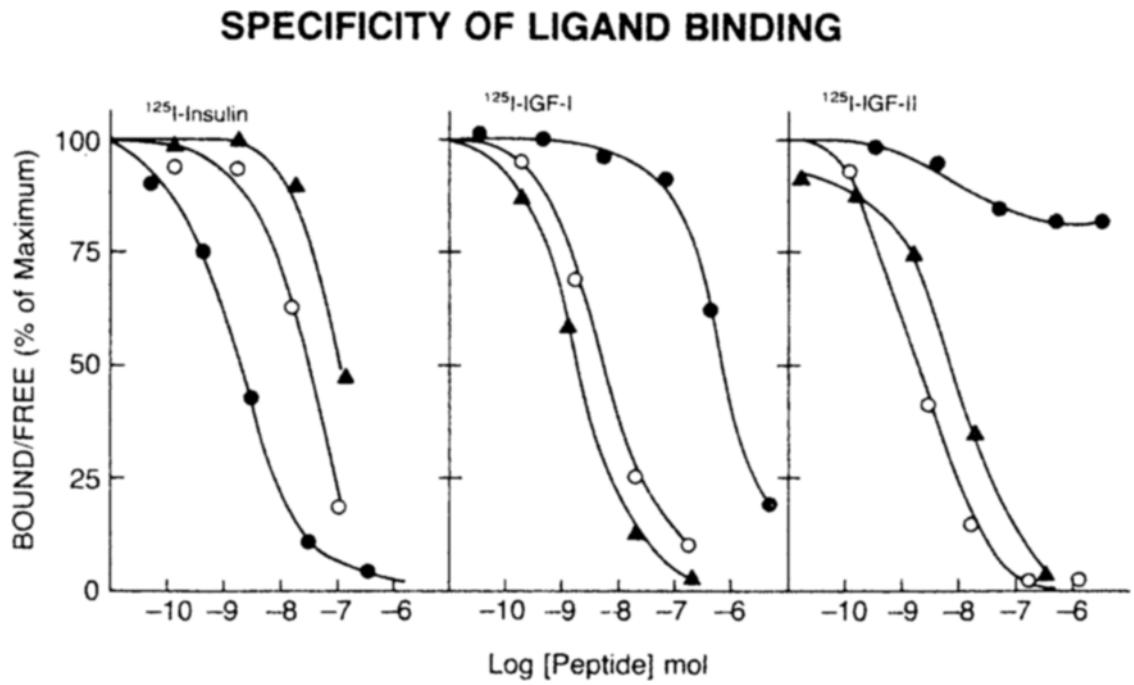

Fig. 2. Competition-inhibition curves for ^{125}I-ligand binding to rat brain membranes. Competition-inhibition experiments were performed on rat brain cortical membranes using ^{125}I-insulin (left), ^{125}I-IGF-I (middle), and ^{125}I-IGF-II (right), with increasing concentrations of unlabeled insulin (•), IGF-I (Δ), and IGF-II (o). Unlabeled insulin produced half-maximal displacement of specific ^{125}I-insulin binding at ~4 n*M*, whereas IGF-II and IGF-I at 50 and 100 n*M*, respectively, were required for a similar degree of inhibition of insulin binding. Similarly, unlabeled IGF-I at 3 n*M* inhibited ^{125}I-IGF-I binding by 50%, whereas 3 n*M* unlabeled IGF-II produced a 50% inhibition of specific ^{25}I-IGF-II binding. Insulin at ~1000 n*M* was required to inhibit specific ^{125}I-IGF-I binding and at this concentration produced only a 20% reduction in specific ^{125}I-IGF-II binding, indicating that insulin has essentially no affinity for IGF-II receptors. Unlabeled IGF-II at 8 n*M* produced 50% inhibition of specific ^{125}I-IGF-I binding. The rank order of the ligands in inhibiting tracer ligand binding is taken as evidence for the specificity of binding and hence the existence of separate receptors. (Adapted from Gammeltoft et al., 1985).

Receptors or Insulin and IGFs in the Brain

Despite similarities between ligand and receptor structure, as well as function, of the family of insulin-related peptides, pharmacological binding studies can be used to distinguish these receptors in the brain. Initially, membranes from homogenates of whole brain were utilized with ^{125}I-insulin or ^{125}I-IGF in in vitro binding assays (Havrankova et al., 1978; Pacold and Blackard, 1979; Gammeltoft et al., 1985; Lowe and LeRoith, 1986). In such systems the degree of inhibition of the binding of labeled ligand by unlabeled hormone can be used to identify specific receptors (Fig. 2). Thus, ^{125}I-insulin specific binding is reduced 10–100 times more by competition with insulin than by IGF-I or IGF-II, whereas ^{125}I-IGF-I is displaced equally well with unlabeled IGF-I or II, but a similar degree of displacement requires a 100-fold molar excess of insulin (Gammeltoft et al., 1985). ^{125}I-IGF-II also binds specifically to brain membrane preparations, with unlabeled IGF-I being required at a five-fold molar excess to achieve a similar level of displacement of specific binding as unlabeled IGF-II. Unlabeled insulin is incapable of competing for ^{125}I-IGF-II

binding sites (Gammeltoft et al., 1985). Such results quantitatively indicate the degree to which insulin and the IGFs can crossreact with the other receptors, and, in this regard, it should be noted that IGF-I showed only 22% of the affinity for the type II receptor as did IGF-II, whereas IGF-II showed 45% of the affinity for type I receptors as did IGF-I (Gammeltoft et al., 1985). This group concluded that type II IGF receptors may be more specific than type I receptors. Owing to the crossreactivity of ligand with receptors, insulin and insulin-like growth factors often share biological responses. However, recent evidence exists indicating that both insulin and IGF-I can elicit the same response through their own respective receptors (Verspohl et al., 1988).

In addition to demonstrating appropriate affinity for insulin and the IGFs, brain insulin receptors behave identically to their peripheral counterparts in terms of time, temperature, and pH dependence of the binding (Havrankova et al., 1978; Pacold and Blackard, 1979). However, there are specific differences between insulin receptor binding in brain preparations vs peripheral preparations. Thus, the affinity of brain insulin receptors for certain insulin analogs has been reported to be higher than that of peripheral receptors (Gammeltoft et al., 1984). Insulin is capable of down-regulating glial cell insulin receptors, but it has no such effect on neuronal cell receptors (Raizada et al., 1987). In fact, one report utilizing cortical cell preparations from mice indicated that a high ambient insulin concentration actually increased insulin receptor number (Van Schravendijk et al., 1984). Furthermore, "negative cooperativity," the mechanism by which the binding of insulin to its receptor progressively lowers the affinity of insulin receptors, was found to be absent in rat brain insulin receptors (Gammeltoft et al., 1984; Van Schravendijk et al., 1984). Despite these important distinctions, classical pharmacological binding studies indicate that specific insulin and IGF-I and -II receptors exist in the brain. Further confirmation of their presence in the brain has come from the use of antibodies specific for insulin and IGF-I and -II receptors, used in conjunction with affinity crosslinking and autophosphorylation experiments, as described below (Heidenreich et al., 1983; Gammeltoft et al., 1985; Lowe et al., 1986; McElduff et al., 1987, 1988).

Structure of Brain Insulin and IGF Receptors

Using membrane preparations from whole brain, chemical, and photoaffinity crosslinking of labeled ligand has been used to determine the size of the ligand binding subunit on reducing SDS-PAGE gels. Despite the initial impression from binding studies that brain insulin- and IGF-I-receptor α subunits were similar to their peripheral, nonneural counterparts, more recent studies have identified certain distinctive aspects. Thus, the specific brain insulin- and IGF-I-receptor α subunits were found to be approximately 10 kDa smaller than those expressed on nonneural tissues, e.g., liver and adipose (Fig. 3; Yip et al., 1980; Heidenreich et al., 1983, 1986; Hendricks et al., 1984a; Ciaraldi et al., 1985; Gammeltoft et al., 1985; Lowe and LeRoith, 1986; Lowe et al., 1986; McElduff et al., 1988). Studies using exo- and endoglycosidases suggest that the main difference between brain receptors and their peripheral counterparts lies in their carbohydrate residues. Thus, neuraminidase, which cleaves terminal sialic acid residues and thereby increases the mobility of the crosslinked liver receptor α subunits on gels, fails to affect brain insulin and IGF-I receptors (Fig. 4; Hendricks et al., 1984a; Heidenreich and Brandenberg, 1986; Lowe et al., 1986; Heidenreich et al., 1986; McElduff et al., 1988). This suggests that brain insulin receptors and IGF-I receptors may differ from those in other tissues in that they either do not express terminal sialic acid residues or, alternatively, they express polysialic acid residues that are neuraminidase resistant. Endoglycosidase H and F digestion suggest that brain receptors, like their periph-

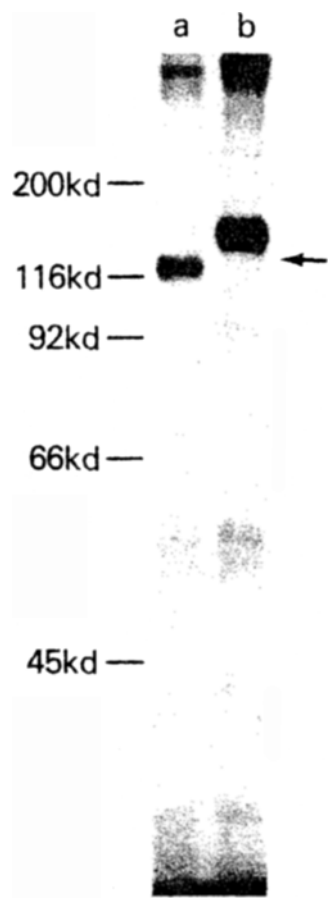

Fig. 3. Crosslinking of ^{125}I-insulin to the insulin receptor of guinea pig brain and liver membranes. Crude microsomal membranes from guinea pig brain (lane a) and liver (lane b) were crosslinked to ^{125}I-insulin using disuccinimidyl suberate as described in Lowe and LeRoith (1986), solubilized and run on SDS/PAGE gels under reducing conditions. Autoradiography of the gels revealed that the ^{125}I-insulin binding α-subunit of brain was approximately 10 kDa smaller than that of liver (as indicated by the arrow). Similar differences between brain and peripheral insulin receptor α-subunits have been observed in mammalian, avian, reptilian, and amphibian species.

eral counterparts, contain high mannose and complex carbohydrate residues that are *N*-linked. Total removal of *N*-linked glycosylation with Endo F results in brain α-subunits with similar Mr as peripheral α-subunits (Heidenreich and Brandenberg, 1986; Heidenreich et al., 1986; McElduff et al., 1988). These results are consistent with the genetic analysis suggesting that the insulin and IGF-I receptors are each expressed from a single gene (Ullrich et al., 1985, 1986). However, tissue specific mRNA splicing variants explaining these differences have not been excluded. Thus, the differences in size between α-subunits in brain and periphery lies

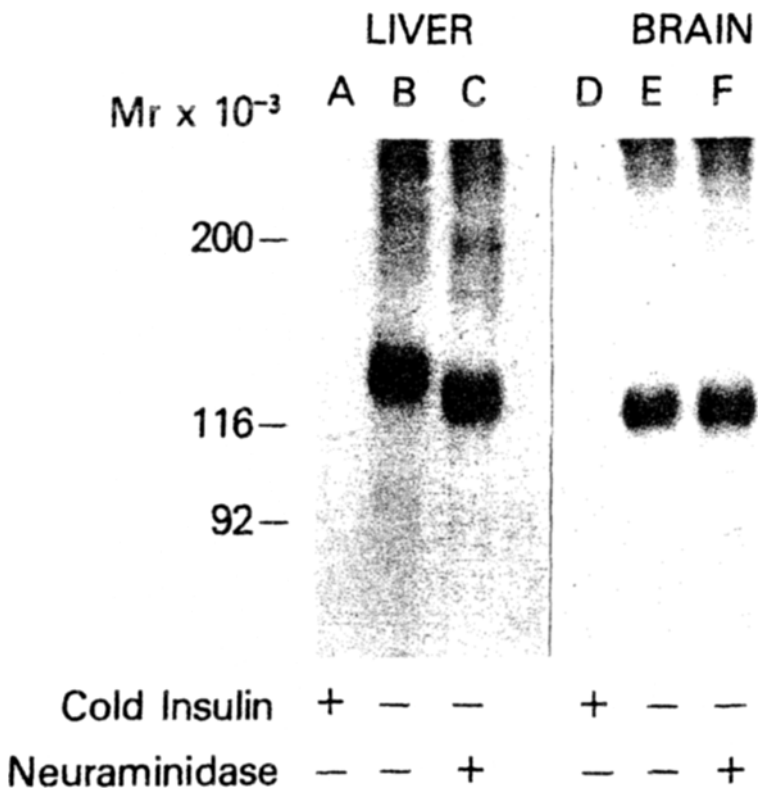

Fig. 4. The effect of neuraminidase digestion on α-subunit size of insulin receptor from liver and brain. ^{125}I-insulin was crosslinked to crude membranes from rat liver (lanes A, B, C) and brain (lanes D, E, F) using DSS. The crosslinked complexes were then incubated in a buffer either without (lanes B and E) or with (lanes C and F) neuraminidase. After enzymatic digestion, samples were solubilized and run on SDS/PAGE gels under reducing conditions. The resultant autoradiogram reveals that neuraminidase digestion lowered the apparent M_r of liver receptor α-subunits by 10 kDa (compare lanes B and C) and was without effect on brain α-subunits (compare lanes E and F). Furthermore, comparison of lane C with lanes E and F reveals that neuraminidase digestion of liver receptors reduced their apparent size to those of brain α-subunits, indicating glycosylation differences (specifically sialation) as an explanation for the difference in molecular weight of brain and peripheral insulin receptor α-subunits.

primarily in the degree of glycosylation, and is probably not owing to differences in the protein core structure (Heidenreich and Brandenberg, 1986; Heidenreich et al., 1986; McElduff et al., 1988). Brain IGF-II receptors also are smaller than their peripheral counterparts and preliminary studies suggest that the low apparent M_r is the result of alterations in *N*-linked glycosylation (McElduff et al., 1987).

Insulin and IGF-I receptors can be partially purified from brain membranes by solubilization followed by affinity chromatography over wheat germ agglutinin agarose. When such preparations are incubated with Mn^{++} and ^{32}P-ATP, the β subunit is autophosphorylated (Rees-Jones et al., 1984) and can be visualized by SDS-PAGE. The apparent M_r of the brain β-subunit thus observed is generally only 2–3 kDa smaller than its peripheral nonneural counterparts (Fig. 5; Lowe and LeRoith, 1986). The reasons for this difference have not been determined. However, in light of the results of alter-

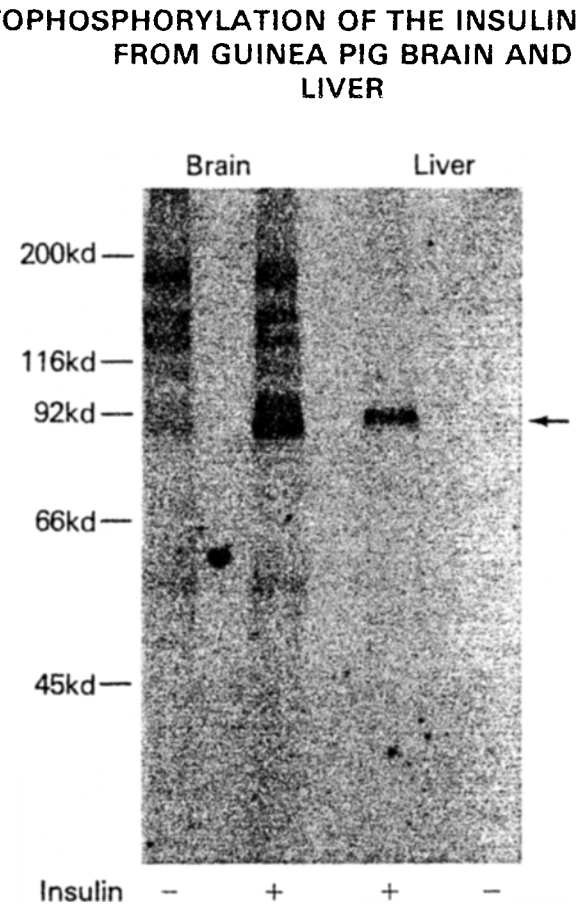

Fig. 5. Autophosphorylation of the insulin receptor from guinea pig brain and liver. Membranes from guinea pig brain and liver were solubilized and insulin receptors were partially purified on wheat germ agglutinin (WGA)–agarose columns. The lectin purified preparation were incubated without (–) and with (+) 100 n*M* insulin for 30 min at 22°C and then ^{32}P-ATP, Mn^{++}, and vanadate were added and phosphorylation was conducted for 10 min. Reaction mixtures were denatured and run on SDS/PAGE gels under reducing conditions. As indicated by the arrow to the right, insulin stimulated autophosphorylation of the 95 kDa receptor β-subunit in liver preparations, while the β-subunit of brain showed an apparent M_r of 92 kDa (reproduced from Lowe and LeRoith, 1986).

ations in the α-subunit glycosylation, these smaller differences in the β subunit are consistent with the cause lying in the carbohydrate moiety of the glycoprotein, since the β subunit has a relatively smaller extracellular domain with fewer *N*-linked glycosylation sites compared with the α subunit (Fig. 1; Ebina et al., 1985).

Ontogeny and Phylogeny of Brain Insulin and IGF-I Receptors

In order to determine the universality of the differences in M_r of brain insulin and IGF-I receptors, ontogenetic and phylogenetic studies have been performed. Brain insulin receptor concentration increased during fetal develop-

ment in rat, rabbit, and chick brains, with peaks in insulin receptor binding described in the perinatal period (Kappy and Raizada, 1982; Kappy et al., 1984; Hendricks et al., 1984b; Devaskar et al., 1986; Lowe et al., 1986; Brennan, 1987). IGF-I receptors in the brain appeared to peak earlier in fetal development (Sara et al., 1983; Bassas et al., 1985), a finding that may be related to the role of IGF-I in stimulating growth and development of brain cells (neurons and glia). Interestingly, insulin receptor binding on chick retinal cells decreased during embryonic development (Peterson et al., 1986). The insulin receptor α-subunit of rat brain is similar in size to the peripheral type at 16 d fetal gestation, with the smaller α-subunit size becoming apparent late in gestation (19–20 d fetuses) and remaining so into neonatal and adult life (Lowe et al., 1986; Brennan, 1987). The shift in molecular size of the α-subunit during development appears to be a result of a loss of sialic acid residues, based on glycosidase studies (Brennan, 1987). In chicks, the α-subunit size difference between brain and peripheral receptors was seen throughout development (Bassas et al., 1987). Phylogenetic conservation of the unique brain receptors strengthens the concept of functional importance. In studies of multiple species, including mammals, aves, amphibian, and reptiles, the unique smaller α-subunit of brain insulin receptors was found (Lowe et al., 1986; Simon et al., 1986; Shemer et al., 1986, 1987b; Hart et al., 1987; Bassas et al., 1987). However, in spite of the lower M_r brain insulin-receptor α-subunit, all the species studied had functional coupling of brain insulin receptor α- and β-subunits, demonstrated by insulin-stimulatable autophosphorylation of the 95kDa receptor β-subunit, as well as tyrosine kinase activity toward exogenous substrates (Lowe et al., 1986; Simon et al., 1986; Shemer et al., 1986; Hart et al., 1987).

Regional Localization of Brain Insulin and IGF Receptors

The brain is a complex organ in terms of regional localization of specific function as well as cell type. Thus, studies on the binding and structure of insulin and IGF receptors, as outlined above, using membranes from whole brain homogenates are limited in what information concerning brain insulin and IGF-I physiology they convey.

Three approaches to determine more finely the distribution of insulin and IGF receptors in the brain, their structural nuances, and their possible actions, have been utilized. First, membranes have been prepared from discrete, dissected brain regions, and in vitro binding assays performed. Such studies have indicated that insulin receptors are widely but unevenly distributed in the brain, with highest concentrations in the hypothalamus and olfactory bulb (Havrankova et al., 1978; Pacold and Blackard, 1979; Goodyer et al., 1984). IGF-I and -II specific receptors have also been determined from membrane binding assays using dissected brain regions. These receptors generally are present in higher concentrations than insulin receptors and show slight differences in distribution (Gammeltoft et al, 1985). Thus, IGF-I receptors are highest in olfactory bulb followed by hippocampus, amygdala, cerebral cortex, and cerebellum, whereas IGF-II receptors were similar in these regions (Gammeltoft et al., 1985). The pituitary gland is especially rich in IGF-II receptors as determined by in vitro binding assays (Goodyer et al., 1984). An even finer resolution of the distribution of insulin and IGF receptors has been accomplished via the use of brain slices in quantitative autoradiographic assay of ^{125}I-ligand binding sites (Table 2). Such studies have indicated that the highest concentrations of insulin and IGF-I receptors are in the olfactory bulb and choroid plexus, and are also fairly high in cerebellum and amygdala (Hill et al., 1986; Baskin et al., 1986; Corp et al., 1986; Lesniak et al., 1988; Bohannon et al., 1986, 1988). Insulin receptor localization, as determined by autoradiography, is typical of other neuropeptide receptor localization, and, importantly, the high insulin receptor concentration in the olfactory-limbic regions may allow for communication of the insulin effects (neurotransmission or neuro-

Table 2
Regional Localization of Brain Insulin and IGF-I and -II Receptors as Determined by Autoradiography

	Insulin	IGF-I	IGF-II
Choroid plexus	++++[b]	++++	++++
Olfactory bulb	++++	++++	++[d]
Median eminence	+[e]	++++	++++
Hypothalamus	++	+++	++
Cerebral cortex	++	++	++
Cerebellum	+++[c]	+++	++
Amygdala	++	++	++
Hippocampus	++	+++	+++
Thalamus	++	+++	++
Anterior pituitary	-[f]	-	++++

[a]Data compiled from Hill et al., 1986; Baskin et al., 1986; Bohannon et al., 1986, 1988; Corp et al., 1986; Lesniak et al., 1988.
[b]++++ = Highest concentration.
[c]+++ = High.
[d]++ = Moderate.
[e]+ = Lower concentration.
[f]- = Extremely low or not present.

modulation), since the regions exhibit extensive neuronal interconnections with other brain regions regulating known behavioral modalities (Hill et al., 1986; Lesniak et al., 1988). IGF-II receptors are high in these regions (Lesniak et al., 1988; Ocrant et al., 1988), but are also high in the median eminence (Lesniak et al., 1988) (one of the CVOs; *see above*), as are IGF-I receptors (Bohannon et al., 1986). Lesniak et al., (1988), have concluded that the overall distribution of insulin and IGF-I and -II receptors is similar, although within a given region different layers may show a different distribution of receptors. As an example, within the olfactory bulb, the olfactory nerve contained IGF-I receptors but no insulin or IGF-II receptors (Lesniak et al., 1988). Studies with anti-IGF-II receptor antibodies used in immunohistochemistry have additionally confirmed high levels of IGF-II receptors in all lobes of the pituitary gland (Valentino et al., 1988). These autoradiographic studies have also suggested that receptors are located on perikarya and are especially abundant in regions rich in dendrites. In vitro binding studies have also demonstrated specific insulin receptors in brain synaptosomes (Raizada et al., 1988).

These techniques thus suggest that insulin (and IGF-I) receptors are located on neurons, but they lack adequate resolution to define specifically brain cell types possessing receptors. Thus, the third method, use of cultured cells, has become increasingly important in determining not only location of receptor, but structure and biological consequence of receptor activation. Cultured cells that have been utilized include primary neuronal and astrocytic glial cells, cultured endothelial cells, retinal cells, and transformed cells of neural origin, such as neuroblastomas.

Primary Neuronal and Glial Cells

Neuronal and glial cells from fetal and neonatal rats have been studied in primary culture systems (Table 3). Specific high affinity insulin and IGF-I receptors are expressed by each type of cell (Lowe et al., 1986; Shemer et al., 1987c; Burgess et al., 1987; Ballotti et al., 1987; Masters et al., 1987). The Mr of the receptors α subunit for both insulin and IGF-I are almost 10 kDa smaller in neuronal than glial cells, suggesting that neuronal cells express brain-type receptors, whereas glial receptors more closely resemble peripheral (i.e., liver and adipose) receptors

Table 3
Comparison of Insulin and IGF-I Receptors in Primary Cultures of Rat Neuronal and Glial Cells[a]

	Neuronal cells		Glial cells	
	Insulin	IGF-I	Insulin	IGF-I
α-Subunit (kDa)	118 kDa	125 kDa	130 kDa	135 kDa
β-Subunit (kDa)[b]	91 kDa	91 kDa (105 kDa)	95 kDa	95 kDa (105 kDa)
Tyrosine kinase activity	+[c]	+	+	+
Neuromodulation	+	-	-	-
Glucose transport	-[d]	-	+	(+)
Thymidine incorporation	(+)[e]	+	(+)	+

[a]Data in Table compiled from Clarke et al., 1984; Boyd et al., 1985; Lowe et al., 1986; Shemer et al., 1987c, 1989; Ballotti et al., 1987; Burgess et al., 1987.

[b]β-subunit sizes determined by in vitro autophosphorylation assays using lectin purified receptor preparations. Values in parentheses represent additional β-subunit subtype, as determined by ligand stimulated phosphorylations in intact cells.

[c]+ = Stimulation by the respective ligand.

[d]- = No effect.

[e](+) = Stimulation by the respective ligand through the other ligands' receptor.

(Table 4) (Lowe et al., 1986; Shemer et al., 1987; Burgess et al., 1987; Masters et al., 1987; Ballotti et al., 1987). Despite these differences in M_r, both cells have functional receptors, as demonstrated by ligand induced autophosphorylation of the receptor β-subunit in both cell free membrane preparations as well as in intact cell phosphorylation experiments (Lowe et al., 1986; Masters et al., 1987; Shemer et al., 1987c, 1989; Ballotti et al., 1987). These latter studies have allowed for the determination of IGF-I receptor β subunit subtypes. Thus, the IGF-I receptor expresses two β-subunit subtypes of 95 and 105 kDa, possibly owing to altered glycosylation of the protein (Fig. 6, *see also* Ota et al., 1988b). Interestingly, the neuronal cells' biological response to insulin differs from that of glial cells; insulin inhibits norepinephrine reuptake in the neuronal cells, whereas glial cells show an insulin-stimulable glucose uptake (Boyd et al., 1985; Clarke et al., 1984; Masters et al., 1987). IGF-I, on the other hand, stimulates ^{3}H-thymidine or uridine incorporation in both neuronal and glial cells (Shemer et al., 1987; Burgess et al., 1987; Ballotti et al., 1987). Insulin at high concentrations can accomplish this by acting through the IGF-I receptor. The finding that insulin and IGF-I can stimulate the same biological responses through their own receptors in Hep G2 cells (Verspohl et al., 1988), however, suggests the possibility of the same effect in brain cells.

IGF-II receptors have been demonstrated on fetal rat astroglial cells, with an apparent size of 250 kDa (Ballotti et al., 1987; Ocrant et al., 1988). Furthermore, recent immunohistochemical studies have revealed that IGF-II receptors are present on only a small number of hypothalamic neurons, whereas astrocytes from this region are rich in these receptors (Ocrant et al., 1988).

Other Neural Derived Cells

Isolated brain microvessels (endothelial cells), which make up the BBB and, as related earlier, may mediate insulin uptake by CSF from plasma, exhibit typical insulin and IGF-I receptor binding (Rosenfeld et al., 1987; Duffy et al., 1988). Their α-subunits exhibit molecular

Table 4A
Biological Actions of Insulin and IGFs (I and II) in the Central and Peripheral Nervous Systems

Metabolic and Neuromodulatory Actions of Insulin	
Action	References
Alters neuronal firing rates	Palovcik et al., 1984; Sakaguchi and Bray, 1987
Promotes electrical coupling	Wolinsky et al., 1985
Inhibits norepinephrine reuptake	Boyd et al., 1985; Masters et al., 1987
Stimulates tryptophane transport and serotonin biosynthesis and uptake	Kwok and Jurio, 1987; Raizada et al., 1987
Maintains Na+/K+ pump in synaptosomes	Dunlop et al., 1987; Bojorge et al., 1987
Down regulates glial cell α_2-adrenergic receptors	Richards et al., 1987
Stimulates synaptogenesis	Puro and Agardh, 1984
Increases choline acetyltransferase activity	Kyriakis et al., 1987
Increases glucose uptake in glial cells	Clarke et al., 1984
Activates pyruvate dehydrogenase	Rinaudo et al., 1987
Stimulates protein, fatty acid, sulfolipid, and cholesterol synthesis	van der Pal et al., 1987; Aiezenman and deVellis, 1987
Central action to lower plasma glucose and free fatty acid levels	Coimbra and Migliorini, 1986; Amir and Shechter, 1987
Promotes neurite outgrowth and survival	Recio-Pinto et al., 1986

Table 4B
Biological Actions of Insulin and IGFs (I and II) in the Central and Peripheral Nervous Systems

Action	Reference
Alteration of feeding behavior	Porte and Woods, 1981; Tannenbaum et al., 1983
Mitogenesis in oligodendrocytes and astrocytes	Shemer et al., 1987c Burgess et al., 1987; Ballotti et al., 1987
Promotes glial cell development	McMorris et al., 1986; van der Pal et al., 1988
Mitogenesis in sympathetic neuroblasts	Di Cicco-Bloom and Black, 1988
Mitogenesis in primary neuronal cells and neuroblastomas	Shemer et al., 1987c; Mattsson et al., 1986; Ota et al., 1988c
Potentiates acetylcholine release from brain slices	Nilsson et al., 1988
Promotes neurite outgrowth and neuronal cell survival	Recio-Pinto and Ishii, 1984; Aizenman and deVellis, 1987
Inhibition of pituitary GH secretion	Yamashita and Melmed, 1986 Rosenfeld and Hoffman, 1987

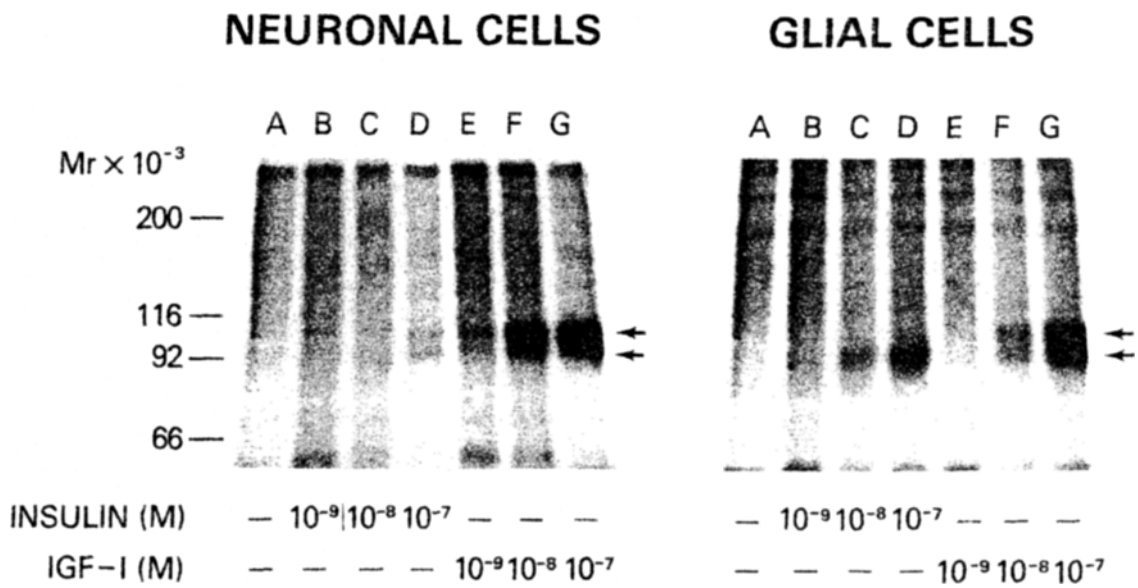

Fig. 6. Stimulation of receptor β-subunit phosphorylation by insulin and IGF-I in intact neuronal and glial cells. Primary cultures of neuronal and glial cells from 1-d-old rat brain were incubated in serum- and phosphate-free medium containing ^{32}P-orthophosphate in order to label intracellular ATP pools. Buffer (lane A), or increasing concentrations of insulin (lanes B, C, D) or IGF-I (lanes E, F, G) were added for 1 min, after which cells were solubilized and insulin and IGF-I receptors partially purified by WGA affinity chromatography. The column effluents were immunoprecipitated using anti-phosphotyrosine antibody and then electrophoresed on SDS/PAGE gels. In neuronal cells (left panel), insulin stimulated ^{32}P incorporation primarily into a 95 kDa protein (β-subunit) with some incorporation into a 105 kDa protein. In glial cells (right panel), insulin stimulated ^{32}P incorporation into a 95 kDa β-subunit. IGF-I, on the other hand, stimulated ^{32}P incorporation into two phosphoproteins from both cells types, one of 95 kDa and one of 105 kDa (indicated by arrows). Thus, neuronal and glial cell IGF-I receptor β-subunits consist of two subtypes of 95 and 105 kDa (reproduced from Shemer et al., 1989).

weights on SDS-PAGE similar to peripheral, nonbrain receptors. IGF-II receptor size appears to be similar on brain microvessels and brain cell membranes (Rosenfeld et al., 1987). In contrast, the retina, including the photoreceptive rod outer segments, express a mixture of insulin receptors, some with the peripheral type α-subunit and some with the brain type, i.e., 10 kDa smaller (Waldbillig et al., 1987a, b). In contrast, IGF-I receptor α-subunits on rod outer segments migrate identically with rat liver plasma membrane receptor α-subunits, suggesting that the IGF-I receptors on these cells are of the "peripheral" type (Zick et al., 1987). Primary cultures of rat pituitary cells exhibit specific insulin and IGF-I-and -II receptor binding, and, like pituitary membrane preparations, IGF-II specific binding predominates (Rosenfeld et al., 1984). Peripheral nerves, such as the superior cervical ganglion and trigeminal ganglion as well as the adrenal medulla, exhibit α-subunits of ~130 kDa, i.e., of the "liver" or peripheral type (Waldbillig and LeRoith, 1987; Heidenreich, 1987). Taken together, these results suggest strongly that only central neurons, and not glial cells, endothelial cells, or peripheral nerves, exhibit altered receptor α-subunit glycosylation, as reflected by lower Mr on reducing gels.

Transformed Cell Lines

Insulin and IGF-I receptors have been studied on several neural-derived clonal cell lines. The human neuroblastoma cell line SK-N-SH and SK-N-MC express specific insulin and IGF-I receptors (Ota et al., 1988a, c). SK-N-MC insu-

lin receptor α-subunits were found to have an Mr of 124 kDa, while that of the IGF-I receptor was found to be 132 kDa. SK-N-SH insulin-receptor and IGF-I receptors α-subunits were found to have Mr of 120 kDa and 126 kDa, respectively. These differences were found to reflect differences in glycosylation. Thus, neuraminidase digestion reduced the Mr of SK-N-MC insulin receptor α-subunits to 120 kDa and did not alter SK-N-SH α-subunit size, suggesting differences in terminal sialation (Ota et al., 1988a). Thus, these cell lines behave much as do whole brain preparations in terms of unique (smaller) insulin and IGF-I receptor α-subunits. In the mouse hybridoma cell line NG-108 (derived from mouse neuroblastoma N18 and rat glioma C6 cell lines), the insulin receptor α-subunit was 134 kDa, similar to the peripheral type size, and neuraminidase digestion also reduced the α-subunit to 120 kDa (Ota et al., 1988a). Heidenreich and Gilmore (1985) found that a number of clonal neuroblastoma and glioma cell lines possessed the "peripheral" type insulin receptor α-subunit, whose electrophoretic mobility could be increased by neuraminidase digestion. *N*-linked glycosylation in these cell lines was demonstrated by a reduced size following endoglycosidase H and F digestion. The rat and mouse cell lines have also been used to study differences in β-subunit glycosylation. Thus, IGF-I receptor β-subunits in N-18, NG-108, and C6 cells consisted of subtypes of 105 and 95 kDa, whereas insulin receptor β-subunits were of a single subtype of 95 kDa (Ota et al., 1988b). The difference in Mr between the IGF-I receptor β-subunit subtypes was found to be primarily a result of differences in glycosylation based on glycosidase and V8 protease mapping studies (Fig. 7; Ota et al., 1988b). Despite these differences, receptors in all cell types exhibited β-subunit autophosphorylation and ligand stimulated tyrosine kinase activity (Ota et al., 1988a,b,c). The phosphorylation of insulin and IGF-I receptors in intact N-18 neuroblastoma cells has also been studied (Shemer et al., 1987a). Thus, after metabolic labeling of intracellular ATP pools with ^{32}P, intact cells were stimulated with insulin or IGF-I. Extracts prepared from the cells when immunoprecipitated with anti-insulin receptor antibodies B10 and B2, as well as antiphosphotyrosine antibody and run on SDS/PAGE, confirmed that in these intact cells, insulin rapidly (within 20 s) stimulated phosphorylation of its receptor β-subunit with an Mr of 95 kDa, whereas IGF-I stimulated autophosphorylation of its two β-subunit subtypes of 95 and 105 kDa (Fig. 8). More important, the use of antiphosphotyrosine antibody indicated that insulin and IGF-I stimulated the phosphorylation of a 185 kDa phosphoprotein. This phosphoprotein was not structurally related to the insulin or IGF-I receptors nor to the epidermal growth factor or platelet derived growth factor receptors, as indicated by the use of appropriate antibodies (Fig. 8). Direct phosphoamino acid analysis of the insulin and IGF-I stimulated 95 and 185 kDa phosphoproteins showed that the ligands stimulated ^{32}P incorporation into serine and tyrosine residues (Fig. 9). Thus, it appears to be an endogenous substrate for the insulin and IGF-I receptor kinases in these cells. A 185 kDa phosphoprotein substrate for the insulin and IGF-I receptor kinase had previously been described in nonneural intact cells (White et al., 1985; Kadowaki et al., 1987), and its existence in neuroblastoma cells suggests that it may represent an ubiquitous substrate for insulin and IGF-I receptor kinases, and that certain early components of the signal transduction pathway for insulin and IGF-I bioeffects may be similar in the central nervous system and the periphery.

Biological Action of Insulin and IGFs in the Nervous System

In classic target tissues, insulin has been considered to be of prime importance in the uptake of plasma glucose and amino acids. These func-

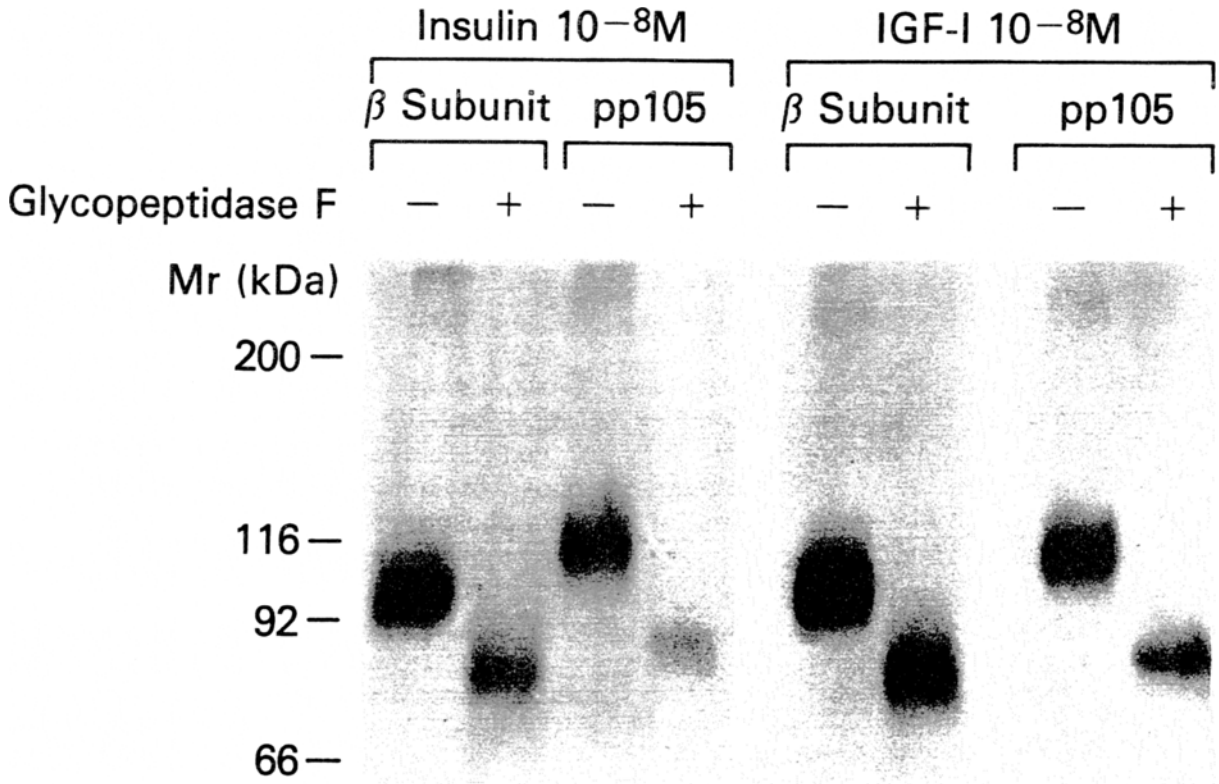

Fig. 7. Glycopeptidase F digestion of β-subunit (95 kDa) and pp105 from N-18 neuroblastoma cells. Wheat germ purified insulin and IGF-I receptors from N-18 cells were incubated with insulin or IGF-I as indicated for 30 min at 22°C and then phosphorylation was carried out by adding Mn^{++} and ^{32}P–ATP. Samples were denatured and run on SDS/PAGE gels under reducing conditions. The bands corresponding to the 95 and 105 kDa phosphoproteins were excised and incubated without (–) or with (+) glycopeptidase F. The digests were then re-electrophoresed. The resulting autoradiogram is shown. As can be seen, complete removal of glycosylation reduced the apparent molecular size of both the 95 kDa β-subunit and pp105 to ~85 kDa, indicating that glycosylation differences and not protein backbone differences explain the different M_rs of the IGF-I β-subunit subtypes (reproduced from Ota et al., 1988b).

tions lead to insulin-induced peripheral utilization of these substrates. Thus, insulin promotes the uptake of glucose and its conversion into glycogen lipid, protein, and energy. The IGFs, on the other hand, have been thought of primarily as regulators of growth and development. The brain, unlike the periphery, has been thought of as insensitive to the metabolic action of insulin. However, insulin and the IGFs exert important biological effects in the central and peripheral nervous system. These effects are summarized in Table 4.

Insulin affects the metabolism of the brain by stimulating glucose uptake into glial cells, activating pyruvate dehydrogenase as well as stimulation of protein and lipid synthesis (Clarke et al., 1984; Rinaudo et al., 1987; Aizenman and de Vellis, 1987; Van der Pal et al., 1988). These effects are seen using nanomolar concentrations of insulin and, thus, are probably mediated by

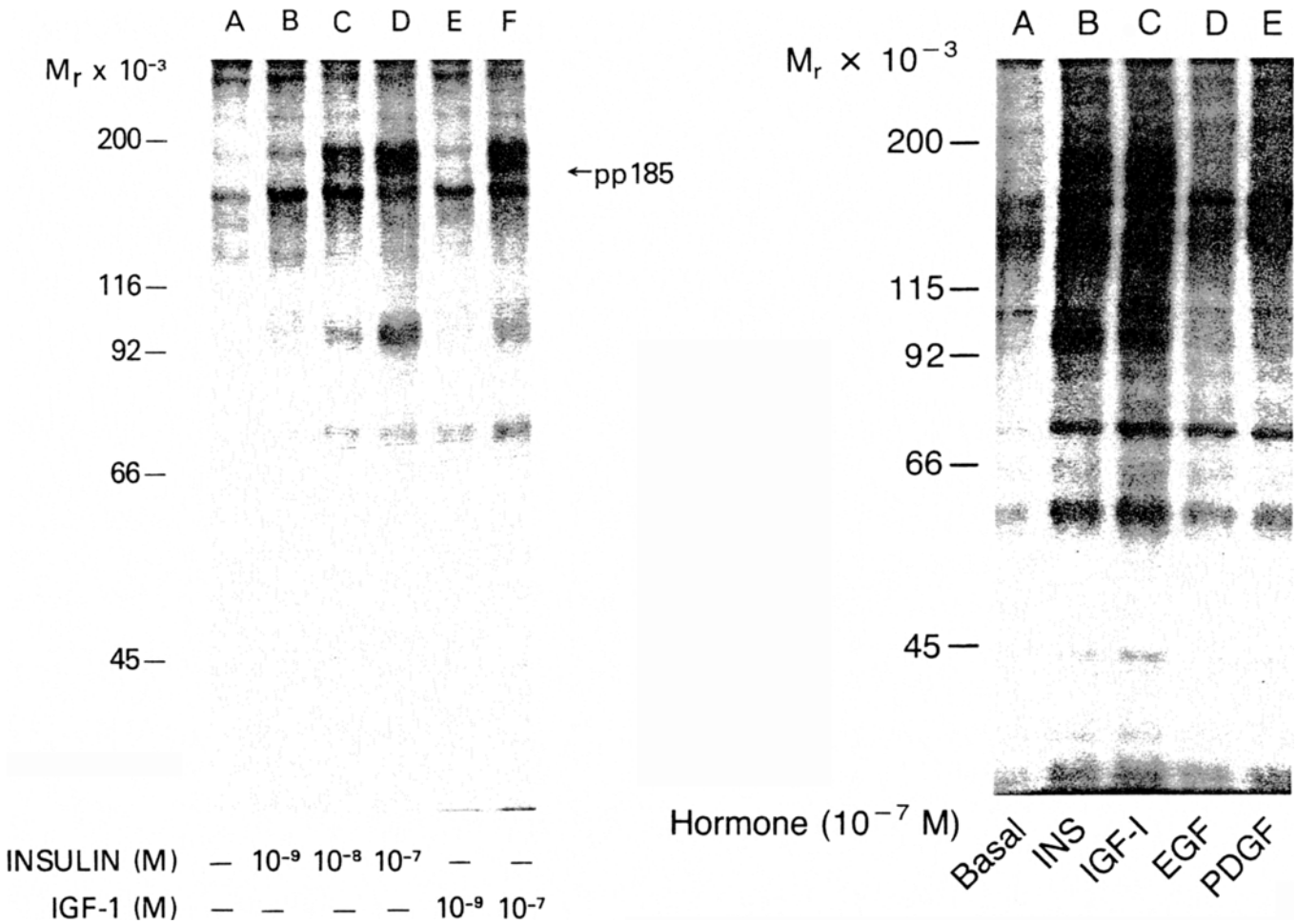

Fig. 8. Insulin and IGF-I stimulated protein phosphorylation in intact N-18 mouse neuroblastoma cells. Left Panel: N-18 mouse neuroblastoma cells were incubated with ^{32}P orthophosphate for 2 h in otherwise phosphate-free and serum-free medium, in order to label intracellular ATP pools. Cells were then incubated without (lane A) or with increasing concentrations of insulin (lanes B, C, D) or IGF-I (lanes E, F) for 1 min. Cells were then solubilized, and whole cell extracts were immunoprecipitated with antiphosphotyrosine antibody. The immunoprecipitates were electrophoresed on SDS/PAGE gels under reducing conditions. Insulin stimulated ^{32}P incorporation into a 95 kDa phosphoprotein (β-subunit), whereas IGF-I stimulated ^{32}P incorporation into 95 and 105 kDa phosphoproteins (β-subunit subtypes). Also, as shown by the arow, insulin and IGF-I stimulated ^{32}P incorporation into a 185 kDa phosphoprotein in these N-18 cells. As seen in the right panel, insulin (lane B) and IGF-I (lane C) but not EGF or PDGF (lanes D, E) stimulated phosphorylation of pp185, identifying it as a specific endogenous substrate for insulin and IGF-I receptor kinases in these neuroblastoma cells (reproduced from Shewer et al. 1987a).

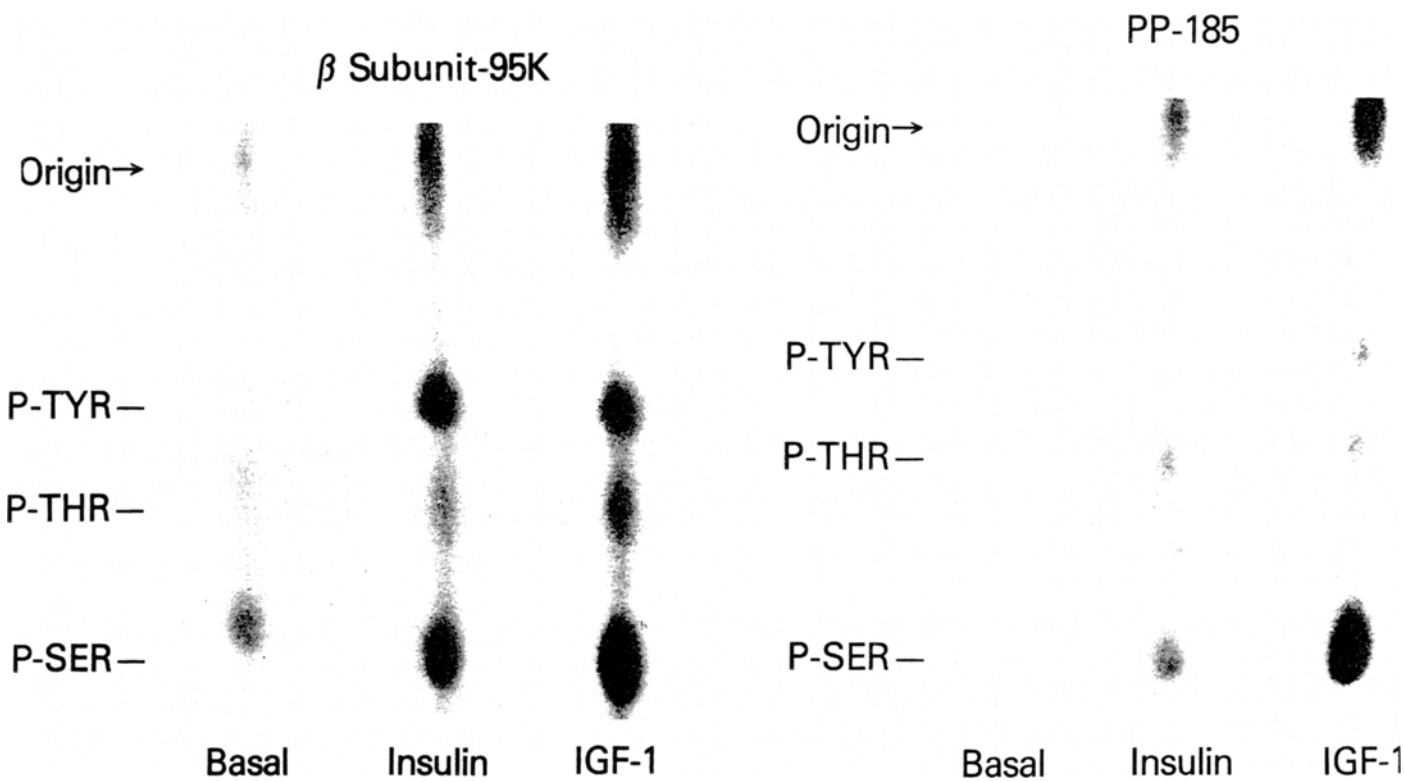

Fig. 9. Phosphoamino acid analysis of 95 kDa β-subunit and pp185 in intact N-18 neuroblastoma cells. N-18 cells were metabolically labeled with ^{32}P, stimulateed with hormones and processed, as described in the legend to Fig. 8. Bands representing the 95 kDa β-subunit (left panel) and the 185kDa phosphoprotein substrate (right panel) were excised, proteins in the gel pieces were hydrolyzed, and phosphoamino acids isolated by ion-exchange chromatography and separated by thin layer electrophoresis. Insulin and IGF-I stimulated ^{32}P incorporation into tyrosine and serine residues and to a lesser extent, threonine residues of both their receptor β-subunits and pp185 (reproduced from Shewer et al. 1987a).

insulin receptors. However, Van der Pal et al., (1988), found that IGF-I could mimic insulin actions on sulfolipid metabolism and specific enzyme activities at lower doses than were optimal for insulin, which suggests that these actions could be mediated by IGF-I receptors. In addition to these metabolic effects, insulin may play an important role as a neuromodulator or neurotransmitter in the central and peripheral nervous system. Thus, it alters neuronal firing rates (Palovcik et al., 1984; Sakaguchi and Bray, 1987), promotes electrical coupling (Wolinsky et al., 1985), and affects other neurotransmitters. Included in the latter are inhibition of norepinephrine reuptake, increased tryptophan and serotonin biosynthesis and transport, and maintenance of synaptosomal Na/K pump activity (Boyd et al., 1985; Raizada et al., 1987; Kwok and Juorio et al., 1987; Bojorge et al., 1987). In cultured chick retinal neurons, insulin stimulation of choline acetyl transferase activity was correlated with insulin receptor binding (Kyriakis et al., 1987). The role of insulin in growth and development includes increased synaptogenesis and neurite formation (Puro and Agardh, 1984; Recio-Pinto and Ishii, 1984; Recio-Pinto et al., 1984, 1986).

The IGFs, on the other hand, are primarily involved in growth and development (Table 4; McMorris et al., 1986; DiCicco-Bloom and Black, 1988), though IGF-I may affect certain metabolic functions, e.g., satiety, either acting through the insulin receptors or via its own receptor (Tannenbaum et al., 1983). Growth and development promoting actions are generally observed at much higher concentrations of insulin as opposed to IGF-I, leading to the suggestion that insulin affects these processes through the IGF-I receptor (McMorris et al. 1986, Ballotti et al., 1987). Both IGFs play an important role in inhibiting GH secretion at the level of the pituitary and hypothalamus (Rosenfeld et al., 1984, 1987), which may represent a physiological negative feedback loop. Although IGF-II may be an important growth factor in fetal brain, as in other fetal tissues, Ballotti et al. (1987), using cultured astroglial cells, have suggested that the mitogenic actions of insulin and both IGFs occur through type I receptors. Thus, despite the presence of an IGF-II receptor protein molecule in the brain (Ocrant et al., 1988; Valentino et al., 1988), it is as yet to be determined if this receptor is involved in signal transduction. Also, as has been discussed by Recio-Pinto and Ishii (1988), crossreactivity of receptor for insulin and the IGFs with pharmacological concentrations of the three ligands may explain overlapping biological functions, although physiological concentrations of each ligand stimulated neurite outgrowth.

As discussed earlier, insulin and IGF-I receptors in the brain differ from their peripheral counterparts chiefly in expressing less glycosylation. In spite of this difference, the brain receptors exhibit normal tyrosine kinase activity, and phosphorylate an endogenous substrate common to peripheral cell types. Thus, both growth promoting and metabolic effects of insulin and IGF-I in the brain are essentially similar to their peripheral actions. However, unique actions of this family of growth factors on brain may lie in a neurotransmitter/neuromodulatory role, and the mechanisms whereby these unique functions are propogated requires further investigation.

Summary

It is apparent from the foregoing review that insulin and the IGFs (I and II) are present in the brain, and that their presence reflects both uptake from the plasma as well as endogenous synthesis. Insulin and IGF-I and -II receptors are present in the brain, on both neuronal and glial cells, as well as brain vasculature, and the neuronal receptors exhibit smaller sized α-subunits owing to altered glycosylation than those on classical targets. These receptors are especially concentrated on the hypothalamus, ol-

factory bulb, and cerebellum, and are generally higher in the perinate than in the adult. Despite slight differences in structure, these receptors possess typical β-subunit autophosphorylation and tyrosine kinase activity and in transformed neural cell lines, phosphorylate a common ubiquitous endogenous substrate. The ligands mediate growth and development (primarily through IGF-I receptors) as well as metabolic function (primarily through insulin receptors). Furthermore, the ligands may further serve as neuromodulators or neurotransmitters and, thus, exert behavioral effects. It is apparent that the brain, which was traditionally thought not to be an insulin target tissue, possesses the requisite "machinery" for insulin and IGF-I signal transduction, and that these signals are essential to the growth, development, and normal functioning of the brain.

Acknowledgments

Work performed in this laboratory was supported in part by grants (to DLR) from the American Diabetes Association (Washington, DC Affiliate) and the Diabetes Research and Education Foundation.

References

Accili D., Perrotti N., Rees-Jones R., and Taylor S. I. (1986) Tissue distribution and subcellular localization of an endogenous substrate (pp120) for the insulin receptor-associated tyrosine kinase. *Endocrinology* **119,** 1274–1280.

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

Agardh C. -D., Lesniak M. A., Gerritsen G. C., and Roth J. (1986) The influence of plasma insulin concentrations on tissue insulin levels in rodents: a study of the diabetic Chinese hamster and ob/ob mouse. *Metabolism* **35,** 244–249.

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

Amir S. and Shechter Y. (1987) Centrally mediated hypoglycemic effect of insulin: apparent involvement of specific insulin receptors. *Brain Res.* **418,** 152–156.

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

Ballotti R., Nielsen F. C., Pringle N., Kowalski A., Richardson W. D., Van Obberghen E., and Gammeltoft S. (1987) Insulin-like growth factor I in cultured rat astrocytes: expression of the gene, and receptor tyrosine kinase. *EMBO J.* **6,** 3633–3639.

Baskin D. G., Brewitt B., Davidson D. A., Corp E., Paquette T., Figlewicz D. P., Lewellen T. K., Graham M. K., Woods S. G., and Dorsa D. M. (1986) Quantitative autoradiographic evidence for insulin receptors in the choroid plexus of the rat brain. *Diabetes* **35,** 246–249.

Baskin D. G., Figlewicz D. P., Woods S. C., Porte D., Jr., and Dorsa D. M. (1987) Insulin in the brain. *Ann. Rev. Physiol.* **49,** 335–347.

Baskin D. G., Porte D., Jr., Guest K., and Dorsa D. M. (1983a) Regional concentrations of insulin in the rat brain. *Endocrinology* **112,** 898–903.

Baskin D. G., Wilcox B. J., Figlewicz D. P., and Dorsa D. M. (1988) Insulin and insulin-like growth factors in the CSN. *Trends Neurosci.* **11,** 107–111.

Baskin D. G., Woods S. C., West D. B., Van Houten M., Posner B. I., Dorsa D. M., and Porte D., Jr. (1983b) Immunocytochemical detection of insulin in rat hypothalamus and its possible uptake from cerebrospinal fluid. *Endocrinology* **113,** 1818–1825.

Bassas L., DePablo F., Lesniak M. A., and Roth J. (1985) Ontogeny of receptors for insulin-like peptides in chick embryo tissues: early dominance of insulin-like growth factor over insulin receptors in brain. *Endocrinology* **117,** 2321–2329.

Bassas L., DePablo F., Lesniak M. A., and Roth J.

(1987) The insulin receptors of chick embryo show tissue-specific structural differences which parallel those of the insulin-like growth factor I receptors. *Endocrinology* **121,** 1468–1476.

Bell G. I., Merryweather J. P., Sanchez-Pescador R., Stempien M. M., Priestly L., Scott J., and Rall L. B. (1984) Sequence of a cDNA clone encoding human preproinsulin-like growth factor II. *Nature* **310,** 775–777.

Bernier M., Frost S. C., Laird D. M., Kohanski R. A., and Lane M. D. (1987) Insulin-activated phosphorylation on tyrosine of a 15 kilodalton cytosolic protein in 3T3 adipocytes, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 11–26.

Binoux M., Hardouin S., Lassarre C., and Hossenlopp P. (1982) Evidence for production by the liver of two IGF binding proteins with similar molecular weights but different affinities for IGF-I and IGF-II. Their relations with serum and cerebrospinal fluid IGF binding proteins. *J. Clin. Endoc. Metab.* **55,** 600–602.

Binoux M., Hossenlopp P., Hardouin S., Seurin D., Lassarre C., and Gourmelen M. (1986) Somatomedin (insulin-like growth factors)-binding proteins. *Horm. Res.* **24,** 141–151.

Binoux M., Hossenlopp P., Lassarre C., and Hardouin N. (1981) Production of insulin-like growth factors and their carrier by rat pituitary gland and brain explants in culture. *FEBS Lett.* **124,** 178–184.

Birch N. P., Christie D. L., and Renwick A. G. C. (1984a) Immunoreactive insulin from mouse brain cells in culture and whole rat brain. *Biochem. J.* **218,** 19–27.

Birch N. P., Christie D. L., and Renwick A. G. C. (1984b) Proinsulin-like material in mouse fetal brain cell cultures. *FEBS Lett.* **168,** 299–302.

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.

Bohannon N. J., Figlewicz D. P., Corp E. S., Wilcox B. J., Porte D., Jr., and Baskin D. G. (1986) Identification of binding sites for an insulin-like growth factor (IGF-I) in the median eminence of the rat brain by quantitative autoradiography. *Endocrinology* **119,** 943–945.

Bojorge G. and deLores Arnaiz G. R. (1987) Insulin modifies Na^+, K^+-ATPase activity of synaptosomal membranes and whole homogenates prepared from rat cerebral cortex. *Neurochem. Int.* **11,** 11–16.

Boyd F. T., Clarke D. W., Muther T. F., and Raizada M. K. (1985) Insulin receptors and insulin modulation of norepinephrine uptake in neuronal cultures from rat brain. *J. Biol. Chem.* **260,** 15880–15884.

Brennan W., Jr. (1987) Insulin receptors in brain development, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 201–208.

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. Biol. Chem.* **261,** 13144–13150.

Budd G. C., Pansky B., and Cordell B. (1986) Detection of insulin synthesis in mammalian anterior pituitary cells by immunohistochemistry and *in situ* RNA-DNA hybridization. *J. Histochem. Cytochem.* **34,** 673–678.

Burgess S. K., Jacobs S., Cuatrecasas P., and Sahyoun N. (1987) Characterization of a neuronal subtype of insulin-like growth factor I receptor. *J. Biol. Chem.* **262,** 1618–1622.

Carlsson-Skwirut C., Jornvall H., Holmgren A., Andersson C., Bergman T., Lundquist G., Sjogren B., and Sara V. R. (1986) Isolation and characterization of variant IGF-I as well as IGF-II from adult human brain. *FEBS Lett.* **201,** 46–50.

Chou C. K., Dull T. J., Russell D. S., Gherzi R., Lebwohl D., Ullrich A., and Rosen O. M. (1987) Human insulin receptors mutated at the ATP-binding site lack protein tyrosine kinase activity and fail to mediate post-receptor effects of insulin. *J. Biol. Chem.* **262,** 1842–1847.

Ciaraldi T., Robbins R., Leidy J. W., Thamm P., and Berhanu P. (1985) Insulin receptors on cultured hypothalamic cells: functional and structural differences from receptors on peripheral target cells. *Endocrinology* **116,** 2179–2185.

Clarke D. W., Boyd F. T., Jr., Kappy M. S., and Raizada M. K. (1984) Insulin binds to specific receptors and stimulates 2-deoxyglucose uptake in cultured glial cells from rat brain. *J. Biol. Chem.* **259,** 11672–11675.

Clarke D. W., Mudd L., Boyd F. T., Jr., Fields M., and Raizada M. K. (1986) Insulin is released from rat

brain neuronal cells in culture. *J. Neurochem.* **47,** 831–836.

Clarke D. W., Poulakos J. J., Mudd, L. M., Raizada M. K., and Cooper D. L. (1987) Evidence for central nervous system insulin synthesis, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips I. M., and LeRoith D., eds.), Plenum Press, New York, pp. 121–130.

Coimbra C. and Migliorini R. H. (1986) Insulin-sensitive glucoreceptors in rat preoptic area that regulate FFA mobilization. *Am J. Physiol.* **251,** E703–E706.

Corp E. S., Woods S. C., Porte D., Jr., Dorsa D. M., Figlewicz D. P., and Baskin D. G. (1986) Localization of ^{125}I-insulin binding sites in the rat hypothalamus by quantitative autoradiography. *Neurosci. Lett.* **70,** 17–22.

DePablo F., Roth J., Hernandez E., and Pruss R. M. (1982) Insulin is present in chicken eggs and early chick embryos. *Endocrinology* **111,** 1909–1916.

D'Ercole A. J., Stiles A. D., and Underwood L. E. (1984) Tissue concentrations of somatomedin C: further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action. *Proc. Natl. Acad. Sci. USA* **81,** 935–939.

Devaskar S. U., Holekamp N., Karycki L., and Devaskar U. P. (1986) Ontogenesis of the insulin receptor in the rabbit brain. *Horm. Res.* **24,** 319–327.

DiCicco-Bloom E. and Black I. B. (1988) Insulin growth factors regulate the mitotic cycle in cultured rat sympathetic neuroblasts. *Proc. Natl. Acad. Sci. USA* **85,** 4066–4070.

Duffy K. R., Pardridge W. M., and Rosenfeld R. G. (1988) Human blood-brain barrier insulin-like growth factor receptor. *Metabolism* **37,** 136–140.

Dunlop M., Dimitriadis E., and Larkins R. G. (1987) Acute changes in myo-inositol uptake and $^{22}Na^+$ flux in murine neuroblastoma cells (N1E-115) following insulin. *FEBS Lett.* **220,** 84–88.

Ebina Y., Araki E., Taira M., Shimada F., Mori M., Craik C. S., Siddle K., Pierce S. B., Roth R. A., and Rutter W. J. (1987) Replacement of lysine residue 1030 in the putative ATP-binding region of the insulin receptor abolishes insulin and antibody stimulated glucose uptake and receptor kinase activity. *Proc. Natl. Acad. Sci. USA* **84,** 704–708.

Ebina Y., Ellis L., Jarnagin K., Edery M., Graf L., Clauser E., Ou H. - H., Masiarz 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 signaling. *Cell* **40,** 747–758.

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

Fujita-Yamaguchi Y., LeBon T. R., Tsubokawa M., Henzel W., Kathuria S., Koyal D., and Ramachandran J. (1986) Comparison of insulin-like growth factor I receptor and insulin receptor purified from human placental membranes. *J. Biol. Chem.* **261,** 16727–16731.

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., Staun-Olsen P., Ottesen B., and Fahrenkrug J. (1984) Insulin receptors in rat brain cortex: kinetic evidence for a receptor subtype in the central nervous system. *Peptides* **5,** 937–944.

Goodyer C. G., DeStephano L., Lai W. H., Guyda H. J., and Posner B. I. (1984) Characterization of insulin-like growth factor receptors in rat anterior pituitary, hypothalamus and brain. *Endocrinology* **114,** 1187–1195.

Characterization of somatomedin/insulin-like growth factor (Sm/IGF) binding proteins (BPs) in conditioned medium from rat astroglia by affinity labeling. *Soc. Neurosci. Abs.* **12,** 1397.

Han V. K. M., D'Ercole A. J., and Lund P. K. (1987) Cellular localization of somatomedin (insulin-like growth factor) messenger RNA in the human fetus. *Science* **236,** 193–197.

Han V. K. M., Lund P. K., Lee D. C., and D'Ercole A. J. (1988) Expression of somatomedin/insulin-like growth factor messenger ribonucleic acids in the human fetus: identification, characterization, and tissue distribution. *J. Clin. Endocrinol. Metab.* **66,** 422–429.

Han V. K. M., Lauder J. M., and D'Ercole A. J. (1988) Fat astrophriel seuroto medium/insulin-like growth factor buildup proteins. Characterization and evidence of biologic function. *J. NeuroScience* **8,** 3135–3143.

Haring H. U., White M. F., Machicao F., Ermel B., Schleicher E., and Obermaier B. (1987) Insulin rap-

idly stimulates phosphorylation of a 46-kDa membrane protein on tyrosine residues as well as phosphorylation of several soluble proteins in intact fat cells. *Proc. Natl. Acad. Sci.* USA **84,** 113–117.

Hart C., Shemer J., Penhos J. C., Lesniak M. A., Roth J., and LeRoith D. (1987) Frog brain and liver show evolutionary conservation of tissue-specific differences among insulin receptors. *Gen. Comp. Endocrinol.* **68,** 170–178.

Haselbacher G. and Humbel R. (1982) Evidence for two species of insulin-like growth factor II (IGF-II and "big" IGF-II) in human spinal fluid. *Endocrinology* **110,** 1822–1824.

Haselbacher G. K., Schway M. E., Pasi A., and Humbel R. E. (1985) Insulin-like growth factor II (IGF-II) in human brain: regional distribution of IGF-II and of higher molecular mass forms. *Proc. Natl. Acad. Sci. USA* **82,** 2153–2157.

Havrankova J., Roth J., and Brownstein M. J. (1979) Concentrations of insulin and insulin receptors in the brain are independent of peripheral insulin levels. Studies of obese and streptozotocin-treated rodents. *J. Clin. Invest.* **64,** 636–642.

Havrankova J., Schmechel D., Roth J., and Brownstein M. (1978) Identification of insulin in rat brain. *Proc. Natl. Acad. Sci. USA* **75,** 5737–5741.

Heidenreich K. A. (1987) Structural evidence for a subtype of insulin receptor in the central nervous system, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 177–190.

Heidenreich K. A. and Brandenberg D. (1986) Oligosaccharide heterogeneity of insulin receptors. Comparison of N-linked glycosylation of insulin receptors in adipocytes and brain. *Endocrinology* **118,** 1835–1842.

Heidenreich K. A., Freidenberg G. R., Figlewicz D. P., and Gilmore P. R. (1986) Evidence for a subtype of insulin-like growth factor I receptor in brain. *Regul. Peptides* **15,** 301–310.

Heidenreich K. A. and Gilmore P. R. (1985) Structural and functional characteristics of insulin receptors in rat neuroblastoma cells. *J. Neurochem.* **45,** 1642–1648.

Heidenreich K. A., Zahniser N. R., Berhanu P., Brandenburg D., and Olefsky J. M. (1983) Structural differences between insulin receptors in the brain and peripheral target tissues. *J. Biol. Chem.* **258,** 8527–8530.

Hendricks S. A., Agardh C. -D., Taylor S. I., and Roth J. (1984a) Unique features of the insulin receptor in rat brain. *J. Neurochem.* **43,** 1302–1309.

Hendricks S. A., DePablo F., and Roth J. (1984b) Early development and tissue specific patterns of insulin binding in chick embryo. *Endocrinology* **118,** 1835–1842.

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.

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.

Jansen M., Van Shaik F. M. A., Ricker A. T., Bullock B., Woods D. E., Gabbay K. H., Nussbaum A. L., Sussenbach J. S., and Van der Brande J. L. (1983) Sequence of a cDNA encoding human insulin-like growth factor I precursor. *Nature* **306,** 609–611.

Kadowaki T., Koyasu S., Nishida E., Tobe K., Izumi T., Takaku F., Sakai H., Yahara I., and Kasuaga M. (1987) Tyrosine phosphorylation of common and specific sets of cellular proteins rapidly induced by insulin, insulin-like growth factor I, and epidermal growth factor in an intact cell. *J. Biol. Chem.* **262,** 7342–7350.

Kappy M. S. and Raizada M. K. (1982) Adult level insulin binding is present in term fetal rat CNS membranes. *Brain Res.* **249,** 390–392.

Kappy M. S., Sellinger, S., and Raizada M. K. (1984) Insulin binding in four regions of the developing rat brain. *J. Neurochem.* **42,** 198–203.

Kasuga M., Van Obberghen E., Nissley S. P., and Rechler M. M. (1982a) Structure of the insulin-like growth factor receptor in chicken embryo fibroblasts. *Proc. Natl. Acad. Sci. USA* **79,** 1864–1868.

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

Kwok R. P. S. and Juorio A. V. (1987) Facilitating effect of insulin on brain 5-hydroxytryptamine metabolism. *Endocrinology* **45,** 267–273.

Kyriakis J. M., Hausman R. E., and Peterson S. W. (1987) Insulin stimulates choline acetyltransferase activity in cultured embryonic chicken retina neurons. *Proc. Natl. Acad. Sci. USA* **84,** 7463–7467.

LeRoith D., Adamo M., Shemer J., Waldbillig R., Lesniak M. A., DePablo F., Hart C., and Roth J. (1988) Insulin-related materials in the nervous system of vertebrates and non-vertebrates: possible extrapancreatic production. *Horm. Metabol. Res.* **20,** 411–420.

LeRoith D., Hendricks S. A., Lesniak M. A., Rishi S., Becher K. L., Havrankova J., Rosenzweig J. L., Brownstein M. J., and Roth J. (1983) Insulin in brain and other extrapancreatic tissues of vertebrate and non-vertebrates, *Advances in Metabolic Diseases,* vol. 10 (Szabo A. J., Levine R., and Luft R., eds.), Academic Press, New York, pp. 303–340.

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.

Lowe W. L., Jr., Boyd F. T., Clarke D. W., Raizada M. K., Hart C., and LeRoith D. (1986) Development of brain insulin receptors: structural and functional studies of insulin receptors from whole brain and primary cell cultures. *Endocrinology* **119,** 25–35.

Lowe W. L., Jr., Lasky S. R., LeRoith D., and Roberts C. T., Jr. (1988) Distribution and regulation of rat insulin-like growth factor I messenger ribonucleic acids encoding alternative carboxyterminal E-peptides: evidence for differential processing and regulation in liver. *Mol. Endocrinol.* **2,** 528–535.

Lowe W. L., Jr. and LeRoith D. (1986) Insulin receptors from guinea pig liver and brain: structural and functional studies. *Endocrinology* **118,** 1669–1677.

Lowe W. L., Jr., Roberts C. T., Jr., Lasky S. R., and LeRoith D. (1987) Differential expression of alternative 5' untranslated regions in mRNAs encoding rat insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **84,** 8946–8950.

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,** 14539–14544.

Machicao F., Haring H., White M. F., Carrascosa J. M., Obermaier B., and Wieland O. H. (1987) An Mr 180,000 protein is an endogenous substrate for the insulin-receptor-associated tyrosine kinase in human placenta. *Biochem. J.* **243,** 797–801.

Madoff D. H., Martensen T. M., and Lane M. D. (1988) Insulin and insulin-like growth factor I stimulate the phosphorylation on tyrosine of a 160 kDa cytosolic protein in 3T3-L1 adipocytes. *Biochem. J.* **252,** 7–15.

Massague J. and Czech M. P. (1982) The subunit structures of two distinct receptors for insulin-like growth factors I and II and their relationship to the insulin receptor. *J. Biol. Chem.* **257,** 5038–5045.

Masters B. A., Shemer J., Judkins J. H., Clarke D. W., LeRoith D., and Raizada M. K. (1987) Insulin receptors and insulin action in dissociated brain cells. *Brain Res.* **417,** 247–256.

Mattsson M. E. K., Enberg G., Ruusala A. -I., Hall K., and Pahlman S. (1986) Mitogenic response of human SH-SY5Y neuroblastoma cells to insulin-like growth factor I and II is dependent on the stage of differentiation. *J. Cell. Biol.* **102,** 1949–1954.

McElduff A., Poronnik P., and Baxter R. C. (1987) The insulin-like growth factor-II (IGF-II) receptor from rat brain is of lower apparent molecular weight than the IGF-II receptor from rat liver. *Endocrinology* **121,** 1306–1311.

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

McElduff A., Watkinson A., Hedo J. A., and Gorden P. (1986) Characterization of the N-linked high-mannose oligosaccharides of the insulin pro-receptor and mature insulin receptor subunits. *Biochem. J.* **239,** 679–683.

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

Morgan D. O., Edman J. C., Standring D. N., Fried V. A., Smith M. C., Roth R. A., and Rutter W. J. (1987) Insulin-like growth factor II receptor as a multifunctional binding protein. *Nature* **329,** 301–307.

Morgan D. O., Jarnagin K., and Roth R. A. (1986) Purification and characterization of the receptor for insulin-like growth factor I. *Biochemistry* **25,** 5560–5564.

Morgan D. O. and Roth R. A. (1987) Acute insulin action requires insulin receptor kinase activity: introduction of an inhibitory monoclonal antibody into mammalian cells blocks the rapid effects of in-

sulin. *Proc. Natl. Acad. Sci. USA* **84**, 41–45.

Murphy L. J., Bell G. I., and Friesen H. G. (1987) Tissue distribution of insulin-like growth factor I and II messenger ribonucleic acid in the adult rat. *Endocrinology* **120**, 1279–1282.

Nilsson L., Sara V. R., and Nordberg A. (1988) Insulin-like growth factor I stimulates the release of acetylcholine from rat cortical slices. *Neurosci. Lett.* **88**, 221–226.

Noguchi T., Kurata L. M., and Sugisaki T. (1987) Presence of somatomedin-C-immunoreactive substances 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. G. (1988) Structural and immunohistochemical characterization of insulin-like growth factor I and II receptors in the murine central nervous system. *Endocrinology* **123**, 1023–1034.

Ota A., Shemer J., Pruss R. M., Lowe W. L., Jr., and LeRoith D. (1988a) Characterization of the altered oligosaccharide composition of the insulin receptor on neural derived cells. *Brain Res.* **443**, 1–11.

Ota A., Wilson G. L., and LeRoith D. (1988b) Insulin-like growth factor I receptors on mouse neuroblastoma cells. Two β subunits are derived from differences in glycosylation. *Eur. J. Biochem.* **174**, 521–530.

Ota A., Wilson G. L., Spilberg O., Pruss R., and LeRoith D. (1988c) Functional insulin-like growth factors I receptors are expressed by neural-derived continuous cell lines. *Endocrinology* **122**, 145–152.

Pacold S. T. and Blackard W. G. (1979) Central nervous system insulin receptors in normal and diabetic rats. *Endocrinology* **105**, 1452–1457.

Palovcik R. A., Phillips M. I., Kappy M. S., and Raizada M. K. (1984) Insulin inhibits pyramidal neurons in hippocampal slices. *Brain Res.* **309**, 187–191.

Pardridge W. F., Eisenberg J., and Young J. (1985) Human blood-brain barrier insulin receptor. *J. Neurochem.* **44**, 1771–1778.

Perdue J. F. (1984) Chemistry, structure and function of insulin-like growth factors and their receptors: a review. *Can. J. Biochem. Cell. Biol.* **62**, 1237–1245.

Peterson S. W., Kyriakis J. M., and Hansmann R. E. (1986) Changes in insulin binding to developing embryonic chick neural retina cells. *J. Neurochem.* **47**, 851–855.

Petruzzelli L., Herrera R., and Rosen O. M. (1984) Insulin receptor is an insulin-dependent tyrosine protein kinase: copurification of insulin-binding activity and protein kinase activity to homogeneity from human placenta. *Proc. Natl. Acad. Sci. USA* **81**, 3327–3331.

Phillips M. I. (1987) Insulin in the brain: a feedback loop involving brain insulin and circumventricular organs, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 163–175.

Porte D. and Woods S. C. (1981) Regulation of food intake and body weight by insulin. *Diabetologia* **20**, 274–280.

Puro D. G. and Agardh E. (1984) Insulin-mediated regulation of neuronal maturation. *Science* **225**, 1170–1172.

Raizada M. K. (1983) Localization of insulin-like immunoreactivity in neurons from primary cultures of rat brain. *Exp. Cell Res.* **143**, 351–357.

Raizada M. K., Boyd F. T., Clarke D. W., and LeRoith D. (1987) Physiologically unique insulin receptors on neuronal cells, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 191–200.

Raizada M. K., Shemer J., Judkins J. H., Clarke D. W., Masters B. A., and LeRoith D. (1988) Insulin receptors in the brain: structural and physiological characterization. *Neurochem. Res.* **13**, 297–303.

Recio-Pinto E. and Ishii D. N. (1984) Effects of insulin, insulin-like growth factor II and nerve growth factor on neurite outgrowth in cultured human neuroblastoma cells. *Brain Res.* **302**, 323–334.

Recio-Pinto E. and Ishii D. N. (1988) Insulin and insulin-like growth factor receptors regulating neurite formation in cultured human neuroblastoma cells. *J. Neurosci. Res.* **19**, 312–320.

Recio-Pinto E., Lang F. F., and Ishii D. N. (1984) Insulin and insulin-like growth factor II permit nerve growth factor binding and the neurite formation response in cultured human neuroblastoma cells. *Proc. Natl. Acad. Sci. USA* **81**, 2562–2566.

Recio-Pinto E., Rechler M. M., and Ishii D. N. (1986) Effects of insulin, insulin-like growth factor II, nerve growth factor on neurite formation and sur-

vival in cultured sympathetic and sensory neurons. *J. Neurosci.* **6**, 1211–1291.

Rees-Jones R. W., Hendricks S. A., Quarum M., and Roth J. (1984) The insulin receptors of rat brain are coupled to tyrosine kinase activity. *J. Biol. Chem.* **259**, 3470–3474.

Rees-Jones R. W. and Taylor S. I. (1985) An endogenous substrate for the insulin receptor-associated tyrosine kinase. *J. Biol. Chem.* **260**, 4461–4467.

Richards E. M., Raizada M. K., and Sumners C. (1987) Insulin down-regulates alpha-2 adrenergic receptors in cultured glial cells, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 209–214.

Rinaudo M. T., Curto M., Bruno R., Marino C., Rossetti V., and Mostert M. (1987) Evidence of an insulin generated pyruvate dehydrogenase stimulating factor in rat brain plasma membranes. *Int. J. Biochem.* **19**, 909–913.

Rosen O. M., Herrera R., Olowe Y., Petruzzelli L. M., and Cobb M. H. (1983) Phosphorylation activates the insulin receptor tyrosine protein kinase. *Proc. Natl. Acad. Sci. USA* **80**, 3237–3240.

Rosenfeld R. G., Ceda G., Wilson D. M., Dollar L. A., and Hoffman A. R. (1984) Characterization of high affinity receptors for insulin-like growth factors, I and II on rat anterior pituitary cells. *Endocrinology* **114**, 1571–1575.

Rosenfeld R. G. and Hoffman A. R. (1987) Insulin-like growth factors and their receptors in the pituitary and hypothamamus, *Insulin, Insulin-like Growth Factors, and Their Receptors in the Central Nervous System* (Raizada M. K., Phillips M. I., and LeRoith D., eds.), Plenum Press, New York, pp. 277–295.

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

Rosenzweig J. L., Havrankova J., Lesniak M. A., Brownstein M., and Roth J. (1980) Insulin is ubiquitous in extrapancreatic tissues of rats and humans. *Proc. Natl. Acad. Sci. USA* **77**, 572–576.

Roth R. A. (1988) Structure of the receptor for insulin-like growth factor II: the puzzle amplified. *Science* **239**, 1269–1271.

Roth R. A. and Cassell D. J. (1983) Insulin receptor: evidence that it is a protein kinase. *Science* **219**, 299–301.

Roth R. A., Stover C., Hari J., Morgan D. O., Smith M. C., Sara V., and Fried V. A. (1987) Interactions of the receptor for insulin-like growth factor II with mannose-6-phosphate and antibodies to the mannose-6-phosphate receptor. *Biochem. Biophys. Res. Comm.* **149**, 600–606.

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

Sakaguchi T. and Bray G. A. (1987) Intrahypothalamic injection of insulin decreases firing rat of sympathetic nerves. *Proc. Natl. Acad. Sci. USA* **84**, 2012–2014.

Sara V. R., Carlsson-Skwirut C., Andersson C., Hall E., Sjogren B., Holmgren A., and Jornvall H. (1986) Characterization of somatomedins from human fetal brain: identification of a variant form of insulin-like growth factor I. *Proc. Natl. Acad. Sci. USA* **83**, 4904–4907.

Sara V. R., Hall K., Misake M., Frylund L., Christensen N., and Wetterberg L. (1983) Ontogenesis of somatomedin and insulin receptors in the human fetus. *J. Clin. Invest.* **71**, 1084–1094.

Sara V. R., Uvnas-Moberg K., Uvnas B., Hall K., Wetterberg L., Posslancec B., and Goiny M. (1982) The distribution of somatomedins in the nervous system of the cat and their release following neural stimulation. *Acta Physiol. Scand.* **115**, 467–470.

Schechter R., Holtzclaw L., Sadiq F., Kahn A., and Devaskar S. (1988) Insulin synthesis by isolated rabbit neurons. *Endocrinology* **123**, 505–513.

Shemer J., Adamo M., Raizada M. K., Heffez D., Zick Y., and LeRoith 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.

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 (pp 185) in intact neuroblastoma cells. *J. Biol. Chem.* **262**, 15476–15482.

Shemer J., Ota A., Adamo M., and LeRoith D. (1988) Insulin-sensitive tyrosine kinase is increased in livers of adult obese Zucker rats: correction with prolonged fasting. *Endocrinology* **123**, 140–148.

Shemer J., Penhos J. C., and LeRoith D. (1986) Insulin receptors in lizard brain and liver: structural and functional studies of α and β subunits demonstrate evolutionary conservation. *Diabetologia* **29,** 321–329.

Shemer J., Raizada M., and LeRoith D. (1987b) Structural and functional studies on insulin receptors from alligator brain and liver. *Comp. Biochem. Physiol.* **85B,** 1–10.

Shemer J., Raizada M. K., Masters B. A., Ota A., and LeRoith D. (1987c) Insulin-like growth factor I receptors in neuronal and glial cells. Characterization and biological effects in primary culture. *J. Biol. Chem.* **262,** 7693–7699.

Shiu R. P. C. and Paterson J. A. (1988) Characterization of insulin-like growth factor II peptides secreted by explants of neonatal brain and of adult pituitary from rats. *Endocrinology* **123,** 1456–1460.

Simon J., Rosebrough R. W., McMurtry J. P., Steele N. C., Roth J., Adamo M., and LeRoith D. (1986) Fasting and refeeding alter the insulin receptor tyrosine kinase in chicken liver but fail to affect brain insulin receptors. *J. Biol. Chem.* **261,** 17081–17088.

Soares M. B., Turken A., Ishii D., Mills L., Episkopou V., Cotter S., Zeitlin S., and Efstratiadis A. (1986) Rat insulin-like growth factor II gene. A single gene with two promoters expressing a multitranscript family. *J. Mol. Biol.* **192,** 737–752.

Stein L. J., Dorsa D. M., Baskin D. G., Figlewicz D. P., Ikeda H., Frankmann S. P., Greenwood M. R. C., Porte D., Jr., and Woods S. P. (1983) Immunoreactive insulin levels are elevated in the cerebrospinal fluid of genetically obese Zucker rats. *Endocrinology* **113,** 2299–2301.

Stylianopoulou F., Herbert J., Soares M. B., and Efstratiatis A. (1988) 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. USA* **85,** 141–145.

Tannenbaum G., Guyda H., and Posner B. (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation *via* brain. *Science* **220,** 77–79.

Tong P. Y., Tollefsen S. E., and Kornfeld S. (1988) The cation-independent mannose-6-phosphate receptor binds insulin-like growth factor II. *J. Biol. Chem.* **263,** 2585–2588.

Ullrich A., Bell J. R., Chen E. Y., Herrera R., Petruzzelli L. M., Dull T. J., Gray A., Coussens L., Liao Y. -C., Toubokawa 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., Tsubokawa M., Collins C., Henzel W., LeBon T., Kathuria S., Chem E., Jacobs S., Francke 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.

Uvnas-Moberg K., Posloncec B., Hagerman M., Castensson S., Rubio C., and Uvnas B. (1982) Occurrence of an insulin-like peptide in extracts of peripheral nerves of the cat and in extracts of human vagal nerves. *Acta Physiol. Scand.* **115,** 471–477.

Uvnas-Moberg K., Posloncec B., Jacobsson B., and Uvnas B. (1988) Occurrence of an insulin-like peptide in extracts of human nervous tissue. *Acta Physiol. Scand.* **133,** 127–130.

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.

Van der Pal R. H. M., Koper J. W., van Golde L. M. G., and Lopes-Cardozo M. (1988) Effects of insulin and insulin-like growth factor (IGF-I) on oligodendrocyte-enriched glial cultures. *J. Neurosci. Res.* **19,** 483–490.

Van Houten M. and Posner B. I. (1979) Insulin binds to brain blood vessels in vivo. *Nature* **282,** 623–625.

Van Houten M., Posner B. I., Kopriwa B. M., and Brawer J. R. (1979) Insulin-binding sites in the rat brain: in vivo localization to the circumventricular organs by quantitative radioautography. *Endocrinology* **105,** 666–673.

Van Houten M., Posner B. I., Kopriwa B. M., and Brawer J. R. (1980) Insulin binding sites localized to nerve terminals in rat median eminence and arcuate nucleus. *Science* **207,** 1081–1083.

Van Schravendijk C. F. H., Hooghe-Peters E. L., DeMeyts P., and Pipeleers D. G. (1984) Identification and characterization of insulin receptors on fetal-mouse brain-cortical cells. *Biochem. J.* **220,** 165–172.

Verspohl R. J., Maddux B. A., and Goldfine I. D.

(1988) Insulin and insulin-like growth factor I regulate the same biological functions in HEP-G2 cells via their own specific receptors. *J. Clin. Endocrinol. Metab.* **67**, 169–174.

Waheed A., Braulke T., Junghans U., and von Figura K. (1988) Mannose-6-phosphate/insulin-like growth factor II receptor: the two types of ligands bind simultaneously to one receptor at different sites. *Biochem. Biophys. Res. Comm.* **152**, 1248–1254.

Waldbillig R. J., Fletcher R. T., Chader G. J., Rajagopalan S., Rodigues M., and LeRoith D. (1987a) Retinal insulin receptors. 1. Structural heterogeneity and functional characterization. *Exp. Eye Res.* **45**, 823–835.

Waldbillig R. J., Fletcher R. T., Chader G. J., Rajagopalan S., Rodrigues M., and LeRoith D. (1987b) Retinal insulin receptors. 2. Characterization and insulin-induced tyrosine kinase activity in bovine retinal rod outer segments. *Exp. Eye Res.* **45**, 837–844.

Waldbillig R. J. and LeRoith D. (1987) Insulin receptors in the peripheral nervous system: a structural and functional analysis. *Brain Res.* **409**, 215–220.

Wallum B. J., Taborsky G. J., Porte D., Jr., Figlewicz D. P., Jacobson L., Beard J. C., Ward W. K., and Dorsa D. (1987) Cerebrospinal fluid insulin levels increase during intravenous insulin infusion in man. *J. Clin. Endoc. Metab.* **64**, 190–194.

Weyhenmeyer J. A. and Fellows R. E. (1983) Presence of immunoreactive insulin in neurons cultured from fetal rat brain. *Cell. Mol. Neurobiol.* **3**, 81–86.

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

Wolinsky E. J., Patterson P. H., and Willard A. L. (1985) Insulin promotes electrical coupling between cultured sympathetic neurons. *J. Neurosci.* **5**, 1675–1679.

Yamashita S. and Melmed S. (1986) Effects of insulin on rat anterior pituitary cells. Inhibition of growth hormone secretion and mRNA levels. *Diabetes* **35**, 440–447.

Yip C. C., Moule M. L., and Yeung C. W. T. (1980) Characterization of insulin receptor subunits in brain and other tissues by photoaffinity labeling. *Biochem. Biophys. Res. Comm.* **96**, 1671–1678.

Young W. S., III (1986) Periventricular hypothalamic cells in the rat brain contain insulin mRNA. *Neuropeptides* **8**, 93–97.

Zafp J., Schmid C. H. and Froesch E. R. (1984) Biological and immunological properties of insulin-like growth factors (IGF) I and II. *Clin. Endocrinol. Metab.* **13**, 3–30.

Zick Y., Grunberger G., Podskalny J. M., Mondada V., Taylor S. I., Gorden P., and Roth J. (1983) Insulin stimulates phosphorylation of serine residues in soluble insulin receptors. *Biochem. Biophys. Res. Comm.* **116**, 1129–1135.

Zick Y., Whittaker J., and Roth J. (1983) Insulin stimulated phosphorylation of its own receptor. Activation of a tyrosine-specific protein kinase that is tightly associated with the receptor. *J. Biol. Chem.* **258**, 3431–3434.

Zick Y., Spiegel A. M., and Sagi-Eisenberg R. (1987) IGF-I receptors in retinal rod outer segments. *J. Biol. Chem.* **262**, 10259–10264.

Zumstein P. R., Luthi C., and Humbel R. E. (1985) Amino acid sequence of a variant pro-form of insulin-like growth factor II. *Proc. Natl. Acad. Sci. USA* **82**, 3169–3174.